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Lessons Learned from Preclinical and Clinical Development of microRNA Therapies

Dr. Denis Drygin, CSO

RNA Leaders World Congress. Basel, March 17h, 2022

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Statements contained in this presentation regarding matters that are not historical facts are "forward-looking statements" within the meaning of the Private Securities Litigation Reform Act of 1995, including statements associated with the Company's RGLS8429 program, including the potential sufficiency of the preclinical data required to support clinical studies, the expected timing for submitting an IND and initiating Phase 1 clinical studies, the expected timing for reporting topline data, and the timing and future occurrence of other preclinical and clinical activities. Because such statements are subject to risks and uncertainties, actual results may differ materially from those expressed or implied by such forward-looking statements. Words such as "believes," "anticipates," "plans," "expects," "intends," "will," "goal," "potential" and similar expressions are intended to identify forward-looking statements. These forward-looking statements are based upon Regulus' current expectations and involve assumptions that may never materialize or may prove to be incorrect. Actual results and the timing of events could differ materially from those anticipated in such forward-looking statements as a result of various risks and uncertainties, which include, without limitation, risks associated with the process of discovering, developing and commercializing drugs that are safe and effective for use as human therapeutics and in the endeavor of building a business around such drugs, and the risk additional toxicology data may be negative. In addition, while Regulus expects the COVID-19 pandemic to adversely affect its business operations and financial results, the extent of the impact on Regulus' ability to achieve its preclinical and clinical development objectives and the value of and market for its common stock, will depend on future developments that are highly uncertain and cannot be predicted with confidence at this time, such as the ultimate duration of the pandemic, travel restrictions, quarantines, social distancing and business closure requirements in the U.S. and in other countries, and the effectiveness of actions taken globally to contain and treat the disease. These and other risks are described in additional detail in Regulus' filings with the Securities and Exchange Commission, including under the "Risk Factors" heading of Regulus most recently quarterly and year end report on Form 10-K. All forward-looking statements contained in this press release speak only as of the date on which they were made. Regulus undertakes no obligation to update such statements to reflect events that occur or circumstances that exist after the date on which they were made.

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Regulus Therapeutics Highlights

Leading microRNA company focused on translating novel technology into innovative therapeutics

Pioneering microRNA therapeutics targeting genetic kidney disease in the clinic with RGLS8429 (IND ready) in ADPKD and Lademirsen/ RG-012 in Alport Syndrome (in partnership with Sanofi)

Advancing broader pipeline targeting novel biology in areas of unmet need

Disciplined discovery and development approach focused on novel targets with validated in-vitro / in-vivo model systems

IONIS PHARMACEUTICALS

Alnylam PHARMACEUTICALS

Expertise in microRNA biology and oligo chemistry based on company foundation through joint venture between Ionis and Alnylam and access to IP/technology

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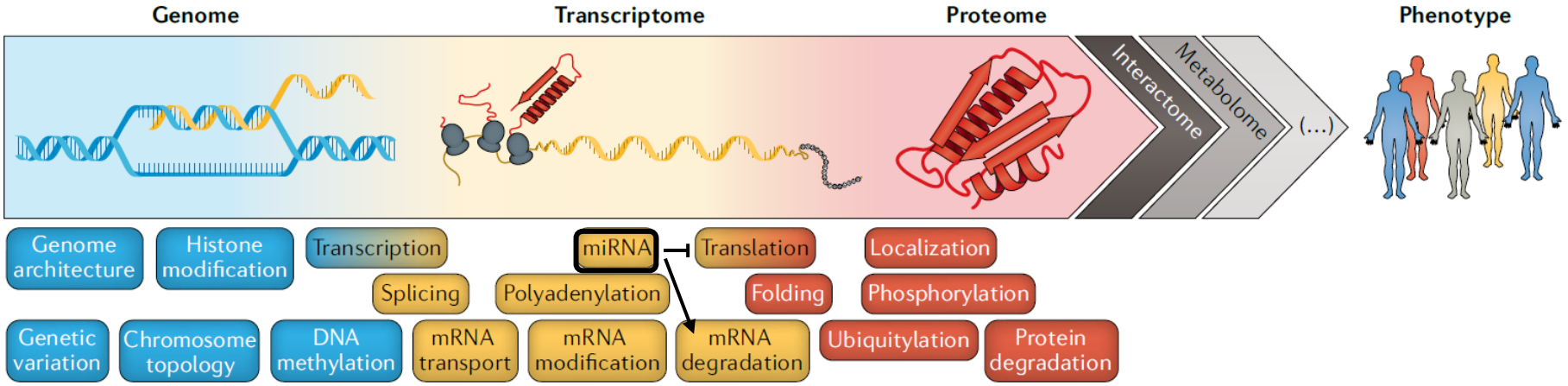
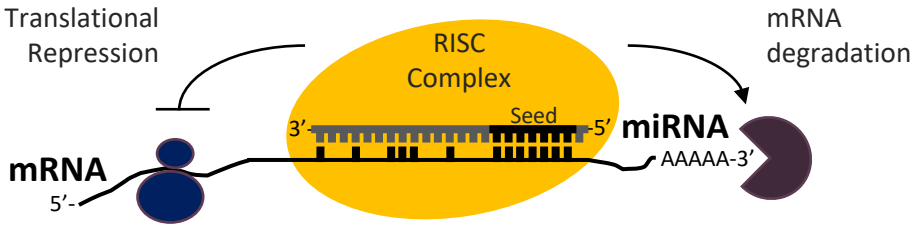
microRNAs - Post-Transcriptional Regulators of Gene Expression



Buccitelli and Selbach, (2020) 21:630 Nat. Rev. Genet

- MicroRNAs are highly conserved, short non-coding RNAs (20-22 nucleotides long) with unique seed sequence of 7-8 nucleotides that bind to complementary target sequences located in the 3' untranslated region (UTR) of targeted mRNAs **and repress mRNA**
- A single microRNA (miRNA) can bind to and repress translation of **multiple** different mRNAs

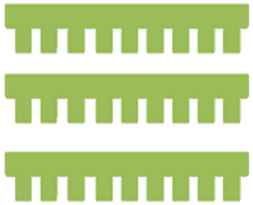


➢ **microRNAs represent promising therapeutic targets for various pathologies**

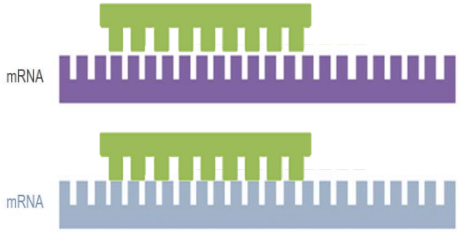
microRNA: The Micro-Manager of Pathways

Disease Phenotype

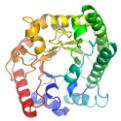
1 microRNA overexpressed in disease state



2 microRNA blocks translation of target mRNA

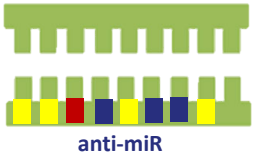


3 Resulting in under expressed protein



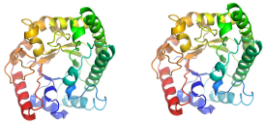
Restoring Phenotype

Chemically modified anti-miR binds to target microRNA



De-repression of target mRNA

Gain of function in pathway



➢ **Targeting microRNA with synthetic microRNA antagonist oligonucleotides represents a potential new class of medicines by improving disease-state pathways towards normal phenotypes**

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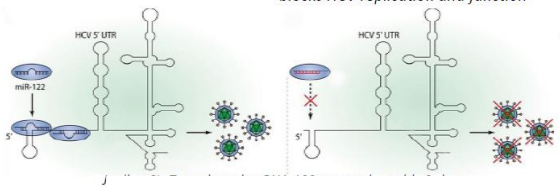
RG-101: Clinically Validated Anti-miR-122

miR-122 is a critical host factor for HCV replication

Inhibition of miR-122 in hepatocytes blocks HCV replication and function

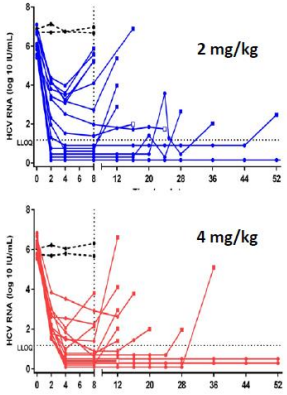


- miR-122 binds to **two seed sequences within IRES that enable HCV replication and function**
- miR-122 seed sequences are **highly conserved across all HCV genotypes**
- Development of escape mutants is highly unlikely

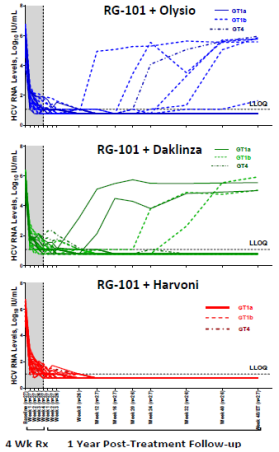
Regulus Controls Key IP Associated with miR-122
US Patent No. 7,307,067, 7,838,504, 8,217,020
EP Patent No. 1,747, 023
Japan Patent No. 4,943,322

