COMPOUND FOR USE IN METHODS FOR TREATMENT OF POLYCYSTIC KIDNEY DISEASE

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims the benefit of priority of US Provisional Patent Application Nos. 63/563,887, filed March 11, 2024, and 63/749,238, filed January 24, 2025, which are incorporated by reference herein in their entirety for any purpose.

SEQUENCE LISTING

[0002] The present application contains a Sequence Listing which has been submitted electronically in XML format. Said XML copy, created on February 26, 2025, is named "01138-0049-00PCT-ST26.xml" and is 104,801 bytes in size. The information in the electronic format of the sequence listing is incorporated herein by reference in its entirety.

FIELD

[0003] Provided herein are methods for the treatment of polycystic kidney disease using a compound comprising a modified oligonucleotide or a pharmaceutically acceptable salt thereof.

BACKGROUND

[0004] Polycystic kidney disease (PKD) is characterized by the accumulation of numerous fluid-filled cysts in the kidney. These cysts are lined by a single layer of epithelial cells called the cyst epithelium. Over time, the cysts increase in size due to elevated cell proliferation and active secretion of fluid by the cyst epithelium. The enlarged cysts compress surrounding normal tissue, resulting in a decline of kidney function. The disease eventually progresses to end-stage renal disease, requiring dialysis or kidney transplant. At this stage, the cysts may be surrounded by areas of fibrosis containing atrophic tubules. Polycystic kidney disease can also cause cysts to develop in the liver and elsewhere in the body.

[0005] A number of genetic disorders can result in PKD. The various forms of PKD are distinguished by the manner of inheritance, for example, autosomal dominant or autosomal recessive inheritance; the involvement of organs and presentation of phenotypes outside of the kidney; the age of onset of end-stage renal disease, for example, at birth, in childhood or adulthood; and the underlying genetic mutation that is associated with the disease. *See*, for example, Kurschat et al., 2014, Nature Reviews Nephrology, 10: 687–699.

SUMMARY

[0006] The present disclosure is directed to methods of treating polycystic kidney disease (PKD), optionally, of treating autosomal dominant polycystic kidney disease (ADPKD), comprising administering to a subject in need thereof a therapeutically-effective amount of a modified oligonucleotide or pharmaceutically acceptable salt thereof.

Embodiment 1. A method of treating polycystic kidney disease comprising administering to a subject in need thereof a modified oligonucleotide or a pharmaceutically acceptable salt thereof at a fixed dose of between about 150 mg and about 350 mg,

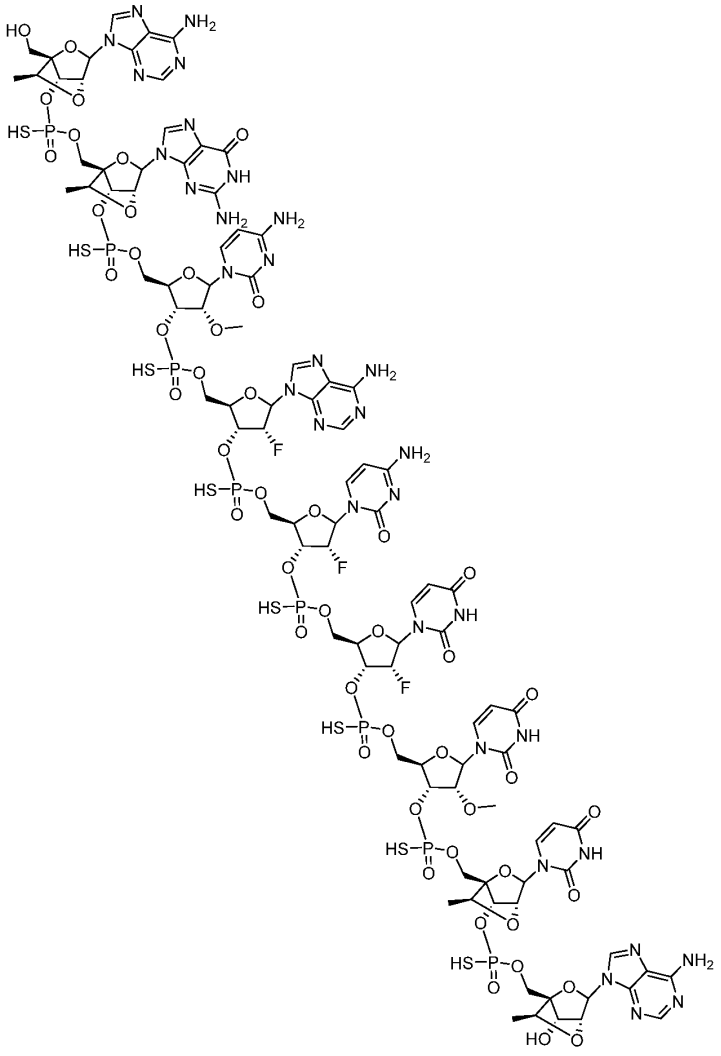
wherein the modified oligonucleotide has the structure 5'-$A_SG_SC_MA_FC_FU_FU_MU_SA_S$-3',

wherein nucleosides followed by subscript "M" are 2'-O-methyl nucleosides; nucleosides followed by subscript "F" are 2'-fluoro nucleosides; and nucleosides followed by subscript "S" are S-cEt nucleosides, and wherein each cytosine is a non-methylated cytosine.

Embodiment 2. The method of embodiment 1, wherein the modified oligonucleotide or a pharmaceutically acceptable salt thereof is administered at a fixed dose of 150 mg, 160 mg, 170 mg, 180 mg, 190 mg, 200 mg, 210 mg, 220 mg, 230 mg, 240 mg, 250 mg, 260 mg, 270 mg, 280 mg, 290 mg, 300 mg, 310 mg, 320 mg, 330 mg, 340 mg, or 350 mg.

Embodiment 3. The method of embodiment 1 or 2, wherein the pharmaceutically acceptable salt is a sodium salt.

Embodiment 4. A method of treating polycystic kidney disease comprising administering to a subject in need thereof a modified oligonucleotide at a fixed dose of between about 150 mg and about 350 mg, wherein the modified oligonucleotide has the structure:



a)

b) or a pharmaceutically acceptable salt thereof.

Embodiment 5. The method of embodiment 4, wherein the modified oligonucleotide is administered at a fixed dose of 150 mg, 160 mg, 170 mg, 180 mg, 190 mg, 200 mg, 210 mg, 220 mg, 230 mg, 240 mg, 250 mg, 260 mg, 270 mg, 280 mg, 290 mg, 300 mg, 310 mg, 320 mg, 330 mg, 340 mg, or 350 mg.

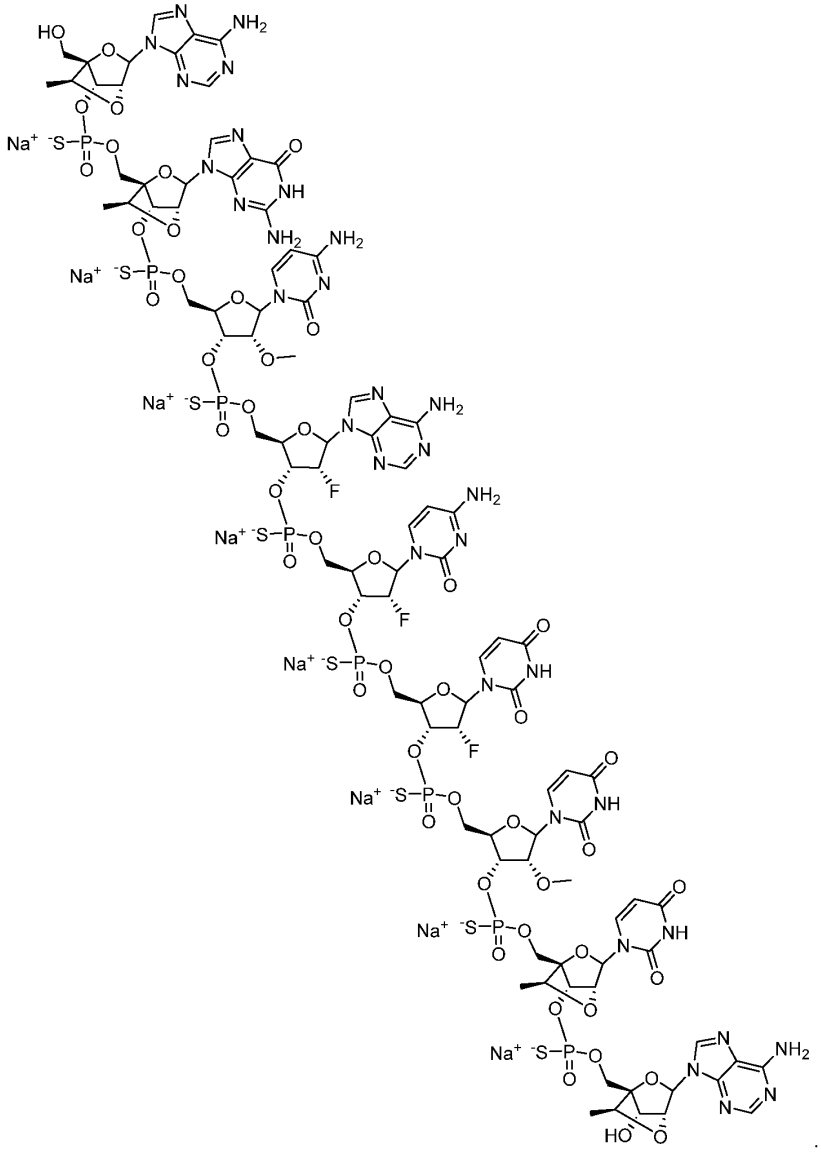
Embodiment 6. The method of embodiment 4 or 5, wherein pharmaceutically acceptable salt is a sodium salt.

Embodiment 7. The method of any one of embodiments 1-6, wherein the modified oligonucleotide is present in a pharmaceutical composition comprising a pharmaceutically acceptable diluent.

Embodiment 8. The method of embodiment 7, wherein the pharmaceutically acceptable diluent is a sterile aqueous solution.

Embodiment 9. The method of embodiment 8, wherein the sterile aqueous solution is a saline solution.

Embodiment 10. A method of treating polycystic kidney disease comprising administering to a subject in need thereof a modified oligonucleotide at a fixed dose of between about 150 mg and about 350 mg, wherein the modified oligonucleotide has the structure:



Embodiment 11. The method of embodiment 10, wherein the modified oligonucleotide is administered at a fixed dose of 150 mg, 160 mg, 170 mg, 180 mg, 190 mg, 200 mg, 210 mg, 220 mg, 230 mg, 240 mg, 250 mg, 260 mg, 270 mg, 280 mg, 290 mg, 300 mg, 310 mg, 320 mg, 330 mg, 340 mg, or 350 mg.

Embodiment 12. The method of embodiment 10 or 11, wherein the modified oligonucleotide is present in a pharmaceutical composition comprising a pharmaceutically acceptable diluent.

Embodiment 13. The method of embodiment 12, wherein the pharmaceutically acceptable diluent is a sterile aqueous solution.

Embodiment 14. The method of embodiment 13, wherein the sterile aqueous solution is a saline solution.

Embodiment 15. The method of any one of embodiments 1-14, wherein the subject has polycystic kidney disease.

Embodiment 16. The method of any one of embodiments 1-15, wherein the subject has been diagnosed as having polycystic kidney disease using clinical, histopathologic, and/or genetic criteria.

Embodiment 17. The method of any one of embodiments 1-16, wherein the polycystic kidney disease is autosomal dominant polycystic kidney disease (ADPKD).

Embodiment 18. The method of embodiment 17, wherein the subject has Mayo Imaging Classification 1C, 1D, or 1E of ADPKD.

Embodiment 19. The method of any one of embodiments 1-18, wherein the subject, prior to administration of the modified oligonucleotide, has an estimated glomerular filtration rate (eGFR) between 30-90 mL/min/1.73 m^2.

Embodiment 20. The method of any one of embodiments 1-19, wherein the subject, prior to administration of the modified oligonucleotide, was determined to have a decreased level of polycystin-1 (PC1) and/or polycystin-2 (PC2) in the kidney, urine or blood of the subject.

Embodiment 21. The method of any one of embodiments 1-20, wherein the subject has a mutation selected from a mutation in the *PKD1* gene or a mutation in the *PKD2* gene.

Embodiment 22. The method of any one of embodiments 1-21, wherein the subject has increased total kidney volume.

Embodiment 23. The method of any one of embodiments 1-22, wherein the subject has hypertension.

Embodiment 24. The method of any one of embodiments 1-23, wherein the subject has impaired kidney function.

Embodiment 25. The method of any one of embodiments 1-24, wherein the method comprises administering the modified oligonucleotide at a fixed dose of 150 mg.

Embodiment 26. The method of any one of embodiments 1-24, wherein the method comprises administering the modified oligonucleotide at a fixed dose of 160 mg.

Embodiment 27. The method of any one of embodiments 1-24, wherein the method comprises administering the modified oligonucleotide at a fixed dose of 170 mg.

Embodiment 28. The method of any one of embodiments 1-24, wherein the method comprises administering the modified oligonucleotide at a fixed dose of 180 mg.

Embodiment 29. The method of any one of embodiments 1-24, wherein the method comprises administering the modified oligonucleotide at a fixed dose of 190 mg.

Embodiment 30. The method of any one of embodiments 1-24, wherein the method comprises administering the modified oligonucleotide at a fixed dose of 200 mg.

Embodiment 31. The method of any one of embodiments 1-24, wherein the method comprises administering the modified oligonucleotide at a fixed dose of 210 mg.

Embodiment 32. The method of any one of embodiments 1-24, wherein the method comprises administering the modified oligonucleotide at a fixed dose of 220 mg.

Embodiment 33. The method of any one of embodiments 1-24, wherein the method comprises administering the modified oligonucleotide at a fixed dose of 230 mg.

Embodiment 34. The method of any one of embodiments 1-24, wherein the method comprises administering the modified oligonucleotide at a fixed dose of 240 mg.

Embodiment 35. The method of any one of embodiments 1-24, wherein the method comprises administering the modified oligonucleotide at a fixed dose of 250 mg.

Embodiment 36. The method of any one of embodiments 1-24, wherein the method comprises administering the modified oligonucleotide at a fixed dose of 260 mg.

Embodiment 37. The method of any one of embodiments 1-24, wherein the method comprises administering the modified oligonucleotide at a fixed dose of 270 mg.

Embodiment 38. The method of any one of embodiments 1-24, wherein the method comprises administering the modified oligonucleotide at a fixed dose of 280 mg.

Embodiment 39. The method of any one of embodiments 1-24, wherein the method comprises administering the modified oligonucleotide at a fixed dose of 290 mg.

Embodiment 40. The method of any one of embodiments 1-24, wherein the method comprises administering the modified oligonucleotide at a fixed dose of 300 mg.

Embodiment 41. The method of any one of claims 1-24, wherein the method comprises administering the modified oligonucleotide at a fixed dose of 310 mg.

Embodiment 42. The method of any one of claims 1-24, wherein the method comprises administering the modified oligonucleotide at a fixed dose of 320 mg.

Embodiment 43. The method of any one of claims 1-24, wherein the method comprises administering the modified oligonucleotide at a fixed dose of 330 mg.

Embodiment 44. The method of any one of claims 1-24, wherein the method comprises administering the modified oligonucleotide at a fixed dose of 340 mg.

Embodiment 45. The method of any one of claims 1-24, wherein the method comprises administering the modified oligonucleotide at a fixed dose of 350 mg.

Embodiment 46. The method of any one of embodiments 1-45, wherein the method comprises administering the modified oligonucleotide once every 2 weeks.

Embodiment 47. The method of any one of embodiments 1-46, wherein the method comprises administering the modified oligonucleotide at least 7 times.

Embodiment 48. The method of any one of embodiments 1-47, wherein the modified oligonucleotide is administered subcutaneously.

Embodiment 49. The method of any one of embodiments 1-48, wherein the treatment reduces total kidney volume in the subject.

Embodiment 50. The method of any one of embodiments 1-49, wherein the treatment slows the rate of increase of total kidney volume in the subject.

Embodiment 51. The method of embodiment 49 or 50, wherein the total kidney volume is height-adjusted total kidney volume (htTKV).

Embodiment 52. The method of any one of embodiments 1-51, wherein the treatment slows the rate of decline of glomerular filtration rate in the subject.

Embodiment 53. The method of any one of embodiments 1-52, wherein the treatment increases glomerular filtration rate in the subject.

Embodiment 54. The method of embodiment 52 or 53, wherein the glomerular filtration rate is estimated glomerular filtration rate.

Embodiment 55. The method of any one of embodiments 1-54, wherein the treatment inhibits or slows the increase in the growth of cysts in the kidney and/or liver of the subject.

Embodiment 56. The method of embodiment 55, wherein the treatment inhibits or slows the increase in total cyst volume, number and/or size distribution.

Embodiment 57. The method of any one of embodiments 1-56 wherein the treatment:

a) improves or slows the rate of decrease of creatinine clearance in the subject;

b) reduces or slows the rate of increase of albumin:creatinine ratio in the subject;

c) reduces or slows the rate of increase of blood urine nitrogen (BUN) level in the subject;

d) reduces or slows the rate of increase of serum creatinine (SCr) level in the subject;

e) increases polycystin-1 (PC1) in the urine of the subject;

f) increases polycystin-2 (PC2) in the urine of the subject;

g) reduces or slows the rate of increase of neutrophil gelatinase-associated lipocalin (NGAL) protein in the urine of the subject; and/or

h) reduces or slows the rate of increase of kidney injury molecule-1 (KIM-1) protein in the urine of the subject.

Embodiment 58. The method of any one of embodiments 1-57 wherein the administering:

a) reduces or slows the rate of increase of monocyte chemoattractant protein-1 (MCP-1) in the urine of the subject;

b) reduces or slows the rate of increase of beta-2 microglobulin (B2M) in the urine of the subject;

c) reduces or slows the rate of increase of complement split products C3a and/or Bb in the plasma of the subject;

d) reduces or slows the rate of increase of serum insulin-like growth factor binding protein acid labile subunit (IGFALS) in the subject;

e) reduces or slows the rate of increase of serum copeptin (CT-proAVP) in the subject;

f) reduces or slows the rate of increase of serum N-acetyl-1-methylhistidine in the subject; and/or

g) reduces or slows the rate of increase of acute phase proteins in the subject.

Embodiment 59. The method of any one of embodiments 1-58, wherein the treatment results in little to no CNS impairment in the subject.

Embodiment 60. The method of embodiment 59, wherein the treatment results in little to no change in the Scale for the Assessment and Rating of Ataxia (SARA) test score for the subject.

Embodiment 61. The method of one of embodiments 1-60, comprising:

a) measuring height-adjusted total kidney volume (HtTKV) in the subject;

b) measuring polycystin-1 (PC1) in the urine of the subject;

c) measuring polycystin-2 (PC2) in the urine of the subject;

d) measuring blood urea nitrogen (BUN) level in the subject;

e) measuring serum creatinine (SCr) level in the subject;

f) measuring creatinine clearance in the subject;

g) measuring urine albumin:creatinine ratio (UACR) in the subject;

h) measuring estimated glomerular filtration rate (eGFR) in the subject;

i) measuring neutrophil gelatinase-associated lipocalin (NGAL) protein in the urine of the subject;

j) measuring kidney injury molecule-1 (KIM-1) protein in the urine of the subject;

k) measuring monocyte chemoattractant protein-1 (MCP-1) in the urine of the subject;

l) measuring beta-2 microglobulin (B2M) in the urine of the subject;

m) measuring serum insulin-like growth factor binding protein acid labile subunit (IGFALS) in the subject;

n) measuring serum copeptin (CT-proAVP) in the subject;

o) measuring serum N-acetyl-1-methylhistidine in the subject;

p) measuring complement split products C3a and/or Bb in the plasma of the subject; and/or

q) measuring total cyst volume, number and/or size distribution in the subject; and/or

r) measuring SARA test score for the subject.

Embodiment 62. The method of any one of embodiments 1-61, wherein the subject is a human subject.

Embodiment 63. The method of any one of embodiments 1-62, which has an acceptable safety and tolerability profile.

Embodiment 64. A modified oligonucleotide or a pharmaceutically acceptable salt thereof for use in treating polycystic kidney disease, wherein the modified oligonucleotide has the structure 5'-$A_SG_SC_MA_FC_FU_FU_MU_SA_S$-3',

wherein nucleosides followed by subscript "M" are 2'-O-methyl nucleosides; nucleosides followed by subscript "F" are 2'-fluoro nucleosides; and nucleosides followed by subscript "S" are S-cEt nucleosides, and wherein each cytosine is a non-methylated cytosine;

and wherein the modified oligonucleotide is administered at a fixed dose of between about 150 mg and about 350 mg.

Embodiment 65. The modified oligonucleotide for use of embodiment 64, wherein the modified oligonucleotide is administered at a fixed dose of 150 mg, 160 mg, 170 mg, 180 mg, 190 mg, 200 mg, 210 mg, 220 mg, 230 mg, 240 mg, 250 mg, 260 mg, 270 mg, 280 mg, 290 mg, 300 mg, 310 mg, 320 mg, 330 mg, 340 mg, or 350 mg.

Embodiment 66. The modified oligonucleotide for use of embodiment 64 or 65, wherein the pharmaceutically acceptable salt is a sodium salt.

Embodiment 67. The modified oligonucleotide for use of any one of embodiments 64-66, wherein the modified oligonucleotide is present in a pharmaceutical composition comprising a sterile saline solution.

Embodiment 68. The modified oligonucleotide for use of any one of embodiments 64-67, wherein the polycystic kidney disease is autosomal dominant polycystic kidney disease (ADPKD).

Embodiment 69. The modified oligonucleotide for use of any one of embodiments 64-68, wherein the modified oligonucleotide is administered once every two weeks.

Embodiment 70. The modified oligonucleotide for use of any one of embodiments 64-69, wherein the modified oligonucleotide is administered at least seven times.

Embodiment 71. Use of a modified oligonucleotide or a pharmaceutically acceptable salt thereof for the preparation of a medicament for treating polycystic kidney disease, wherein the modified oligonucleotide has the structure 5'-$A_SG_SC_MA_FC_FU_FU_MU_SA_S$-3',

wherein nucleosides followed by subscript "M" are 2'-O-methyl nucleosides; nucleosides followed by subscript "F" are 2'-fluoro nucleosides; and nucleosides followed by subscript "S" are S-cEt nucleosides, and wherein each cytosine is a non-methylated cytosine;

wherein the modified oligonucleotide is formulated for administration at a fixed dose of between about 150 mg and about 350 mg.

Embodiment 72. The use of embodiment 71, wherein the modified oligonucleotide is administered at a fixed dose of 150 mg, 160 mg, 170 mg, 180 mg, 190 mg, 200 mg, 210 mg, 220 mg, 230 mg, 240 mg, 250 mg, 260 mg, 270 mg, 280 mg, 290 mg, 300 mg, 310 mg, 320 mg, 330 mg, 340 mg, or 350 mg.

Embodiment 73. The use of embodiment 71 or 72, wherein the pharmaceutically acceptable salt is a sodium salt.

Embodiment 74. The use of any one of embodiments 71-73, wherein the modified oligonucleotide is present in a pharmaceutical composition comprising a sterile saline solution.

Embodiment 75. The use of any one of embodiments 71-74, wherein the polycystic kidney disease is autosomal dominant polycystic kidney disease (ADPKD).

Embodiment 76. The use of any one of embodiments 71-75, wherein the modified oligonucleotide is administered at least once every two weeks.

Embodiment 77. The use of any one of embodiments 71-76, wherein the modified oligonucleotide is administered at least seven times.

BRIEF DESCRIPTION OF FIGURES

[0007] **FIG. 1.** Purine nucleobase structures.

[0008] **FIG. 2A-2C**. Efficacy of RG-NG-1015 in the *Pkd1*-F/RC model of PKD. Effects of treatment on (2A) kidney-to-body weight ratio, (2B) blood urea nitrogen (BUN) level, and (2C) blood creatinine level.

[0009] **FIG. 3**. Maximum Tolerated Dose (MTD) study and Comparative Dose Assessment of RG-NG-1001, RGLS4326, and RG-NG-1017. 6–7-week-old male C57BL/6J mice were dosed with a single intracerebroventricular (ICV) injection of RG-NG-1001 and RGLS4326 (anti-miR-17 oligos that inhibit AMPA-R) and RG-NG-1017 (anti-miR-17 oligos that does not inhibit AMPA-R; RG-NG-1017) at different dose levels in 4 µLvolume and monitored for 7 days. Mortality of the mice is indicated for the three different compounds at different dosages.

[00010] **FIG. 4A-4F**. Assessment of activity of RG-NG-1015 and RGLS4326 against miR-17 (4A), miR-20a (4B), miR-93 (4C), and miR106(a) (4D) luciferase sensors activity in HeLa cells in vitro is set forth. Assessment of activity of RG-NG-1015 and RGLS4326 against luciferase sensors containing full length 3' untranslated region (UTR) of the miR-17 direct target genes PKD1 (4E) and PKD2 (4F) is set forth.

[00011] **FIG. 5A-5D**. Pharmacokinetic and target engagement (as measured by miPSA) of RGLS4326 and RG-NG-1015 following a single subcutaneous administration in C57BL6 mice were measured. Plasma concentration (5A), tissue concentration (5B), kidney target engagement (5C), and liver target engagement (5D) are shown.

[00012] **FIG. 6A-6E.** Effect of RG-NG-1015 at different dosages and regimens and in combination with tolvaptan on *Pcy*/DBA mouse model of PKD was measured. The dosing schedule is shown in FIG. 6A, and the key to the graphs in FIG. 6C-6E is shown in FIG. 6B. Kidney weight/body weight (6C), cystic area (%) (6D), and urine Ngal/Cr (6E) are shown. Error bars represent standard deviations. *p<0.05, **p<0.01, ***p<0.001, ****p<0.001, (ns)p>0.05 compared to Pcy vehicle treated group; One-way ANOVA Bonferroni's multiple comparison test. #p<0.05, ##p<0.01, ###p<0.001, ####p<0.001, (ns)p>0.05 compared to Tolvaptan alone treated group; One-way ANOVA Sadik's multiple comparison test. $p<0.05, $$p<0.01, $$$p<0.001, $$$$p<0.001, (ns)p>0.05 compared to dose-matched RG-NG-1015 alone treated group; One-way ANOVA Sadik's multiple comparison test.

[00013] **FIG. 7A-7D**. Urinary polycystin-1 (PC1; FIG. 7A) and polycystin-2 (PC2; FIG. 7C) levels (shown as PC1/CD133 or PC2/CD133 ratios) across healthy patients and patients with chronic kidney disease and ADPKD were measured. HV: Healthy volunteers; CKD: Chronic Kidney Disease including T1D (Type 1 Diabetes), T2D (Type 2 Diabetes), AKF (Acute Kidney Failure), HT (Hypertension) and COPD patient samples (Chronic Obstructive Pulmonary Disease) with CKD stages 2-4; ADPKD: Autosomal Dominate Polycystic Kidney Disease, where Mayo Imaging Classification were based on htTKV and age; CD133: Prominin-1, has been shown to co-localized on urinary exosome-like vesicles with PC1 and PC2 in ADPKD patients samples (Hogan et al, J Am Soc Nephrol. 2009 Feb; 20(2):278-288). *p-values, One-way ANOVA compared to HV with Dunnett's correction. Urinary PC1 (FIG. 7B) and PC2 (FIG. 7D) levels (shown as PC1/CD133 or PC2/CD133 ratios) across placebo and 1 mg/kg and 2 mg/kg RG-NG-1015 (RGLS8429) baseline and $Mean_{D85\text{-}D113}$ values were measured.

[00014] **FIG. 8A-8C**. Absolute changes in urinary PC1/CD133 ratios from individual baseline were measured for 2 mg/kg RG-NG-1015 (RGLS8429) (FIG. 8A), 1 mg/kg RG-NG-1015 (FIG. 8B), and placebo (FIG. 8C).

[00015] **FIG. 9A-9C**. Absolute changes in urinary PC2/CD133 ratios from individual baseline were measured for 2 mg/kg RG-NG-1015 (FIG. 9A), 1 mg/kg RG-NG-1015 (FIG. 9B), and placebo (FIG. 9C).

[00016] **FIG. 10A-10B**. Exploratory regression analysis of absolute changes in urinary PC1/CD133 (FIG. 10A) and PC2/CD133 (FIG. 10B) ratios from baseline were performed for 2 mg/kg RG-NG-1015, 1 mg/kg RG-NG-1015, and placebo. Solid circle represents RG-NG-1015 treatment (1 mg/kg Q2W x7), solid square represents RG-NG-1015 treatment (2 mg/kg Q2W x7). Open circle represents placebo (cohort 1+2). For RG-NG-1015 (1mg/kg): #, Change from Baseline for PC1; Statistical Significance by Wilcoxon Matched-pairs Signed Rank Test based on PC1/CD133 ratios at Day 85 and 86. #, Change from Baseline for PC2; Statistical Significance by Wilcoxon Matched-pairs Signed Rank Test based on PC2/CD133 ratios at Day 113. For RG-NG-1015 (2mg/kg): #, Change from Baseline for PC1; Statistical Significance by Wilcoxon Matched-pairs Signed Rank Test based on PC1/CD133 ratios at Day 57, 86, 99 and 113. #, Change from Baseline for PC2; Statistical Significance by Wilcoxon Matched-pairs Signed Rank Test based on PC2/CD133 ratios at Day 57. Exploratory regression analysis by non-linear regression (2^{nd} order polynomial). One subject with absolute increase in PC1/CD133=3.85 and PC2/CD133=0.033 at Day 113 was excluded for curve fitting purposes.

