Abstract

Background
Autosomal dominant polycystic kidney disease (ADPKD), caused by mutations in the PKD1 or PKD2 gene (which encodes for the proteins polycystin-1 (PC1) and polycystin-2 (PC2)), is among the most common monogenetic disorders and a leading genetic cause of end-stage renal disease. Kidney-specific over-expression of miR-17-92 produce kidney cysts in mice, whereas genetic knockdown of miR-17-92 attenuates disease progression in multiple mouse models of PKD. RGLS4326 is designed to specifically bind to miR-17 family of microRNAs, antagonize miR-17 activity and reduce disease progression in mouse models of PKD. In this study, we investigate the effect of RGLS4326 treatment on 3D growth of human primary ADPKD cysts derived from ADPKD donor samples.

Methods
Primary human ADPKD cyst cells (HuADPKD) were transfected with RGLS4326 or control oligo at 20nM, 100nM or 300nM for 24h. RNA samples were harvested for confirmation of miR-17 inhibition by measuring de-repression of a selected set of direct miR-17 target genes (PD-Sig) and RNA sequencing. Following 24h transfection, cells were seeded and further cultured in a 3D cyst formation assay for 8 additional days.

Results
RGLS4326 inhibits 3D cyst growth and proliferation of HuADPKD cells in vitro compared to oligo control. Our preclinical data supports the clinical development of RGLS4326 for the treatment of ADPKD.

Characteristics of Autosomal Dominant Polycystic Kidney Disease (ADPKD)

| Renal phenotype | Normal | Focal | Multicystic | Adult | Infratentorial | Enzymatic
d|---|---|---|---|---|---|
| Examples of hyperechoic pelvic tubular dilatations | R227C | V268C | | | |
| Examples of associated anomalies | Normal | Heterozygous (fully penetrant allele) | Heterozygous (partially penetrant allele) | Homozygous (fully penetrant allele) |
| Level of functional PKC (%) | 100 | 75 | 50 | 25 |

RGLS4326 de-represses miR-17 downstream target genes in primary ADPKD cyst derived from human donors in vitro

• RNA-sequencing expression profiling was used to assess inhibition of miR17 in primary human ADPKD cyst cells following RGLS4326 treatment by transfection for 24h.

• Kolmogorov-Smirnov test statistics comparing log2FC cumulative distribution indicated significant shift towards upregulation (i.e. de-repression) of predicted miR-17 target genes following RGLS4326 treatment (100nM) in vitro.

• For each subsequent experiment, functional inhibition of miR-17 was measured and shown by de-repression of a human miR-17 PD-Sig in representative cyst samples (N=1 for each treatment and dose) after 24h treatment and prior to further culturing in 3D cyst formation assay for 8 additional days.

• Efficacy of RGLS4326 in Human Primary 3D-Cyst Cultures derived from Autosomal Dominant Polycystic Kidney Disease (ADPKD) Donors

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Conclusion
• Our data from primary human ADPKD donors cysts demonstrated a correlation between miR-17 inhibition and cyst growth reduction across both RGLS4326 and control oligo treatment. Low magnitude of non-specific and idiosyncratic changes were observed from control oligo in the assay.

• Importantly, miR-17 inhibition-driven cyst growth reduction was specific to proliferating ADPKD cells, as no cytotoxic activity was observed in non-ADPKD collecting duct cells such as IMCD3 following RGLS4326 treatment in vitro or genetic knockdown of miR-17–92 cluster in vivo [Patel et al. (2013) PNAS. Jun 25;110(26):10765].

• Our preclinical data supports the clinical development of RGLS4326 for the treatment of human ADPKD.