Viral Load Reduction in HCV Patients

Single SC Dose of RG-101 in GT1, 3, and 4 HCV Patients



HCV Patients: RG-101 + 4 weeks DAA

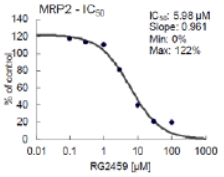


- **Potent and durable viral response after a single dose with 3 of 28 patients being HCV negative after 1 year**
- **RG-101 demonstrated significant virologic response in combination with direct-acting antiviral medications (100% response + 4 weeks of Harvoni; 80% response + 4 weeks of Olysio or Daklinza)**
- **Ten of 200 RG-101 treated subjects (5%) experienced transient hyperbilirubinemia with two cases of Grade 3 (jaundice) with unexplained etiology**
- **Despite no Hy's Law cases, in June 2016 FDA placed RG-101 on clinical hold requesting identification of potential mechanism of hyperbilirubinemia**

Adapted From: Neben. TIDES Conference 2018

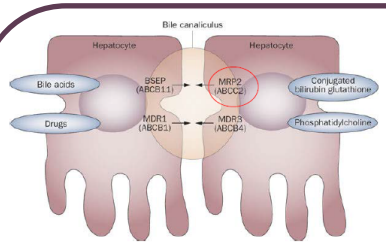
The Identified Culprit of RG-101 Toxicity: Inhibition of MRP2, a Transporter for Conjugated Bilirubin

Transporter	Drug	IC_{50} (µM)
BSEP	RG-101	>100
MRP2		**5, 6, 20, 62 (n=4)**
MRP3		>100
MRP4		>100



Based on preclinical data estimated RG-101 liver concentration is 12 µM when dosed at 4 mg/kg

Adapted From: Neben. TIDES Conference 2018



- Bilirubin is produced as a byproduct of heme metabolism
- Direct (or Conjugated) bilirubin is produced in the liver by glucuronidation
- Conjugated bilirubin is excreted into the bile by Multidrug Resistance-Associated Protein-2 (MRP2), which is expressed on the apical side of hepatocytes
- Interference with MRP2-mediated bilirubin excretion leads to elevated levels in blood

- Known MRP2 inhibitors causing hyperbilirubinemia (examples): ritonavir, abacavir, tenofovir, probenecid, furosemide, cyclosporin A
- Genetic defects in MRP2: Dubin Johnson Syndrome is a human autosomal recessive genetic deficiency of MRP2 associated with episodic jaundice due to conjugated hyperbilirubinemia; otherwise, a benign clinical condition with no long-term liver sequelae[1]
- ***MRP2 expression is lower in humans compared with rodents and monkeys and is further reduced (by approx. 70%) in HCV patients[2,3], which makes HCV patients more sensitive than normal animals***

1. Keppler, Drug Metab Dispos (2014), 2. Li et al., Drug Metab Dispos (2009), 3. Hinoshita et al., J Hepatol (2001)

➢ **Contrary to popular belief oligonucleotide-based therapeutics can have off-target effects similarly to small molecule drugs**

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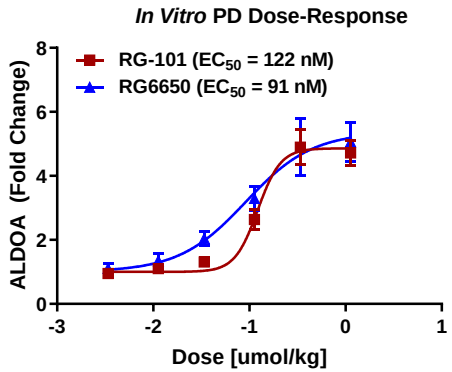
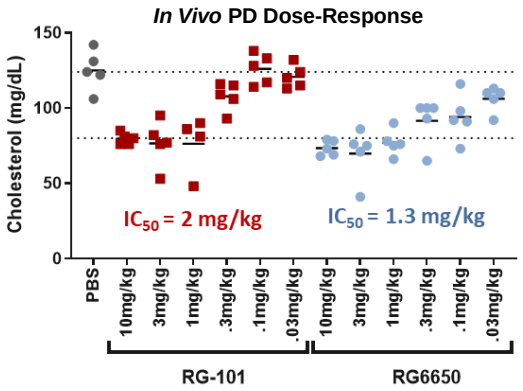
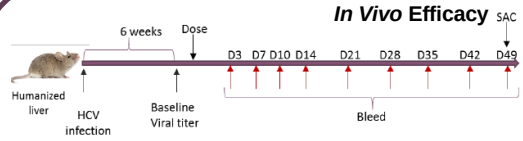
RGLS6650 – 2nd Generation miR-122 Antagonist Devoid of MRP2 Inhibitory Activity

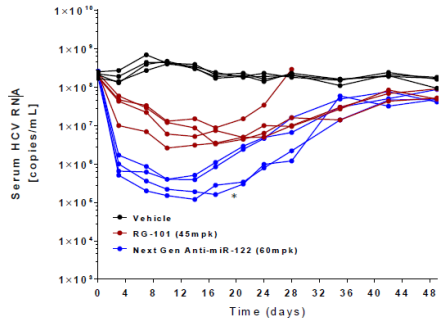
Compound	MRP2 IC50 (μM)
RG-101	31.0
RG6650	>150





We were able to address the shortcoming of RG-101 with 2nd generation compound, RG6650, which exhibited activity similar to RG-101, but was devoid of MRP2 inhibitory activity





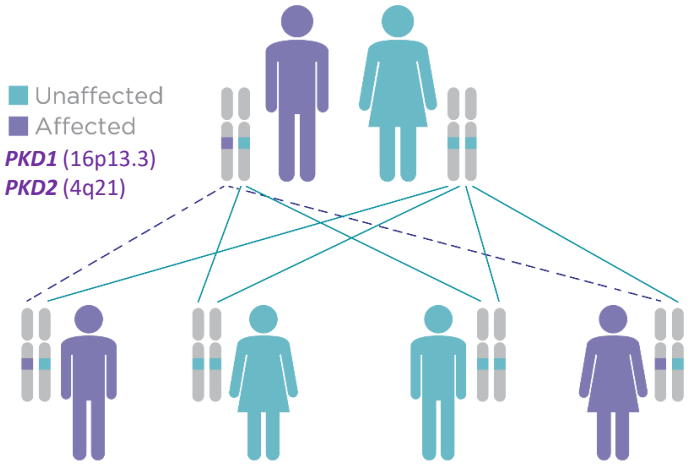
- **PXB Chimeric mouse with humanized liver:**
  - >80% of liver is human hepatocytes
  - Liver secretes human albumin and human-like bile
  - >95% of mRNAs in human liver are expressed in the PXB-mouse

Adapted From: Neben. TIDES Conference 2018

➢ **Using proprietary platform, Regulus was able to quickly develop second generation miR-122 antagonist RGLS6650, which is equipotent to RG-101 but is devoid of MRP2 inhibitory activity**

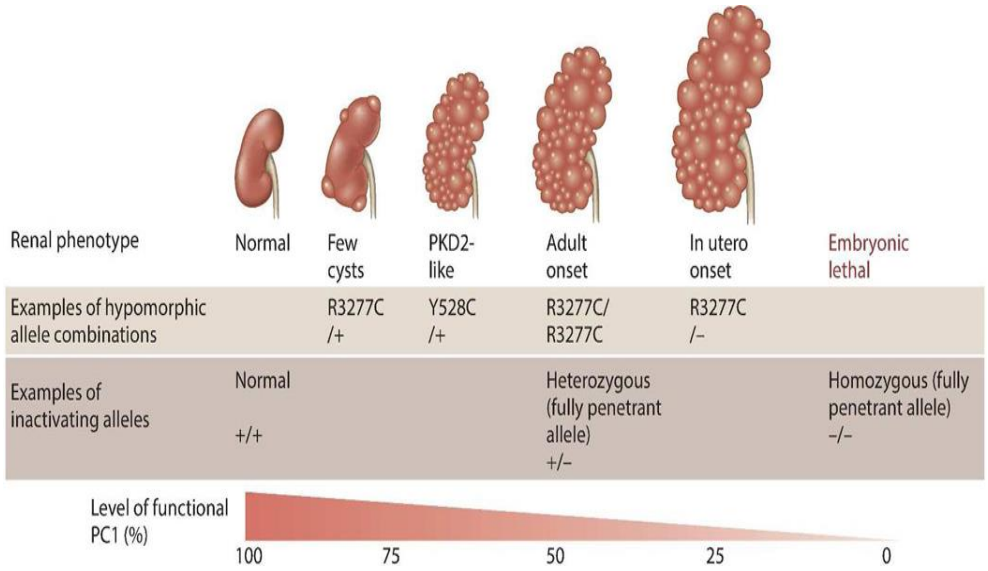
Autosomal Dominant Polycystic Kidney Disease (ADPKD)

- ADPKD is a monogenetic disorder with 160,000 patients diagnosed in US alone that is 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. 50% of patients develop ESRD by age of 60 with estimated annual cost of renal replacement therapy in U.S. being > $3.5B.



Cloutier et al. (2020) BMC Health Serv. Res. 20:126
Graphics adapted from PKD Foundation

- 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.