[00017] **FIG. 11A-11B**. % changes in urinary PC1/CD133 (FIG. 11A) and PC2/CD133 (FIG. 11B) ratios from baseline was measured for 2 mg/kg RG-NG-1015, 1 mg/kg RG-NG-1015, and placebo, and shown in group mean plots. Urinary polycystin levels before, during, and 28 days after last (and 7^{th}) dose of treatment with 1mg/kg and 2 mg/kg of RG-NG-1015 once every two weeks over 113 days were measured. Urinary PC1 level is shown in FIG. 11A, and urinary PC2 level is shown in FIG. 11B. Solid circle represents RG-NG-1015 treatment (1 mg/kg Q2W x7) (Total N=9 subjects, with Mayo Imaging Class 1C/1D/1E=5/3/1). Open circle represents placebo cohort 1+2 (Q2W x7) (Total N=6 subjects, with Mayo Imaging Class 1C/1D/1E=1/4/1). Solid square represents RG-NG-1015 treatment (2 mg/kg Q2W x7) (Total N=11 subjects, with Mayo Imaging Class 1C/1D/1E=5/4/1).

[00018] **FIG. 12A-12B**. Mean changes in polycystin levels after 3 months of Q2W dosing (average of all available measurements between Day 85 to Day 116) compared to baseline was shown for 2 mg/kg RG-NG-1015, 1 mg/kg RG-NG-1015, and placebo, and FIG. 12A shows absolute change of urinary PC1/CD133 ratios and FIG. 12B shows absolute change of urinary PC2/CD133 Ratios.

[00019] **FIG. 13A-13B**. Mean changes in polycystin levels after 3 months of Q2W dosing (average of all available measurements between Day 85 to Day 116) compared to baseline was shown for 2 mg/kg RG-NG-1015, 1 mg/kg RG-NG-1015, and placebo, and FIG. 13A shows % change of urinary PC1/CD133 ratios and FIG. 13B shows % change of urinary PC2/CD133 Ratios.

[00020] **FIG. 14A-14B.** Correlation between polycystin levels and pharmacokinetics (PK) parameters is shown. Correlation between urine PC1 and single dose C_{max} is shown in FIG. 14A. Correlation between urine PC1 and single dose AUC_{last} is shown in FIG. 14B.

[00021] **FIG. 15A-15C**. Changes in height-adjusted total kidney volume (htTKV) (FIG. 15A) and total kidney cyst volume (TKCV) (FIG. 15B) at the end of study compared to baseline were measured for 2 mg/kg RG-NG-1015, 1 mg/kg RG-NG-1015, and placebo. Correlation between % change of TKCV and % change of htTKV was also measured (FIG. 15C).

[00022] **FIG. 16A-16C.** Changes in total liver volume (TLV) (FIG. 16A) and total liver cyst volume (TLCV) (FIG. 16B) at the end of study compared to baseline were measured for 2 mg/kg RG-NG-1015, 1 mg/kg RG-NG-1015, and placebo. Correlation between absolute change of TLCV and % change of TLV was also measured (FIG. 16C).

[00023] **FIG. 17A-17D.** Exploratory correlation between change in PC1 (shown as absolute change of PC1/CD133, $Mean_{D85\text{-}D113}$ from baseline) and change in htTKV (FIG. 17A) and exploratory correlation between change in PC1 (shown as absolute change of PC1/CD133, $Mean_{D85\text{-}D113}$ values) and change in eGFR (FIG. 17B) were measured. Exploratory correlation between change in PC2 (shown as absolute change of PC1/CD133, $Mean_{D85\text{-}D113}$ from baseline) and change in htTKV (FIG. 17C) and exploratory correlation between change in PC2 (shown as absolute change of PC1/CD133, $Mean_{D85\text{-}D113}$ values) and change in eGFR (FIG. 17D) were measured.

[00024] **FIG. 18A-18C.** RG-NG-1015 inhibits miR-17 and confers efficacy in a dose-responsive manner. Cross-sections of kidneys from *Pkd1*$^{F/RC}$ mice administered RG-NG-1015 (RGLS8429) injected at various doses, PBS, or 20 mg/kg control oligonucleotides are shown in FIG. 18A. *, Cross-section of kidney from age-matched wild type C57BL6 mice (Lakhia et al. 2022 Nat Commun. 2022 Aug 15;13(1):4765) is shown for reference purposes only. $, *Pkd1*$^{F/RC}$ mice in this treatment group were dosed only on post-natal day (P)8 and 12. All other *Pkd1*$^{F/RC}$ mice in the study were dosed on P8, 10, 12 and 15. Calculated % inhibition of kidney weight/body weight (KW/BW) for various *Pkd1*$^{F/RC}$ mice kidney concentrations of RG-NG-1015 (RGLS8429) is shown in FIG. 18B. Calculated % inhibition of miR-17 for various WT-C57BL6 mice kidney concentrations of RGLS-4326 and RG-NG-1015 (RGLS8429) are shown in FIG. 18C.

[00025] **FIG. 19A-19B.** Individual subplots of urinary PC1/CD133 ratio (FIG. 19A) and PC2/CD133 ratio (FIG. 19B), at baseline and at the end of the study, for all cohorts in Part A. Baseline value was calculated as the average of screening and pre-dose values. End of study (EOS) is the mean of values from D85 through D113 (D85-113).

[00026] **FIG. 20A-20B.** Absolute changes in urinary PC1/CD133 (FIG. 20A) and PC2/CD133 (FIG. 20B) during the course of treatment. Absolute change based on raw data (non-transformed). # indicates statistical significance evaluated at 0.05 significance level by Wilcoxon matched-pairs (one-tailed) signed-rank test compared to baseline within treatment group. "Scr1" and "Scr2" indicate data from the subjects' first and second screening visits. In FIG. 20A-20B, each set of four bars is, from left to right: placebo, 1 mg/kg RGLS8429, 2 mg/kg RGLS8429, and 3 mg/kg RGLS8429.

[00027] **FIG. 21A-21B.** Percent changes in urinary PC1/CD133 (FIG. 21A) and urinary PC2/CD133 (FIG. 21B) from baseline to end of the study. Percent change data and mixed model random coefficients regression analysis were performed on a log scale transformation to account for non-normal distribution.

[00028] **FIG. 22A-22C.** Individual subplots of changes in height-adjusted total kidney volume (htTKV) (FIG. 22A), total kidney cyst volume (TKCV) (FIG. 22B), for all cohorts in Part A. Individual subplots of correlation between percent change of TKCV and percent change of htTKV (FIG. 22C), for all cohorts in Part A.

[00029] **FIG. 23A-23B.** Correlation between the absolute change in urinary PC1/CD133 ratio ($Mean_{D85-D113}$ from baseline) (FIG. 23A) and urinary PC2/CD133 ratio ($Mean_{D85-D113}$ from baseline) (FIG. 23B) and the percent change in htTKV (EOS from Baseline). Absolute change (PC) and percent change (htTKV) data and statistical analyses performed on raw data without log scale transformation.

[00030] **FIG. 24.** Correlation between the absolute change of urinary PC1/CD133 ($Mean_{D85-D113}$ from baseline) and the absolute change of eGFR (EOS from baseline). Absolute change (PC and eGFR) and statistical analyses performed on raw data without log scale transformation.

[00031] **FIG. 25A-25B.** Percentage change in urinary PC1/CD133 ($Mean_{D85-D113}$ from baseline) (FIG. 25A) and PC2/CD133 ($Mean_{D85-D113}$ from baseline) (FIG. 25B) for all cohorts in Parts A and B. Geometric least squares mean percent change data shown. Error bars represent standard errors. ANCOVA analyses were performed on log scale transformation to account for non-normal distribution. Data was not available for one subject in each of 3 mg/kg and 300 mg/kg fixed groups.

[00032] **FIG. 26.** Percent change in htTKV for each treatment group in Parts A and B. Geometric least squares mean percent change data shown. Error bars represent standard errors. ANCOVA analyses were performed on log scale transformation to account for non-normal distribution. Data was not available for two subjects in 3 mg/kg group and one subject in 300 mg fixed group. One subject in 300 mg/kg fixed group experienced renal cyst rupture thus only contralateral kidney results were included in the analysis.

DETAILED DESCRIPTION

[00033] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the arts to which the invention belongs. Unless specific definitions are provided, the nomenclature utilized in connection with, and the procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well-known and commonly used in the art. In the event that there is a plurality of definitions for terms herein, those in this section prevail. Standard techniques may be used for chemical synthesis, chemical analysis, pharmaceutical preparation, formulation and delivery, and treatment of subjects. Certain such techniques and procedures may be found for example in "Carbohydrate Modifications in Antisense Research" Edited by Sanghvi and Cook, American Chemical Society, Washington D.C., 1994; and "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, Pa., 18th edition, 1990; and which is hereby incorporated by reference for any purpose. Where permitted, all patents, patent applications, published applications and publications, GENBANK sequences, websites and other published materials referred to throughout the entire disclosure herein, unless noted otherwise, are incorporated by reference in their entirety. Where reference is made to a URL or other such identifier or address, it is understood that such identifiers can change and particular

information on the internet can change, but equivalent information can be found by searching the internet. Reference thereto evidences the availability and public dissemination of such information.

[00034] Before the present compositions and methods are disclosed and described, it is to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting. It must be noted that, as used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise.

Definitions

[00035] "Polycystic kidney disease" or "PKD" is a cystic kidney disease characterized by the accumulation of numerous fluid-filled cysts in the kidney. Multiple cysts form in at least one kidney, frequently leading to enlargement of the affected kidney(s) and progressive loss of kidney function.

[00036] "Marker of polycystic kidney disease" means a medical parameter that is used to assess severity of polycystic kidney disease, kidney function, and/or response of a subject having polycystic kidney disease to treatment. Non-limiting examples of markers of polycystic kidney disease include total kidney volume, hypertension, glomerular filtration rate, and kidney pain.

[00037] "Marker of kidney function" means a medical parameter that is used to assess kidney function in a subject. Non-limiting examples of markers of kidney function include glomerular filtration rate, blood urea nitrogen level, and serum creatinine level.

[00038] "Autosomal dominant polycystic kidney disease" or "ADPKD" is a polycystic kidney disease caused by one or more genetic mutations in the *PKD1* and/or *PKD2* gene. 85% of ADPKD is caused by mutations in *PKD1*, which is located on chromosome 16, with the majority of the remaining ADPKD cases caused by mutations in *PKD2*, which is located on chromosome 4.

[00039] "Autosomal recessive polycystic kidney disease" or "ARPKD" is a polycystic kidney disease caused by one or more genetic mutations in the *PKHD1* gene, which is located on chromosome 6. Up to 50% of neonates with ARPKD die from complications of intrauterine kidney disease, and about a third of those who survive develop end stage renal disease (ESRD) within 10 years.

[00040] "Nephronophthisis" or "NPHP" means an autosomal recessive cystic kidney disease characterized by corticomedullary cysts, tubular basement membrane disruption, and tubulointerstitial nephropathy.

[00041] "Total kidney volume" or "TKV" is a measurement of total kidney volume. Total kidney volume may be determined by Magnetic Resonance Imaging (MRI), Computed Tomography (CT) scan, or ultrasound (US) imaging, and the volume calculated by a standard methodology, such as an ellipsoid volume equation (for ultrasound), or by quantitative stereology or boundary tracing (for CT/MRI).

[00042] "Height-adjusted total kidney volume" or "HtTKV" is a measure of total kidney volume per unit height. Patients with an HtTKV value $\geq$ 600 ml/m are predicted to develop stage 3 chronic kidney disease within 8 years.

[00043] "Kidney pain" means clinically significant kidney pain necessitating medical leave, pharmacologic treatment (narcotic or last-resort analgesic agents), or invasive intervention.

[00044] "Worsening hypertension" means a change in blood pressure that requires initiation of or an increase in hypertensive treatment.

[00045] "Fibrosis" means the formation or development of excess fibrous connective tissue in an organ or tissue. In certain embodiments, fibrosis occurs as a reparative or reactive process. In certain embodiments, fibrosis occurs in response to damage or injury. The term "fibrosis" is to be understood as the formation or development of excess fibrous connective tissue in an organ or tissue as a reparative or reactive process, as opposed to a formation of fibrous tissue as a normal constituent of an organ or tissue.

[00046] "Hematuria" means the presence of red blood cells in the urine.

[00047] "Albuminuria" means the presence of excess albumin in the urine, and includes without limitation, normal albuminuria, high normal albuminuria, microalbuminuria and macroalbuminuria. Normally, the glomerular filtration permeability barrier, which is composed of podocyte, glomerular basement membrane and endothelial cells, prevents serum protein from leaking into urine. Albuminuria may reflect injury of the glomerular filtration permeability barrier. Albuminuria may be calculated from a 24-hour urine sample, an overnight urine sample or a spot-urine sample.

[00048] "High normal albuminuria" means elevated albuminuria characterized by (i) the excretion of 15 to <30 mg of albumin into the urine per 24 hours and/or (ii) an albumin/creatinine ratio of 1.25 to <2.5 mg/mmol (or 10 to <20 mg/g) in males or 1.75 to <3.5 mg/mmol (or 15 to <30 mg/g) in females.

[00049] "Microalbuminuria" means elevated albuminuria characterized by (i) the excretion of 30 to 300 mg of albumin into the urine per 24 hours and/or (ii) an albumin/creatinine ratio of 2.5 to <25 mg/mmol (or 20 to <200 mg/g) in males or 3.5 to <35 mg/mmol (or 30 to <300 mg/g) in females.

[00050] "Macroalbuminuria" means elevated albuminuria characterized by the excretion of more than 300 mg of albumin into the urine per 24 hours and/or (ii) an albumin/creatinine ratio of >25 mg/mmol (or >200 mg/g) in males or >35 mg/mmol (or >300 mg/g) in females.

[00051] "Albumin/creatinine ratio" means the ratio of urine albumin (mg/dL) per urine creatinine (g/dL) and is expressed as mg/g. In certain embodiments, albumin/creatinine ratio may be calculated from a spot-urine sample and may be used as an estimate of albumin excretion over a 24-hour period.

[00052] "Glomerular filtration rate" or "GFR" means the flow rate of filtered fluid through the kidney and is used as an indicator of kidney function in a subject. In certain embodiments, a subject's GFR is determined by calculating an estimated glomerular filtration rate. In certain embodiments, a subject's GFR is directly measured in the subject, using the inulin method.

[00053] "Estimated glomerular filtration rate" or "eGFR" means a measurement of how well the kidneys are filtering creatinine, and is used to approximate glomerular filtration rate. As the direct measurement of GFR is complex, eGFR is frequently used in clinical practice. Normal results may range from 90-120 mL/min/1.73 m^2. Levels below 60 mL/min/1.73 m^2 for 3 or more months may be an indicator chronic kidney disease. Levels below 15 mL/min/1.73 m^2 may be an indicator of kidney failure.

[00054] "Proteinuria" means the presence of an excess of serum proteins in the urine. Proteinuria may be characterized by the excretion of > 250 mg of protein into the urine per 24 hours and/or a urine protein

to creatinine ratio of ≥ 0.20 mg/mg. Serum proteins elevated in association with proteinuria include, without limitation, albumin.

[00055] "Blood urea nitrogen level" or "BUN level" means a measure of the amount of nitrogen in the blood in the form of urea. The liver produces urea in the urea cycle as a waste product of the digestion of protein, and the urea is removed from the blood by the kidneys. Normal human adult blood may contain between 7 to 21 mg of urea nitrogen per 100 ml (7–21 mg/dL) of blood. Measurement of blood urea nitrogen level is used as an indicator of renal health. If the kidneys are not able to remove urea from the blood normally, a subject's BUN level rises.

[00056] "Elevated" means an increase in a medical parameter that is considered clinically relevant. A health professional may determine whether an increase is clinically significant.

[00057] "End stage renal disease (ESRD)" means the complete or almost complete failure of kidney function.

[00058] "Quality of life" means the extent to which a subject's physical, psychological, and social functioning are impaired by a disease and/or treatment of a disease. Quality of life may be reduced in subjects having polycystic kidney disease.

[00059] "Impaired kidney function" means reduced kidney function, relative to normal kidney function.

[00060] "Slow the worsening of" and "slow worsening" mean to reduce the rate at which a medical condition moves towards an advanced state.

[00061] "Delay time to dialysis" means to maintain sufficient kidney function such that the need for dialysis treatment is delayed.

[00062] "Delay time to renal transplant" means to maintain sufficient kidney function such that the need for a kidney transplant is delayed.

[00063] "Improves life expectancy" means to lengthen the life of a subject by treating one or more symptoms of a disease in the subject.

[00064] "Subject" means a human or non-human animal selected for treatment or therapy.

[00065] "Subject in need thereof" means a subject that is identified as in need of a therapy or treatment.

[00066] "Subject suspected of having" means a subject exhibiting one or more clinical indicators of a disease.

[00067] "Disease associated with miR-17" means a disease or condition that is modulated by the activity of one or more miR-17 family members.

[00068] "Administering" means providing a pharmaceutical agent or composition to a subject, and includes, but is not limited to, administering by a medical professional and self-administering.

[00069] "Parenteral administration" means administration through injection or infusion. Parenteral administration includes, but is not limited to, subcutaneous administration, intravenous administration, and intramuscular administration.

[00070] "Subcutaneous administration" means administration just below the skin.

[00071] "Intravenous administration" means administration into a vein.

[00072] "Administered concomitantly" refers to the co-administration of two or more agents in any manner in which the pharmacological effects of both are manifest in the patient at the same time. Concomitant administration does not require that both agents be administered in a single pharmaceutical composition, in the same dosage form, or by the same route of administration. The effects of both agents need not manifest themselves at the same time. The effects need only be overlapping for a period and need not be coextensive.

[00073] "Duration" means the period during which an activity or event continues. In certain embodiments, the duration of treatment is the period during which doses of a pharmaceutical agent or pharmaceutical composition are administered.

[00074] "Therapy" means a disease treatment method. In certain embodiments, therapy includes, but is not limited to, administration of one or more pharmaceutical agents to a subject having a disease.

[00075] "Treat" means to apply one or more specific procedures used for the amelioration of at least one indicator of a disease. In certain embodiments, the specific procedure is the administration of one or more pharmaceutical agents. In certain embodiments, treatment of PKD includes, but is not limited to, reducing total kidney volume, improving kidney function, reducing hypertension, and/or reducing kidney pain.

[00076] "Ameliorate" means to lessen the severity of at least one indicator of a condition or disease. In certain embodiments, amelioration includes a delay or slowing in the progression of one or more indicators of a condition or disease. The severity of indicators may be determined by subjective or objective measures which are known to those skilled in the art.

[00077] "At risk for developing" means the state in which a subject is predisposed to developing a condition or disease. In certain embodiments, a subject at risk for developing a condition or disease exhibits one or more symptoms of the condition or disease, but does not exhibit a sufficient number of symptoms to be diagnosed with the condition or disease. In certain embodiments, a subject at risk for developing a condition or disease exhibits one or more symptoms of the condition or disease, but to a lesser extent required to be diagnosed with the condition or disease.

[00078] "Prevent the onset of" means to prevent the development of a condition or disease in a subject who is at risk for developing the disease or condition. In certain embodiments, a subject at risk for developing the disease or condition receives treatment similar to the treatment received by a subject who already has the disease or condition.

[00079] "Delay the onset of" means to delay the development of a condition or disease in a subject who is at risk for developing the disease or condition. In certain embodiments, a subject at risk for developing the disease or condition receives treatment similar to the treatment received by a subject who already has the disease or condition.

[00080] "Dose" means a specified quantity of a pharmaceutical agent provided in a single administration. In certain embodiments, a dose may be administered in two or more boluses, tablets, or injections. For example, in certain embodiments, where subcutaneous administration is desired, the desired dose requires a volume not easily accommodated by a single injection. In such embodiments, two or more

injections may be used to achieve the desired dose. In certain embodiments, a dose may be administered in two or more injections to minimize injection site reaction in an individual. In certain embodiments, a dose is administered as a slow infusion.

[00081] "Dosage unit" means a form in which a pharmaceutical agent is provided. In certain embodiments, a dosage unit is a vial containing lyophilized oligonucleotide. In certain embodiments, a dosage unit is a vial containing reconstituted oligonucleotide.

[00082] "Therapeutically effective amount" refers to an amount of a pharmaceutical agent that provides a therapeutic benefit to an animal.

[00083] "Pharmaceutical composition" means a mixture of substances suitable for administering to an individual that includes a pharmaceutical agent. For example, a pharmaceutical composition may comprise a sterile aqueous solution.

[00084] "Pharmaceutical agent" means a substance that provides a therapeutic effect when administered to a subject.

[00085] "Active pharmaceutical ingredient" means the substance in a pharmaceutical composition that provides a desired effect.

[00086] "Pharmaceutically acceptable salt" means a physiologically and pharmaceutically acceptable salt of a compound provided herein, *i.e.*, a salt that retains the desired biological activity of the compound and does not have undesired toxicological effects when administered to a subject. Nonlimiting exemplary pharmaceutically acceptable salts of compounds provided herein include sodium and potassium salt forms. The terms "compound," "oligonucleotide," and "modified oligonucleotide" as used herein include pharmaceutically acceptable salts thereof unless specifically indicated otherwise.

[00087] "Saline solution" means a solution of sodium chloride in water.

[00088] "Improved organ function" means a change in organ function toward normal limits. In certain embodiments, organ function is assessed by measuring molecules found in a subject's blood or urine. For example, in certain embodiments, improved kidney function is measured by a reduction in blood urea nitrogen level, a reduction in proteinuria, a reduction in albuminuria, etc.

[00089] "Acceptable safety profile" means a pattern of side effects that is within clinically acceptable limits.

[00090] "Side effect" means a physiological response attributable to a treatment other than desired effects. In certain embodiments, side effects include, without limitation, injection site reactions, liver function test abnormalities, kidney function abnormalities, liver toxicity, renal toxicity, central nervous system abnormalities, and myopathies. Such side effects may be detected directly or indirectly. For example, increased aminotransferase levels in serum may indicate liver toxicity or liver function abnormality. For example, increased bilirubin may indicate liver toxicity or liver function abnormality.

[00091] The term "blood" as used herein, encompasses whole blood and blood fractions, such as serum and plasma.

[00092] "Anti-miR" means an oligonucleotide having a nucleobase sequence complementary to a microRNA. In certain embodiments, an anti-miR is a modified oligonucleotide.

[00093] "Anti-miR-17" means a modified oligonucleotide having a nucleobase sequence complementary to one or more miR-17 family members. In certain embodiments, an anti-miR-17 is fully complementary (i.e., 100% complementary) to one or more miR-17 family members. In certain embodiments, an anti-miR-17 is at least 80%, at least 85%, at least 90%, or at least 95% complementary to one or more miR-17 family members.

[00094] "miR-17" means the mature miRNA having the nucleobase sequence 5'-CAAAGUGCUUACAGUGCAGGUAG-3' (SEQ ID NO: 1).

[00095] "miR-20a" means the mature miRNA having the nucleobase sequence 5'-UAAAGUGCUUAUAGUGCAGGUAG-3' (SEQ ID NO: 2).

[00096] "miR-20b" means the mature miRNA having the nucleobase sequence 5'-CAAAGUGCUCAUAGUGCAGGUAG -3' (SEQ ID NO: 3).

[00097] "miR-93" means the mature miRNA having the nucleobase sequence 5'-CAAAGUGCUGUUCGUGCAGGUAG-3' (SEQ ID NO: 4).

[00098] "miR-106a" means the mature miRNA having the nucleobase sequence 5'-AAAAGUGCUUACAGUGCAGGUAG-3' (SEQ ID NO: 5).

[00099] "miR-106b" means the mature miRNA having the nucleobase sequence 5'-UAAAGUGCUGACAGUGCAGAU-3' (SEQ ID NO: 6).

[000100] "miR-17 seed sequence" means the nucleobase sequence 5'-AAAGUG-3,' which is present in each of the miR-17 family members.

[000101] "miR-17 family member" means a mature miRNA having a nucleobase sequence comprising the miR-17 seed sequence, and which is selected from miR-17, miR-20a, miR-20b, miR-93, miR-106a, and miR-106b.

[000102] "miR-17 family" means the following group of miRNAs: miR-17, miR-20a, miR-20b, miR-93, miR-106a, and miR-106b, each having a nucleobase sequence comprising the miR-17 seed sequence.

[000103] "Target nucleic acid" means a nucleic acid to which an oligomeric compound is designed to hybridize.

[000104] "Targeting" means the process of design and selection of nucleobase sequence that will hybridize to a target nucleic acid.

[000105] "Targeted to" means having a nucleobase sequence that will allow hybridization to a target nucleic acid.

[000106] "Modulation" means a perturbation of function, amount, or activity. In certain embodiments, modulation means an increase in function, amount, or activity. In certain embodiments, modulation means a decrease in function, amount, or activity.

[000107] "Expression" means any functions and steps by which a gene's coded information is converted into structures present and operating in a cell.

[000108] "Nucleobase sequence" means the order of contiguous nucleobases in an oligomeric compound or nucleic acid, typically listed in a 5' to 3' orientation, and independent of any sugar, linkage, and/or nucleobase modification.

[000109] "Contiguous nucleobases" means nucleobases immediately adjacent to each other in a nucleic acid.

[000110] "Nucleobase complementarity" means the ability of two nucleobases to pair non-covalently via hydrogen bonding.

[000111] "Complementary" means that one nucleic acid is capable of hybridizing to another nucleic acid or oligonucleotide. In certain embodiments, complementary refers to an oligonucleotide capable of hybridizing to a target nucleic acid.

[000112] "Fully complementary" means each nucleobase of an oligonucleotide is capable of pairing with a nucleobase at each corresponding position in a target nucleic acid. In certain embodiments, an oligonucleotide is fully complementary (also referred to as 100% complementary) to a microRNA, i.e. each nucleobase of the oligonucleotide is complementary to a nucleobase at a corresponding position in the microRNA. A modified oligonucleotide may be fully complementary to a microRNA, and have a number of linked nucleosides that is less than the length of the microRNA. For example, an oligonucleotide with 16 linked nucleosides, where each nucleobase of the oligonucleotide is complementary to a nucleobase at a corresponding position in a microRNA, is fully complementary to the microRNA. In certain embodiments, an oligonucleotide wherein each nucleobase has complementarity to a nucleobase within a region of a microRNA stem-loop sequence is fully complementary to the microRNA stem-loop sequence.

[000113] "Percent complementarity" means the percentage of nucleobases of an oligonucleotide that are complementary to an equal-length portion of a target nucleic acid. Percent complementarity is calculated by dividing the number of nucleobases of the oligonucleotide that are complementary to nucleobases at corresponding positions in the target nucleic acid by the total number of nucleobases in the oligonucleotide.

[000114] "Percent identity" means the number of nucleobases in a first nucleic acid that are identical to nucleobases at corresponding positions in a second nucleic acid, divided by the total number of nucleobases in the first nucleic acid. In certain embodiments, the first nucleic acid is a microRNA and the second nucleic acid is a microRNA. In certain embodiments, the first nucleic acid is an oligonucleotide and the second nucleic acid is an oligonucleotide.

[000115] "Hybridize" means the annealing of complementary nucleic acids that occurs through nucleobase complementarity.

[000116] "Mismatch" means a nucleobase of a first nucleic acid that is not capable of Watson-Crick pairing with a nucleobase at a corresponding position of a second nucleic acid.

[000117] "Identical" in the context of nucleobase sequences, means having the same nucleobase sequence, independent of sugar, linkage, and/or nucleobase modifications and independent of the methylation state of any pyrimidines present.

[000118] "MicroRNA" means an endogenous non-coding RNA between 18 and 25 nucleobases in length, which is the product of cleavage of a pre-microRNA by the enzyme Dicer. Examples of mature

microRNAs are found in the microRNA database known as miRBase (microrna.sanger.ac.uk/). In certain embodiments, microRNA is abbreviated as "miR."

[000119] "microRNA-regulated transcript" means a transcript that is regulated by a microRNA.

[000120] "Seed match sequence" means a nucleobase sequence that is complementary to a seed sequence, and is the same length as the seed sequence.

[000121] "Oligomeric compound" means a compound that comprises a plurality of linked monomeric subunits. Oligomeric compounds include oligonucleotides.

[000122] "Oligonucleotide" means a compound comprising a plurality of linked nucleosides, each of which can be modified or unmodified, independent from one another.

[000123] "Naturally occurring internucleoside linkage" means a 3' to 5' phosphodiester linkage between nucleosides.

[000124] "Natural sugar" means a sugar found in DNA (2'-H) or RNA (2'-OH).

[000125] "Internucleoside linkage" means a covalent linkage between adjacent nucleosides.

[000126] "Linked nucleosides" means nucleosides joined by a covalent linkage.

[000127] "Nucleobase" means a heterocyclic moiety capable of non-covalently pairing with another nucleobase.

[000128] "Nucleoside" means a nucleobase linked to a sugar moiety.

[000129] "Nucleotide" means a nucleoside having a phosphate group covalently linked to the sugar portion of a nucleoside.

[000130] "Compound comprising a modified oligonucleotide consisting of" a number of linked nucleosides means a compound that includes a modified oligonucleotide having the specified number of linked nucleosides. Thus, the compound may include additional substituents or conjugates. Unless otherwise indicated, the modified oligonucleotide is not hybridized to a complementary strand and the compound does not include any additional nucleosides beyond those of the modified oligonucleotide.

[000131] "Modified oligonucleotide" means a single-stranded oligonucleotide having one or more modifications relative to a naturally occurring terminus, sugar, nucleobase, and/or internucleoside linkage. A modified oligonucleotide may comprise unmodified nucleosides.

[000132] "Modified nucleoside" means a nucleoside having any change from a naturally occurring nucleoside. A modified nucleoside may have a modified sugar and an unmodified nucleobase. A modified nucleoside may have a modified sugar and a modified nucleobase. A modified nucleoside may have a natural sugar and a modified nucleobase. In certain embodiments, a modified nucleoside is a bicyclic nucleoside. In certain embodiments, a modified nucleoside is a non-bicyclic nucleoside.

[000133] "Modified internucleoside linkage" means any change from a naturally occurring internucleoside linkage.

[000134] "Phosphorothioate internucleoside linkage" means a linkage between nucleosides where one of the non-bridging atoms is a sulfur atom.