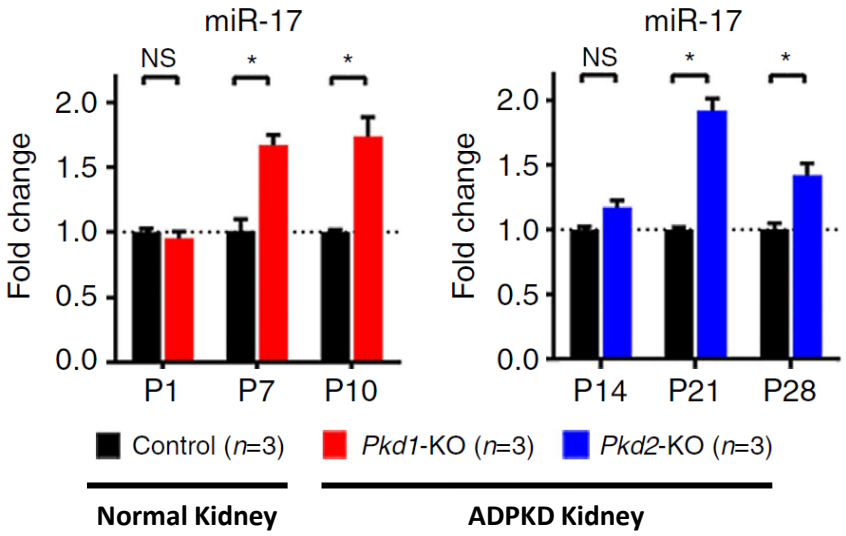


Dosage Model of Cystogenesis in ADPKD

Ong et al, Kidney Int 2015
Bergmann et al, Nat Reve Dis Primers 2019

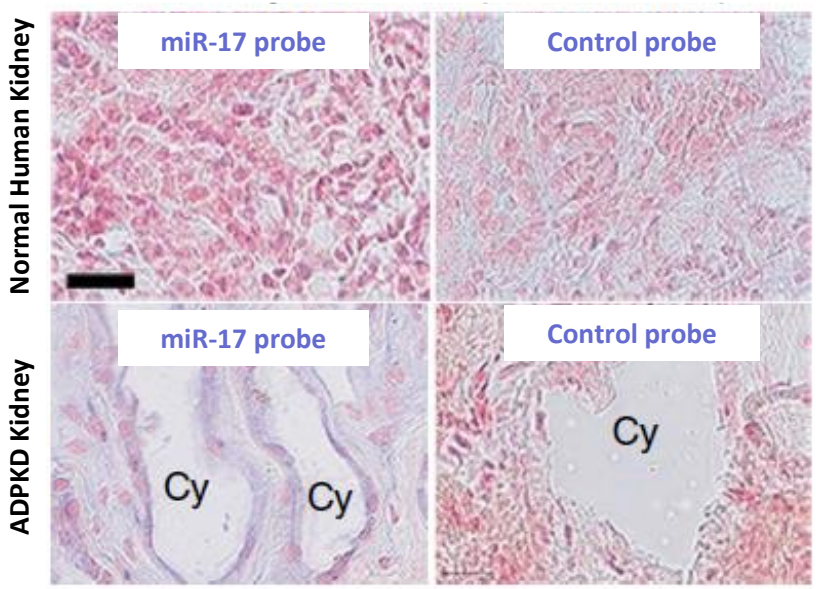
miR-17 is Upregulated in Mouse Kidney Cysts and Human ADPKD Cyst Cells

Mouse Kidney Samples
qPCR analysis



Samples taken at postnatal day (P)1, P7 and P10 for *Pkd1*-KO or Control; and P14, P21 and 28 for *Pkd2*-KO or Control

Human Kidney Samples
In-situ hybridization



Patel et al. (2013) PNAS Jun 25;110(26):10765
Hajarnis et al. (2017) Nat. Commun. Feb 16;8:14395

➢ **miR-17 upregulation also observed in other PKD mouse models**

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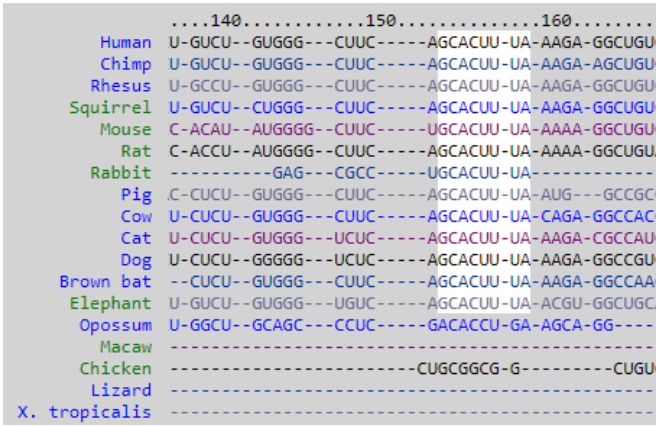
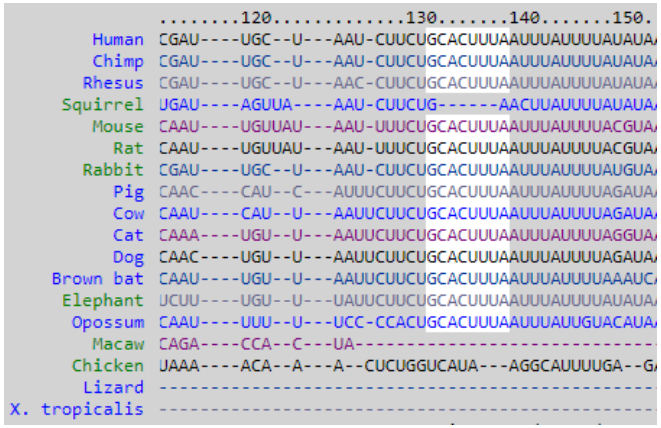
miR-17 Directly Binds *PKD1* & *PKD2* Genes Mediating ADPKD

- 3'UTRs of *PKD1* and *PKD2* contain conserved miR-17 binding sequences

***PKD1*: 3'UTR conserved binding site for miR-17**

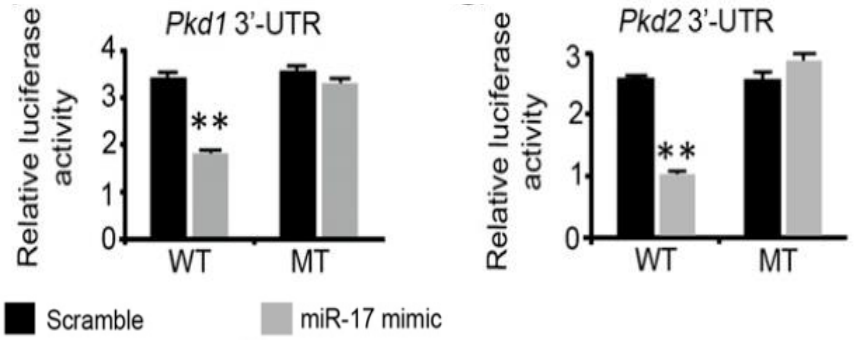


***PKD2*: 3'UTR conserved binding site for miR-17**



PNAS 2013 June;110(26): 10765-10770

- miR-17 mimic represses *Pkd1* and *Pkd2* in mouse collecting duct (IMCD3) cells



WT: wildtype 3'UTR
MT: mutated 3'UTR

➢ **miR-17 regulates expression of PC1/PC2 across multiple species**

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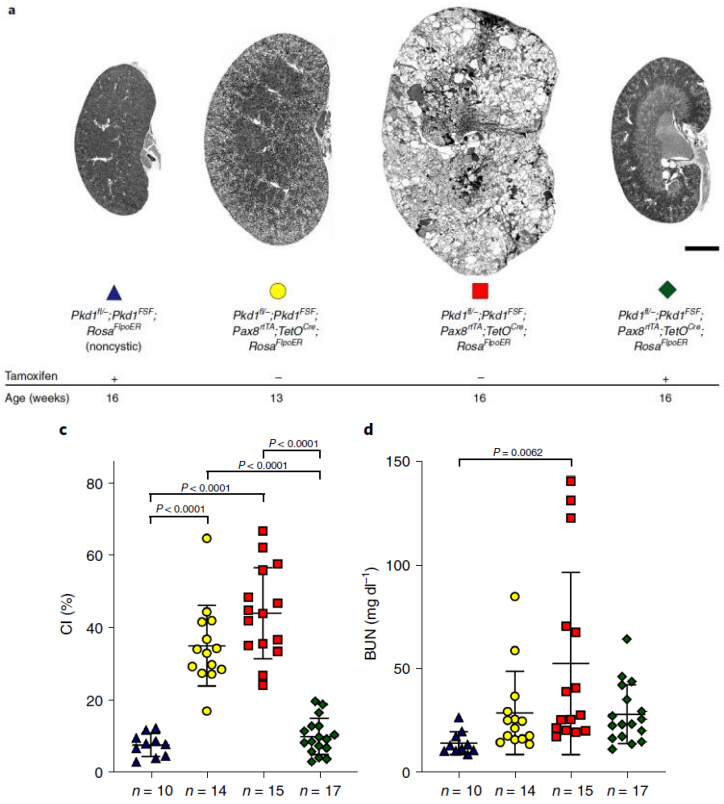
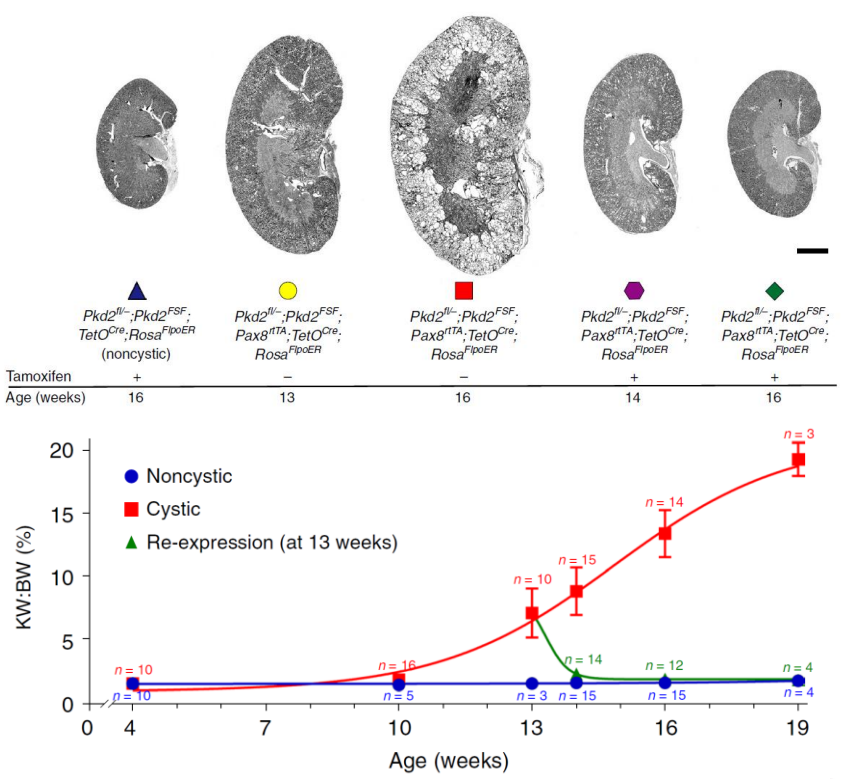
Re-activation of *Pkd2 (or Pkd1)* Gene Expression Rapidly Reverse ADPKD in Mouse Models of ADPKD

- Restoration of *Pkd1* or *Pkd2* gene expression, and thereby increase of PC1 or PC2 protein levels, in mouse models of ADPKD rapidly reduced KW/BW, cyst formation, and serum BUN levels.

Tamoxifen-inducible **increase of PC1 expression** starting at 13w in *Pkd1*$^{Cre/Flpo}$ mouse model of ADPKD.



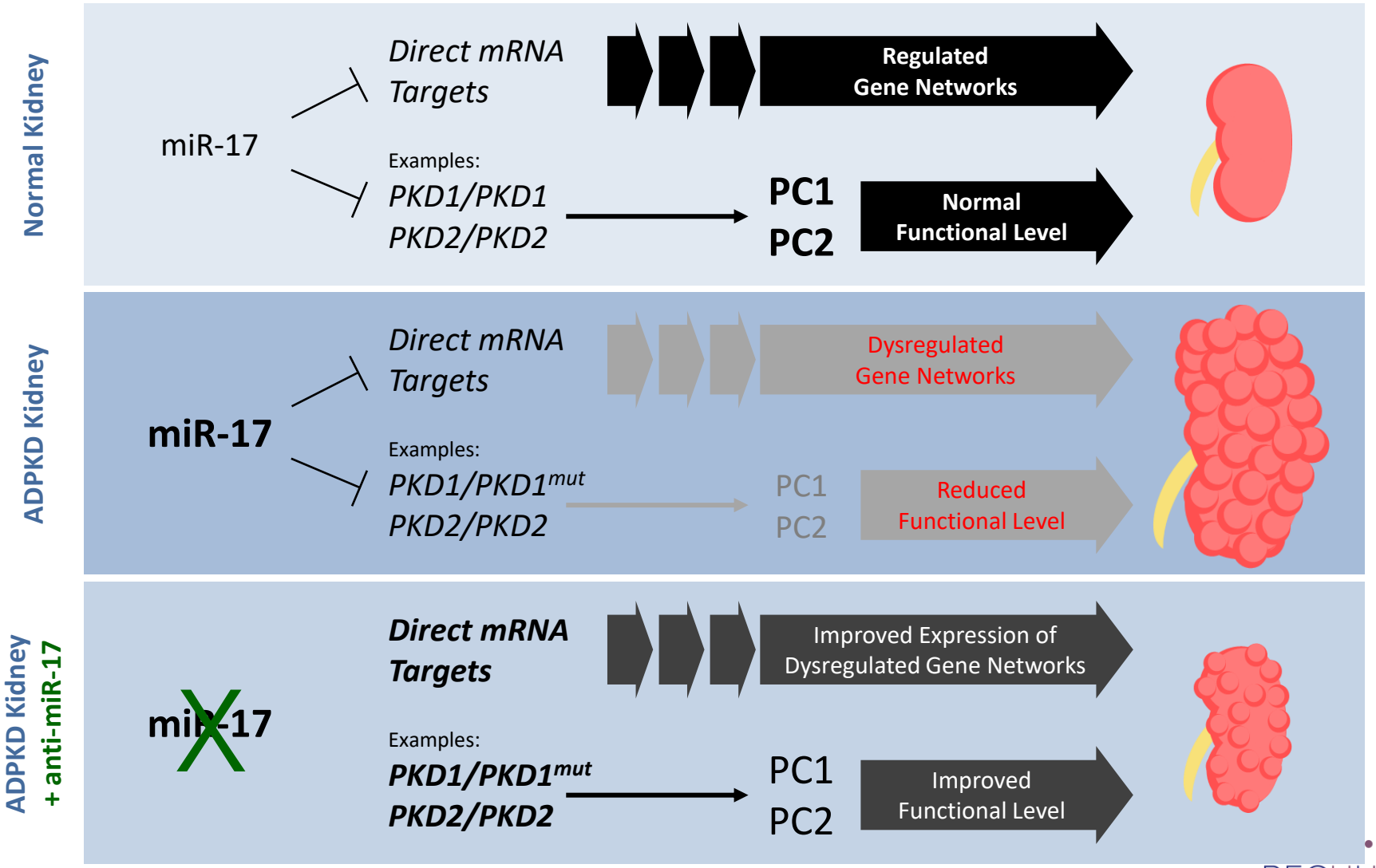
Tamoxifen-inducible **increase of PC2 expression** starting at 13w in *Pkd2*$^{Cre/Flpo}$ mouse model of ADPKD.



[1]*Dong 2021; Nat Genet*

➢ **PC1 and PC2 regulate kidney plasticity**

miR-17 Antagonism as a Therapeutic Strategy for Treatment of ADPKD



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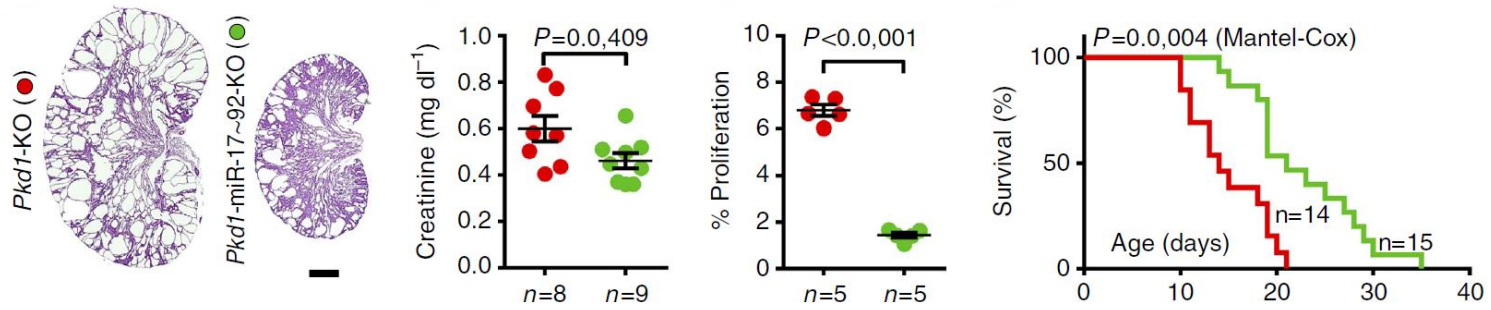
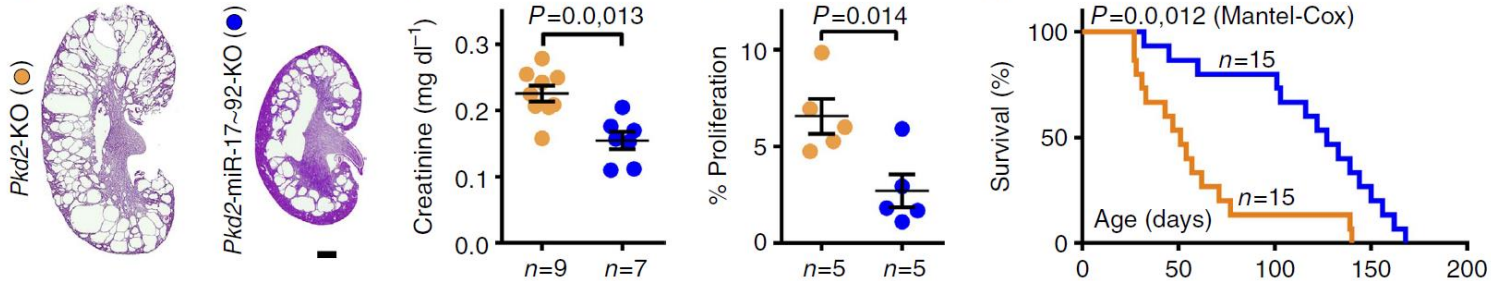
Kidney-specific Knockdown of miR-17~92 Cluster Attenuates Disease in Multiple ADPKD Mouse Models, Including *Pkd1*-KO, *Pkd2*-KO and *Pkd1*$^{F/RC}$