[000135] "Modified sugar moiety" means substitution and/or any change from a natural sugar.

[000136] "Unmodified nucleobase" means the naturally occurring heterocyclic bases of RNA or DNA: the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) (including 5-methylcytosine), and uracil (U).

[000137] "5-methylcytosine" means a cytosine comprising a methyl group attached to the 5 position.

[000138] "Non-methylated cytosine" means a cytosine that does not have a methyl group attached to the 5 position.

[000139] "Modified nucleobase" means any nucleobase that is not an unmodified nucleobase.

[000140] "Sugar moiety" means a naturally occurring furanosyl or a modified sugar moiety.

[000141] "Modified sugar moiety" means a substituted sugar moiety or a sugar surrogate.

[000142] "2'-O-methyl sugar" or "2'-OMe sugar" means a sugar having an O-methyl modification at the 2' position.

[000143] "2'-O-methoxyethyl sugar" or "2'-MOE sugar" means a sugar having an O-methoxyethyl modification at the 2' position.

[000144] "2'-fluoro" or "2'-F" means a sugar having a fluoro modification of the 2' position.

[000145] "Bicyclic sugar moiety" means a modified sugar moiety comprising a 4 to 7 membered ring (including by not limited to a furanosyl) comprising a bridge connecting two atoms of the 4 to 7 membered ring to form a second ring, resulting in a bicyclic structure. In certain embodiments, the 4 to 7 membered ring is a sugar ring. In certain embodiments, the 4 to 7 membered ring is a furanosyl. In certain such embodiments, the bridge connects the 2'-carbon and the 4'-carbon of the furanosyl. Nonlimiting exemplary bicyclic sugar moieties include LNA, ENA, cEt, S-cEt, and R-cEt.

[000146] "Locked nucleic acid (LNA) sugar moiety" means a substituted sugar moiety comprising a (CH_2)-O bridge between the 4' and 2' furanose ring atoms.

[000147] "ENA sugar moiety" means a substituted sugar moiety comprising a $(CH_2)_2$-O bridge between the 4' and 2' furanose ring atoms.

[000148] "Constrained ethyl (cEt) sugar moiety" means a substituted sugar moiety comprising a $CH(CH_3)$-O bridge between the 4' and the 2' furanose ring atoms. In certain embodiments, the $CH(CH_3)$-O bridge is constrained in the S orientation. In certain embodiments, the $CH(CH_3)$-O is constrained in the R orientation.

[000149] "S-cEt sugar moiety" means a substituted sugar moiety comprising an S-constrained $CH(CH_3)$-O bridge between the 4' and the 2' furanose ring atoms.

[000150] "R-cEt sugar moiety" means a substituted sugar moiety comprising an R-constrained $CH(CH_3)$-O bridge between the 4' and the 2' furanose ring atoms.

[000151] "2'-O-methyl nucleoside" means a 2'-modified nucleoside having a 2'-O-methyl sugar modification.

[000152] "2'-O-methoxyethyl nucleoside" means a 2'-modified nucleoside having a 2'-O-methoxyethyl sugar modification. A 2'-O-methoxyethyl nucleoside may comprise a modified or unmodified nucleobase.

[000153] "2'-fluoro nucleoside" means a 2'-modified nucleoside having a 2'-fluoro sugar modification. A 2'-fluoro nucleoside may comprise a modified or unmodified nucleobase.

[000154] "Bicyclic nucleoside" means a 2'-modified nucleoside having a bicyclic sugar moiety. A bicyclic nucleoside may have a modified or unmodified nucleobase.

[000155] "cEt nucleoside" means a nucleoside comprising a cEt sugar moiety. A cEt nucleoside may comprise a modified or unmodified nucleobase.

[000156] "S-cEt nucleoside" means a nucleoside comprising an S-cEt sugar moiety.

[000157] "R-cEt nucleoside" means a nucleoside comprising an R-cEt sugar moiety.

[000158] "β-D-deoxyribonucleoside" means a naturally occurring DNA nucleoside.

[000159] "β-D-ribonucleoside" means a naturally occurring RNA nucleoside.

[000160] "LNA nucleoside" means a nucleoside comprising a LNA sugar moiety.

[000161] "ENA nucleoside" means a nucleoside comprising an ENA sugar moiety.

[000162] "Hydrogen bond acceptor" means the component of a hydrogen bond that does not supply the shared hydrogen atom.

[000163] "Hydrogen bond donor" means the bond or molecule that supplies the hydrogen atom of a hydrogen bond.

[000164] Reference to "about" a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter per se. In certain embodiments, the term "about" includes the indicated amount ± 10%. In other embodiments, the term "about" includes the indicated amount ± 5%. In certain other embodiments, the term "about" includes the indicated amount ± 1%. Also, the term "about X" includes description of "X". Further, the singular forms "a" and "the" include plural references unless the context clearly dictates otherwise. Thus, e.g., reference to "the compound" includes a plurality of such compounds.

Overview

[000165] Polycystic kidney disease (PKD) is an inherited form of kidney disease in which fluid-filled cysts develop in the kidneys, leading to renal insufficiency, and often end-stage renal disease (ESRD). Certain PKDs are also characterized by kidney enlargement. The excessive proliferation of cysts is a hallmark pathological feature of PKD. In the management of PKD, the primary goal for treatment is to manage symptoms such as hypertension and infections, maintain kidney function and prevent or delay the onset of ESRD, which in turn improves life expectancy of subjects with PKD.

[000166] miR-17 has been identified as a target for the treatment of PKD. The anti-miR-17 compound RGLS4326 was discovered by screening a chemically diverse and rationally designed library of anti-miR-17 oligonucleotides for optimal pharmaceutical properties. 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 attenuates cyst growth in human *in vitro* autosomal dominant polycystic kidney disease (ADPKD) models and multiple PKD mouse models after subcutaneous administration. A Phase 1 single ascending

dose (SAD) clinical trial in healthy volunteers (HV) was initiated in December 2017, followed by a Phase 1 multiple ascending dose (MAD) study in HV that was initiated in May 2018.

[000167] Subsequent to the initiation of the phase 1b MAD clinical trial, nonclinical toxicology studies revealed CNS-related findings, including abnormal gait, reduced motor activity, and/or prostration, at high doses of RGLS4326 in mice. RGLS4326 was found to be an antagonist of the AMPA receptor (AMPA-R), a glutamate receptor and ion channel on excitatory synapses in the central nervous system (CNS) that mediates fast excitatory neurotransmission and, therefore, is a key component of all neuronal networks. Antagonism of the AMPA receptor could explain the CNS-mediated findings observed at high doses of RGLS4326 in nonclinical toxicology models. While no such CNS-related findings were observed in human subjects, it is nonetheless preferable to avoid antagonism of the AMPA receptor. Accordingly, a library of anti-miR-17 compounds was screened to identify compounds with physicochemical and pharmacological properties comparable to RGLS4326, that also have a more favorable safety profile (for example, able to avoid antagonism of AMPA receptor).

[000168] One such compound, RG-NG-1015, was identified and selected as a candidate therapeutic agent for the treatment of ADPKD. The structure names "RG-NG-1015" and "RGLS8429" are used interchangeably herein.

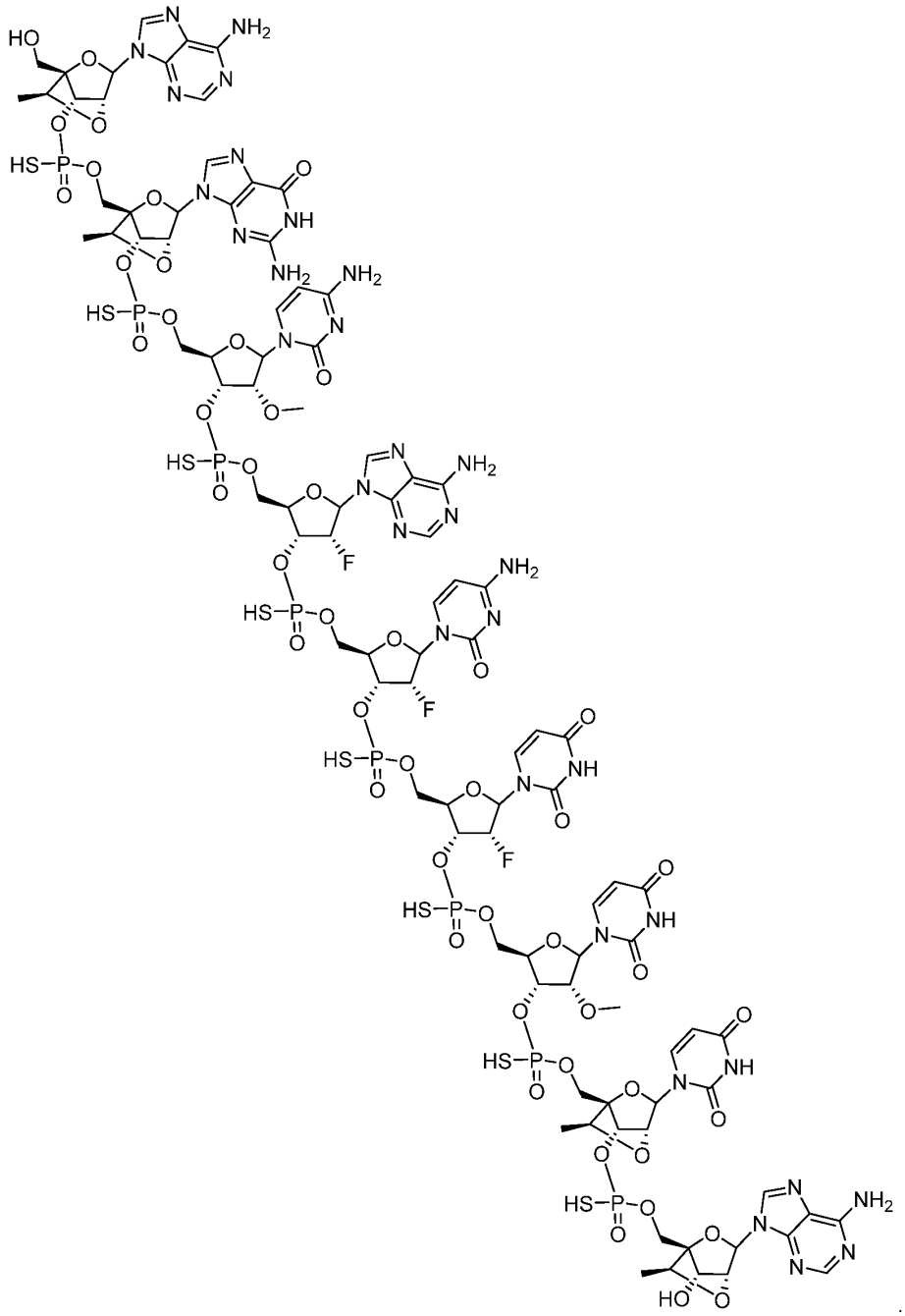
RG-NG-1015 and Related Compounds

[000169] Provided herein is a compound comprising a modified oligonucleotide, wherein the modified oligonucleotide has the following structure 5'-$A_SG_SC_MA_FC_FU_FU_MU_SA_S$-3', wherein each cytosine is a non-methylated cytosine.

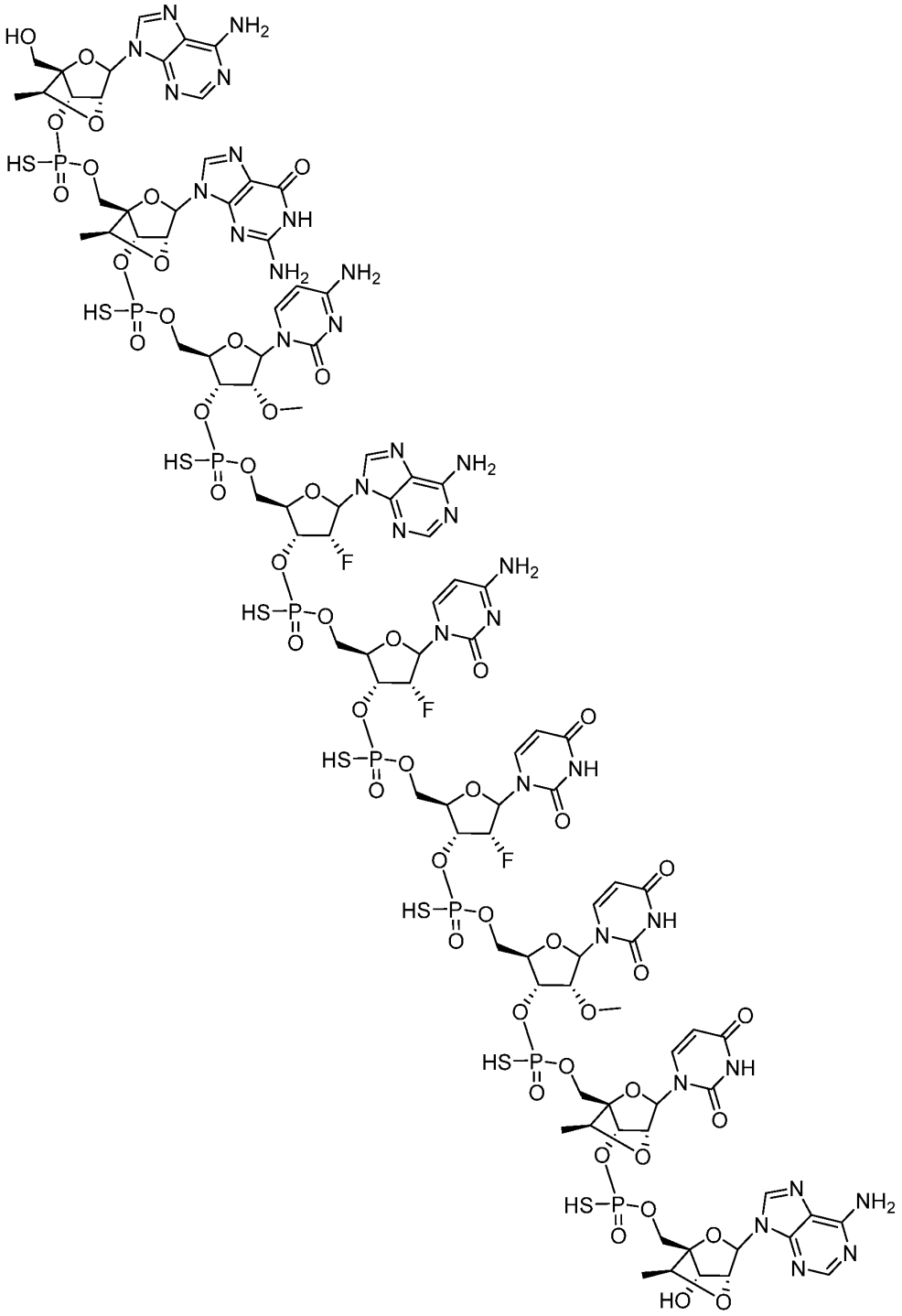
[000170] In certain embodiments, the compound consists of the modified oligonucleotide.

[000171] In certain embodiments, the pharmaceutically acceptable salt is a sodium salt.

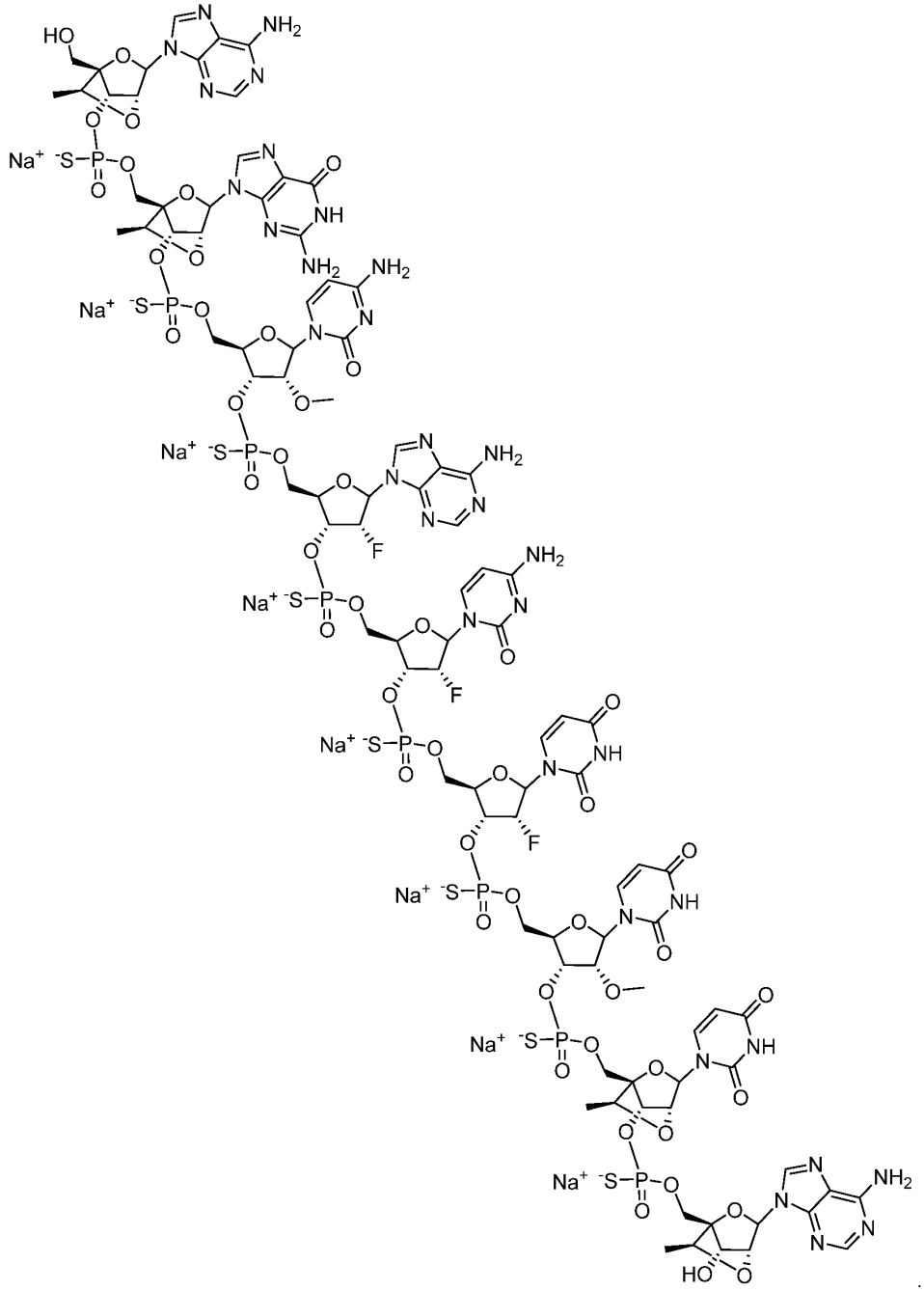
[000172] Provided herein is a modified oligonucleotide named RG-NG-1015, wherein the structure of the modified oligonucleotide is:



[000173] Provided herein are also pharmaceutically acceptable salts of modified oligonucleotide RG-NG-1015. Thus, in some embodiments, a modified oligonucleotide has the structure:



or a pharmaceutically acceptable salt thereof. A nonlimiting exemplary pharmaceutically acceptable salt of RG-NG-1015 has the structure:



[000174] In some embodiments, a pharmaceutically acceptable salt of a modified oligonucleotide comprises fewer cationic counterions (such as Na^+) than there are phosphorothioate and/or phosphodiester linkages per molecule (i.e., some phosphorothioate and/or phosphodiester linkages are protonated). In some embodiments, a pharmaceutically acceptable salt of RG-NG-1015 comprises fewer than 8 cationic counterions (such as Na^+) per molecule of RG-NG-1015. That is, in some embodiments, a pharmaceutically acceptable salt of RG-NG-1015 may comprise, on average, 1, 2, 3, 4, 5, 6, or 7

cationic counterions per molecule of RG-NG-1015, with the remaining phosphorothioate groups being protonated.

[000175] As used herein, and in the absence of a specific reference to a particular pharmaceutically acceptable salt of RG-NG-1015, any dosages, whether expressed in e.g. mg/kg, mg, or as a % by weight, should be taken as referring to the amount of RG-NG-1015, in its protonated form (*i.e.*, not as a salt).

[000176] In some embodiments, RG-NG-1015 is formulated for subcutaneous administration. In some such embodiments, RG-NG-1015 is provided in a pre-filled vial containing sufficient volume to extract 1 mL of 150 mg/mL RG-NG-1015. In some embodiments, the RG-NG-1015 is in a solution comprising 0.3% saline.

Methods of Treating Polycystic Kidney Disease

[000177] Provided herein are methods for inhibiting the activity of one or more members of the miR-17 family in a cell, comprising contacting a cell with a compound provided herein, which comprises a nucleobase sequence complementary to the miR-17 seed sequence.

[000178] Provided herein are methods for inhibiting the activity of one or more members of the miR-17 family in a subject, comprising administering to the subject a pharmaceutical composition provided herein. In certain embodiments, the subject has a disease associated with one or more members of the miR-17 family.

[000179] Provided herein are methods for the treatment of polycystic kidney disease (PKD), comprising administering to a subject in need thereof a compound provided herein, which comprises a nucleobase sequence complementary to the miR-17 seed sequence. In certain embodiments, the subject has a polycystic kidney disease. In certain embodiments, the polycystic kidney disease is selected from autosomal dominant polycystic kidney disease (ADPKD), autosomal recessive polycystic kidney disease (ARPKD), and nephronophthisis (NPHP). In certain embodiments, the polycystic kidney disease is selected from autosomal dominant polycystic kidney disease (ADPKD) and autosomal recessive polycystic kidney disease (ARPKD).

[000180] In certain embodiments, the subject has a disorder that is characterized by multiple non-renal indicators, and also by polycystic kidney disease. Such disorders include, for example, Joubert syndrome and related disorders (JSRD), Meckel syndrome (MKS), or Bardet-Biedl syndrome (BBS). Accordingly, provided herein are methods for the treatment of polycystic kidney disease (PKD), comprising administering to a subject a compound provided herein, which comprises a nucleobase sequence complementary to the miR-17 seed sequence, wherein the subject has Joubert syndrome and related disorders (JSRD), Meckel syndrome (MKS), or Bardet-Biedl syndrome (BBS). Provided herein are methods for the treatment of polycystic kidney disease (PKD), comprising administering a compound provided herein, which comprises a nucleobase sequence complementary to the miR-17 seed sequence, wherein the subject is suspected of having Joubert syndrome and related disorders (JSRD), Meckel syndrome (MKS), or Bardet-Biedl syndrome (BBS).

[000181] In certain embodiments, the polycystic kidney disease is autosomal dominant polycystic kidney disease (ADPKD). ADPKD is caused by mutations in the *PKD1* or *PKD2* gene. ADPKD is a progressive disease in which cyst formation and renal enlargement lead to renal insufficiency and eventually end-stage renal disease in 50% of patients by age 60. ADPKD patients may require lifelong dialysis and/or kidney transplant. ADPKD is the most frequent genetic cause of kidney failure. The excessive proliferation of cysts is a hallmark pathological feature of ADPKD. In the management of PKD, the primary goal for treatment is to maintain kidney function and prevent or delay the onset of end-stage renal disease (ESRD), which in turn improves life expectancy of subjects with PKD. Total kidney volume generally increases steadily in ADPKD patients, with increases correlating with a decline in kidney function. Provided herein are methods for the treatment of ADPKD, comprising administering to a subject having or suspected of having ADPKD a compound provided herein, which comprises a nucleobase sequence complementary to the miR-17 seed sequence.

[000182] In certain embodiments, the polycystic kidney disease is autosomal recessive polycystic kidney disease (ARPKD). ARPKD is caused by mutations in the *PKHD1* gene, and is a cause of chronic kidney disease in children. A typical renal phenotype of ARPKD is enlarged kidneys; however, ARPKD has notable effects on other organs, particularly the liver. Patients with ARPKD progress to end-stage renal disease and require a kidney transplant as young as 15 years of age. Provided herein are methods for the treatment of ARPKD, comprising administering to a subject having or suspected of having ARPKD a compound provided herein, which comprises a nucleobase sequence complementary to the miR-17 seed sequence.

[000183] In certain embodiments, the polycystic kidney disease is nephronophthisis (NPHP). Nephronophthisis is an autosomal recessive cystic kidney disease that is a frequent cause of ESRD in children. NPHP is characterized by kidneys of normal or reduced size, cysts concentrated at the corticomedullary junction, and tubulointerstitial fibrosis. Mutations in one of several NPHP genes, for example, *NPHP1*, have been identified in patients with NPHP. Provided herein are methods for the treatment of NPHP, comprising administering to a subject having or suspected of having NPHP a compound provided herein, which comprises a nucleobase sequence complementary to the miR-17 seed sequence.

[000184] In certain embodiments, a subject having polycystic kidney disease has Joubert syndrome and related disorders (JSRD). JSRD includes a broad range of hallmark features, including brain, retinal, and skeletal abnormalities. Certain subjects with JSRD have polycystic kidney disease, in addition to hallmark features of JSRD. Accordingly, provided herein are methods for the treatment of polycystic kidney disease in a subject having JSRD, comprising administering to a subject having JSRD a compound provided herein, which comprises a nucleobase sequence complementary to the miR-17 seed sequence. In certain embodiments, a subject is suspected of having JSRD.

[000185] In certain embodiments, a subject having polycystic kidney disease has Meckel syndrome (MKS). MKS is a disorder with severe signs and symptoms in many parts of the body, including the central nervous system, skeletal system, liver, kidney, and heart. Common features of MKS is the

presence of numerous fluid-filled cysts in the kidney, and kidney enlargement. Accordingly, provided herein are methods for the treatment of MKS, comprising administering to a subject having MKS a compound provided herein, which comprises a nucleobase sequence complementary to the miR-17 seed sequence. In certain embodiments, the subject is suspected of having MKS.

[000186] In certain embodiments, a subject having polycystic kidney disease has Bardet-Biedl syndrome (BBS). BBS is disorder affecting many parts of the body, including the eye, heart, kidney, liver and digestive system. A hallmark feature of BBS is the presence of renal cysts. Accordingly, provided herein are methods for the treatment of polycystic kidney disease in a subject having BBS, comprising administering to a subject having BBS a compound provided herein, which comprises a nucleobase sequence complementary to the miR-17 seed sequence. In certain embodiments, the subject is suspected of having BBS.

[000187] In certain embodiments, the subject has been diagnosed as having PKD prior to administration of the compound comprising the modified oligonucleotide. Diagnosis of PKD may be achieved through evaluation of parameters including, without limitation, a subject's family history, clinical features (including without limitation hypertension, albuminuria, hematuria, and impaired GFR), kidney imaging studies (including without limitation MRI, ultrasound, and CT scan), and/or histological analysis.

[000188] In some embodiments, the subject with ADPKD is classified as class 1C, 1D, or 1E according to Mayo Imaging Classification of ADKPD. In some embodiments, the subject with ADPKD is defined as a subject having estimated glomerular filtration rate (eGFR) between 30 to 90 mL/min/1.73 m^2.

[000189] In some embodiments, a method of treating ADPKD is provided, comprising administering to a subject in need thereof a fixed dose of between about 150 mg and about 350 mg, between about 150 mg and about 300 mg, between about 150 mg and about 250 mg, between about 150 mg and about 200 mg, between about 200 mg and about 350 mg, between about 200 mg and about 300 mg, between about 200 mg and about 250 mg, between about 250 mg and about 350 mg, or between about 250 mg and about 300 mg of a compound comprising a modified oligonucleotide as discussed herein, such as RG-NG-1015, or a pharmaceutically acceptable salt thereof, such as sodium salt of RG-NG-1015. In some embodiments, the compound comprising a modified oligonucleotide or pharmaceutically acceptable salt thereof is administered at a fixed dose of between 150 mg and 350 mg, between 150 mg and 300 mg, between 150 mg and 250 mg, between 150 mg and 200 mg, between 200 mg and 350 mg, between 200 mg and 300mg, between 200 mg and 250 mg, between 250 mg and 350 mg, or between 250 mg and 300 mg. In some embodiments, the compound comprising a modified oligonucleotide or pharmaceutically acceptable salt thereof is administered at a fixed dose of about 150 mg, about 160 mg, about 170 mg, about 180 mg, about 190 mg, about 200 mg, about 210 mg, about 220 mg, about 230 mg, about 240 mg, about 250 mg, about 260 mg, about 270 mg, about 280 mg, about 290 mg, about 300 mg, about 310 mg, about 320 mg, about 330 mg, about 340 mg, or about 350 mg. In some embodiments, the compound comprising a modified oligonucleotide or pharmaceutically acceptable salt thereof is administered at a fixed dose of 150 mg, 160 mg, 170 mg, 180 mg, 190 mg, 200 mg, 210 mg, 220 mg, 230 mg, 240 mg, 250 mg, 260 mg, 270 mg, 280 mg, 290 mg, 300 mg, 310 mg, 320 mg, 330 mg, 340 mg, or 350 mg. In some

embodiments, the compound comprising a modified oligonucleotide or pharmaceutically acceptable salt thereof is administered at a fixed dose of about 150 mg or 150 mg. In some embodiments, the compound comprising a modified oligonucleotide or pharmaceutically acceptable salt thereof is administered at a fixed dose of about 160 mg or 160 mg. In some embodiments, the compound comprising a modified oligonucleotide or pharmaceutically acceptable salt thereof is administered at a fixed dose of about 170 mg or 170 mg. In some embodiments, the compound comprising a modified oligonucleotide or pharmaceutically acceptable salt thereof is administered at a fixed dose of about 180 mg or 180 mg. In some embodiments, the compound comprising a modified oligonucleotide or pharmaceutically acceptable salt thereof is administered at a fixed dose of about 190 mg or 190 mg. In some embodiments, the compound comprising a modified oligonucleotide or pharmaceutically acceptable salt thereof is administered at a fixed dose of about 200 mg or 200 mg. In some embodiments, the compound comprising a modified oligonucleotide or pharmaceutically acceptable salt thereof is administered at a fixed dose of about 210 mg or 210 mg. In some embodiments, the compound comprising a modified oligonucleotide or pharmaceutically acceptable salt thereof is administered at a fixed dose of about 220 mg or 220 mg. In some embodiments, the compound comprising a modified oligonucleotide or pharmaceutically acceptable salt thereof is administered at a fixed dose of about 230 mg or 230 mg. In some embodiments, the compound comprising a modified oligonucleotide or pharmaceutically acceptable salt thereof is administered at a fixed dose of about 240 mg or 240 mg. In some embodiments, the compound comprising a modified oligonucleotide or pharmaceutically acceptable salt thereof is administered at a fixed dose of about 250 mg or 250 mg. In some embodiments, the compound comprising a modified oligonucleotide or pharmaceutically acceptable salt thereof is administered at a fixed dose of about 260 mg or 260 mg. In some embodiments, the compound comprising a modified oligonucleotide or pharmaceutically acceptable salt thereof is administered at a fixed dose of about 270 mg or 270 mg. In some embodiments, the compound comprising a modified oligonucleotide or pharmaceutically acceptable salt thereof is administered at a fixed dose of about 280 mg or 280 mg. In some embodiments, the compound comprising a modified oligonucleotide or pharmaceutically acceptable salt thereof is administered at a fixed dose of about 290 mg or 290 mg. In some embodiments, the compound comprising a modified oligonucleotide or pharmaceutically acceptable salt thereof is administered at a fixed dose of about 300 mg or 300 mg. In some embodiments, the compound comprising a modified oligonucleotide or pharmaceutically acceptable salt thereof is administered at a fixed dose of about 310 mg or 310 mg. In some embodiments, the compound comprising a modified oligonucleotide or pharmaceutically acceptable salt thereof is administered at a fixed dose of about 320 mg or 320 mg. In some embodiments, the compound comprising a modified oligonucleotide or pharmaceutically acceptable salt thereof is administered at a fixed dose of about 330 mg or 330 mg. In some embodiments, the compound comprising a modified oligonucleotide or pharmaceutically acceptable salt thereof is administered at a fixed dose of about 340 mg or 340 mg. In some embodiments, the compound comprising a modified oligonucleotide or pharmaceutically acceptable salt thereof is administered at a fixed dose of about 350 mg or 350 mg. In some embodiments, the compound

comprising a modified oligonucleotide or pharmaceutically acceptable salt thereof is administered subcutaneously.