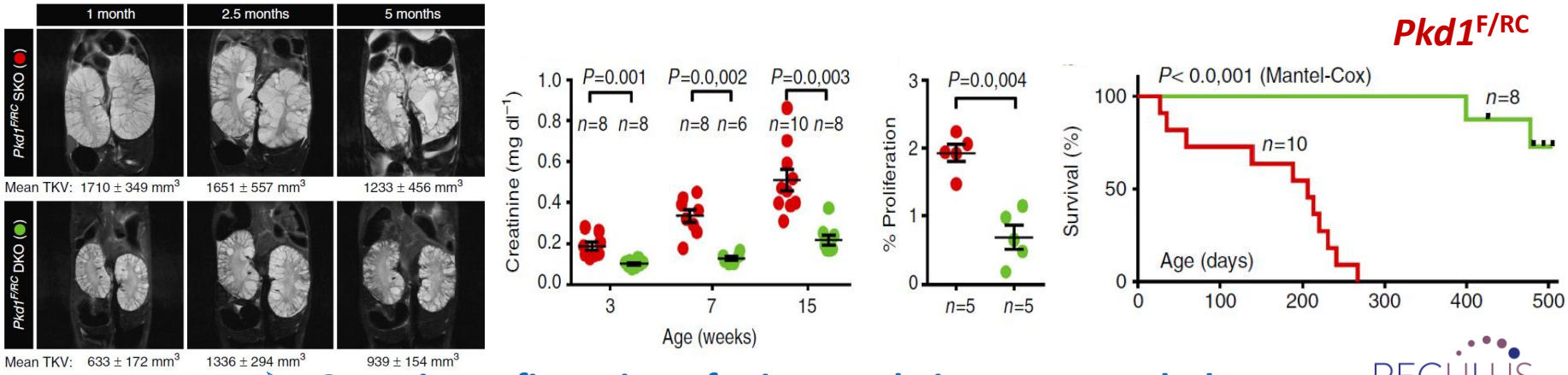
Pkd1-KO



Pkd2-KO



Pkd1$^{F/RC}$



➢ **Genetic confirmation of miR-17 role in ADPKD Pathology**

Hajarnis et al. (2017) Nat. Commun. Feb 16;8:14395

RGLS4326: First Generation Inhibitor of miR-17

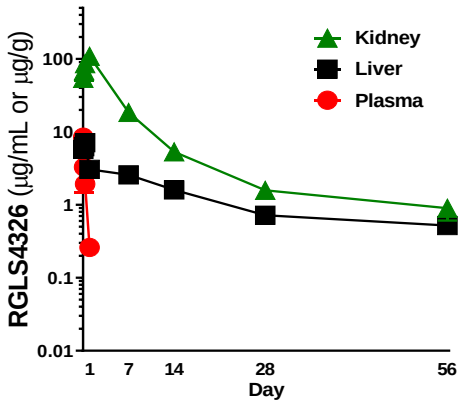
RGLS4326 chemical modifications, base sequence and corresponding complementarity to the miR-17 family of mature microRNAs

Seed

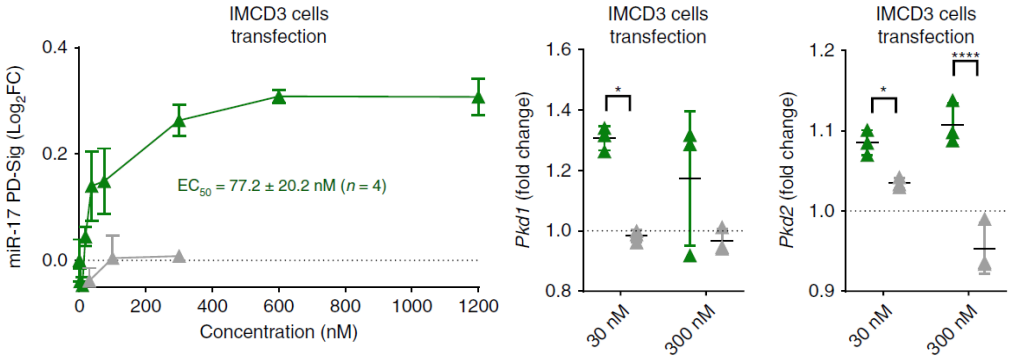
mature miR-106b-5p: 5′-U**AAAGUGC**UGACAGUGCAGAU -3′
mature miR-106a-5p: 5′-A**AAAGUGC**UUACAGUGCAGGUAG-3′
mature miR-93-5p: 5′-C**AAAGUGC**UCUUCGUGCAGGUAG-3′
mature miR-20b-5p: 5′-C**AAAGUGC**UCAUAGUGCAGGUAG-3′
mature miR-20a-5p: 5′-U**AAAGUGC**UUAUAGUGCAGGUAG-3′
mature miR-17-5p: 5′-C**AAAGUGC**UUACAGUGCAGGUAG-3′
RGLS4326: 3′-GUUUCACGA-5′

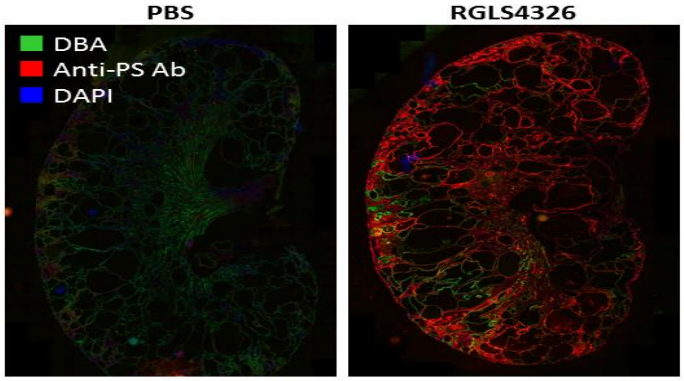
Red: (S)-constrained ethyl; Blue: 2′-O-methyl; Green: 2′-deoxy-2′-fluoro

Single subcutaneous dose at 30 mg/kg in WT-C57BL6 mice



RGLS4326 treatment de-represses multiple miR-17 target genes, including *Pkd1* and *Pkd2*

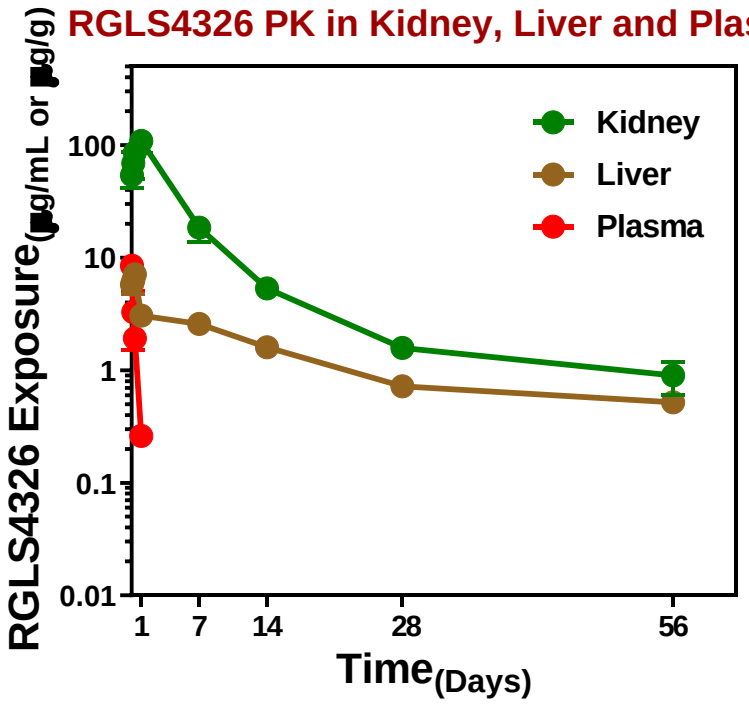


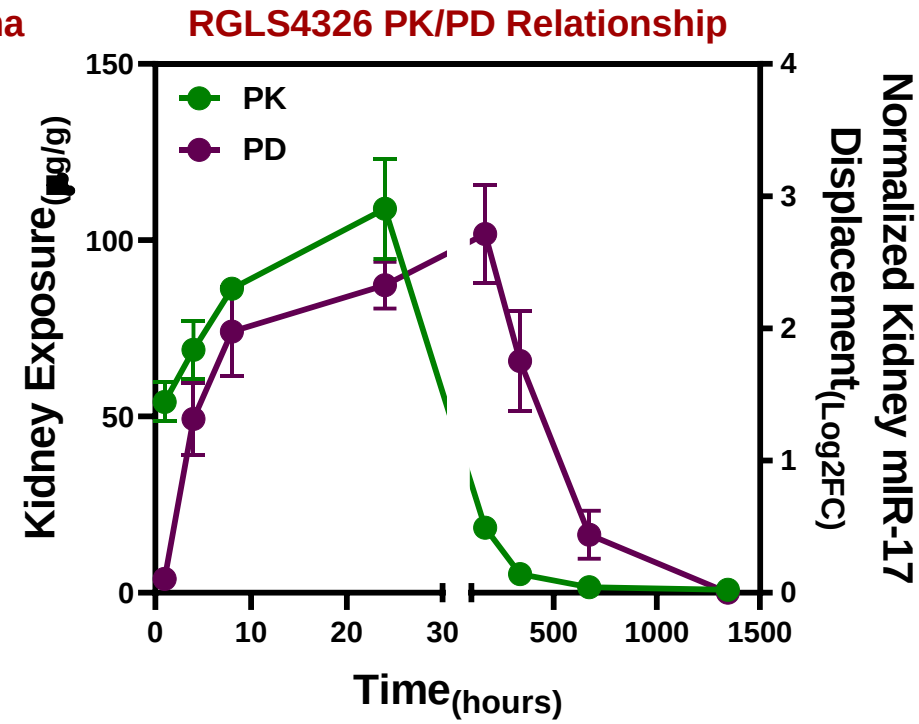


➢ **PK/PD properties of RGLS4326 make it particularly suited for treatment of ADPKD**

[1]Lee 2019, Nat Commun.