[000190] In some embodiments, the compound comprising a modified oligonucleotide or pharmaceutically acceptable salt thereof is administered every 2 weeks (14 days). In some embodiments, the compound comprising a modified oligonucleotide or pharmaceutically acceptable salt thereof is administered at least once, at least twice, at least 3 times, at least 4 times, at least 5 times, at least 6 times, at least 7 times, or more. In some embodiments, the subject is not administered tolvaptan in the 28 days before administration of the compound comprising a modified oligonucleotide as discussed herein or pharmaceutically acceptable salt thereof.

[000191] In certain embodiments, diagnosis of PKD includes screening for mutations in one or more of the *PKD1* or *PKD2* genes. In certain embodiments, diagnosis of ARPKD includes screening for mutations in the *PKHP1* gene. In certain embodiments, diagnosis of NPHP includes screening for one or more mutations in one or more of the *NPHP1*, *NPHP2*, *NPHP3*, *NPHP4*, *NPHP5*, *NPHP6*, *NPHP7*, *NPHP8*, or *NPHP9* genes. In certain embodiments, diagnosis of JSRD includes screening for mutations in the *NPHP1, NPHP6, AHI1, MKS3, or RPGRIP1L* genes. In certain embodiments, diagnosis of MKS includes screening for mutations in the *NPHP6, MKS3, RPGRIP1L, NPHP3, CC2D2A, BBS2, BBS4, BBS6,* or *MKS1* genes. In certain embodiments, diagnosis of BBS includes screening for mutations in *BBS2, BBS4, BBS6, MKS1, BBS1, BBS3, BBS5, BBS7, BBS7, BBS8, BBS9, BBS10, BBS11,* or *BBS12* genes.

[000192] In certain embodiments, the subject has an increased total kidney volume. In certain embodiments, the total kidney volume is height-adjusted total kidney volume (HtTKV). In certain embodiments, the subject has hypertension. In certain embodiments, the subject has impaired kidney function. In certain embodiments, the subject is in need of improved kidney function. In certain embodiments, the subject is identified as having impaired kidney function.

[000193] In certain embodiments, levels of one or more miR-17 family members are increased in the kidney of a subject having PKD. In certain embodiments, prior to administration, a subject is determined to have an increased level of one or more miR-17 family members in the kidney. The level of a miR-17 family member may be measured from kidney biopsy material. In certain embodiments, prior to administration, a subject is determined to have an increased level of one or more miR-17 family members in the urine or blood of the subject. In certain embodiments, prior to administration, a subject is determined to have a decreased level of polycystin-1 (PC1) or polycystin-2 (PC2) in the urine of the subject. In certain embodiments, prior to administration, a subject is determined to have a decreased level of polycystin-1 (PC1) or polycystin-2 (PC2) in the urine of the subject. In certain embodiments, prior to administration, a subject is determined to have a decreased level of polycystin-1 (PC1) and/or polycystin-2 (PC2) in the urine of the subject.

[000194] In some embodiments, prior to administration, a subject is determined to have an increased level of neutrophil gelatinase-associated lipocalin (NGAL) and/or kidney injury molecule-1 (KIM-1) in the urine of the subject. In some embodiments, prior to administration, a subject is determined to have an

increased level of neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule-1 (KIM-1) in the urine of the subject. In some embodiments, prior to administration, a subject is determined to have an increased level of neutrophil gelatinase-associated lipocalin (NGAL) or kidney injury molecule-1 (KIM-1) in the urine of the subject.

[000195] In any of the embodiments provided herein, a subject may undergo certain tests prior to administration, during administration, and/or after administration to diagnose polycystic kidney disease in the subject, for example, to determine the cause of the polycystic kidney disease, to evaluate the extent of polycystic kidney disease in the subject, and/or to determine the subject's response to treatment. Such tests may assess markers of polycystic kidney disease. Certain of these tests, such as glomerular filtration rate (GFR) and blood urea nitrogen (BUN) level, are also indicators of kidney function. Markers of polycystic disease include, without limitation: measurement of total kidney volume and height-adjusted total kidney volume (htTKV) in the subject; measurement of hypertension in the subject; assessment of kidney pain the in the subject; measurement of fibrosis in the subject; measurement of polycystin-1 (PC1) in the urine of the subject; measurement of polycystin-2 (PC2) in the urine of the subject; measurement of blood urea nitrogen (BUN) level in the subject; measurement of serum creatinine (SCr) level in the subject; measuring creatinine clearance in the subject; measuring albuminuria in the subject; measuring albumin:creatinine ratio in the subject; measuring glomerular filtration rate (GFR) and estimated GFR (eGFR) in the subject; measuring hematuria in the subject; measurement of NGAL protein in the urine of the subject; and/or measurement of KIM-1 protein in the urine of the subject. Unless indicated otherwise herein, blood urea nitrogen (BUN) level, serum creatinine (SCr) level, creatinine clearance, albuminuria, albumin:creatinine ratio, glomerular filtration rate (GFR), and hematuria refer to a measurement in the blood (such as whole blood or serum) of a subject.

[000196] In some embodiments, subject may also undergo additional tests prior to administration, during administration, and/or after administration, such as measurement of monocyte chemoattractant protein-1 (MCP-1) and/or beta-2 microglobulin (B2M) in urine of the subject; measurement of insulin-like growth factor binding protein acid labile subunit (IGFALS), copeptin (CT-proAVP), and/ or N-acetyl-1-methylhistidine in serum of the subject; measurement of complement split products C3a and/or Bb in plasma of the subject; and/or measurement of total cyst volume, number and/or size distribution of the cyst in the subject.

[000197] Markers of polycystic kidney disease are determined by laboratory testing. The reference ranges for individual markers may vary from laboratory to laboratory. The variation may be due to, for example, differences in the specific assays used. Thus, the upper and lower limits of the normal distribution of the marker within a population, also known as the upper limit of normal (ULN) and lower limit of normal (LLN), respectively, may vary from laboratory to laboratory. For any particular marker, a health professional may determine which levels outside of the normal distribution are clinically relevant and/or indicative of disease. For example, a health professional may determine the glomerular filtration rate that may be indicative of a decline in the rate of kidney function in a subject with polycystic kidney disease.

[000198] In certain embodiments, administration of a compound provided herein results in one or more clinically beneficial outcomes. In certain embodiments, the administration improves kidney function in the subject. In certain embodiments, the administration slows the rate of decline of kidney function in the subject. In certain embodiments, the administration reduces total kidney volume in the subject. In certain embodiments, the administration slows the rate of increase in total kidney volume in the subject. In certain embodiments, the administration reduces height-adjusted total kidney volume (HtTKV). In certain embodiments, the administration slows the rate of increase in HtTKV.

[000199] In certain embodiments, the administration increases polycystin-1 (PC1) in the urine of the subject. In certain embodiments, the administration increases polycystin-2 (PC2) in the urine of the subject. In certain embodiments, the administration increases polycystin-1 (PC1) and polycystin-2 (PC2) in the urine of the subject.

[000200] In certain embodiments, the administration inhibits cyst growth (total cyst volume, number, and/or size distribution) in the subject. In certain embodiments, the administration slows rate of increase in cyst growth (total cyst volume, number, and/or size distribution) in the subject. In some embodiments, a cyst is present in the kidney of a subject. In some embodiments, a cyst is present in an organ other than the kidney, for example, the liver.

[000201] In certain embodiments, the administration alleviates kidney pain in the subject. In certain embodiments, the administration slows the increase in kidney pain in the subject. In certain embodiments, the administration delays the onset of kidney pain in the subject.

[000202] In certain embodiments, the administration reduces hypertension in the subject. In certain embodiments, the administration slows the worsening of hypertension in the subject. In certain embodiments, the administration delays the onset of hypertension in the subject.

[000203] In certain embodiments, the administration reduces fibrosis in kidney of the subject. In certain embodiments, the administration slows the worsening of fibrosis in the kidney of the subject.

[000204] In certain embodiments, the administration delays the onset of end stage renal disease in the subject. In certain embodiments, the administration delays time to dialysis for the subject. In certain embodiments, the administration delays time to renal transplant for the subject. In certain embodiments, the administration improves life expectancy of the subject.

[000205] In certain embodiments, the administration reduces albuminuria in the subject. In certain embodiments, the administration slows the worsening of albuminuria in the subject. In certain embodiments, the administration delays the onset of albuminuria in the subject. In certain embodiments, the administration reduces hematuria in the subject. In certain embodiments, the administration slows the worsening of hematuria in the subject. In certain embodiments, the administration delays the onset of hematuria in the subject. In certain embodiments, the administration reduces or slows the rate of increase of blood urea nitrogen (BUN) level in the subject. In certain embodiments, the administration reduces or slows the rate of increase of serum creatinine (SCr) level in the subject. In certain embodiments, the administration improves or slows the rate of decrease of creatinine clearance in the subject. In certain

embodiments, the administration reduces or slows the rate of increase of urine albumin:creatinine ratio in the subject.

[000206] In certain embodiments, the administration improves glomerular filtration rate in the subject. In certain embodiments, the administration slows the rate of decline of glomerular filtration rate in the subject. In certain embodiments, the glomerular filtration rate is an estimated glomerular filtration rate (eGFR). In certain embodiments, the glomerular filtration rate is a measured glomerular filtration rate (mGFR).

[000207] In certain embodiments, the administration reduces or slows the rate of increase of neutrophil gelatinase-associated lipocalin (NGAL) protein in the urine of the subject. In certain embodiments, the administration reduces or slows the rate of increase of kidney injury molecule-1 (KIM-1) protein in the urine of the subject.

[000208] In certain embodiments, the administration reduces or slows the rate of increase of monocyte chemoattractant protein-1 (MCP-1) in the urine of the subject. In certain embodiments, the administration reduces or slows the rate of increase of beta-2 microglobulin (B2M) in the urine of the subject.

[000209] In certain embodiments, the administration reduces or slows the rate of increase of complement split products C3a and/or Bb in plasma of the subject.

[000210] In certain embodiments, the administration reduces or slows the rate of increase of acute phase proteins (i.e., Alb, fibrinogen, and/or high sensitivity C-reactive proteins).

[000211] In certain embodiments, the administration reduces or slows the rate of increase of insulin-like growth factor binding protein acid labile subunit (IGFALS) in the serum of the subject. In certain embodiments, the administration reduces or slows the rate of increase of copeptin (CT-proAVP) in the serum of the subject. In certain embodiments, the administration reduces or slows the rate of increase of N-acetyl-1-methylhistidine in the serum of the subject.

[000212] In any of the embodiments, provided herein, a subject may be subjected to certain tests to evaluate the extent of disease in the subject. Such tests include, without limitation, measurement of total kidney volume in the subject; measurement of htTKV in the subject; measurement of hypertension in the subject; measurement of kidney pain in the subject; measurement of fibrosis in the kidney of the subject; measurement of blood urea nitrogen (BUN) level in the subject; measuring serum creatinine (SCr) level in the subject; measuring creatinine clearance in the blood of the subject; measuring albuminuria in the subject; measuring albumin:creatinine ratio in the subject; measuring glomerular filtration rate (GFR) in the subject, wherein the glomerular filtration rate is estimated or measured; measurement of polycystin-1 (PC1) and/or measurement of polycystin-2 (PC2) in the urine of the subject; measurement of neutrophil gelatinase-associated lipocalin (NGAL) protein in the urine of the subject; and/or measurement of kidney injury molecule-1 (KIM-1) protein in the urine of the subject; measurement of MCP-1 and/or measurement of B2M in the urine of the subject; measurement of IGFALS, measurement of CT-proAVP, and/or measurement of N-acetyl-1-methylhistidine in the serum of the subject; measurement of total cyst volume, number and/or size distribution of the cysts.

[000213] In some embodiments, administration of the compound comprising a modified oligonucleotide as discussed herein or a pharmaceutically acceptable salt thereof results in little to no CNS impairment in the subject. In some embodiments, CNS impairment is measured by the Scale for the Assessment and Rating of Ataxia (SARA) test. The SARA is a tool for assessing ataxia. In some embodiments, prior to administration, a SARA test is performed on the subject. In some embodiments, administration results in little or no change in Scale for the Assessment and Rating of Ataxia (SARA) test scores for the subject compared to before treatment.

[000214] In some embodiments, before administration, during administration, and/or after administration, a subject may be subjected to certain tests to evaluate the pharmacokinetics of the compound comprising a modified oligonucleotide as discussed herein or a pharmaceutically acceptable salt thereof. A pharmacokinetic analysis is performed to measure and compare one or more of the following parameters: maximum observed concentration (C_{max}), time to maximum observed concentration (T_{max}), area under the concentration–time curve up to 24 h post dose (AUC_{0-24}), area under the concentration–time curve up to the last quantifiable concentration (AUC_{0-t}), area under the concentration–time curve over the dosing interval (AUC_{tau}), area under the concentration–time curve extrapolated to infinity (AUC_{inf}), half-life ($t_{½}$), Apparent clearance (CL/F), apparent volume of distribution (V_z/F), Fraction of unchanged compound thereof excreted in the urine (fe), and/or total amount of unchanged compound thereof excreted in the urine (Ae).

[000215] In some embodiments, during and/or after administration of the compound comprising a modified oligonucleotide as discussed herein or a pharmaceutically acceptable salt thereof, a subject may be subjected to certain tests to evaluate the development of anti-drug antibodies (ADA) in the plasma of the subject.

[000216] In certain embodiments, a subject having polycystic kidney disease experiences a reduced quality of life. For example, a subject having polycystic kidney disease may experience kidney pain, which may reduce the subject's quality of life. In certain embodiments, the administration improves the subject's quality of life.

[000217] In any of the embodiments provided herein, the subject is a human subject. In certain embodiments, the human subject is an adult. In certain embodiments, an adult is at least 21 years of age. In certain embodiments, the human subject is a pediatric subject, i.e. the subject is less than 21 years of age. Pediatric populations may be defined by regulatory agencies. In certain embodiments, the human subject is an adolescent. In certain embodiments, an adolescent is at least 12 years of age and less than 21 years of age. In certain embodiments, the human subject is a child. In certain embodiments, a child is at least two years of age and less than 12 years of age. In certain embodiments, the human subject is an infant. In certain embodiments, and infant is at least one month of age and less than two years of age. In certain embodiments, the subject is a newborn. In certain embodiments, a newborn is less than one month of age. In certain embodiments, the subject is between 18 to 70 years of age.

[000218] Any of the compounds described herein may be for use in therapy. Any of the compounds provided herein may be for use in the treatment of polycystic kidney disease. In certain embodiments, the

polycystic kidney disease is autosomal dominant polycystic kidney disease. In certain embodiments, the polycystic kidney disease is autosomal recessive polycystic kidney disease. In certain embodiment, the polycystic kidney disease is nephronophthisis. In certain embodiments, the subject has Joubert syndrome and related disorders (JSRD), Meckel syndrome (MKS), or Bardet-Biedl syndrome (BBS).

[000219] Any of the modified oligonucleotides described herein may be for use in therapy. Any of the modified oligonucleotides provided herein may be for use in the treatment of polycystic kidney disease.

[000220] Any of the compounds provided herein may be for use in the preparation of a medicament. Any of the compounds provided herein may be for use in the preparation of a medicament for the treatment of a polycystic kidney disease.

[000221] Any of the modified oligonucleotides provided herein may be for use in the preparation of a medicament. Any of the modified oligonucleotides provided herein may be for use in the preparation of a medicament for the treatment of polycystic kidney disease.

[000222] Any of the pharmaceutical compositions provided herein may be for use in the treatment of polycystic kidney disease.

[000223] In some embodiments, the method of treatment has an acceptable safety and tolerability profile. In some embodiments, the methods of treatment are generally safe and well-tolerated.

Certain Additional Therapies

[000224] Treatments for polycystic kidney disease or any of the conditions listed herein may comprise more than one therapy. As such, in certain embodiments, provided herein are methods for treating a subject having or suspected of having polycystic kidney disease comprising administering at least one therapy in addition to administering compound provided herein, which comprises a nucleobase sequence complementary to the miR-17 seed sequence.

[000225] In certain embodiments, the at least one additional therapy comprises a pharmaceutical agent. In certain embodiments, a pharmaceutical agent is an anti-hypertensive agent. Anti-hypertensive agents are used to control blood pressure of the subject.

[000226] In certain embodiments, a pharmaceutical agent is a vasopressin receptor 2 antagonist. In certain embodiments, a vasopressin receptor 2 antagonist is tolvaptan.

[000227] In certain embodiments, pharmaceutical agents include angiotensin II receptor blockers (ARB). In certain embodiments, an angiotensin II receptor blocker is candesartan, irbesartan, olmesartan, losartan, valsartan, telmisartan, or eprosartan.

[000228] In certain embodiments, pharmaceutical agents include angiotensin II converting enzyme (ACE) inhibitors. In certain embodiments, an ACE inhibitor is captopril, enalapril, lisinopril, benazepril, quinapril, fosinopril, or ramipril.

[000229] In certain embodiments, a pharmaceutical agent is a diuretic. In certain embodiments, a pharmaceutical agent is a calcium channel blocker.

[000230] In certain embodiments, a pharmaceutical agent is a glucosylceramide synthase inhibitor. In certain embodiments, a glucosylceramide synthase inhibitor is venglustat.

[000231] In certain embodiments, a pharmaceutical agent is an antihyperglycemic agent. In certain embodiments, an antihyperglycemic agent is a biguanide. In certain embodiments, a biguanide is metformin.

[000232] In certain embodiments, a pharmaceutical agent is a kinase inhibitor. In certain embodiments, a kinase inhibitor is bosutinib or KD019.

[000233] In certain embodiments, a pharmaceutical agent is an adrenergic receptor antagonist.

[000234] In certain embodiments, a pharmaceutical agent is an aldosterone receptor antagonist. In certain embodiments, an aldosterone receptor antagonist is spironolactone. In certain embodiments, spironolactone is administered at a dose ranging from 10 to 35 mg daily. In certain embodiments, spironolactone is administered at a dose of 25 mg daily.

[000235] In certain embodiments, a pharmaceutical agent is a mammalian target of rapamycin (mTOR) inhibitor. In certain embodiments, an mTOR inhibitor is everolimus, rapamycin, or sirolimus.

[000236] In certain embodiments, a pharmaceutical agent is a hormone analogue. In certain embodiments, a hormone analogue is somatostatin or adrenocorticotrophic hormone.

[000237] In certain embodiments, a pharmaceutical agent is an anti-fibrotic agent. In certain embodiments, an anti-fibrotic agent is a modified oligonucleotide complementary to miR-21.

[000238] In certain embodiments, an additional therapy is dialysis. In certain embodiments, an additional therapy is kidney transplant.

[000239] In certain embodiments, pharmaceutical agents include anti-inflammatory agents. In certain embodiments, an anti-inflammatory agent is a steroidal anti-inflammatory agent. In certain embodiments, a steroid anti-inflammatory agent is a corticosteroid. In certain embodiments, a corticosteroid is prednisone. In certain embodiments, an anti-inflammatory agent is a non-steroidal anti-inflammatory drug. In certain embodiments, a non-steroidal anti-inflammatory agent is ibuprofen, a COX-I inhibitor, or a COX-2 inhibitor.

[000240] In certain embodiments, a pharmaceutical agent is a pharmaceutical agent that blocks one or more responses to fibrogenic signals.

[000241] In certain embodiments, an additional therapy may be a pharmaceutical agent that enhances the body's immune system, including low-dose cyclophosphamide, thymostimulin, vitamins and nutritional supplements (e.g., antioxidants, including vitamins A, C, E, beta-carotene, zinc, selenium, glutathione, coenzyme Q-10 and echinacea), and vaccines, e.g., the immunostimulating complex (ISCOM), which comprises a vaccine formulation that combines a multimeric presentation of antigen and an adjuvant.

[000242] In certain embodiments, the additional therapy is selected to treat or ameliorate a side effect of one or more pharmaceutical compositions provided herein. Such side effects include, without limitation, injection site reactions, liver function test abnormalities, kidney function abnormalities, liver toxicity, renal toxicity, central nervous system abnormalities, and myopathies. For example, increased aminotransferase levels in serum may indicate liver toxicity or liver function abnormality. For example, increased bilirubin may indicate liver toxicity or liver function abnormality.

Certain MicroRNA Nucleobase Sequences

[000243] The miR-17 family includes miR-17, miR-20a, miR-20b, miR-93, miR-106a, and miR-106b. Each member of the miR-17 family has a nucleobase sequence comprising the nucleobase sequence 5'-AAAGUG-3,' or the miR-17 seed sequence, which is the nucleobase sequence at positions 2 through 7 of SEQ ID NO: 1. Additionally, each member of the miR-17 family shares some nucleobase sequence identity outside the seed region. Accordingly, a modified oligonucleotide comprising a nucleobase sequence complementary to the miR-17 seed sequence may target other microRNAs of the miR-17 family, in addition to miR-17.

[000244] In certain embodiments, a modified oligonucleotide comprises the nucleobase sequence 5'-AGCACUUUA-3'.

[000245] In certain embodiments, each cytosine is independently selected from a non-methylated cytosine and a 5-methylcytosine. In certain embodiments, at least one cytosine is a non-methylated cytosine. In certain embodiments, each cytosine is a non-methylated cytosine. In certain embodiments, at least one cytosine is a 5-methylcytosine. In certain embodiments, each cytosine is a 5-methyl cytosine.

[000246] In certain embodiments, the number of linked nucleosides of a modified oligonucleotide is less than the length of its target microRNA. A modified oligonucleotide having a number of linked nucleosides that is less than the length of the target microRNA, wherein each nucleobase of the modified oligonucleotide is complementary to a nucleobase at a corresponding position of the target microRNA, is considered to be a modified oligonucleotide having a nucleobase sequence that is fully complementary (also referred to as 100% complementary) to a region of the target microRNA sequence. For example, a modified oligonucleotide consisting of 9 linked nucleosides, where each nucleobase is complementary to a corresponding position of miR-17, is fully complementary to miR-17.

[000247] In certain embodiments, a modified oligonucleotide has a nucleobase sequence having one mismatch with respect to the nucleobase sequence of a target microRNA. In certain embodiments, a modified oligonucleotide has a nucleobase sequence having two mismatches with respect to the nucleobase sequence of a target microRNA. In certain such embodiments, a modified oligonucleotide has a nucleobase sequence having no more than two mismatches with respect to the nucleobase sequence of a target microRNA. In certain such embodiments, the mismatched nucleobases are contiguous. In certain such embodiments, the mismatched nucleobases are not contiguous.

[000248] Although the sequence listing accompanying this filing identifies each nucleobase sequence as either "RNA" or "DNA" as required, in practice, those sequences may be modified with a combination of chemical modifications specified herein. One of skill in the art will readily appreciate that in the sequence listing, such designation as "RNA" or "DNA" to describe modified oligonucleotides is somewhat arbitrary. For example, a modified oligonucleotide comprising a nucleoside comprising a 2′-O-methoxyethyl sugar moiety and a thymine base may described as a DNA residue in the sequence listing, even though the nucleoside is modified and is not a natural DNA nucleoside.

[000249] Accordingly, nucleic acid sequences provided in the sequence listing are intended to encompass nucleic acids containing any combination of natural or modified RNA and/or DNA,

including, but not limited to such nucleic acids having modified nucleobases. By way of further example and without limitation, a modified oligonucleotide having the nucleobase sequence "ATCGATCG" in the sequence listing encompasses any oligonucleotide having such nucleobase sequence, whether modified or unmodified, including, but not limited to, such compounds comprising RNA bases, such as those having sequence "AUCGAUCG" and those having some DNA bases and some RNA bases such as "AUCGATCG" and oligonucleotides having other modified bases, such as "AT^{me}CGAUCG," wherein ^{me}C indicates a 5-methylcytosine.

Certain Modifications

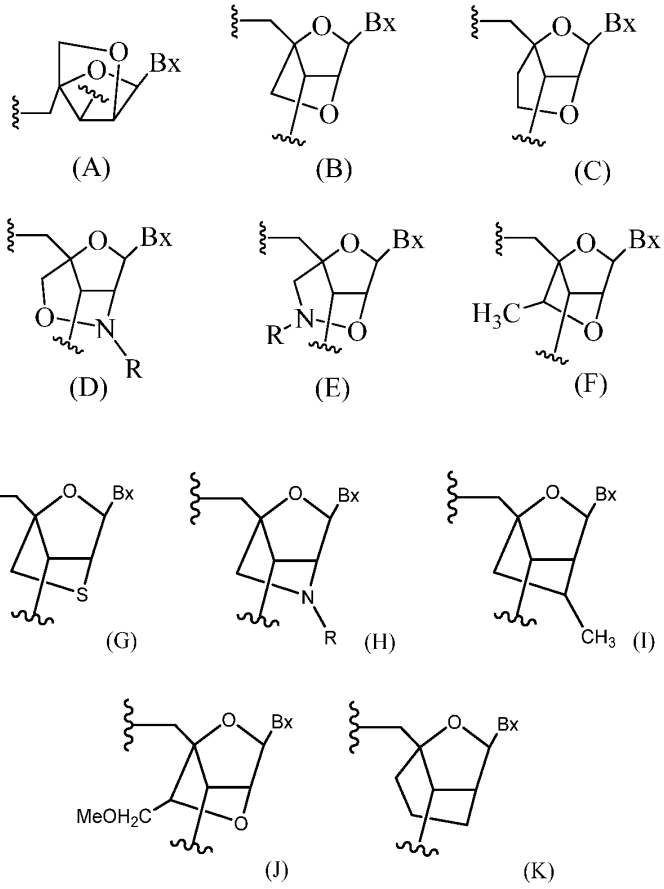
[000250] In certain embodiments, oligonucleotides provided herein may comprise one or more modifications to a nucleobase, sugar, and/or internucleoside linkage, and as such is a modified oligonucleotide. A modified nucleobase, sugar, and/or internucleoside linkage may be selected over an unmodified form because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for other oligonucleotides or nucleic acid targets and increased stability in the presence of nucleases.

[000251] In certain embodiments, a modified oligonucleotide comprises one or more modified nucleosides.

[000252] In certain embodiments, a modified nucleoside is a sugar-modified nucleoside. In certain such embodiments, the sugar-modified nucleosides may further comprise a natural or modified heterocyclic base moiety and/or may be connected to another nucleoside through a natural or modified internucleoside linkage and/or may include further modifications independent from the sugar modification. In certain embodiments, a sugar modified nucleoside is a 2'-modified nucleoside, wherein the sugar ring is modified at the 2' carbon from natural ribose or 2'-deoxy-ribose.

[000253] In certain embodiments, a 2'-modified nucleoside has a bicyclic sugar moiety. In certain such embodiments, the bicyclic sugar moiety is a D sugar in the alpha configuration. In certain such embodiments, the bicyclic sugar moiety is a D sugar in the beta configuration. In certain such embodiments, the bicyclic sugar moiety is an L sugar in the alpha configuration. In certain such embodiments, the bicyclic sugar moiety is an L sugar in the beta configuration.

[000254] Nucleosides comprising such bicyclic sugar moieties are referred to as bicyclic nucleosides or BNAs. In certain embodiments, bicyclic nucleosides include, but are not limited to, (A) α-L-methyleneoxy (4'-CH_2-O-2') BNA; (B) β-D-methyleneoxy (4'-CH_2-O-2') BNA; (C) ethyleneoxy (4'-$(CH_2)_2$-O-2') BNA; (D) aminooxy (4'-CH_2-O-N(R)-2') BNA; (E) oxyamino (4'-CH_2-N(R)-O-2') BNA; (F) methyl(methyleneoxy) (4'-$CH(CH_3)$-O-2') BNA (also referred to as constrained ethyl or cEt); (G) methylene-thio (4'-CH_2-S-2') BNA; (H) methylene-amino (4'-CH2-N(R)-2') BNA; (I) methyl carbocyclic (4'-CH_2-$CH(CH_3)$-2') BNA; (J) c-MOE (4'-$CH(CH_2$-OMe)-O-2') BNA and (K) propylene carbocyclic (4'-$(CH_2)_3$-2') BNA as depicted below.



wherein Bx is a nucleobase moiety and R is, independently, H, a protecting group, or C_1-C_{12} alkyl.

[000255] In certain embodiments, a 2'-modified nucleoside comprises a 2'-substituent group selected from F, OCF_3, O-CH_3 (also referred to as "2'-OMe"), $OCH_2CH_2OCH_3$ (also referred to as "2'-O-methoxyethyl" or "2'-MOE"), 2'-$O(CH_2)_2SCH_3$, O-$(CH_2)_2$-O-$N(CH_3)_2$, -$O(CH_2)_2O(CH_2)_2N(CH_3)_2$, and O-$CH_2$-C(=O)-$N(H)CH_3$.

[000256] In certain embodiments, a 2'-modified nucleoside comprises a 2'-substituent group selected from F, O-CH_3, and $OCH_2CH_2OCH_3$.

[000257] In certain embodiments, a sugar-modified nucleoside is a 4'-thio modified nucleoside. In certain embodiments, a sugar-modified nucleoside is a 4'-thio-2'-modified nucleoside. A 4'-thio modified nucleoside has a β-D-ribonucleoside where the 4'-O replaced with 4'-S. A 4'-thio-2'-modified nucleoside is a 4'-thio modified nucleoside having the 2'-OH replaced with a 2'-substituent group. Suitable 2'-substituent groups include 2'-OCH_3, 2'-$OCH_2CH_2OCH_3$, and 2'-F.

[000258] In certain embodiments, a modified oligonucleotide comprises one or more internucleoside modifications. In certain such embodiments, each internucleoside linkage of a modified oligonucleotide is a modified internucleoside linkage. In certain embodiments, a modified internucleoside linkage comprises a phosphorus atom.

[000259] In certain embodiments, a modified oligonucleotide comprises at least one phosphorothioate internucleoside linkage. In certain embodiments, each internucleoside linkage of a modified oligonucleotide is a phosphorothioate internucleoside linkage.

[000260] In certain embodiments, a modified oligonucleotide comprises one or more modified nucleobases. In certain embodiments, a modified nucleobase is selected from 5-hydroxymethyl cytosine, 7-deazaguanine and 7-deazaadenine. In certain embodiments, a modified nucleobase is selected from 7-deaza-adenine, 7-deazaguanosine, 2-aminopyridine and 2-pyridone. In certain embodiments, a modified nucleobase is selected from 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2 aminopropyladenine, 5-propynyluracil and 5-propynylcytosine.

[000261] In certain embodiments, a modified nucleobase comprises a polycyclic heterocycle. In certain embodiments, a modified nucleobase comprises a tricyclic heterocycle. In certain embodiments, a modified nucleobase comprises a phenoxazine derivative. In certain embodiments, the phenoxazine can be further modified to form a nucleobase known in the art as a G-clamp.

[000262] In certain embodiments, a modified oligonucleotide is conjugated to one or more moieties which enhance the activity, cellular distribution or cellular uptake of the resulting antisense oligonucleotides. In certain such embodiments, the moiety is a cholesterol moiety. In certain embodiments, the moiety is a lipid moiety. Additional moieties for conjugation include carbohydrates, peptides, antibodies or antibody fragments, phospholipids, biotin, phenazine, folate, phenanthridine, anthraquinone, acridine, fluoresceins, rhodamines, coumarins, and dyes. In certain embodiments, the carbohydrate moiety is N-acetyl-D-galactosamine (GalNac). In certain embodiments, a conjugate group is attached directly to an oligonucleotide. In certain embodiments, a conjugate group is attached to a modified oligonucleotide by a linking moiety selected from amino, azido, hydroxyl, carboxylic acid, thiol, unsaturations (e.g., double or triple bonds), 8-amino-3,6-dioxaoctanoic acid (ADO), succinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), 6-aminohexanoic acid (AHEX or AHA), substituted C1-C10 alkyl, substituted or unsubstituted C2-C10 alkenyl, and substituted or unsubstituted C2-C10 alkynyl. In certain such embodiments, a substituent group is selected from hydroxyl, amino, alkoxy, azido, carboxy, benzyl, phenyl, nitro, thiol, thioalkoxy, halogen, alkyl, aryl, alkenyl and alkynyl.

[000263] In certain such embodiments, the compound comprises a modified oligonucleotide having one or more stabilizing groups that are attached to one or both termini of a modified oligonucleotide to enhance properties such as, for example, nuclease stability. Included in stabilizing groups are cap structures. These terminal modifications protect a modified oligonucleotide from exonuclease degradation, and can help in delivery and/or localization within a cell. The cap can be present at the 5'-terminus (5'-cap), or at the 3'-terminus (3'-cap), or can be present on both termini. Cap structures include, for example, inverted deoxy abasic caps.

Certain Pharmaceutical Compositions

[000264] Provided herein are pharmaceutical compositions comprising a compound or modified oligonucleotide provided herein, and a pharmaceutically acceptable diluent. In certain embodiments, the

pharmaceutically acceptable diluent is an aqueous solution. In certain embodiments, the aqueous solution is a saline solution. As used herein, pharmaceutically acceptable diluents are understood to be sterile diluents. Suitable administration routes include, without limitation, intravenous and subcutaneous administration. In certain embodiments, administration is intravenous administration. In certain embodiments, administration is subcutaneous administration. In certain embodiments, administration is oral administration.

[000265] In certain embodiments, a pharmaceutical composition is administered in the form of a dosage unit. For example, in certain embodiments, a dosage unit is in the form of a tablet, capsule, or a bolus injection.

[000266] In certain embodiments, a pharmaceutical agent is a modified oligonucleotide which has been prepared in a suitable diluent, adjusted to pH 7.0-9.0 with acid or base during preparation, and then lyophilized under sterile conditions. The lyophilized modified oligonucleotide is subsequently reconstituted with a suitable diluent, e.g., aqueous solution, such as water or physiologically compatible buffers such as saline solution, Hanks's solution, or Ringer's solution. The reconstituted product is administered as a subcutaneous injection or as an intravenous infusion. The lyophilized drug product may be packaged in a 2 mL Type I, clear glass vial (ammonium sulfate-treated), stoppered with a bromobutyl rubber closure and sealed with an aluminum overseal.

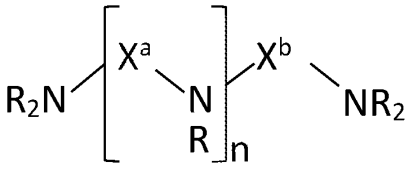
[000267] In certain embodiments, the pharmaceutical compositions provided herein may additionally contain other adjunct components conventionally found in pharmaceutical compositions, at their art-established usage levels. Thus, for example, the compositions may contain additional, compatible, pharmaceutically-active materials such as, for example, antipruritics, astringents, local anesthetics or anti-inflammatory agents.

[000268] In some embodiments, the pharmaceutical compositions provided herein may contain additional materials useful in physically formulating various dosage forms of the compositions provided herein, such as dyes, flavoring agents, preservatives, antioxidants, opacifiers, thickening agents and stabilizers; such additional materials also include, but are not limited to, excipients such as alcohol, polyethylene glycols, gelatin, lactose, amylase, magnesium stearate, talc, silicic acid, viscous paraffin, hydroxymethylcellulose and polyvinylpyrrolidone. In various embodiments, such materials, when added, should not unduly interfere with the biological activities of the components of the compositions provided herein. The formulations can be sterilized and, if desired, mixed with auxiliary agents, e.g., lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, colorings, flavorings and/or aromatic substances and the like which do not deleteriously interact with the oligonucleotide(s) of the formulation. Certain pharmaceutical compositions for injection are suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Certain solvents suitable for use in pharmaceutical compositions for injection include, but are not limited to, lipophilic solvents and fatty oils, such as sesame oil, synthetic fatty acid esters, such as ethyl oleate or triglycerides, and liposomes. Aqueous injection suspensions may contain substances that increase the viscosity of the suspension, such as

sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, such suspensions may also contain suitable stabilizers or agents that increase the solubility of the pharmaceutical agents to allow for the preparation of highly concentrated solutions.

[000269] Lipid moieties have been used in nucleic acid therapies in a variety of methods. In one method, the nucleic acid is introduced into preformed liposomes or lipoplexes made of mixtures of cationic lipids and neutral lipids. In another method, DNA complexes with mono- or poly-cationic lipids are formed without the presence of a neutral lipid. In certain embodiments, a lipid moiety is selected to increase distribution of a pharmaceutical agent to a particular cell or tissue. In certain embodiments, a lipid moiety is selected to increase distribution of a pharmaceutical agent to fat tissue. In certain embodiments, a lipid moiety is selected to increase distribution of a pharmaceutical agent to muscle tissue.

[000270] In certain embodiments, a pharmaceutical composition provided herein comprise a polyamine compound or a lipid moiety complexed with a nucleic acid. In certain embodiments, such preparations comprise one or more compounds each individually having a structure defined by formula (Z) or a pharmaceutically acceptable salt thereof,



wherein each X^a and X^b, for each occurrence, is independently C_{1-6} alkylene; n is 0, 1, 2, 3, 4, or 5; each R is independently H, wherein at least n + 2 of the R moieties in at least about 80% of the molecules of the compound of formula (Z) in the preparation are not H; m is 1, 2, 3 or 4; Y is O, NR^2, or S; R^1 is alkyl, alkenyl, or alkynyl; each of which is optionally substituted with one or more substituents; and R^2 is H, alkyl, alkenyl, or alkynyl; each of which is optionally substituted each of which is optionally substituted with one or more substituents; provided that, if n = 0, then at least n + 3 of the R moieties are not H. Such preparations are described in PCT publication WO/2008/042973, which is herein incorporated by reference in its entirety for the disclosure of lipid preparations. Certain additional preparations are described in Akinc et al., *Nature Biotechnology* **26**, 561 - 569 (01 May 2008), which is herein incorporated by reference in its entirety for the disclosure of lipid preparations.

[000271] In certain embodiments, a pharmaceutical composition provided herein is prepared using known techniques, including, but not limited to mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or tableting processes.

[000272] In certain embodiments, a pharmaceutical composition provided herein is a solid (e.g., a powder, tablet, and/or capsule). In certain of such embodiments, a solid pharmaceutical composition comprising one or more oligonucleotides is prepared using ingredients known in the art, including, but not limited to, starches, sugars, diluents, granulating agents, lubricants, binders, and disintegrating agents.

[000273] In certain embodiments, a pharmaceutical composition provided herein is formulated as a depot preparation. Certain such depot preparations are typically longer acting than non-depot preparations. In certain embodiments, such preparations are administered by implantation (for example subcutaneously or

intramuscularly) or by intramuscular injection. In certain embodiments, depot preparations are prepared using suitable polymeric or hydrophobic materials (for example an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

[000274] In certain embodiments, a pharmaceutical composition provided herein comprises a delivery system. Examples of delivery systems include, but are not limited to, liposomes and emulsions. Certain delivery systems are useful for preparing certain pharmaceutical compositions including those comprising hydrophobic compounds. In certain embodiments, certain organic solvents such as dimethylsulfoxide are used.

[000275] In certain embodiments, a pharmaceutical composition provided herein comprises one or more tissue-specific delivery molecules designed to deliver the one or more pharmaceutical agents provided herein to specific tissues or cell types. For example, in certain embodiments, pharmaceutical compositions include liposomes coated with a tissue-specific antibody.

[000276] In certain embodiments, a pharmaceutical composition provided herein comprises a sustained-release system. A non-limiting example of such a sustained-release system is a semi-permeable matrix of solid hydrophobic polymers. In certain embodiments, sustained-release systems may, depending on their chemical nature, release pharmaceutical agents over a period of hours, days, weeks or months.

[000277] Certain pharmaceutical compositions for injection are presented in unit dosage form, e.g., in ampoules or in multi-dose containers.

[000278] In certain embodiments, a pharmaceutical composition provided herein comprises a modified oligonucleotide in a therapeutically effective amount. In certain embodiments, the therapeutically effective amount is sufficient to prevent, alleviate or ameliorate symptoms of a disease or to prolong the survival of the subject being treated.

[000279] In certain embodiments, one or more modified oligonucleotides provided herein is formulated as a prodrug. In certain embodiments, upon *in vivo* administration, a prodrug is chemically converted to the biologically, pharmaceutically or therapeutically more active form of an oligonucleotide. In certain embodiments, prodrugs are useful because they are easier to administer than the corresponding active form. For example, in certain instances, a prodrug may be more bioavailable (e.g., through oral administration) than is the corresponding active form. In certain instances, a prodrug may have improved solubility compared to the corresponding active form. In certain embodiments, prodrugs are less water soluble than the corresponding active form. In certain instances, such prodrugs possess superior transmittal across cell membranes, where water solubility is detrimental to mobility. In certain embodiments, a prodrug is an ester. In certain such embodiments, the ester is metabolically hydrolyzed to carboxylic acid upon administration. In certain instances the carboxylic acid containing compound is the corresponding active form. In certain embodiments, a prodrug comprises a short peptide (polyaminoacid) bound to an acid group. In certain of such embodiments, the peptide is cleaved upon administration to form the corresponding active form.

[000280] In certain embodiments, a prodrug is produced by modifying a pharmaceutically active compound such that the active compound will be regenerated upon *in vivo* administration. The prodrug

can be designed to alter the metabolic stability or the transport characteristics of a drug, to mask side effects or toxicity, to improve the flavor of a drug or to alter other characteristics or properties of a drug. By virtue of knowledge of pharmacodynamic processes and drug metabolism *in vivo*, those of skill in this art, once a pharmaceutically active compound is known, can design prodrugs of the compound (see, e.g., Nogrady (1985) Medicinal Chemistry A Biochemical Approach, Oxford University Press, New York, pages 388-392).

[000281] Additional administration routes include, but are not limited to, oral, rectal, transmucosal, intestinal, enteral, topical, suppository, through inhalation, intrathecal, intracardiac, intraventricular, intraperitoneal, intranasal, intraocular, intratumoral, intramuscular, and intramedullary administration. In certain embodiments, pharmaceutical intrathecals are administered to achieve local rather than systemic exposures. For example, pharmaceutical compositions may be injected directly in the area of desired effect (e.g., into the kidney).

Certain Kits

[000282] Kits are also provided. In some embodiments, the kits comprise one or more compounds comprising a modified oligonucleotide disclosed herein. In some embodiments, the kits may be used for administration of the compound to a subject.

[000283] In certain embodiments, the kit comprises a pharmaceutical composition ready for administration. In certain embodiments, the pharmaceutical composition is present within a vial. A plurality of vials, such as 10, can be present in, for example, dispensing packs. In some embodiments, the vial is manufactured so as to be accessible with a syringe. The kit can also contain instructions for using the compounds.

[000284] In some embodiments, the kit comprises a pharmaceutical composition present in a pre-filled syringe (such as a single-dose syringes with, for example, a 27 gauge, ½ inch needle with a needle guard), rather than in a vial. A plurality of pre-filled syringes, such as 10, can be present in, for example, dispensing packs. The kit can also contain instructions for administering the compounds comprising a modified oligonucleotide disclosed herein.

[000285] In some embodiments, the kit comprised a modified oligonucleotide provided herein as a lyophilized drug product, and a pharmaceutically acceptable diluent. In preparation for administration to a subject, the lyophilized drug product is reconstituted in the pharmaceutically acceptable diluent.

[000286] In some embodiments, in addition to compounds comprising a modified oligonucleotide disclosed herein, the kit can further comprise one or more of the following: syringe, alcohol swab, cotton ball, and/or gauze pad.

Certain Experimental Models

[000287] In certain embodiments, methods are provided of using and/or testing modified oligonucleotides provided herein in an experimental model. Those having skill in the art are able to select

and modify the protocols for such experimental models to evaluate a pharmaceutical agent provided herein.

[000288] Generally, modified oligonucleotides are first tested in cultured cells. Suitable cell types include those that are related to the cell type to which delivery of a modified oligonucleotide is desired *in vivo*. For example, suitable cell types for the study of the methods described herein include primary or cultured cells.

[000289] In certain embodiments, the extent to which a modified oligonucleotide interferes with the activity of one or more miR-17 family members is assessed in cultured cells. In certain embodiments, inhibition of microRNA activity may be assessed by measuring the level of one or more of a predicted or validated microRNA-regulated transcript. An inhibition of microRNA activity may result in the increase in the miR-17 family member-regulated transcript, and/or the protein encoded by miR-17 family member-regulated transcript (i.e., the miR-17 family member-regulated transcript is de-repressed). Further, in certain embodiments, certain phenotypic outcomes may be measured.

[000290] Several animal models are available to the skilled artisan for the study of one or more miR-17 family members in models of human disease. Models of polycystic kidney disease include, but are not limited to, models with mutations and/or deletions in *Pkd1* and/or *Pkd2*; and models comprising mutations in other genes. Nonlimiting exemplary models of PKD comprising mutations and/or deletions in *Pkd1* and/or *Pkd2* include hypomorphic models, such as models comprising missense mutations in *Pkd1* and models with reduced or unstable expression of *Pkd2*; inducible conditional knockout models; and conditional knockout models. Nonlimiting exemplary PKD models comprising mutations in genes other than *Pkd1* and *Pkd2* include models with mutations in *Pkhd1*, *Nek8*, Kif3a, and/or *Nphp3*. PKD models are reviewed, *e.g.*, in Shibazaki *et al.*, *Human Mol. Genet.*, 2008; 17(11): 1505-1516; Happe and Peters, *Nat Rev Nephrol.*, 2014; 10(10): 587-601; and Patel *et al.*, *PNAS*, 2013; 110(26): 10765-10770.

Certain Quantitation Assays

[000291] In certain embodiments, microRNA levels are quantitated in cells or tissues *in vitro* or *in vivo*. In certain embodiments, changes in microRNA levels are measured by microarray analysis. In certain embodiments, changes in microRNA levels are measured by one of several commercially available PCR assays, such as the TaqMan® MicroRNA Assay (Applied Biosystems).

[000292] Modulation of microRNA activity with an anti-miR or microRNA mimic may be assessed by microarray profiling of mRNAs. The sequences of the mRNAs that are modulated (either increased or decreased) by the anti-miR or microRNA mimic are searched for microRNA seed sequences, to compare modulation of mRNAs that are targets of the microRNA to modulation of mRNAs that are not targets of the microRNA. In this manner, the interaction of the anti-miR with its target microRNA, or a microRNA mimic with its targets, can be evaluated. In the case of an anti-miR, mRNAs whose expression levels are increased are screened for the mRNA sequences that comprise a seed match to the microRNA to which the anti-miR is complementary.

[000293] Modulation of microRNA activity with an anti-miR compound may be assessed by measuring the level of a messenger RNA target of the microRNA, either by measuring the level of the messenger RNA itself, or the protein transcribed therefrom. Antisense inhibition of a microRNA generally results in the increase in the level of messenger RNA and/or protein of the messenger RNA target of the microRNA, *i.e.*, anti-miR treatment results in de-repression of one or more target messenger RNAs.

EXAMPLES

[000294] The following examples are presented in order to more fully illustrate some embodiments of the invention. They should in no way be construed, however, as limiting the broad scope of the invention.

[000295] Those of ordinary skill in the art will readily adopt the underlying principles of this discovery to design various compounds without departing from the spirit of the current invention.

Example 1: The role of miR-17 in PKD

[000296] miR-17 family members of the miR-17~92 cluster of microRNAs are upregulated in mouse models of PKD. Genetic deletion of the miR-17~92 cluster in a mouse model of PKD reduces kidney cyst growth, improves renal function, and prolongs survival (Patel *et al.*, *PNAS*, 2013; 110(26): 10765-10770). The miR-17~92 cluster contains 6 different microRNAs, each with a distinct sequence: miR-17, miR-18a, miR-19a, miR-19-b-1 and miR-92a-1.

[000297] The miR-17~92 cluster includes two microRNAs, miR-17 and miR-20a, that are members of the miR-17 family of microRNAs. Each member of this family shares seed sequence identity, and varying degrees of sequence identity outside the seed region. The other members of the miR-17 family are miR-20b, miR-93, miR-106a, and miR-106b. miR-20b and miR-106a reside within the miR-106a~363 cluster on the human X chromosome, and miR-93 and miR-106b reside within the miR-106b~25 cluster on human chromosome 7. The sequences of the miR-17 family members are shown in Table 1.

Table 1: miR-17 family of microRNAs

microRNA	SEQUENCE (5' TO 3') seed region in bold	SEQ ID NO:
miR-17	C**AAAGUG**CUUACAGUGCAGGUAG	1
miR-20a	U**AAAGUG**CUUAUAGUGCAGGUAG	2
miR-20b	C**AAAGUG**CUCAUAGUGCAGGUAG	3
miR-93	C**AAAGUG**CUGUUCGUGCAGGUAG	4
miR-106a	A**AAAGUG**CUUACAGUGCAGGUAG	5
miR-106b	U**AAAGUG**CUGACAGUGCAGAU	6

[000298] The anti-miR-17 compound RGLS4326 was discovered by screening a chemically diverse and rationally designed library of anti-miR-17 oligonucleotides for optimal pharmaceutical properties. 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 attenuates cyst growth in human *in vitro* ADPKD models and multiple PKD mouse models after subcutaneous administration. A phase 1 single ascending dose (SAD) clinical trial of RGLS4326 in healthy volunteers was initiated in December 2017, followed by a phase 1 multiple ascending dose (MAD) clinical trial in healthy volunteers that was initiated in May 2018. A phase 1b clinical trial of RGLS4326 for the treatment of patients with autosomal dominant polycystic kidney disease (ADPKD) was initiated in October 2020.

[000299] Subsequent to the initiation of the phase 1 MAD clinical trial, nonclinical toxicology studies revealed central nervous system (CNS)-related findings, including abnormal gait, reduced motor activity, and/or prostration, at high doses of RGLS4326. To identify potential candidates for off-target pharmacology, a panel of 174 targets including G-protein coupled receptors, transporters, ion channels, nuclear receptors, and cytokine receptors was evaluated *in vitro* for possible interactions with RGLS4326. RGLS4326 was found to be an antagonist of the AMPA glutamate receptor, with a 50% inhibitory concentration (IC50) of 4.6 uM (14.2 μg/mL) based on ligand binding and a functional IC50 of 300-600 nM (0.9-1.8 μg/mL) based on patch clamp activity. AMPA receptors are ion channels on excitatory synapses in the CNS that mediate fast excitatory neurotransmission and, therefore, are key components of all neuronal networks. Such an interaction with the AMPA receptor could explain the CNS-mediated findings observed at high doses of RGLS4326 in nonclinical toxicology models.

Example 2: Screen for anti-miR-17 Compounds with Reduced AMPA Receptor Binding

[000300] RGLS4326 has the following sequence and chemical modification pattern: $A_SG_SC_MA_FC_FU_FU_MU_SG_S$ where nucleosides followed by subscript "M" are 2'-O-methyl nucleosides, nucleosides followed by subscript "F" are 2'-fluoro nucleosides, nucleosides followed by subscript "S" are S-cEt nucleosides, each cytosine is a non-methylated cytosine, and all linkages are phosphorothioate linkages. Chemical modification and length variants of RGLS4326 were designed and screened to identify a compound that retains the potency and pharmacokinetic profile of RGLS4326 and exhibits reduced binding to the AMPA receptor (AMPA-R).

[000301] A library of compounds was designed with varying chemical modifications, nucleobase sequence, and length, relative to RGLS4326.

Table 2: anti-miR-17 Library

Compound #	Chemical Notation	Nucleobase Sequence	SEQ ID NO	Length
RG-NG-1001	$A_SG_SC_SA_SC_SU_SU_SU_SG_S$	AGCACUUUG		9
RG-NG-1002	$A_SG_SC_SA_SC_SU_SU_SU_S$	AGCACUUU-		8

RG-NG-1003	$A_SG_SC_MA_SC_MU_SU_MU_SG_S$	AGCACUUUG		9
RG-NG-1004	$U_SA_SA_SG_SC_SA_SC_SU_SU_SU_SG_S$	UAAGCACUUUG	12	11
RG-NG-1005	$U_SA_SA_MG\underline{C}_SA_SC_SU_MU_MU_SG_S$	UAAGCACUUUG	13	11
RG-NG-1006	$A_LG_L\underline{C}_LA_L\underline{C}_LT_LT_LT_LG_L$	AGCACTTTG		9
RG-NG-1007	$A_SG_SC_FA_FC_FU_FU_FU_SG_S$	AGCACUUUG		9
RG-NG-1008	$A_SG_EC_MA_FC_FU_FU_MT_EG_S$	AGCACUUTG		9
RG-NG-1009	$A_EG_EC_MA_FC_FU_FU_MT_EG_E$	AGCACUUTG		9
RG-NG-1010	$A_EG_EC_MA_FC_FU_FU_MU_SG_S$	AGCACUUUG		9
RG-NG-1011	$A_LG_LC_MA_FC_FU_FU_MU_SG_S$	AGCACUUUG		9
RG-NG-1012	$A_SG_LC_MA_FC_FU_FU_MT_LG_S$	AGCACUUTG		9
RG-NG-1013	$A_SA_SG_SC_MA_FC_FU_FU_MU_S$	AAGCACUUU-		9
RG-NG-1014	$A_SG_SC_MA_FC_FU_FU_MU_S$	AGCACUUU-		8
RG-NG-1015	$A_SG_SC_MA_FC_FU_FU_MU_SA_S$	AGCACUUUA		9
RG-NG-1016	$A_SG_SC_MA_FC_FU_FU_MU_SC_S$	AGCACUUUC		9
RG-NG-1017	$A_SG_SC_MA_FC_FU_FU_MU_SU_S$	AGCACUUUU		9
RG-NG-1018	$A_F\underline{C}_ET_EG_ST_EAA_SG_SC_MA_FC_FU_FU_MU_SC_S$	ACTGTAAGCACUUUC	14	15
RG-NG-1019	$A_SC_STG_SU_SAA_SG_SC_MA_FC_FU_FU_MU_SC_S$	ACTGUAAGCACUUUC	15	15
RG-NG-1020	$T_EG_ST_EAA_SG_SC_MA_FC_FU_FU_MU_SC_S$	TGTAAGCACUUUC	16	13
RG-NG-1021	$T_EAA_SG_SC_MA_FC_FU_FU_MU_SC_S$	TAAGCACUUUC	17	11
RG-NG-1022	$T_EA_EA_SG_SC_MA_FC_FU_FU_MU_SC_S$	TAAGCACUUUC	18	11
RG-NG-1023	$A_EG_SC_MA_FC_FU_FU_MU_SG_E$	AGCACUUUG		9
RG-NG-1024	$A_SG_SC_MA_FC_FU_FU_MT_EG_E$	AGCACUUTG		9
RG-NG-1025	$A_SG_SC_MA_FC_FU_FU_MU_SG_E$	AGCACUUUG		9
RG-NG-1026	$A_SG_SC_MA_LC_FU_FU_MU_SC_S$	AGCACUUUC		9
RG-NG-1027	$A_SG_SC_SA_FC_FU_FU_MU_SC_S$	AGCACUUUC		9
RG-NG-1028	$A_SG_SC_SA_LC_FU_FU_MU_SC_S$	AGCACUUUC		9
RG-NG-1029	$A_MG_L\underline{C}_LA_L\underline{C}_LU_MT_LU\underline{C}_M$	AGCACUTUC		9
RG-NG-1030	$A_MA_MG_L\underline{C}_LA_L\underline{C}_LU_MT_LU\underline{C}_M$	AAGCACUTUC	19	10
RG-NG-1031	$A_MA_MG_L\underline{C}_LA_L\underline{C}_LU_MT_LU_M$	AAGCACUTU-		9
Nucleosides followed by subscript "M" are 2'-O-methyl nucleosides; nucleosides followed by subscript "F" are 2'-fluoro nucleosides; nucleosides followed by subscript "S" are S-cEt nucleosides; nucleosides followed by subscript "E" are 2'-O-methoxyethyl (2'-MOE) nucleosides; and nucleosides followed by subscript "L" are LNA nucleosides.				

[000302] The activity of anti-miR-17 compounds was evaluated in a radioligand binding assay which measured the binding of the [^{3}H] AMPA ligand to the AMPA-R present on rat brain synaptic membranes, in the presence of increasing concentrations of anti-miR-17 compound. anti-miR-17 compounds with affinity for the AMPA-R will bind to and compete with the binding of the [^{3}H] AMPA ligand.

[000303] The assay was performed according to previously published methods (Honore et al., *J Neurochem.*, 1982, 38(1):173-178; Olsen et al., *Brain Res.*, 1987, 402(2):243-254). 5.0 nM of the ligand [^{3}H] AMPA, 1.0 mM of the non-specific ligand L-Glutamic acid, and anti-miR compound at uM concentrations were incubated with synaptic membranes prepared from Wistar rat cerebral cortex for 90 minutes. The compounds shown in Table 2 were tested in three experiments. Anti-miRs targeted to microRNAs other than miR-17 were used as control compounds (RG5124 targeted to miR-33a; RG5365 targeted to let-7a; RG8093 targeted to miR-214). RGLS4326 and RG-NG-1001 were also tested in each

experiment, as it was demonstrated to bind to and inhibit the activity of the AMPA-R. The amount of the [^{3}H] AMPA ligand was quantitated by radioligand binding, and is shown in Tables 3, 4, and 5. As illustrated by the data, the compounds vary in their ability to inhibit binding of the radiolabeled ligand to the AMPA-R.