RGLS4326 Shows Favorable PK/PD Profiles After a Single Subcutaneous (SC) Dose of 30 mg/kg in Mice





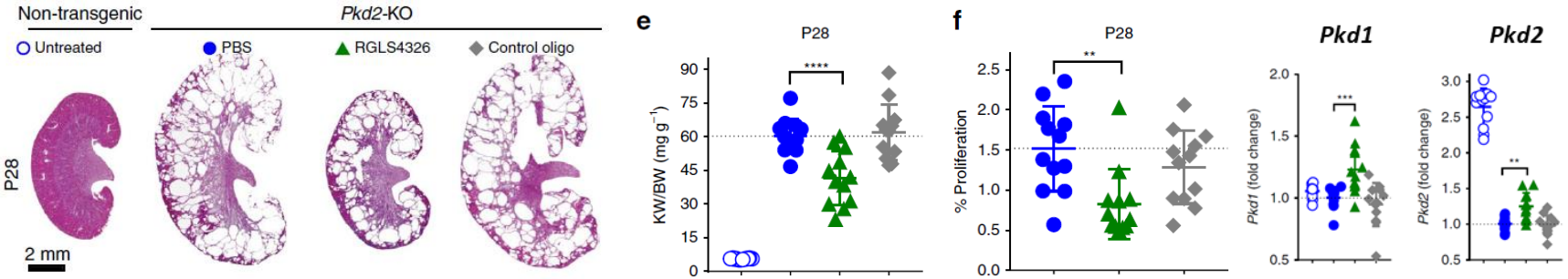
(A) RGLS4326 was rapidly absorbed into and cleared from plasma.
RGLS4326 distributed primarily to kidney, with kidney-to-liver ratio of >10-fold by Cmax.

(B) RGLS4326 potently engaged kidney miR-17, with peak target engagement (by miPSA) observed at Day 7

➢ **Strong correlation in PK/PD response**

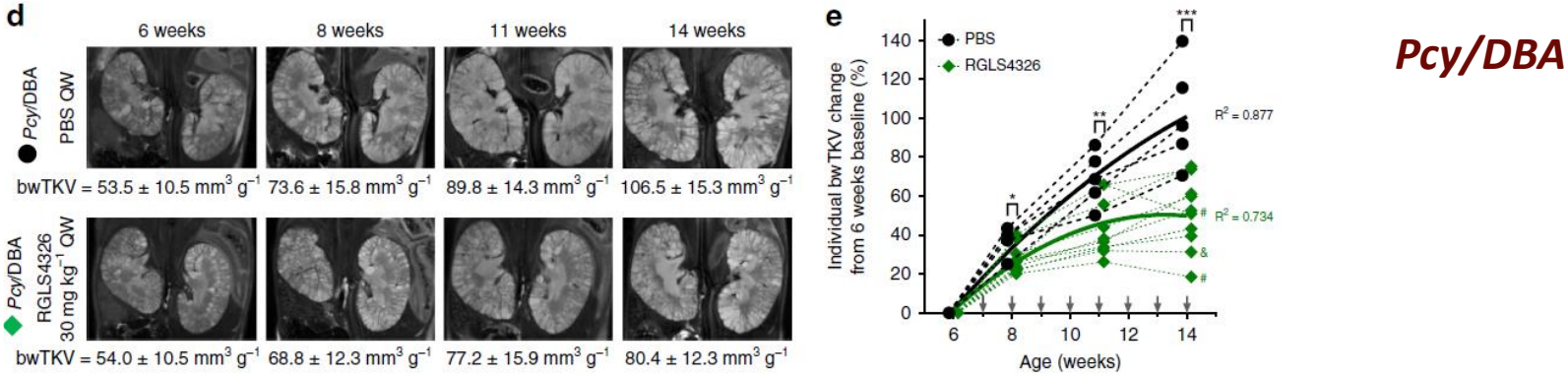
Lee et al. (2018) ASN-Kidney Week; Poster Presentation

RGLS4326 Is Pharmacologically Potent in Multiple Mouse Models of PKD



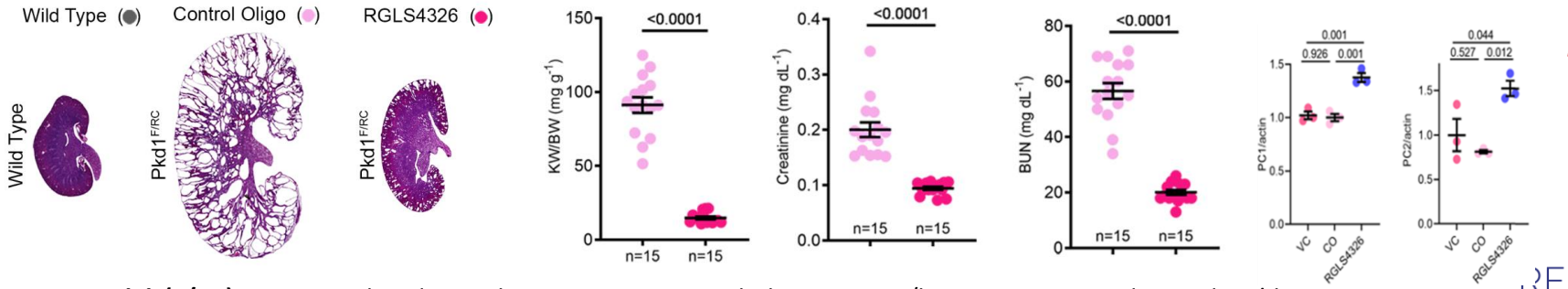
Pkd2-KO

***Pkd2*-KO** mice were dosed SC with PBS, RGLS4326 or Control Oligo at 20 mg/kg on P10, 11, 12, 19 and sacrificed at P28



Pcy/DBA

***Pcy*/DBA** mice were dosed SC QW starting at 7 w.o. with PBS or RGLS4326 at 30 mg/kg



Pkd1F/RC

***Pkd1*(F/RC)** mice were dosed SC with RGLS4326 or Control Oligo at 20 mg/kg on P8, 10, 12 and 15 and sac'd at P18

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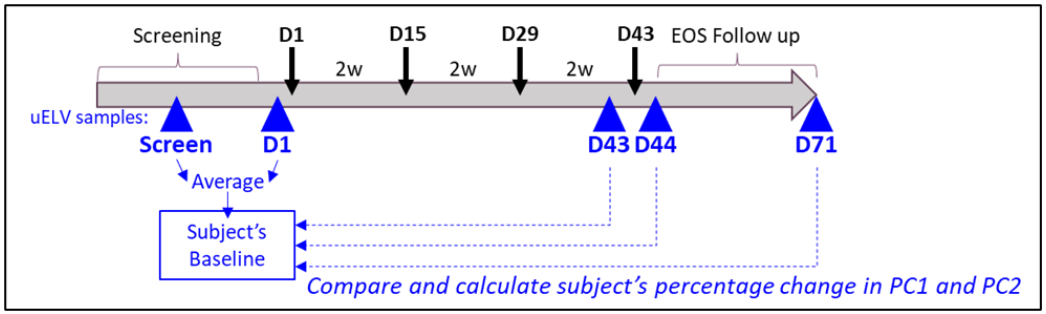
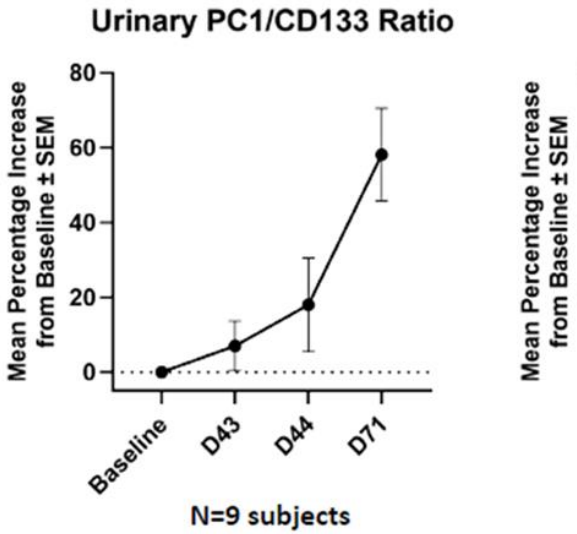
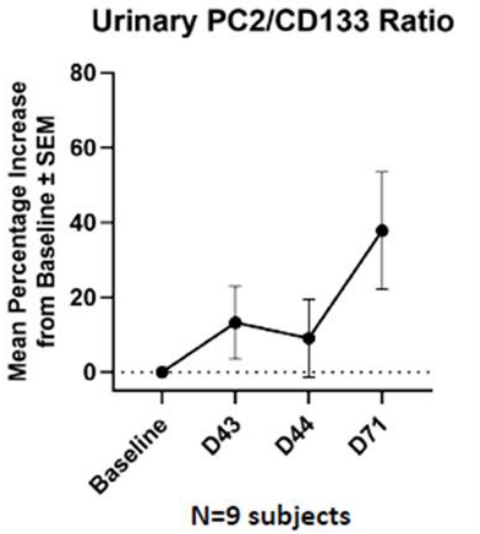
Lee et al. (2019) Nat. Commun. 10:4148