Table 3: Inhibition of Ligand Binding to AMPA-R Experiment #1

	SEQ ID NO		% Inhibition @ uM				
Compound #		**Nucleobase Sequence & Chemistry (5' to 3')**	100	10	1	0.1	IC_{50} (uM)
RG-NG-1001		$A_SG_SC_SA_SC_SU_SU_SU_SG_S$	91.3	89.1	76.2	41.1	0.17
RGLS4326		$A_SG_SC_MA_FC_FU_FU_MU_SG_S$	104.1	93	55.5	17.6	0.70
RG-NG-1002		$A_SG_SC_SA_SC_SU_SU_SU_S$	24.0	-16.9	-2.0	0	>100
RG-NG-1003		$A_SG_SC_MA_SC_MU_SU_MU_SG_S$	77.8	56.9	13.9	5.7	9.26
RG-NG-1004	20	$U_SA_SA_SG_SC_SA_SC_SU_SU_SU_SG_S$	76.1	44.2	4.7	-4.7	17.28
RG-NG-1005	21	$U_SA_SA_MG\underline{C}_SA_SC_SU_MU_MU_SG_S$	28.3	0.8	-1.3	10.8	>100
RG-NG-1006		$A_LG_L\underline{C}_LA_L\underline{C}_LT_LT_LT_LG_L$	93.9	68.9	35.3	6.7	2.77
RG5124		$A_SC_SA_MA_FU_FG_FC_MA_SC_S$ (anti-miR-33a)	-5.1	-3.7	12.9	0.4	>100
RG5365		anti-let7a	-4	6.5	-6.3	2.4	>100
RG8093		anti-miR-214	-9.8	-2	-11.5	-6.9	>100

Table 4: Inhibition of Ligand Binding to AMPA-R Experiment #2

	SEQ ID NO		% Inhibition @ uM				
Compound #		**Chemical Notation**	100	10	1	0.1	IC_{50} (uM)
RG-NG-1001		$A_SG_SC_SA_SC_SU_SU_SU_SG_S$	93.5	90.1	68.3	42.2	0.19
RGLS4326		$A_SG_SC_MA_FC_FU_FU_MU_SG_S$	96.8	93.6	73.1	31.5	0.27
RG-NG-1002		$A_SG_SC_SA_SC_SU_SU_SU_S$	24.6	-2.1	4.6	-5.5	>100
RG-NG-1007		$A_SG_SC_FA_FC_FU_FU_FU_SG_S$	94.3	83.2	39.7	3.1	1.69
RG-NG-1008		$A_SG_EC_MA_FC_FU_FU_MT_EG_S$	59.6	22.1	13.6	-22.4	56.58
RG-NG-1009		$A_EG_EC_MA_FC_FU_FU_MT_EG_E$	32.6	13	2.7	-11.8	>100
RG-NG-1010		$A_EG_EC_MA_FC_FU_FU_MU_SG_S$	86.4	70.1	31.3	0.7	3.44
RG-NG-1011		$A_LG_LC_MA_FC_FU_FU_MU_SG_S$	94	78.5	30.6	-2.2	2.56
RG-NG-1012		$A_SG_LC_MA_FC_FU_FU_MT_LG_S$	88.3	69	38.5	9.9	2.56
RG-NG-1013		$A_SA_SG_SC_MA_FC_FU_FU_MU_S$	37.2	3.7	-5.6	-3.8	>100
RG-NG-1014		$A_SG_SC_MA_FC_FU_FU_MU_S$	20.4	8.6	-5.5	14.9	>100
RG-NG-1015		$A_SG_SC_MA_FC_FU_FU_MU_SA_S$	19.9	6.5	11	2.3	>100
RG-NG-1016		$A_SG_SC_MA_FC_FU_FU_MU_SC_S$	5.9	12.3	10.4	4.7	>100
RG-NG-1017		$A_SG_SC_MA_FC_FU_FU_MU_SU_S$	8.1	4.3	-1.3	0.2	>100
RG-NG-1026		$A_SG_SC_MA_LC_FU_FU_MU_SC_S$	2.3	-3.5	-1.1	-3.3	>100
RG-NG-1027		$A_SG_SC_SA_FC_FU_FU_MU_SC_S$	3	-2.2	2.3	-2.2	>100
RG-NG-1028		$A_SG_SC_SA_LC_FU_FU_MU_SC_S$	2.7	2	2.1	15.7	>100
RG-NG-1029		$A_MG_L\underline{C}_LA_L\underline{C}_LU_MT_LU\underline{C}_M$	38.4	7	15.9	0.9	>100

RG-NG-1030	19	$A_MA_MG_LC_LA_LC_LU_MT_LUC_M$	1.9	15	6.5	2.9	>100
RG-NG-1031		$A_MA_MG_LC_LA_LC_LU_MT_LU_M$	16.7	2.7	1	-4.3	>100
RG5124		$A_SC_SA_MA_FU_FG_FC_MA_SC_S$ (anti-miR-33a)	7.2	-4.2	1.6	-1.2	>100

Table 5: Inhibition of Ligand Binding to AMPA-R Experiment #3

	SEQ ID NO		% Inhibition @ uM				
Compound #		**Chemical Notation**	100	10	1	0.1	IC_{50} (uM)
RG-NG-1001		$A_SG_SC_SA_SC_SU_SU_SU_SG_S$	96.6	94.6	84.8	59.4	0.10
RGLS4326		$A_SG_SC_MA_FC_FU_FU_MU_SG_S$	97.2	89	55.8	14.6	0.78
RG-NG-1018	22	$A_EC_ET_EG_ST_EAA_SG_SC_MA_FC_FU_FU_MU_SC_S$	12.3	1	8.1	3	>100
RG-NG-1019	23	$A_SC_ST_EG_SU_SAA_SG_SC_MA_FC_FU_FU_MU_SC_S$	21	15.6	17.2	12.9	>100
RG-NG-1020	24	$T_EG_ST_EAA_SG_SC_MA_FC_FU_FU_MU_SC_S$	-7.9	-23.3	6.6	17	>100
RG-NG-1021	25	$T_EAA_SG_SC_MA_FC_FU_FU_MU_SC_S$	-1.5	-11.5	-10.9	-2	>100
RG-NG-1022	26	$T_EA_EA_SG_SC_MA_FC_FU_FU_MU_SC_S$	-10.9	-7	-5.4	-23.5	>100
RG-NG-1023		$A_EG_SC_MA_FC_FU_FU_MU_SG_E$	95.3	75.2	25.6	3.1	3.12
RG-NG-1024		$A_SG_SC_MA_FC_FU_FU_MT_EG_E$	35.2	6.5	-4.7	-17	>100
RG-NG-1025		$A_SG_SC_MA_FC_FU_FU_MU_SG_E$	96.4	84.1	36.5	-3.8	1.85
RG5124		$A_SC_SA_MA_FU_FG_FC_MA_SC_S$ (anti-miR-33a)	23	16.1	9.3	5.1	>100

[000304] To evaluate functional antagonism of anti-miR-17 oligonucleotides towards the AMPA-R, certain oligonucleotides were tested using the manual whole-cell patch clamp technique, which records membrane currents as a measure of AMPA-R activity.

[000305] Manual whole-cell patch clamp studies were performed by Metrion Biosciences (Cambridge, UK). Whole-cell voltage clamp experiments were performed at room temperature (18 - 21 °C) using an EPC10 patch clamp amplifier using Patchmaster software (HEKA Elektronik). Glass patch pipettes were fabricated from borosilicate glass capillaries (Harvard Apparatus) to resistances between 1.4 and 2.5 MΩ. Membrane currents were recorded using the whole-cell patch clamp technique. ChanTest® GluA1/GluA4 EZCells were clamped at a holding potential of -80 mV and membrane currents elicited by 10 µM (S)-AMPA delivered using a VC38 perfusion system (ALA Scientific Instruments). The minimal current amplitude values were measured with each application of 10 µM (S)-AMPA. The fractional change of current amplitude produced by each concentration of compound was calculated relative to the control current (pre-compound) and expressed as percentage change (% inhibition) for each cell. The compounds tested are shown in Table 6. RGLS4326 was tested in a separate study from all other compounds in Table 6.

[000306] As shown in Table 6, relative to RGLS4326, compounds RG-NG-1015, RG-NG-1016, and RG-NG-1017 exhibited reduced functional antagonism towards AMPA-R based on the manual whole-cell patch clamp studies in human ChanTest® GluA1/GluA4 EZ-Cells.

Table 6: Functional Antagonism of AMPA-R in Whole-Cell Patch Clamp Studies

Compound ID	Sequence & Chemistry (5'-3')	Target	%Inhibition on membrane currents evoked with 10uM (s)-AMPA (Mean ± SD)	
			0.3 uM	3 uM
RG-NG-1001	$A_SG_SC_SA_SC_SU_SU_SU_SG_S$	miR-17	80.5 ± 6.3	88.8 ± 4.5
RGLS4326	$A_SG_SC_MA_FC_FU_FU_MU_SG_S$	miR-17	46.9 ± 9.8	87.4 ± 7.9
RG-NG-1015	$A_SG_SC_MA_FC_FU_FU_MU_SA_S$	miR-17	16.0 ± 5.0	39.5 ± 5.4
RG-NG-1016	$A_SG_SC_MA_FC_FU_FU_MU_SC_S$	miR-17	16.0 ± 6.2	27.1 ± 9.8
RG-NG-1017	$A_SG_SC_MA_FC_FU_FU_MU_SU_S$	miR-17	20.0 ± 6.3	28.2 ± 5.5
RG5124	$A_SC_SA_MA_FU_FG_FC_MA_SC_S$	miR-33a	14.1 ± 7.2	42.1 ± 9.4

Example 3: Relationship Between Nucleobase Properties and AMPA-R Binding

[000307] As illustrated by the AMPA-R binding and whole-cell patch clamp studies, the presence of guanosine at the 3'-terminus of an anti-miR-17 oligonucleotide, at the position complementary to the first nucleotide of miR-17, influences the functional antagonism of the AMPA-R. Like guanosine, adenosine is a purine, however adenosine did not inhibit the AMPA-R. Guanosine and adenosine are similar with regard to several properties except for hydrogen bonding, thus the differences in hydrogen bonding at positions 1, 2, and 6 of the purine base were evaluated. The purine nucleobases tested are shown in FIG. 1 and Table 7. In the "Purine Position" column of Table 7, "A" indicates a position of the purine that is hydrogen acceptor and "D" indicates a position of the purine that is a hydrogen donor. In the "Purine Position" column of Table 7, "N" indicates a neutral position that is neither a hydrogen acceptor or donor. Also tested were varying 2'-sugar moieties on the purine nucleobase, to evaluate the influence of 2'-sugar moiety chemistry on the ability of the purine nucleobase to inhibit the AMPA-R.

Table 7: Anti-miR-17 Compounds With Varying Nucleobase and Sugar Moiety Chemistry

Compound ID	Nucleobase at 3-terminus (single letter notation)	2'-Sugar Moiety at 3'-terminus	SEQ ID NO	Sequence & Chemistry (5'-3')	Purine Position		
					# 6	# 1	# 2
RG-NG-1001	guanosine (G)	S-cEt		$A_SG_SC_SA_SC_SU_SU_SU_SG_S$	A	D	D
RG-NG-1040	guanosine (G)	S-cEt	27	$\underline{C}_ET_EG_S\underline{C}_EA_E\underline{C}_ET_EG_ST_EA_D$ $A_SG_SC_MA_FC_FU_FU_MU_SG_S$	A	D	D
RG-NG-1032	guanosine (G)	S-cEt	28	$G_S\underline{C}_EA_E\underline{C}_ET_EG_ST_EA_D$ $A_SG_SC_MA_FC_FU_FU_MU_SG_S$	A	D	D
RG4326	guanosine (G)	S-cEt		$A_SG_SC_MA_FC_FU_FU_MU_SG_S$	A	D	D
RG-NG-1033	guanosine (G)	DNA		$A_SG_SC_MA_FC_FU_FU_MU_SG_D$	A	D	D
RG-NG-1034	guanosine (G)	2'-O-methyl		$A_SG_SC_MA_FC_FU_FU_MU_SG_M$	A	D	D
RG-NG-1035	inosine (I)	S-cEt		$A_SG_SC_MA_FC_FU_FU_MU_SI_M$	A	D	N
RG-NG-1036	2-aminopurine (N)	2'-O-methyl		$A_SG_SC_MA_FC_FU_FU_MU_SN_M$	N	A	D
RG-NG-1037	2,6-diaminopurine (D)	2'-O-methyl		$A_SG_SC_MA_FC_FU_FU_MU_SD_M$	D	A	D

RG-NG-1039	isoguanine (F)	DNA		$A_SG_SC_MA_FC_FU_FU_MU_SF_D$	D	D	A
RG-NG-1038	adenosine (A)	2'-O-methyl		$A_SG_SC_MA_FC_FU_FU_MU_SA_M$	D	A	N
RG-NG-1015	adenosine (A)	S-cEt		$A_SG_SC_MA_FC_FU_FU_MU_SA_S$	D	A	N
RG5124	cytidine (C)	S-cEt		$A_SC_SA_MA_FU_FG_FC_MA_SC_S$			

[000308] The compounds were tested in the radioligand binding assay described herein, to determine the ability of the anti-miR-17 compounds to and compete with the binding of the [^{3}H] AMPA ligand. As shown in Table 8, a correlation was observed between inhibition of ligand binding to the +AMPA-R and the presence of a hydrogen bond acceptor at purine position #6 of the nucleobase at the 3'-terminus of the oligonucleotide. For example, compounds having guanosine or inosine at the 3'-terminus resulted in inhibition of ligand binding to the AMPA-R. Compounds with a 3'-terminal nucleobase having a hydrogen bond acceptor at purine position #6, for example RG-NG-1037 and RG-NG-1039, were less likely to inhibit ligand binding to the AMPA-R.

Table 8: Inhibition of Ligand Binding to AMPA-R

ID	Nucleobase at 3-terminus	2'-Sugar Moiety at 3'-terminus	Purine Position			% Inhibition @ Concentration				IC50 (uM)
			#6	#1	#2	100 uM	10 uM	1 uM	0.1 uM	
RG-NG-1001	guanosine	S-cEt	A	D	D	102.9	99.3	81.3	41.9	0.15
RG-NG-1040	guanosine	S-cEt	A	D	D	64.1	38.9	14.4	10.4	28.61
RG-NG-1032	guanosine	S-cEt	A	D	D	92	78.3	43.8	24.3	1.17
RG-NG-4326	guanosine	S-cEt	A	D	D	99.7	83.5	40.8	-0.1	1.61
RG-NG-1033	guanosine	DNA	A	D	D	97.3	96.5	80.9	44.9	0.13
RG-NG-1034	guanosine	2'-O-methyl	A	D	D	99.7	85.2	44.8	14.3	1.20
RG-NG-1035	inosine	S-cEt	A	D	N	98.5	91.3	46.3	27.2	0.76
RG-NG-1036	2-aminopurine	2'-O-methyl	N	A	D	78.1	36.2	11.3	19.2	18.61
RG-NG-1037	2,6-diaminopurine	2'-O-methyl	D	A	D	10.7	2.4	9.2	11.1	100.00
RG-NG-1039	isoguanine	DNA	D	D	A	30.2	35.8	27	27.3	100.00
RG-NG-1038	adenosine	2'-O-methyl	D	A	N	81.3	47.4	25.4	17.9	8.23
RG-NG-1015	adenosine	S-cEt	D	A	N	5.6	-7	-10.3	-0.8	100.00
RG5124	cytidine	S-cEt				10.1	4.4	-0.6	-8.6	100.00

Example 4: Anti-miR-17 Compounds with Reduced Binding and Inhibition of AMPA-R Showed No CNS Toxicity in High Dose Studies

[000280] RG-NG-1015, RG-NG-1016, and RG-NG-1017 were tested in high-dose mouse toxicity studies. Each compound was tested in a single dose at 2000 mg/kg, and at escalating doses (100, 450, and 2000 mg/kg). As shown in Table 9, while escalating doses of RG-NG-1001 and RGLS4326 resulted in ataxia, lethargy, and in the case of RGLS4326, unconsciousness at the highest dose, no CNS-toxicity were observed for RG-NG-1015, RG-NG-1016, or RG-NG-1017.

Table 9: Anti-miR-17 Compounds and CNS-related findings

	Mice/ group	SC Dose (mg/kg/dose)	Schedule	CNS-related findings noted:
RG-NG-1001	8	100, 450, 2000	QDx3	Mild Body Scratching and Ataxia observed at 100 and increase at 450 mg/kg; Lethargy observed at 2000 mg/kg
RGLS4326	10	100, 450, 2000	QDx4	Ataxia and/or lethargy observed at 450 mg/kg; Ataxia, lethargy and/or unconsciousness observed at 2000 mg/kg
RG-NG-1015	7	2000	Single	No
	6	100, 450, 2000	QDx4	No
RG-NG-1016	10	2000	Single	No
	6	100, 450, 2000	QDx4	No
RG-NG-1017	10	2000	Single	No
	6	100, 450, 2000	QDx4	No
RG5124	7-10	100, 450, 2000	QDx3	Mild ataxia observed at 2000 mg/kg

Example 5: Maximum Tolerated Dose (MTD) Study and Comparative Dose Assessment of Different Compounds

[000309] Data from below studies further support that AMPA-R antagonism is responsible for CNS toxicity and mortality observed in previous toxicity studies of RGLS4326.

[000310] **Study 1: Maximum Tolerated Dose (MTD) Study and Comparative Dose Assessment of RG-NG-1017, RGLS4326 and RG-NG-1001**

[000311] Compounds (RG-NG-1017, RGLS4326, RG-NG-1001) were evaluated in a pilot maximum tolerated dose (MTD) study (discussed below). RG-NG-1017, RGLS4326 and RG-NG-1001 were initially evaluated at 4 dose-levels each. RG-NG-1017 was included for evaluation as a non-AMPA-R binding compound, as compared to RGLS4326 and RG-NG-1001, which bind AMPA-R. C57Bl/6J male mice (Jackson Laboratories), age 6-7 weeks, were used in this study. Mice were assigned randomly to treatment groups, and the study was blinded. Animals were allowed to acclimate for no less than 5 days and housed on a 12 hr light/dark cycle (lights on 7:00 AM). No more than 4 mice were house in each cage in a ventilated cage rack system. The diet consisted of standard rodent chow and water ad libitum.

[000312] **MTD pilot study**

[000313] The following parameters were used for this study:

1. Route(s) of administration: intracerebroventricular (ICV) dosing of RG-NG-1017, RG-NG-1001, RGLS4326
2. Dose Volume(s): 4 µL
3. Formulation(s): vehicle, Ca^{2+} and Mg^{2+} free dPBS

4. Dose Frequency: Once
5. Study duration: 8 Days
6. Number of Groups: 3
7. Number of animals per group: (2-4 each group)
8. Total number of animals: 54

[000314] For the ICV administration, mice were anesthetized and positioned for injections. The skin over the skull was incised, and a small hole was made in the skull above the target using a microdrill. The stereotactic coordinates were anteroposterior (AP), −0.4 mm; mediolateral (ML), +/- 1.0-1.5 mm; dorsoventral (DV), −3.0 mm from the bregma for injection into both the right and left lateral cerebral ventricles (Hironaka et al, 2015). Animals were injected unilaterally with 4 µl into the right lateral cerebral ventricle. Compounds were injected over 1-2 min, and the needle was left in place for 0.5-1 min prior to withdrawal. The incision was closed with sutures, wound clips, or VetBond.

[000315] Following ICV treatment (Day 0), animals were monitored for 7 days in which daily health checks, body weight, and mortality was recorded. On Day 7, brain and kidney were collected and fixed (10% formalin) and stored pending histology.

[000316] Results from the MTD study are shown in Table 10 and FIG. 3. All animal deaths were reported to occur within the first 5-8 hours post-ICV injection. Mice injected with 2.5 µg RGLS4326 were reported to display some immediate signs of respiratory distress and were provided heating pads. RG-NG-1017 (non-AMPA-R binding compound) was well-tolerated at high doses, with no established MTD for this compound (0 deaths at 600 µg, 100 µg, or 50 µg; 1 death at 300 µg). 100% mortality was observed at high doses for RGLS4326 and RG-NG-1001 (*e.g.*, 600, 300, 100 µg), in addition to 100% mortality observed at 50 µg and 25 µg for both AMPA-R binding compounds. RG-NG-1001 MTD was not attained in this study, and was predicted to be under 2.5 µg. The MTD for RGLS4326 was predicted at ~2.5 < 5.0 µg by ICV. All animals were reported to fully recover on Day 2 of observation.

Table 10: Summary Results from 7-day MTD Study

	Mortality/total number of mice		
Dose (µg)	RG-NG-1017	RGLS4326	RG-NG-1001
600	0/2	2/2	2/2
300	1/2	2/2	2/2
100	0/2	2/2	2/2
50	0/2	3/3	3/3
25		3/3	3/3
10		3/4	3/4
5		2/4	4/4
2.5		0/ 3	1/3

[000317] **Maximum Tolerated Dose (MTD) Study for RGLS4326**

[000318] A second MTD study for RGLS4326 by ICV was conducted to assess dose selection for evaluating the compound in disease models (Table 11). In this study, a different mouse strain was evaluated (Swiss:Rjorl male mice, age 5 weeks, sourced from Janvier). Mice were placed under isoflurane anaesthesia (5% for induction and 2% for maintenance, under 100% O_2) and given 5 mg/kg s.c. carprofen (Rimadyl®). They were then placed in a stereotaxic frame. A midline sagittal incision was made in the scalp and a hole was drilled in the skull over the left lateral ventricle. A stainless-steel cannula (external diameter 0.51 mm) was placed stereotaxically into the left lateral ventricle at the following coordinates: +0.5 posterior to Bregma, L ± 0.7 mm, V = -2.7 mm. After a 2-minute delay to allow the brain tissue to slide over the cannula, 4 µL of a solution containing 0.625 mg/mL of RGLS4326 was slowly infused over 2 minutes. After infusion, the cannula was left in place for a further 5 minutes to prevent backflow of the solution along the cannula track. Mice were given 5 mg/kg s.c. carprofen (Rimadyl®) at 24 and 48 hours, after surgery. Mice were monitored during 3-7 days after surgery (starting 24 h after ICV administration) and their body weight was taken daily to check their health status. For mice monitored over 7 days, body weight was taken on Day 1 and on Day 7 after surgery to check their health status.

Table 11: Design of MTD Study for RGLS4326

Group	Number of animals	Treatment (RG4326)	Dose-level	Concentration (mg/mL)	Administration Volume
1	4 males	RGLS4326 (i.c.v.)	3 mg/mouse	0.75 mg/mL	4 mL/mouse
2	4 males	RGLS4326 (i.c.v.)	4 mg/mouse	1 mg/mL	4 mL/mouse
3	4 males	RGLS4326 (i.c.v.)	5 mg/mouse	1.25 mg/mL	4 mL/mouse
4	4 males	RGLS4326 (i.c.v.)	7.5 mg/mouse	1.875 mg/mL	4 mL/mouse

[000319] In Study 1, 6 mice were injected with 4 µL of a solution at 0.625 mg/mL (2.5 µg total per ICV; Table 10). At the end of anesthesia, the mice remained lying on one side. They were quiet with some periods of scratching during the first hours after surgery. No toxic effects were observed at 24, 48 or 72 hours in the 6 mice administered. In Study 2, four mice were injected with 4 different doses of RGLS4326 (0.75, 1.0, 1.25 and 1.875 mg/mL, volume of 4 µL). One mouse that received the highest dose (1.875 mg/mL, i.e., 7.5 µg/mouse) was found dead around 24 hours after ICV injection. All other mice were in good health, until the end of the pilot study (7 days after administration).

[000320] Combined results from Studies 1 and 2 demonstrated that RGLS4326 was generally well-tolerated in test subjects, but only at doses considerably lower than RG-NG-1017 (*see* FIG. 3).

[000321] Table 12 summarizes the MTD data for RGLS4326 for the Study 1 and 2 mouse models. Based on these results from Study 2, an MTD of ~ 4 µg was predicted for RGLS4326 in the Swiss:Rjorl mouse strain.

Table 12: 7-Day Survival Data for MTD Studies 1 and 2

	Dead/Total Mice	
Dose (μg) RGLS4326	Study 1	Study 2
600	2/2	
300	2/2	
100	2/2	
50	3/3	
25	3/3	
10	3/4	
7.5	-	1/4
5	2/4	0/4
4		0/4
3		0/4
2.5	0/3	0/6

[000322] In summary, compounds RG-NG-1017, RGLS4326 and RG-NG-1001 were evaluated across two MTD studies, demonstrating significant differences in tolerability between non-AMPA-R binding (RG-NG-1017) and AMPA-R binding compounds (RGLS4326, RG-NG-1001) (*see* FIG. 3). Despite 1 death at the ICV dose of 300 μg, an MTD was not established for RG-NG-1017, as no deaths occurred at the higher tested dose of 600 μg. Additionally, no impact on mortality was observed at doses of 100 and 50 μg for RG-NG-1017. By comparison, a clear impact on mortality was evident for the AMPA-R binding compounds RGLS4326 and RG-NG-1001, with no surviving animals across the tested dosing range of 25 μg to 600 μg. A trend for improved survival was seen at lower doses of RGLS4326 (10 μg), with 50% survival in RGLS4326 treated animals at 5 μg, and 100% survival at 2.5 μg. Similarly, in the case of RG-NG-1001 (which shows stronger AMPA-R binding compared to RGLS4326), 100% mortality was evident at the low dose of 5 μg, with a trend toward improved survival at 2.5 μg. The results for RGLS43426 from Study 1 were further confirmed in a second MTD study (Study 2), utilizing a different mouse strain. This study found that modest differences may exist for RGLS4326 tolerability between strains, with survival observed to only impact mice at the top dose of 7.5 μg versus at 5 μg in Study 1 using C57/Bl/6J. However, these results still support that MTD for the AMPA-R binding RGLS4326 occurs between ~2.5 μg and 5-7.5 μg (depending on strain), as compared to a significantly higher MTD for non-AMPA-R binding RG-NG-1017 (at least >40-fold, or higher) (FIG. 3).

Example 6: In Vitro and In Vivo Potency of anti-miR-17 Compounds

[000323] The *in vitro* potency of certain compounds was evaluated using a miR-17 luciferase sensor assay which uses a luciferase reporter vector for miR-17, with two fully complementary miR-17 binding sites in tandem in the 3'-UTR of the luciferase gene. HeLa cells were co-transfected with the luciferase reporter vector and an exogenous miR-17-expression vector that acted to repress the luciferase signal. HeLa cells were then individually treated with anti-miR-17 oligonucleotides at concentrations of 0.045,

0.137. 0.412, 1.23, 3.70, 11.1, 33.3, 100, and 300 nM. At the end of the 18- to 24-hour transfection period, luciferase activity was measured. RG5124 was included as a control compound. As shown in Table 13, these compounds inhibited miR-17 function and de-repressed miR-17 luciferases reporter activity with similar EC_{50} values compared to RGLS4326 *in vitro*.

Table 13: Inhibition of miR-17 in Luciferase Assay

Compound ID	EC50 (nM)	Log2FC (37.5 nM)
RGLS4326	18.50	2.70
RG-NG-1032	< 1	3.85
RG-NG-1015	22.40	2.51
RG-NG-1016	20.30	2.32
RG-NG-1017	15.00	2.67
RG5124	n/a	0.40

[000324] As shown in FIG. 4, RG-NG-1015 inhibited miR-17, as well as miR-20a, miR-106a, and miR-93 in a luciferase assay in HeLa cells, with similar EC_{50} values compared to RGLS4326 *in vitro.*

[000325] RG-NG-1015 also de-repressed luciferase sensors containing full-length 3' untranslated region (UTR) of the miR-17 direct target genes PKD1 and PKD2, with similar EC_{50} values compared to RGLS4326 *in vitro*.

[000326] The activity of certain compounds was evaluated using a mouse miR-17 Pharmacodynamic-Signature (miR-17 PD-Sig), which consists of the expression of 18 unique miR-17 target genes normalized by six reference housekeeping genes, to provide an unbiased and comprehensive assessment of miR-17 activity. The mouse miR-17 PD-Sig score was the calculated average of the 18 genes' individual log2 fold changes (normalized by six housekeeping genes) compared to mock transfection (Lee et al., *Nat. Commun*., 2019, 10, 4148).

[000327] As shown in Table 13, the tested oligonucleotides inhibited miR-17 function and de-repressed expression of multiple direct miR-17 target genes (as measured by miR-17 PD-signature) in normal and PKD kidney cell lines (both mouse and human) with similar EC_{50} values compared to RGLS4326 *in vitro*. The PD-Sig for RGLS4326 in mIMCD3 cells (77.2, indicated by "*") was not generated in this experiment; the value in Table 14 is that reported by Lee et al., *Nat. Commun*., 2019, 10, 4148. Blank cells in the table indicate that a compound was not tested in a particular cell line.

Table 14: miR-17 PD-Sig in Normal and PKD Cell Lines

Cell Line	**Species**	**Derived from**	**Anti-miR**				
			RGLS4326	RG-NG-1015	RG-NG-1016	RG-NG-1017	RG5124
mIMCD3	mouse	Normal	77.2*	168	146.3	134.5	n/a
D52B5	mouse	PKD	92.8	82.4		74.1	506.6
HK-2	human	Normal	47.8	55.6		49.2	
WT9-7	human	PKD	17.3	21.2		20.1	

[000328] *In vivo* potency was evaluated using the microRNA polysome shift assay (miPSA). This assay was used to determine the extent to which compounds directly engage the miR-17 target in the kidney in normal and PKD mice. The miPSA relies on the principle that active miRNAs bind to their mRNA targets in translationally active high molecular weight (HMW) polysomes, whereas the inhibited miRNAs reside in the low MW (LMW) polysomes. Treatment with anti-miR results in a shift of the microRNA from HMW polysomes to LMW polysomes. Thus, the miPSA provides a direct measurement of microRNA target engagement by a complementary anti-miR (Androsavich et al., *Nucleic Acids Research*, 2015, 44: e13).