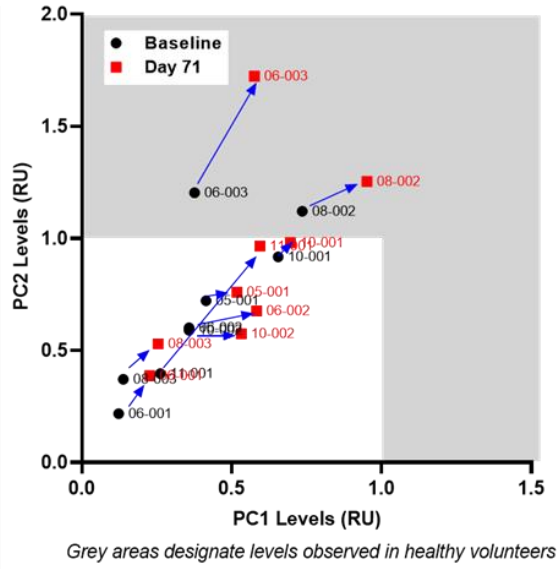
RGLS4326-03 Trial: Biomarker Analysis

Subjects dosed Q2W x 4 at 1mg/kg









- **Urine samples were collected at several time points. Urinary exosome-like vesicle (uELV) PC1 and PC2 levels were compared between Day 71 and Baseline to analyze PD response**
- **Statistically significant increase in uELV PC1 and PC2 ($p<0.05$ for both), with mean percent increase of 58.4% and 38.4%, respectively.**

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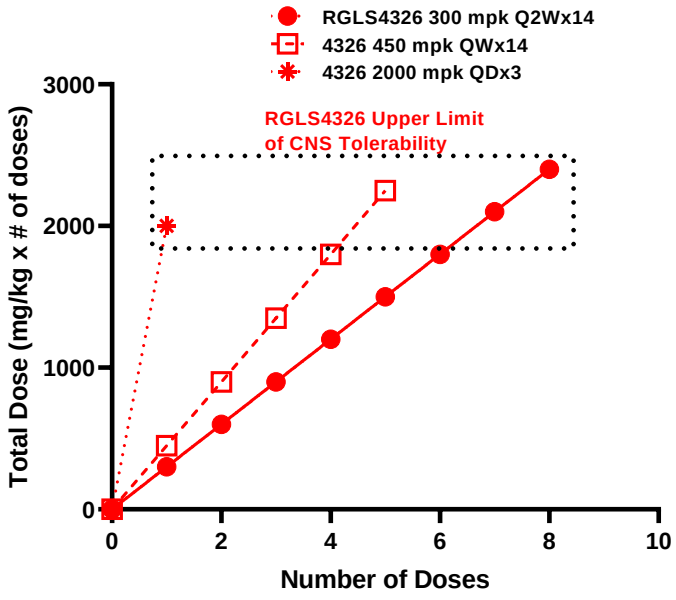
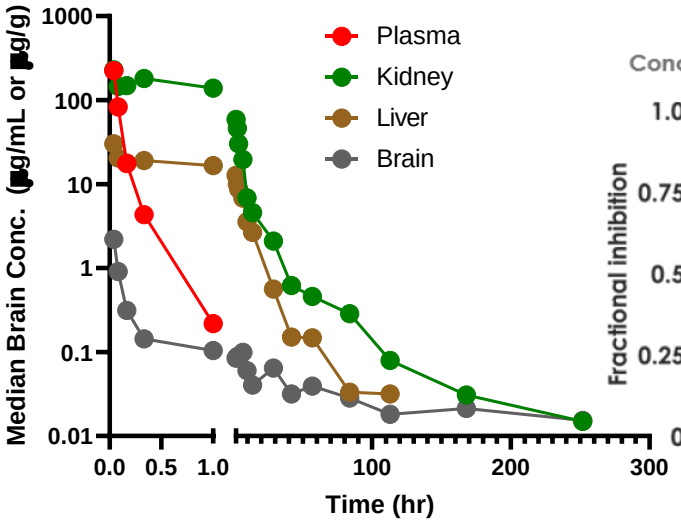
CNS Toxicity through AMPA-R Antagonism

Mouse Tolerability

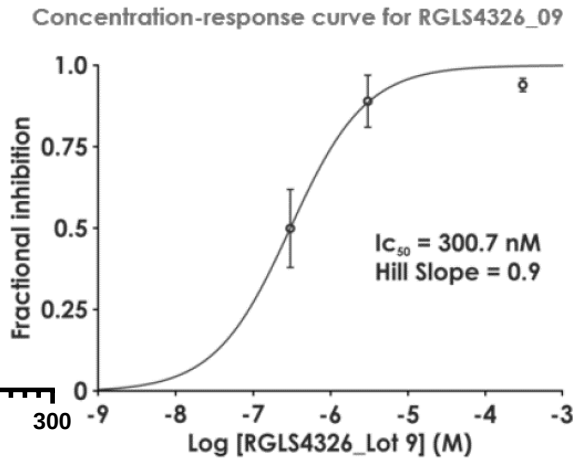


Mouse PK Profile

150 mg/kg SC



GluA1/A4 Patch-Clamp Assay



- Mice developed CNS toxicity after the administration of ≥ 2000 mg/kg cumulative dose
- Long tissue half-life in CNS leads to accumulation in the mouse brain posing a theoretical problem with multi-year dosing
- RGLS4326 inhibits human GluA1:A4 AMPA-R, with functional IC50 of ~437nM among 2 studies
- Additional Schild regression analysis indicated RGLS4326 is a competitive antagonist of AMPA-R

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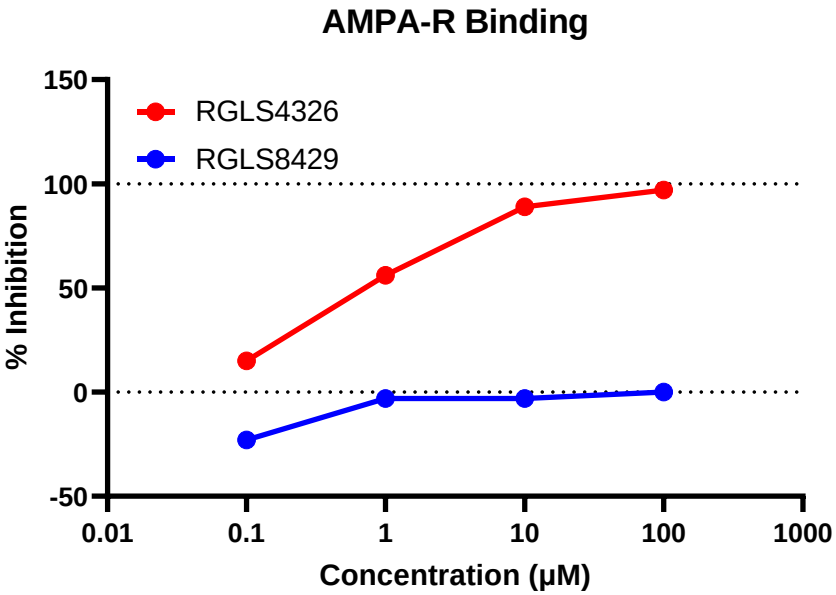
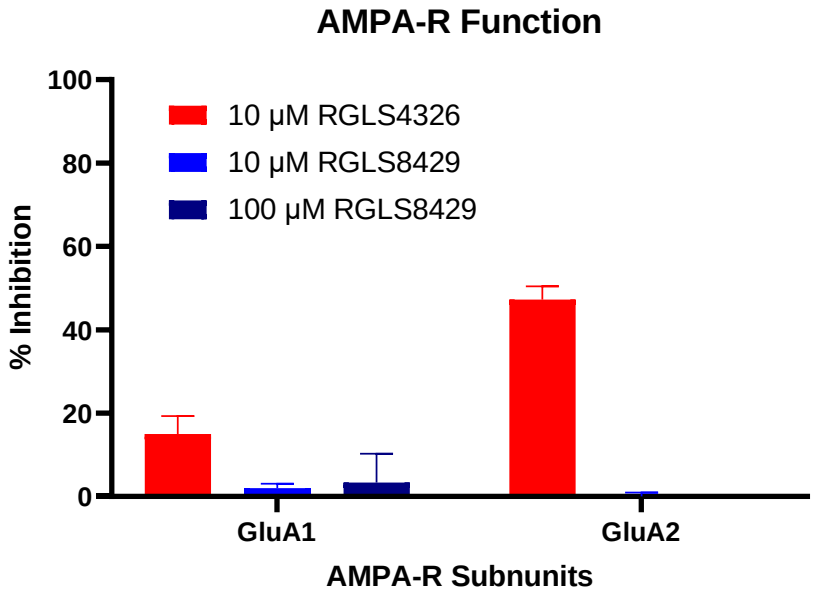
RGLS8429 Does Not Inhibit AMPA Glutamate Receptors (AMPA-R)





- **RGLS4326, but not RGLS8429, inhibits [^{3}H] AMPA ligand binding to synaptic membranes from rat cerebral cortex**
- **RGLS4326, but not RGLS8429, inhibits whole-cell patch-clamp studies in HEK293 cells overexpressing rGluA1 or rGluA2 in response to 3mM Glutamate**

Unpublished Preliminary Data Sobolevsky et al. Columbia University

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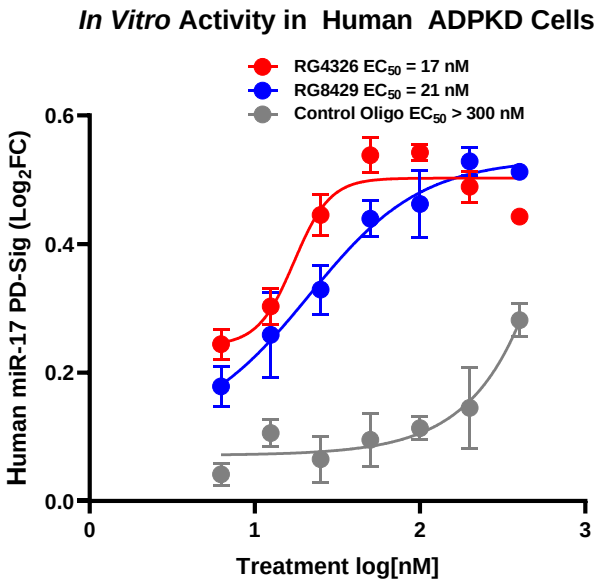
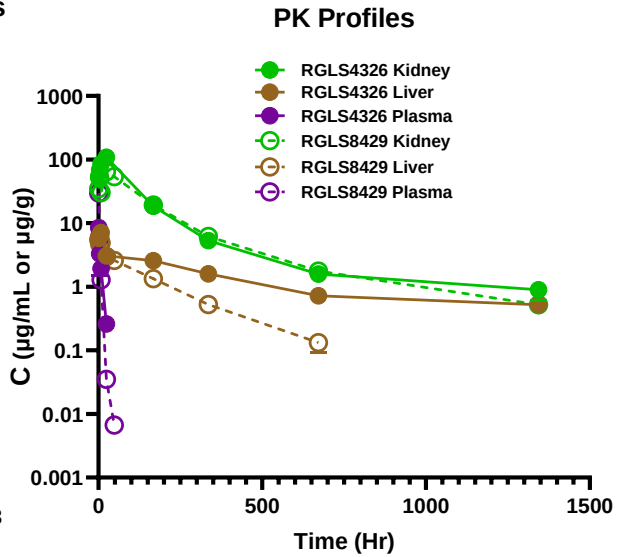
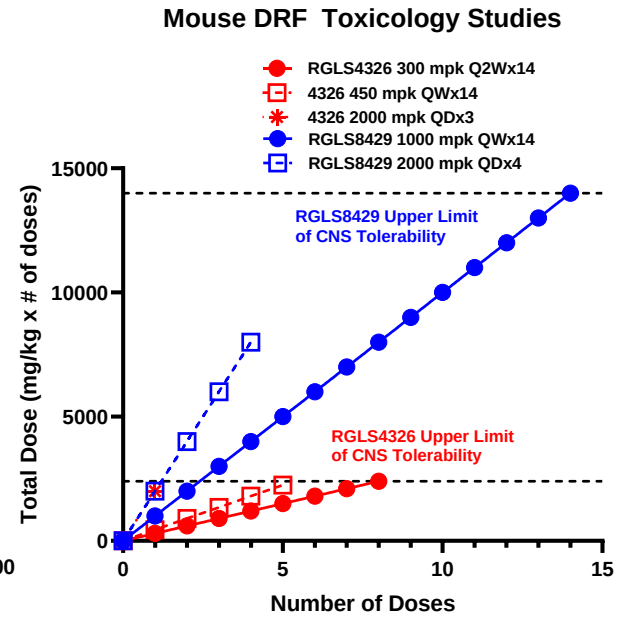
RGLS8429 Maintains Potency and PK Attributes of RGLS4326 and is Devoid of CNS Toxicity







- **RGLS8429 is equipotent to RGLS4326 in inhibiting miR-17 in human ADPKD cells**
- **RGLS8429 and RGLS4326 demonstrate very similar PK profiles with both compounds being preferentially distributed to the kidneys**
- **Administration of RGLS8429 up to 14,000 mg/kg cumulative dose does not produce CNS toxicity in mice**

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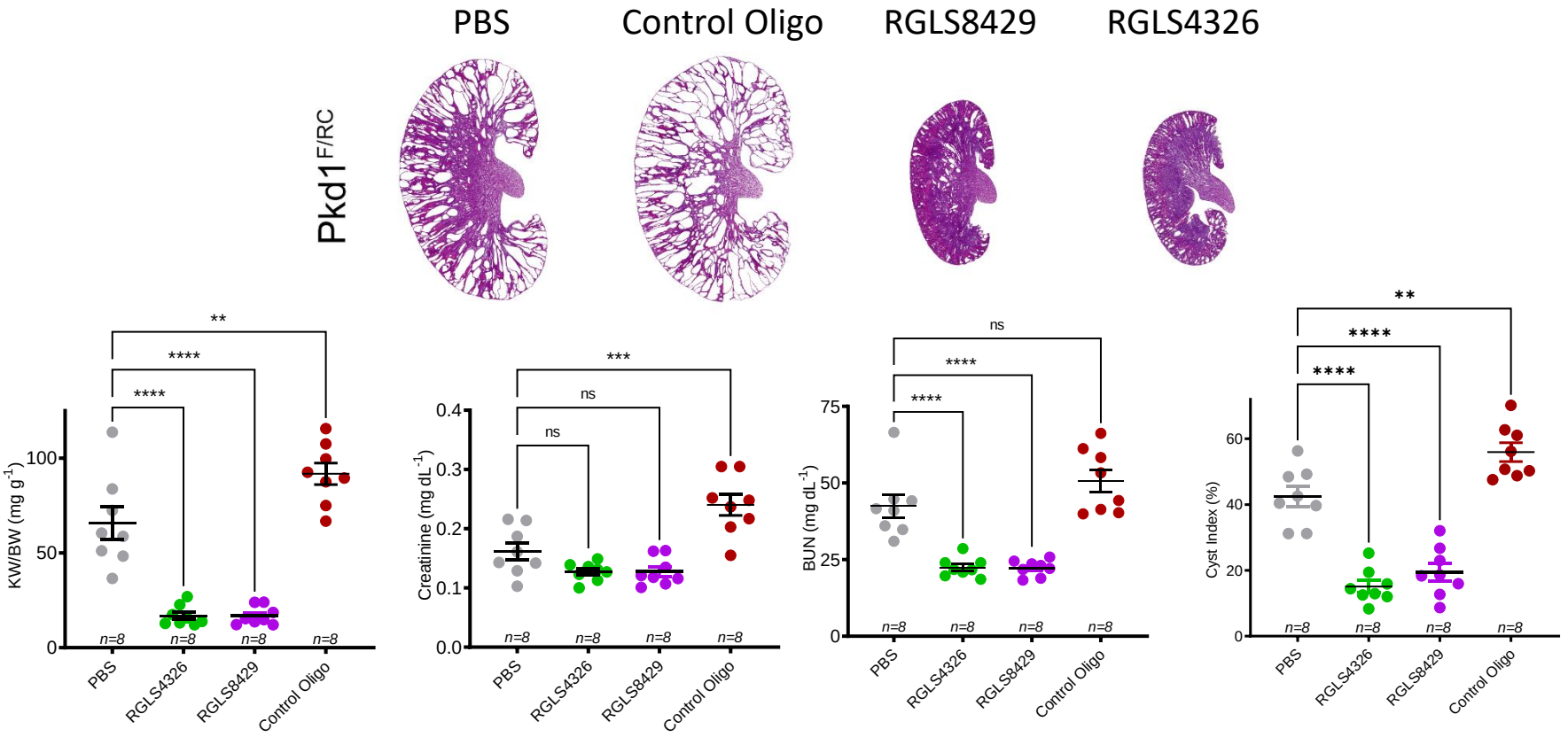
RGLS8429 has Similar Efficacy in *Pkd1*$^{F/RC}$ Mouse Model of ADPKD Compared with RGLS4326

- *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



➢ **RGLS8429 demonstrates similar therapeutic activity compared to RGLS4326 in *Pkd1*$^{F/RC}$ mouse model of ADPKD *in vivo***

Summary

- **MicroRNAs (miRs) are small non-coding RNAs that play an important role in the regulation of gene expression and are known to be involved in the pathogenesis of numerous diseases**
- **Targeting excessive expression of pathogenic miRs with anti-miRs represents a promising therapeutic strategy**
- **Clinical trial of RG-101 in patients with chronic HCV infection and of RGLS4326 in patients with ADPKD demonstrated beneficial therapeutic and pharmacodynamic responses, respectively, but also exposed unwanted off-target side effects**
- **Additional preclinical safety pharmacology screening of oligonucleotide-based therapeutics against transporters, ion channels and GPCRs can help avoid unwanted off-target effects**
- **Successfully executed SAR campaign allowed us to optimize clinical candidate profile for RGLS8429, which is planned to enter clinical development for the treatment of ADPKD in Q2 2022**

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Thank You!


A cure is our finish line.
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Walk for PKD
Wednesday, October 13, 2021
Torrey Pines Hike
PKD FOUNDATION
WALK FOR PKD