[000329] Wild type mice were administered a single dose of 0.3mg/kg, 3 mg/kg, or 30 mg/kg. Kidney tissue was collected seven days later and subjected to the miPSA. The mean displacement score for each treatment is shown in Table 15 (PBS, n = 17; RGLS4326 30 mg/kg, n = 10; all other treatments, n = 4-5). The tested oligonucleotides displaced miR-17 from translationally active polysome (as measured by miPSA) in normal mouse kidneys.

Table 15: miPSA Displacement Scores

			anti-miR Dose		
			0.3 m/kg	3 mg/kg	30 mg/kg
RG-NG-1015	Mean		2.238	2.562	2.718
	SEM		0.1242	0.1303	0.118
RG-NG-1016	Mean		1.892	2.263	1.134
	SEM		0.1088	0.1716	0.5324
RG-NG-1017	Mean		2.077	2.292	2.793
	SEM		0.1513	0.08974	0.09685
RGLS4326	Mean		1.846	2.247	2.362
	SEM		0.1234	0.0478	0.1439
Vehicle (PBS)	Mean	0			
	SEM	0.06318			
RG5124	Mean				0.5935
	SEM				0.1784

[000330] Furthermore, as shown in Table 16 and FIG. 5A-5D, RGLS4326 and RG-NG-1015 have similar pharmacokinetic and Target Engagement (as measured by miPSA) profiles following a single subcutaneous administration in C57BL6 mice.

Table 16: Pharmacokinetic and Target Engagement profiles

Tissue	Test Article	Dose	T_{max}	C_{max}	AUC_{last}	$T_{1/2}$	Kidney-to-Liver Ratio by AUC_{last}
Plasma	RGLS4326	26.0 mg/kg	1 h	32 mg/mL	95.3 (mg*h)/mL	2.9 h	-
	RG-NG-1015	26.9 mg/kg	1 h	26 mg/mL	86.9 (mg*h)/mL	2.6 h	-
Kidney	RGLS4326	26.0 mg/kg	8 h	54 mg/g	395 (mg*d)/g	9.4 d	23.9
	RG-NG-1015	26.9 mg/kg	8 h	53 mg/g	447 (mg*d)/g	9.9 d	22.6
Liver	RGLS4326	26.0 mg/kg	1 h	4.8 mg/g	16.5 (mg*d)/g	4.7 d	-

	RG-NG-1015	26.9 mg/kg	1 h	5.2 mg/g	19.8 (mg*d)/g	6.2 d	-

Example 7: Efficacy of RG-NG-1015 in an Experimental Model of ADPKD

[000331] The efficacy of RG-NG-1015 was evaluated in the KspCre/Pkd1F/RC (*Pkd1*-F/RC) mouse model. *Pkd1*-F/RC is an orthologous ADPKD model that contains a germline hypomorphic Pkd1 mutation (the mouse equivalent of the human PKD1-R3277C (RC mutation) on one allele and loxP sites flanking Pkd1 exons 2 and 4 on the other allele. KspCre-mediated recombination was used to delete the floxed Pkd1 exons and produce a compound mutant mouse with a renal tubule-specific, somatic null mutation on one allele and a germline hypomorphic mutation on the other. This is an aggressive, but long-lived model of ADPKD (Hajarnis et al., *Nat. Commun*., 2017, 8, 14395).

[000332] On each of days 8, 10, 12, and 15 of age, sex-matched of *Pkd1*-F/RC mice were administered a subcutaneous injection of RGLS4326 at a dose of 20 mg/kg (n = 8; 4 males and 4 females per treatment group), RG5124 at a dose of 20 mg/kg (n = 8), or RG-NG-1015 at a dose of 20 mg/kg (n = 8), or PBS (n = 8). Mice were sacrificed at 18 days of age, and kidney weight, body weight, cyst index, serum creatinine level, and blood urea nitrogen (BUN) level were measured. BUN level is a marker of kidney function. A higher BUN level correlates with poorer kidney function, thus a reduction in BUN level is an indicator of reduced kidney injury and damage and improved function. Statistical significance was calculated by one-way ANOVA with Dunnett's multiple correction.

[000333] Results are shown in Table 17 and FIG. 2 (**** = $p<0.0001$; *** = $p<0.001$; ** = $p<0.01$; ns = not significant). The efficacy of RG-NG-1015 was similar to that of RGLS4326. The mean ratio of kidney weight to body weight (KW/BW ratio) was significantly lower in *Pkd1*-F/RC mice treated with RGLS4326 and RG-NG-1015, respectively, than the mean KW/BW ratio in *Pkd1*-F/RC mice administered PBS (FIG. 2A). Mean BUN levels were significantly reduced in *Pkd1*-F/RC mice treated with RGLS4326 and RG-NG-1015, respectively, compared to mice treated with PBS (FIG. 2B). Mean serum creatinine levels in *Pkd1*-F/RC mice were reduced in mice treated with RGLS4326 and RG-NG-1015, respectively, relative to mice treated with PBS, however the reduction was not statistically significant (FIG. 2C). Treatment with the control oligonucleotide, RG5124, did not reduce kidney weight to body weight ratio, serum creatinine, or serum BUN, demonstrating that the results observed with RGLS4329 and RG-NG-1015 were specific to the inhibition of miR-17.

Table 17: Efficacy of RG-NG-1015 in a Mouse Model of ADPKD

	PBS	**RGLS4326**	**RG-NG-1015**	**RG5124**
Mean BW/KW Ratio	65.61	16.81	16.83	91.75
Mean Difference vs. PBS		-48.8	-48.79	26.14
Adjusted P-Value		<0.0001	<0.0001	0.0045

Mean Serum BUN	42.48	22.41	22.26	50.61
Mean Difference vs. PBS		-20.06	-20.21	+8.138
Adjusted P-Value		<0.0001	<0.0001	0.1142
Mean Serum Creatine	0.1619	0.1273	0.1279	0.2403
Mean Difference vs. PBS		-0.03463	-0.03400	+0.07838
Adjusted P-Value		0.1456	0.1557	0.0004

[000334] The efficacy of RG-NG-1015 was also evaluated in the *Pcy*/DBA mouse model of PKD alone and in combination with tolvaptan. Pcy/DBA mice exhibit slowly progressing PKD caused by a missense mutation in the Nphp3 gene, which is responsible for adolescent nephronophthisis in humans (Takahashi et al., *J Am Soc Nephrol* 1991, 1:980–989; Olbrich et al., *Nat Genet* 2003, 34:455–459). In *Pcy* mice, cysts are derived from distal tubules, and whole-nephron segments become diffusely occupied by cysts accompanying disease progression by 30 weeks of age, often with the occurrence of ESRD (Nagao et al., *Exp Anim* 2012, 61:477–488). In particular, male *Pcy*/DBA mice has been used to characterize the pharmacological profiles of many investigational products for ADPKD treatment, including tolvaptan and RGLS4326, the first-generation anti-miR-17 (Aihara et al., *J Pharmacol Exp Ther* 2014 May;349(2):258-67 and Lee et al., *Nat. Commun*., 2019, 10, 4148). Studies in these mice typically involve initiation of treatment at ~5weeks of age and continues through to 15-30 weeks of age.

[000335] As outlined in FIG. 6A and 6B, five groups of male Pcy/DBA mice (n=13 per treatment group) were treated subcutaneously with PBS or RG-NG-1015 at 25, 5, 1, or 0.2 mg/kg once every two weeks (Q2W). Two groups of male Pcy/DBA mice (n=13 per group) were also treated with RG-NG-1015 at 50 mg/kg once every four weeks (Q4W) or at 12.5 mg/kg once weekly (QW). Another four groups of male Pcy/DBA mice (n=13 per group) were treated subcutaneously with PBS or RG-NG-1015 at 25, 5, or 1 mg/kg Q2W in combination with tolvaptan at 0.3% (w/w chow) *ad libitum*. A group of male WT-BDA/2J mice received subcutaneous injections of PBS Q2W was included in the study as a normal range reference. Mice were randomized into treatment groups at 5 weeks of age, and treatment started at 6 weeks of age for 17 weeks and were sacrificed 7 days after the final treatment. Kidney weight, body weight, kidney cyst index, urine Ngal-to-Creatinine ratio (Ngal/Cr) were measured. Urine Ngal/Cr is a marker of kidney injuries.

[000336] As seen in FIG. 6C-6E and Tables 18-20, RG-NG-1015 is effective in the Pcy/DBA mouse model of PKD at various dosages and regiments, and also provides additive or synergistic effects when used in combination with tolvaptan. In particular, RG-NG-1015 treatment significantly reduced mean KW/BW, urine Ngal/Cr, and kidney cyst index in Pcy/DBA mice in a dose-dependent manner (Table 18 and FIG. 6C-6E). In addition, RG-NG-1015 treatment by similar total dosage (total of 212.5-250 mg per

mouse for the duration of the study) but different dosing regiments (including QW, Q2W, and Q4W) reduced mean KW/BW, urine Ngal/Cr, and kidney cyst index at similar levels in Pcy/DBA mice (Table 19; FIG. 6C-6E). Treatment with tolvaptan alone reduced mean KW/BW, urine Ngal/Cr and kidney cyst index in Pcy/DBA mice, and combination of RG-NG-1015 plus tolvaptan further reduced mean KW/BW, urine Ngal/Cr, and kidney cyst index (Table 20; FIG. 6C-6E). The observed effects of the drug combination on KW/BW, urine Ngal/Cr, and kidney cyst index were synergistic, mostly additive, and less than additive, respectively, as indicated by Bliss additivity analysis (Table 20).

Table 18: Effects of Dose

Test Article 1 SC Dosing	Test Article 2 Chow Supl.	Dose	Regimen	Mean KW/BW		Mean Urine Ngal/Cr		Mean Cystic Area	
				mg/kg	% Inhibition	mg/mg	% Inhibition	%	% Inhibition
Vehicle	-	-	Q2W	91.3	0	21.07	0	60.62	0
RG-NG-1015	-	25 mg/kg	Q2W	66.87	34.3	11.36	48.8	46.46	26.0
RG-NG-1015	-	5 mg/kg	Q2W	70.02	29.9	14.65	32.3	44.58	29.4
RG-NG-1015	-	1 mg/kg	Q2W	82.43	12.5	15.18	29.6	50.58	18.4
RG-NG-1015	-	0.2 mg/kg	Q2W	78.02	18.7	18.38	13.5	47.09	24.8

Table 19: Effects of Regimen

Test Article 1 SC Dosing	Test Article 2 Chow Supl.	Dose	Regimen	Mean KW/BW		Mean Urine Ngal/Cr		Mean Cystic Area		Number of doses	Total doses (mg)
				mg/kg	% Inhibition	mg/mg	% Inhibition	%	% Inhibition		
Vehicle	-	-	Q2W	91.3	0	21.07	0	60.62	0	17	
RG-NG-1015	-	25 mg/kg	Q2W	66.87	34.3	11.36	48.8	46.46	26.0	9	225
RG-NG-1015	-	50 mg/kg	Q4W	67.09	34.0	9.32	59.1	47.35	24.3	5	250
RG-NG-1015	-	12.5 mg/kg	QW	74.52	23.6	8.82	61.6	44.99	28.7	17	212.5

Table 20: Effects of Combination

Test Article 1 SC Dosing	Test Article 2 Chow Suppl.	Dose	Regimen	Mean KW/BW			Mean Urine Ngal/Cr			Mean Cystic Area		
				mg/kg	% Inhibition	Bliss Analysis	mg/mg	% Inhibition	Bliss Analysis	%	% Inhibition	Bliss Analysis
Vehicle	-	-	Q2W	91.3	0	-	21.07	0	-	60.62	0	-
Vehicle	0.3% Tolvaptan	-	-	59.26	44.9	-	8.73	62.1	-	44.68	29.2	-
RG-NG-1015	-	25 mg/kg	Q2W	66.87	34.3	-	11.36	48.8	-	46.46	26.0	-
RG-NG-1015	0.3% Tolvaptan	25 mg/kg	Q2W	42.21	68.9	Synergistic	5.04	80.6	Additive	38.61	40.4	<Additive
RG-NG-1015	-	5 mg/kg	Q2W	70.02	29.9	-	14.65	32.3	-	44.58	29.4	-
RG-NG-1015	0.3% Tolvaptan	5 mg/kg	Q2W	45.62	64.1	Synergistic	5.84	76.6	Additive	37.47	42.5	<Additive
RG-NG-1015	-	1 mg/kg	Q2W	82.43	12.5	-	15.18	29.6	-	50.58	18.4	-
RG-NG-1015	0.3% Tolvaptan	1 mg/kg	Q2W	47.11	62.0	Synergistic	11.16	49.8	<Additive	41.91	34.3	<Additive

Example 8: Metabolites of RG-NG-1015

[000337] *In vitro* and *in vivo* studies were conducted to investigate the metabolism of RG-NG-1015. For both *in vitro* and *in vivo* samples, tissue samples were homogenized in lysis buffer on ice and RG-NG-1015 and/or metabolites were isolated from plasma, tissue homogenates, or urine via liquid-liquid extraction and solid-phase extraction steps. Calibration samples containing known amounts of RG-NG-1015 were extracted in parallel with test tissue homogenates, plasma, or urine samples. The molecular weight (MW) of RG-NG-1015 and potential metabolites were calculated from the MS signal and compared with theoretical values.

[000338] The *in vitro* metabolic stability of RG-NG-1015 was assessed in mouse, monkey, and human tissues (i.e., kidney and liver lysates) as well as serum. RG-NG-1015 was incubated in these matrices at a concentration of 5 μM with kidney and liver homogenates (corresponding to 307 μg/g tissue) or serum samples (corresponding to 15.3 μg/mL) for 24 hours at 37°C. RG-NG-1015 and metabolites were then extracted and analyzed by HPLC-TOF.

[000339] *In vivo* metabolism was evaluated in liver and kidney after a single dose of RG-NG-1015 to CD-1 mice, and in plasma, tissues, and urine after single and/or repeated administration to monkeys. CD-1 mice received a single SC dose of RG-NG-1015 at 2000 mg/kg, and monkeys received up to 5 weekly SC doses of RG-NG-1015 at 15, 75, or 150 mg/kg. RG-NG-1015 and metabolites were then extracted and analyzed by HPLC-TOF.

[000340] RG-NG-1015 undergoes sequential hydrolysis from both 3' and 5' ends to produce chain-shortened metabolites (*See* Table 21). Nine potential metabolites were identified: 5' N-1, 5' N-2, 5' N-3, 5' N-4, 3' N-1, 3' N-2, 3' N-3, 3' N-4, and 3' N-5 as set forth below in Table 21. All metabolites differed from RG-NG-1015 by sequential removal of terminal nucleotides and are terminated in hydroxyl groups on the 3′ and 5′ ends. 5' end shortmers (from N-5 to N-8) and 3' end shortmers (from N-6 to N-8) were not observed.

Table 21: Sequences, exact mass of neutral molecule, m/z and charge state of RG-NG-1015 and its potential metabolites

Compound Name	SEQ ID NO	Compound Sequence (5'->3')*	Exact Mass of Neutral Molecule	Mass to Charge Ratio (m/z)	Charge State	Comment
RG-NG-1015		$A_SG_SC_MA_FC_FU_FU_MU_SA_S$	3064.31	1531.15	-2	Parent Molecule
3'N-1		$A_SG_SC_MA_FC_FU_FU_MU_S$	2693.26	1345.63	-2	Metabolite
3'N-2		$A_SG_SC_MA_FC_FU_FU_M$	2345.24	1171.62	-2	Metabolite
3'N-3		$A_SG_SC_MA_FC_FU_F$	2009.22	1003.61	-2	Metabolite
3'N-4		$A_SG_SC_MA_FC_F$	1685.23	841.61	-2	Metabolite
3'N-5		$A_SG_SC_MA_F$	1362.21	680.11	-2	Metabolite
3'N-6		$A_SG_SC_M$	1015.19	506.59	-2	Not Observed
3'N-7		A_SG_S	680.15	339.08	-2	Not Observed
3'N-8		A_S	293.11	145.56	-2	Not Observed

5'N-1		$G_SC_MA_FC_FU_FU_MU_SA_S$	2693.26	1345.63	-2	Metabolite
5'N-2		$C_MA_FC_FU_FU_MU_SA_S$	2306.22	1152.11	-2	Metabolite
5'N-3		$A_FC_FU_FU_MU_SA_S$	1971.19	984.59	-2	Metabolite
5'N-4		$C_FU_FU_MU_SA_S$	1624.16	811.08	-2	Metabolite
5'N-5		$U_FU_MU_SA_S$	1301.15	649.57	-2	Not Observed
5'N-6		$U_MU_SA_S$	977.15	487.57	-2	Not Observed
5'N-7		U_SA_S	641.13	319.57	-2	Not Observed
5'N-8		A_S	293.11	145.56	-2	Not Observed
5'N+1	29	$A_SA_SG_SC_MA_FC_FU_FU_MU_SA_S$	3435.35	1717.17	-2	Synthetic Impurity

Example 9: Clinical study to evaluate the safety, tolerability, pharmacodynamics, and pharmacokinetics of RG-NG-1015 (RGLS8429) **in patients with autosomal dominant polycystic kidney disease**

A. Overview of study design

[000341] This study consists of part A and part B. The drug product names "RG-NG-1015" and "RGLS8429" are used interchangeably herein.

[000342] Part A is a phase Ib, double blind, placebo-controlled multiple ascending dose (MAD) study, where RG-NG-1015 (RGLS8429) or placebo is administered via subcutaneous (SC) injection to approximately 36 subjects with a diagnosis of Mayo Imaging Classification (MIC) 1C, 1D or 1E autosomal dominant polycystic kidney disease (ADPKD) (based upon either the MRI obtained during screening or a prior MRI obtained within 5 years of screening with documented Mayo Imaging Classification). Subjects are required to sign an informed consent form (ICF) and are evaluated against the inclusion/exclusion criteria during a screening period.

[000343] Those who meet all inclusion/ exclusion criteria are randomized centrally 3:1 to receive RG-NG-1015 (RGLS8429) or placebo by subcutaneous (SC) injection every other week (Q2W) x 7 doses:

Cohort 1 (approximately 12 subjects): 1 mg/kg RG-NG-1015 (RGLS8429) or placebo

Cohort 2 (approximately 12 subjects): 2 mg/kg RG-NG-1015 (RGLS8429) or placebo

Cohort 3 (approximately 12 subjects): 3 mg/kg RG-NG-1015 (RGLS8429) or placebo.

[000344] Part B is an open-label fixed-dosing study. Part B consists of one cohort of up to 30 subjects who meet all inclusion/ exclusion criteria to receive RG-NG-1015 (RGLS8429) by SC injection Q2W x 7 doses:

Cohort 4 (up to 30 subjects): 300 mg RG-NG-1015 (RGLS8429).

[000345] The study drug is administered by SC injection in the clinic by the Investigator, or another qualified and trained site staff member with safety monitoring for at least 4 hours by site staff.

[000346] Subjects participate in the study for up to 141 days. The study consists of up to 28 days of screening (day -28 through day -1), followed by 85 days of treatment period (day 1 through day 86), and followed by 28 days of follow-up period (day 92 through day 113). During the treatment period, clinic

visits take place on day 1 and/or day 2 (dose 1 on day 1), day 15 (dose 2), day 29 (dose 3), day 43 (dose 4), day 57 (dose 5), day 71 (dose 6), and day 85 and/or day 86 (dose 7 on day 85). During the follow up period, clinic visits take place on day 92, day 99, and day 113 (end of study visit).

B. Subject population

[000347] This study consists of part A: three sequential cohorts of approximately 12 subjects each randomized centrally 3:1 to receive RG-NG-1015 (RGLS8429) 1 mg/kg, 2 mg/kg, or 3 mg/kg or placebo by SC injection every other week (Q2W) x 7 doses (36 subjects total), and part B: one cohort of up to 30 subjects to receive fixed dose of 300 mg of RG-NG-1015 (RGLS8429) by SC injection Q2W x 7 doses.

Inclusion criteria

[000348] Subjects must meet all of the following inclusion criteria to participate in the study:

1) age 18 to 70 years, inclusive at the time of signing the informed consent;

2) diagnosed with ADPKD (1C, 1D, or 1E according to Mayo Imaging Classification based upon either the Magnetic Resonance Imaging [MRI] obtained during screening, or a prior MRI obtained within 5 years of screening with documented Mayo Imaging Classification);

3) estimated glomerular filtration rate (eGFR) between 30-90 mL/min/1.73 M^2;

3) body mass index (BMI) 18 to 35 kg/m^2;

4) if the subject has hypertension, the antihypertensive regimen must be stable for at least 28 days prior to randomization (part A) or Day 1 (part B) and the blood pressure adequately controlled prior to randomization (part A) or Day 1 (part B);

5) screening hematology and clinical chemistries of following:

a) platelets within the normal range,

b) total and direct bilirubin <1.5x upper limit of normal (ULN), unless elevated bilirubin is associated with a known benign condition (e.g., Gilbert's syndrome),

c) Alanine Aminotransferase (ALT) <1.5x ULN,

d) Aspartate Aminotransferase (AST) <1.5x ULN,

e) Alkaline Phosphatase (ALP) <1.5x ULN,

f) Gamma-Glutamyl Transferase (GGT) <1.5x ULN.

6) must understand and consent to the study procedures explained in the Informed Consent Form (ICF) and be willing and able to comply with the protocol.

7) Female subjects of childbearing potential must not be lactating and must have no plans to become pregnant during the course of the study through 28 days after the last dose of study drug. Female subjects of childbearing potential who are heterosexual must agree to use one of the following methods of contraception considered to be highly effective (i.e., results in <1% failure rate when used consistently and correctly) from screening through 28 days after the last dose of study drug:

a) Intrauterine device (IUD) or intrauterine systems (IUS) in place for at least 3 months prior to randomization (Part A) or Day 1 (Part B)

b. Partner has had a vasectomy. Vasectomy in the partner is considered to be highly effective only if the partner is the sole sexual partner of the female subject of childbearing potential and had his vasectomy performed 6 months or more prior to randomization (Part A) or Day 1 (Part B)

c. Stable hormonal contraception associated with inhibition of ovulation (with approved oral, transdermal, or depot regimen) for at least 3 months prior to randomization (Part A) or Day 1 (Part B)

8) A female subject of non-childbearing potential must have undergone one of the following sterilization procedures at least 6 months prior to the first dose of study drug:

a. Hysterectomy

b. Bilateral oophorectomy

c. Bilateral tubal occlusion

d. Bilateral salpingectomy

or be postmenopausal with no menstrual periods for at least 1 year prior to the first dose of study drug.

9) Any non-vasectomized heterosexual male subjects must agree to use a condom with spermicide. (No restrictions are required for a vasectomized heterosexual male provided his vasectomy was performed 6 months or more prior to study start. A heterosexual male who has been vasectomized less than 6 months prior to study start must follow the same restrictions as a non-vasectomized heterosexual male.)

10) Male and female subjects must agree not to donate sperm or preserve eggs (ova), respectively, from Day 1 until 28 days after the last dose of study drug

11) Must agree to not donate blood in the 28 days prior to randomization (Part A) or Day 1 (Part B) or plasma in the 7 days prior to randomization (Part A) or Day 1 (Part B) through the End of Study Visit (EOS).

Exclusion Criteria

[000349] Subjects who meet any of the following criteria are excluded from the study:

1) administration of tolvaptan in the 28 days before randomization (part A) or Day 1 (part B);

2) subject is mentally incapacitated or has significant emotional problems;

3) any medical condition or social circumstance that, in the opinion of the Investigator, may make the subject unlikely to complete the study or comply with study procedures and requirements, or may pose a risk to the subject's safety;

4) history or presence of alcoholism or drug abuse within the past 2 years prior to screening;

5) active infection of the urinary tract (e.g., kidney, bladder, etc.);

6) known hepatitis B, hepatitis C, or human immunodeficiency virus (HIV) infection;

7) only one kidney or kidney transplant recipient;

8) history of malignancy, except for successfully treated squamous or basal cell carcinoma skin cancer;

9) history of a clinically significant reaction to an oligonucleotide compound in the opinion of the Investigator;

10) tattoo(s) or scarring at or near the site of SC injection or any other condition that may interfere with injection site examination(s), in the opinion of the Investigator;

11) participation in another clinical trial and/or exposure to any investigational drug or approved therapy for investigational use within 28 days or 5 half-lives of the investigational drug's dosing, whichever is longer, prior to dosing. The 28-day or 5-half-life windows will be calculated from the date of the last dosing in the previous study to Day 1 of the current study.

C. Drug Product

[000350] RG-NG-1015 (RGLS8429) is provided in 2 mL clear glass vials containing sufficient volume to extract the labeled volume of 1 mL of 150 mg/mL of RG-NG-1015 in 0.3% saline. Placebo injection is provided in 2 mL clear glass vials containing sufficient volume to extract the labeled volume of 1 mL of 1.5 µg/mL of riboflavin in 0.9% sodium chloride. Both RG-NG-1015 (RGLS8429) and placebo solutions are clear and colorless to pale yellow.

D. Administration

[000351] RG-NG-1015 (RGLS8429) and placebo are administered via subcutaneous (SC) injection as a bolus in the anterior abdominal wall in accordance with the standard-of-care procedures at the site. The injection(s) on each dosing day is rotated to different quadrant of the abdomen. Study drug is administered by a qualified and trained site staff member. In Part A, because the volume of study drug varies considerably depending on the dose level, the following guideline must be followed: the maximum volume for each injection must not exceed 2 mL (e.g., a 6-mL dose would require three 2-mL injections in the same quadrant of the abdomen). In Part B, the volume shall not exceed 2 mL. Cohort 4 will get a fixed dose of active drug, and thus the volume does not vary.

E. Endpoints

[000352] The primary objectives and endpoints of this study are:

Primary Objectives	Primary Endpoints
To evaluate the safety and tolerability of RG-NG-1015 (RGLS8429)	Incidence of adverse events (AEs) and change in safety laboratory test results, vital signs, Scale for the Assessment and Rating of Ataxia [SARA], and 12-lead electrocardiogram (ECG) over time
To assess the impact of RG-NG-1015 (RGLS8429) on ADPKD biomarkers	Change from baseline in polycystin-1 (PC1), polycystin-2 (PC2), neutrophil gelatinase-associated lipocalin (NGAL), and kidney injury molecule-1 (KIM-1) biomarkers

[000353] The secondary objections and endpoints of this study are:

Secondary Objectives	Secondary Endpoints

To assess the impact of RG-NG-1015 on height-adjusted total kidney volume (htTKV)	Change from baseline in htTKV
To characterize the pharmacokinetic (PK) properties of RG-NG-1015 (RGLS8429)	PK parameters of RG-NG-1015 including: • Maximum observed concentration (C_{max}) • Time to maximum observed concentration (T_{max}) • Area under the concentration–time curve up to 24 h post dose (AUC_{0-24}) • Area under the concentration–time curve up to the last quantifiable concentration (AUC_{0-t}) • Area under the concentration–time curve over the dosing interval (AUC_{tau}) • Area under the concentration–time curve extrapolated to infinity (AUCinf) • Half-life ($t_{½}$) • Apparent clearance (CL/F) • Apparent volume of distribution (V_z/F) • Fraction of unchanged RG-NG-1015 excreted in the urine (fe) • Total amount of unchanged RG-NG-1015 excreted in the urine (Ae) Presence of anti-drug antibodies (ADA)
To assess the impact of RG-NG-1015 (RGLS8429) on renal function	Change from baseline in eGFR, urine albumin to creatinine ratio (UACR), serum creatinine (SCr), and BUN

[000354] The exploratory objectives and endpoints of this study are:

Exploratory Objectives	**Exploratory Endpoints**
To characterize the effects of RG-NG-1015 on potential renal biomarkers	• Change from baseline in monocyte chemoattractant protein-1 (MCP-1) and beta-2 microglobulin (B2M) renal biomarkers in urine • Change from baseline in potential renal biomarkers (e.g., Insulin-like Growth Factor Binding Protein Acid Labile Subunit [IGFALS], copeptin [CT-proAVP], N-acetyl-1-methylhistidine, and others) in serum
To characterize the effects of RG-NG-1015 (RGLS8429) on potential image-based biomarkers	Changes from baseline in additional image-based biomarkers (e.g., total cyst volume, number and/or size distribution)
To characterize the relationship between ADPKD gene mutations and biomarker response	Relationship between ADPKD genetic mutations (e.g., PKD1 or PKD2 mutation, truncation, or missense mutation, etc.) and biomarker response (e.g., PC1 and PC2 levels)

F. Assessments

[000355] Clinical and safety assessments at screening and at prespecified timepoints during the study include:

- demographics, medical history, and concomitant medication,
- height and weight measurements,

- vital signs (body temperature, seated systolic and diastolic BP, HR, and RR),
- physical examinations (full and limited),
- SARA assessment to detect possible CNS impairment by the study drug,
- Safety laboratory testing (hematology complete blood count, chemistry metabolic panel, urinalysis, coagulation, and lipids),
- 12- lead ECGs,
- ADPKD genetic testing,
- plasma sample testing for C3a and Bb complement,
- plasma sample testing for antidrug antibodies,
- urine biomarker testing (PC1, PC2, NGAL, KIM-1),
- renal function testing (eGFR calculation and UACR, SCr, and BUN testing),
    - eGFR is calculated using the 2021 CKD-EPI Creatinine-Cystatin C Age, Sex Equation (Inker, 2021) during screening.
- plasma and urine pharmacokinetic testing
    - the plasma and urine concentration versus time data are used to derive the following PK parameters: C_{max}, T_{max}, AUC_{0-24}, AUC_{inf} (where calculable), AUC_{tau}, $t_{½}$, CL/F, V_z/F, fe, and Ae, and additional PK parameters as appropriate),
    - Pre-dose plasma PK sample on Days 1and 85 should be obtained within 60 minutes prior to dosing. Post-dose plasma samples on Days 1 and 2 and Days 85 and 86 should be obtained within the following time margins: collections at 2, 4, 6, and 8 hr ±15 minutes, 12 hr ±30 minutes, and 24 hr ±60 minutes.
    - Pre-dose PK samples are collected on Day 15, Day 43, and Day 71. Pre-dose and 4hr ±15 minutes post-dose samples are collected on Day 29 and Day 57. PK samples are collected on Day 99 and Day 113 EOS Visits.
    - 24-hr urine collection for PK analysis begins immediately after study drug dosing on Day 1 (0-24 hr) and Day 71 (0-24 hr). The subject should void immediately before dosing (before the 24-hr urine collection is started). Last void for the 24-hour collection is 24 hours post dose.
- MRI to determine change in htTKV from baseline.
- exploratory renal biomarkers testing in residual urine (MCP-1 and B2M) and residual serum (e.g., IGFALS, CT-proAVP, N-acetyl-1-methylhistidine, and others); as well as exploratory image-based biomarkers (e.g., total cyst volume, number and/or size distribution, etc.).
- acute phase response (Alb, fibrinogen, and high-sensitivity C-reactive protein) testing in plasma samples.

G. Data Analysis/ Statistical Methods

[000356] Tabular summaries and analysis results will be generated by dose level for RG-NG-1015 versus placebo (pooled across all cohorts). Descriptive statistics by dose level and treatment group will be tabulated by visit.

[000357] The following analyses are performed:

[000358] Safety analysis: safety data (frequencies of TEAEs (treatment emergent adverse event) and SAEs (serious adverse event), safety laboratory tests, vital signs, ECGs, and SARA test scores) are summarized descriptively by dose level and treatment group, as appropriate. The analysis of safety laboratory tests, vital signs and ECGs includes summary statistics over time, and changes from baseline are summarized descriptively. Changes from baseline in the SARA test scores are summarized descriptively over time for each category and the total score;

- The SARA test has eight categories with accumulative score ranging from 0 (no ataxia) to 40 (most severe ataxia). When completing the outcome measure, each category is assessed and scored accordingly. Scores for the eight items range as follows:
  1. Gait (0-8 points),
  2. Stance (0-6 points),
  3. Sitting (0-4 points),
  4. Speech disturbance (0-6 points),
  5. Finger chase (0-4 points),
  6. Nose-finger test (0-4 points),
  7. Fast alternating hand movement (0-4 points),
  8. Heel-shin slide (0-4 points).
- Once each of the 8 categories has been assessed, the total will be calculated to determine the severity of ataxia.

[000359] Phamacodynamic (biomarker) analysis: urine biomarker (PC1, PC2, NGAL, KIM-1) response are characterized. In part A, changes in urine biomarkers (i.e., PC1, PC2, NGAL, KIM-1) over time are compared to baseline (at screening and day 1 sample) and are compared between each RG-NG-1015 (RGLS8429) dose level and placebo (placebo pooled across cohort 1-3) using analysis of covariance and adjusting for baseline biomarker value and treatment group. Analysis is performed for data collected at Day 29, Day 57, Day 85, Day 86, Day 92, Day 99, and Day 113, but the main comparisons will be made at Day 86 and Day 113 time points. In Part B, changes from baseline in urine biomarker values (PC1, PC2, NGAL, KIM-1) will be compared using two-sided paired t-test. Analyses will be performed for data collected at Day 29, Day 57, Day 85, Day 86, Day 92, Day 99, and Day 113, but the main comparisons will be made at Day 86 and Day 113 time points. P-values < 0.05 will be considered statistically significant with no adjustment for multiplicity. Plots will be used to evaluate the relationships between changes from baseline in biomarkers (PC1, PC2, NGAL, KIM-1) and htTKV versus plasma exposure (e.g., AUC_{tau}, C_{max}, C_{min}) and dose level.

[000360] MRI analysis: The absolute and percent change from baseline in htTKV will be summarized using descriptive statistics. In Part A, changes from baseline in htTKV values are compared between

treatment groups at each dose level, as well as all subjects receiving RG-NG-1015 (RGLS8429) versus placebo (placebo pooled across cohort 1-3) using analysis of covariance and adjusting for baseline htTKV value, baseline Mayo Imaging Classification (MIC) (1C, 1D, or 1E), and treatment group. In Part B, changes from baseline in htTKV will be compared using two-sided paired t-test. P-values < 0.05 will be considered statistically significant with no adjustment for multiplicity.

[000361] Renal function analysis: changes from baseline in eGRF (calculated using the CKD-EPI equation using creatinine and cystatin-C and without race), UACR, SCr, and BUN are analyzed and summarized descriptively.

[000362] Pharmacokinetic analysis: the laboratory analyzing the PK samples will be unblinded, so PK analysis is performed only on subjects receiving RG-NG-1015 (RGLS8429). The plasma and urine concentration versus time data is used to derive the following PK parameters: C_{max}, T_{max}, AUC_{0-24}, AUC_{inf} (where calculable), AUC_{tau}, $t_{½}$, CL/F, V_z/F, fe, and Ae. Descriptive statistics for plasma concentrations are summarized by timepoint and cohort. Using non-compartmental methods, the plasma and urine concentration versus time data are used to derive the following PK parameters: Cmax, Tmax, AUC0-24, AUCinf (where calculable), AUCtau, t½, CL/F, Vz/F, fe, and Ae. Dose proportionality is explored using the power model for C_{max}, AUC_{0-24}, AUC_{inf}, and AUC_{tau} as data permit.

[000363] ADA analysis: The incidence and titer of ADA for subjects who develop ADAs at any time during the study is tabulated by cohort. ADA impact on the PK parameters will be evaluated by subgroup analysis (e.g., calculate PK parameters in subjects with and without ADAs).

[000364] Exploratory analysis: descriptive statistics is tabulated by visit for the exploratory renal biomarkers in urine (MCP-1 and B2M) and serum (e.g., IGFALS, CT-proAVP, N-acetyl-1-methylhistidine, and others); as well as exploratory image-based biomarkers (e.g., total cyst volume, number and/or size distribution, etc.). The relationship between ADPKD genetic mutations (e.g., PKD1 or PKD2 mutation, truncation, or missense mutation, etc.) and biomarker response (e.g., PC1 and PC2 levels) is explored.

[000365] Interim analysis: Part A: after the subjects in each cohort complete dosing of study drug and the EOS Visit, the Sponsor analyzes unblinded safety, biomarker, renal function, and PK data. Part B: after the subjects in cohort 4 complete dosing of RG-NG-1015 and the EOS Visit, the Sponsor will analyze all available unblinded safety, biomarkers, efficacy, and PK data.

[000366] A Clinical Research Organization (CRO) medical monitor reviews safety data on an ongoing basis. The Sponsor and CRO Medical Monitor will perform monthly blinded safety reviews of the AEs and safety laboratory test results to monitor safety during the study. After the last subject in the enrolling cohort receives their first dose administration (Day 1), a cohort dose-escalation meeting will occur at least 4 weeks later. The Sponsor will review the available safety data to make the dose escalation decision for Cohort 2 and the process will be repeated to make the dose escalation decision for Cohort 3.

H. Part A Results

[000367] Treatment with RG-NG-1015 achieves one or more of the primary, secondary, and/or exploratory endpoints, while having acceptable safety and tolerability.

[000368] Table 22 provides baseline characteristics for Cohort 1 and Cohort 2:

Baseline Characteristics	RG-NG-1015 (2 mg/kg) N=11	RG-NG-1015 (1 mg/kg) N=9	Placebo (Cohort 1 & 2) N=6
Age (years) mean (SD)	46 (12)	52 (12)	45 (12)
Female n (%)	5 (46%)	5 (56%)	3 (50%)
White n (%)	10 (91%)	9 (100%)	4 (67%)
BMI mean (SD)	26 (3)	30 (5)	28 (5)
Tolvaptan use in prior 3 months n (%)	1 (10%)	2 (22%)	1 (17%)
eGFR (mL/min/1.73m2)) mean (SD)	68 (19)	47 (20)	52 (18)
htTKV(mL/m) mean (SD)	1264 (567)	1698 (737)	1684 (575)
Mayo Imaging Classification 1C/1D/1E (%)	46%/36%/18%	56%/33%/11%	17%/67%/17%
Genetic Mutation *PKD1/PKD2* (%)	82%/18%	56%/33%	67%/0%

The enrolled population represents significant disease burden by kidney size and reduced eGFR.

[000369] The study showed that 1 mg/kg and 2 mg/kg RG-NG-1015 (RGLS8429) administered once every two weeks over 12 weeks were well-tolerated, with no significant safety findings.

[000370] Table 23 provides adverse events seen for Cohort 1 and Cohort 2:

	RG-NG-1015 (2 mg/kg) N=11	RG-NG-1015 (1 mg/kg) N=9	Placebo (Cohort 1 & 2) N=6
Any Treatment Emergent Adverse Events (TEAEs)	7 (64%)	7 (78%)	3 (50%)
Any Treatment Related TEAEs	5 (46%)	1 (11%)	0
Any Treatment Emergent Serious Adverse Events (TESAEs)	0	1 (11%)*	0
Any Treatment Related TESAEs	0	0	0
Any TEAEs leading to early withdrawal**	1 (9%)**	0	0
Any TEAEs leading to death	0	0	0

[000371] No accumulation of RG-NG-1015 was observed in plasma or urine with repeat dosing every other week. The AUC plasma exposure in patients administered 1 mg/kg RG-NG-1015 was nearly twice that of healthy volunteers and consistent with ~35% reduction in renal excretion. AUC plasma exposure increased at 2 mg/kg relative to 1 mg/kg.

Urinary polycystin (PC) measurements:

[000372] PC1 and PC2 measurement in urinary exosomes has demonstrated a clear distinction between healthy subjects and patients with ADPKD and inversely correlates with disease severity. *See* FIG. 7A and 7C.

[000373] Multiple analytic methodologies were used to assess urinary PC levels:

- Absolute change in urinary PC1 and PC2 levels from baseline, including best-fit regression models over the duration of treatment (*see* FIG. 7B and 7D, 8A-8C, 9A-9C, and 10A-10B);
- Percent change in urinary PC1 and PC2 levels from baseline (*see* FIG. 11A and 11B);
- Mean change in urinary PC1 and PC2 levels from baseline after three months of dosing of RG-NG-1015 (*see* FIG. 12A-12B and 13A-13B).

[000374] The study showed increases in absolute and % change in urinary polycystin (PC1 and PC2) levels with treatment of 1 mg/kg and 2 mg/kg RG-NG-1015 (RGLS8429) from baseline. *See* FIG. 7B and 7D, 8A-C, 9A-9C, 10A-10B, and 11A-11B. As shown in FIG. 11A, for 1 mg/kg of RG-NG-1015, statistically significant increases from baseline (average of 3 separate samplings prior to treatment) in urinary PC1 were noted at 12 weeks of dosing (36 and 41% on days 85 and 86, respectively). As seen in FIG. 11B, for 1 mg/kg of RG-NG-1015, increases from baseline were also observed in urinary PC2 at 12 weeks, however, the changes did not reach statistical significance (Please refer to FIG. 10A and 10B for statistical analysis). The polycystin pattern is consistent with tissue PK profile in pre-clinical studies. The study also shows increased absolute changes in PC1 and PC2 levels with treatment of 2 mg/kg RG-NG-1015compared to 1 mg/kg RG-NG-1015. *See* FIG. 8A-C, 9A-9C, and 10A-10B. In FIG. 10A, for example, for 2 mg/kg of RG-NG-1015, statistically significant increases from baseline in urinary PC1 were noted at days 57, 86, 99, and 113 of dosing. In FIG. 10B, for 2 mg/kg of RG-NG-1015, statistically significant increases from baseline in urinary PC2 were noted at day 57.

[000375] The study also showed increases in mean polycystin levels after 3 months of dosing (Q2W) with treatment of 1 mg/kg and 2 mg/kg RG-NG-1015 (RGLS8429), for absolute change of PC1 and PC2 (Fig. 12A-B) and % change of PC1 and PC2 (Fig. 13A-B). 2 mg/kg of RG-NG-1015 showed a greater increase of mean polycystin levels compared to 1 mg/kg of RG-NG-1015.

[000376] Urinary measurement of PC1 and PC2 demonstrated greater biological activity of RG-NG-1015 (RGLS8429) at 2 mg/kg compared to placebo, which was most evident after three months of dosing. There was a mechanistic dose response at 2mg/kg dose level based on urinary polycystin analyses.

[000377] The study also demonstrated that polycystin is a valid pharmacodynamic marker (i.e., for dose-ranging) as urinary polycystin exhibited appropriate PK/PD correlation to serve as a pharmacodynamic biomarker in ADPKD. *See* FIG. 14A and 14B. As shown in FIG. 14A-14B, an emerging dose response is observed at 0.3mg/kg and 1mg/kg RGLS4326 (NCT04536688) as well as at 1mg/kg RG-NG-1015 (RGLS8429). A positive correlation was observed between PC1 and both PK parameters measured (C_{max} and AUC_{last}) when combining RGLS4326 & RG-NG-1015 datasets. Also, a similar pharmacodynamic response was observed between both RGLS 4326 and RG-NG-1015 at 1 mg/kg dose level.

Renal function parameters and renal MRI measures:

[000378] Patients with ADPKD experience ~6% growth in their kidneys annually based on published longitudinal studies, so there is an expected progression of ~1-2% growth in kidney volume over 12 weeks.

[000379] An end-of-study MRI was used to evaluate the impact on novel imaging biomarkers that characterize cystic architecture. The exploratory results of MRI image analysis are shown in FIG. 15A-C and 16A-C.

[000380] FIG. 15A-15B show changes in height-adjusted total kidney volume (htTKV) and total kidney cyst volume (TKCV) for subjects receiving 1 mg/kg RG-NG-1015, 2 mg/kg RG-NG-1015, and placebo. Fig. 15C shows correlation between changes in TKCV and changes in htTKV.

[000381] Table 24 shows the mean %htTKV change in 2 mg/kg group, 1 mg/kg group, and placebo:

%HtTKV	2mpk	1mpk	Pb-All
Mean	-0.84	+1.79	+0.52
SD	3.11	5.90	3.13

Table 25 shows the mean %TKCV change in 2 mg/kg group, 1 mg/kg group, and placebo:

% TKCV	2mpk	1mpk	Pb-All
Mean	-0.53	+4.36	+0.47
SD	3.90	8.21	1.94

[000382] FIG. 16A and B show changes in total liver volume (TLV) and total liver cyst volume TLCV) for subjects receiving 1 mg/kg RG-NG-1015, 2 mg/kg RG-NG-1015, and placebo. Fig. 16C shows correlation between changes in TLCV and changes in TLV.

[000383] Table 26 shows the mean %TLV change in 2 mg/kg group, 1 mg/kg group, and placebo:

%TLV	2mpk	1mpk	Pb-All
Mean	-1.03	-2.99	+5.03
SD	7.91	7.65	5.61

Table 27 shows the mean absolute TLCV change in 2 mg/kg group and placebo:

Abs TLCV (mL)	2mpk	Pb
Mean	-1.72	+0.71
SD	14.95	1.966

[000384] FIG. 17A shows exploratory correlation between change in PC1 compared to change in HtTKV. Table 28 shows the simple linear regression for placebo, 1 mg/kg, and 2 mg/kg RG-NG-1015:

Simple Linear Regression	R^2	P-value
Placebo	0.0092	0.857
RGLS8429 (1mg/kg)	0.0211	0.709
RGLS8429 (2 mg/kg)	0.0719	0.425

[000385] FIG. 17B shows exploratory correlation between change in PC1 compared to change in eGFR. Table 29 shows the simple linear regression for placebo, 1 mg/kg, and 2 mg/kg RG-NG-1015:

Simple Linear Regression	R^2	P-value
Placebo	0.0021	0.930

RGLS8429 (1mg/kg)	0.2418	0.179
RGLS8429 (2 mg/kg)	0.4027	0.036

[000386] FIG. 17C shows exploratory correlation between change in PC2 compared to change in HtTKV. Table 30 shows the simple linear regression for placebo, 1 mg/kg, and 2 mg/kg RG-NG-1015:

Simple Linear Regression	R^2	P-value
Placebo	0.0728	0.605
RGLS8429 (1mg/kg)	0.0639	0.570
RGLS8429 (2 mg/kg)	0.0373	0.512

[000387] FIG. 17D shows exploratory correlation between change in PC2 compared to change in eGFR. Table 31 shows the simple linear regression for placebo, 1 mg/kg, and 2 mg/kg RG-NG-1015:

Simple Linear Regression	R^2	P-value
Placebo	0.0766	0.596
RGLS8429 (1mg/kg)	0.5902	0.197
RGLS8429 (2 mg/kg)	0.2249	0.006

[000388] For cohort 1, renal function parameters did not demonstrate significant changes over the short term, 12-week dosing. Baseline measurements were consistent with stage of ADPKD Mayo Imaging Classification. No notable changes in renal function measures were seen over 12 weeks (i.e., eGFR, UACR, SCr , BUN, U-NGAL, U-KIM 1

[000389] For cohort 2, the exploratory results of MRI image analysis are as follows:

- htTKV mean change of -0.84% in 2 mg/kg group vs. +0.52% in placebo;
- 4/11 subjects receiving 2 mg/kg demonstrated reductions in htTKV>2%, reductions in TKCV, and increases in both urinary PC1 & PC2;
- Changes in TKCV correlated with changes in htTKV;
- Reductions in liver volume and liver cyst volume were noted among some patients treated with RG-NG-1015;
- No notable changes in renal function measures were seen over 12 weeks (i.e., eGFR, UACR, SCr, BUN, U-NGAL, U-KIM 1), which are as expected based on short-term treatment and the small number of subjects.

[000390] Some case highlights are set forth below:

- Subject 1: highest increase in PC1 & PC2*
    - 47 y/o male diagnosed in 2006
    - Baseline eGFR 66 mL/min and htTKV941 mL/m

- D113 MRI: htTKV reduced by 4.96%; TKCV reduced by 4.34%;

- Subject 2: 2nd highest increase in PC1*
  - 44 y/o female diagnosed in 2019
  - Baseline eGFR 65 mL/min and htTKV1253 mL/m
  - D113 MRI: htTKV reduced by 6.28%; TKCV reduced by 6.93%;
- Subject 3: 2nd highest increase in PC2*
  - 29 y/o male diagnosed in 2020
  - Baseline eGFR 88 mL/min and htTKV1162 mL/m
  - D113 MRI: htTKV reduced by 4.22%; TKCV reduced by 2.73%.
- Among the 4 active subjects in cohort 2 with reductions in htTKV>2%, all 4 had increases in PC1 & PC2.

*Mean of % change between Day 85 and Day 113 of Polycystin (PC).

[000391] Results from Cohort 2 suggest a numerical improvement in total volume and cyst volume in both kidneys and livers of patients with ADPKD compared to placebo.

[000392] Table 32 provides baseline characteristics for Cohort 3, and for an updated Placebo group that includes additional subjects relative to the Placebo group described in Table 22:

Baseline Characteristics	RG-NG-1015 (3 mg/kg) N=12	Placebo (Cohort 1 & 2) N=10
Age (years) mean (SD)	48 (11)	45 (12)
Female n (%)	4 (33%)	4 (40%)
White n (%)	11 (92%)	7 (70%)
BMI mean (SD)	27 (4)	27 (4)
Tolvaptan use in prior 3 months n (%)	2 (17%)	1 (10%)
eGFR (mL/min/1.73m2)) mean (SD)	61 (16)	55 (17)
htTKV(mL/m) mean (SD)	1393 (459)	1763 (859)
Mayo Imaging Classification 1C/1D/1E (%)	58%/25%/17%	20%/60%/20%
Genetic Mutation *PKD1/PKD2/Neither* (%)	75%/8%/17%	80%/0%/20%

[000393] Analyses for subjects in Cohort 3 were conducted as described herein for Cohorts 1 and 2. FIG. 19 and 20 show data for placebo, and Cohorts 1, 2, and 3. The data for Cohorts 1 and 2 is the data presented above. The placebo group has additional subjects relative to the data presented above.

[000394] FIG. 19A and 19B show individual subplots of urinary PC1/CD133 ratio and urinary PC2/CD133 ratio, at baseline and at the end of the study, for all cohorts in Part A. Increases in urinary

PC1/CD133 and PC2/CD133 were observed following the 3 mg/kg treatment, and these increases were more consistent than those observed following the 1 mg/kg and 2 mg/kg treatments.

[000395] Also measured in Cohort 3 subjects were the absolute changes in urinary PC1/CD133 and PC2/CD133 ratios during the course of treatment. As shown in FIG. 20A and 20B, the magnitude of mean absolute increases was generally similar in the 2 mg/kg and 3 mg/kg groups through Day 99. Further, the 3 mg/kg group showed a more consistent response with statistical significance at all post-baseline visits.

[000396] Analysis of percent changes in urinary PC1/CD133 and PC2/CD133 ratios from baseline to the end of the study revealed a dose-responsive increase in these ratios, with the greatest increases observed in the 3 mg/kg cohort. *See* FIG. 21A and 21B.

[000397] FIG. 22A and 22B show changes in height-adjusted total kidney volume (htTKV) and total kidney cyst volume (TKCV) for subjects receiving 1 mg/kg RG-NG-1015, 2 mg/kg RG-NG-1015, 3 mg/kg, and placebo. The data for the 1 mg/kg and 2 mg/kg groups are also shown in FIG. 15A-15C. FIG. 22C shows the correlation between changes in TKCV and changes in htTKV.

[000398] FIG. 23A shows the correlation between the change in PC1 to the change in htTKV. Table 33 shows, for the data in FIG. 23A, the simple linear regression for the updated placebo group and all RGLS8429 Part A cohorts:

Simple Linear Regression	R^2	P-value
Placebo	0.0207	0.6920
RGLS8429 (1mg/kg)	0.0211	0.7092
RGLS8429 (2 mg/kg)	0.0720	0.4250
RGLS8429 (3 mg/kg)	0.1683	0.2390

[000399] FIG. 23B shows the correlation between the change in PC2 compared and changes in htTKV. Table 34 shows, for the data in FIG. 23B, the simple linear regression for the updated placebo group and all RGLS8429 Part A cohorts:

Simple Linear Regression	R^2	P-value
Placebo	0.0820	0.4226
RGLS8429 (1mg/kg)	0.0639	0.5117
RGLS8429 (2 mg/kg)	0.0373	0.5694
RGLS8429 (3 mg/kg)	0.2581	0.1399

[000400] FIG. 24 shows the correlation between change in PC1 compared and change in eGFR. Table 35 shows, for the data in FIG. 24, the related simple linear regression for the updated placebo group and all RGLS8429 Part A cohorts:

Simple Linear Regression	R^2	P-value
Placebo	0.0110	0.7734
RGLS8429 (1mg/kg)	0.2418	0.1787
RGLS8429 (2 mg/kg)	0.4027	0.0360
RGLS8429 (3 mg/kg)	0.0984	0.3476

[000401] The 3 mg/kg dose of RGLS8429 demonstrated more consistent increases in urinary PC1 and PC2 across patients, compared to the lower dose cohorts. Urinary PC1 and PC2 from baseline to end of the study were changed in a dose-responsive manner, with statistical significance seen at the 3 mg/kg dose (compared to placebo). Further, exploratory MRI imaging analysis suggested that three months of dosing at 3 mg/kg RGLS8429 reduced htTKV.

[000402] As was the case for the 1 mg/kg and 2 mg/kg doses, the 3 mg/kg dose exhibited a favorable safety and tolerability profile.

I. Part B Results

[000403] Analysis of results was conducted for 14 subjects of the up to 30 subjects in Cohort 4. Table 36 provides baseline characteristics for Cohort 4:

Baseline Characteristics of Cohort 4 subjects (N=14)	
Age (years) mean	47
eGFR (mL/min/1.73m2)) mean	59
htTKV(mL/m) mean	1479
Mayo Class 1C/1D/1E (%)	7 (50%) / 4 (30%) / 3 (20%)
Genetic Mutation *PKD1/PKD2/Neither* (%)	9 (64%) / 1 (7%) / 4 (29%)

[000404] A primary endpoint of the study is to assess the impact of RG-NG-1015 (RGLS8429) on ADPKD biomarkers, including the change in urinary PC1 and PC2 from baseline to the end of participation in the study (day 113) for each of the 14 subjects. Geometric least squares mean percent change and statistical analyses were performed on log-transformed data. CD133 was used as a normalization control. Placebo results were pooled from each of the subjects receiving placebo in Part A. Table 37 shows the geometric least square mean and statistical analyses of percent change in the ratio of PC1 to CD133, at the end of study relative to baseline:

PC1/CD133 Ratio	Geometric Mean	Log SE	Upper Limit	Lower Limit	Upper Standard Error	Lower Standard Error
300 mg (N = 14)	36.85	0.0733	47.26	27.18	10.41	9.67
Placebo (pooled, N = 10)	5.09	0.0851	14.42	-3.48	9.33	8.57

The data in Table 37, along with the corresponding data for the 1 mg/kg, 2 mg/kg, and 3 mg/kg doses, are also shown in FIG. 25A. Geometric least squares mean percent change data are shown. Error bars represent standard errors. ANCOVA analyses was performed on log scale transformation to account for non-normal distribution. Data was not available for one subject in each of 3 mg/kg and 300 mg/kg fixed groups.

[000405] Table 38 shows the geometric least square mean and statistical analyses of percent change in the ratio of PC2 to CD133, at the end of the study relative to baseline:

PC2/CD133 Ratio	Geometric Mean	Log SE	Upper Limit	Lower Limit	Upper Standard Error	Lower Standard Error
300 mg (N = 14)	31.11	0.0900	43.46	19.83	12.35	11.28
Placebo (pooled, N = 10)	-9.9	0.1030	-0.12	-18.72	9.78	8.82

The data in Table 38, along with the corresponding data for the 1 mg/kg, 2 mg/kg, and 3 mg/kg doses, are also shown in FIG. 25B. Geometric least squares mean percent change data are shown. Error bars represent standard errors. ANCOVA analyses was performed on log scale transformation to account for non-normal distribution. Data was not available for one subject in each of 3 mg/kg and 300 mg/kg fixed groups.

A secondary objective of the study was to assess the impact of RG-NG-1015 (RGLS8429) on height-adjusted total kidney volume (htTKV) from baseline to the end of participation in the study (day 113) for each of the 14 subjects. Geometric least squares mean percent change and statistical analyses were performed on log-transformed data. Placebo results were pooled from each of the subjects receiving placebo in Part A. Table 39 shows the geometric least squares mean and statistical analyses of the % change in htTKV at the end of the study relative to baseline:

% change htTKV	Geometric Mean	Log SE	Upper Limit	Lower Limit	Upper Standard Error	Lower Standard Error
300 mg (N = 14)	0.38	0.01	1.65	-0.87	1.27	1.25
Placebo (pooled, N = 10)	2.56	0.01	4.04	1.10	1.48	1.46

The data in Table 39, along with the corresponding data for the 1 mg/kg, 2 mg/kg, and 3 mg/kg cohorts, are also shown in FIG. 26. Geometric least squares mean percent change data are shown. Error bars represent standard errors. ANCOVA analyses was on log scale transformation to account for non-normal distribution. Data was not available for two subjects in 3 mg/kg group and one subject in 300 mg fixed group. One subject in 300 mg/kg fixed group experienced a renal cyst rupture and thus only contralateral kidney results for this subject are included. Due to the small sizes of each cohort, the length of the dosing period, and the inherent fluctuations in eGFR, variability across the dosing groups was expected.

When the percent change in htTKV was analyzed according to MIC, it was observed that the 300 mg dose of RG-NG-1015 (RGLS8429) consistently impacted the percent change in htTKV regardless of MIC. Table 40 shows the % change in htTKV for each individual subject who received placebo or the 300 mg dose, according to MIC:

% change htTKV					
MIC 1C		**MIC 1D**		**MIC 1E**	
Placebo	**300 mg**	**Placebo**	**300 mg**	**Placebo**	**300 mg**
3.97	3.45	0.29	-3.26	0.48	-2.92
2.49	-4.1	-4.49	0.97	7.97	2.19
	-2.79	3.68	3.98		0.93
	10.27	-0.82			
	-1.79	3.98			
	2.25	10.06			
	-2.21				

No notable changes in renal function measures were seen over 12 weeks (e.g., eGFR, UACR, SCr, cystatin-C, BUN, U-KIM 1), which is as expected based on short-term treatment and the small number of subjects.

Table 41 shows the rate of change of eGFR for the 300 mg group, as compared to placebo:

	Rate of Change of eGFR (mL/min/m^2/year)		
	Annual Mean Change	Lower 95% CL Change	Upper 95% CL Change
300 mg (N = 14)	**-0.899**	-3.072	1.274
Placebo (N = 10)	**-2.270**	-4.886	0.345

[000406] In summary, the 300 mg fixed dose of RG-NG-1015 (RGLS8429) was found to have a favorable safety and tolerability profile. PC1 and PC2 levels in urine were consistent with optimal inhibition of miR-17. Notably, measurement of TKV suggested an effect on disease progression following three months of treatment with 300 mg RG-NG-1015 (RGLS8429). Similar results are expected for the remaining subjects of Cohort 4.

Example 10: Modelling of target engagement studies data for RG-NG-1015 (RGLS8429)

[000407] The efficacy of RG-NG-1015 (RGLS8429) was evaluated in the KspCre;*Pkd1*F/RC (*Pkd1*-F/RC) mouse model. On each of post-natal days 8, 10, 12, and 15 of age, *Pkd1*-F/RC mice were administered a subcutaneous injection of RG-NG-1015 at various doses, control oligonucleotide at 20 mg/kg, or PBS (N=8-13 per group). A separate group of Pkd1-F/RC mice were administered a subcutaneous injection of RG-NG-1015 on post-natal days 8 and 12 at 20 mg/kg. Mice were sacrificed at 18 days of age. Left kidney was perfused with cold PBS and 4% PFA prior to collection. All other mouse

kidneys were collected, fixed in 10% formalin, dehydrated, and embedded in paraffin using a standard protocol. Samples were sectioned at 5 μm and subjected to hematoxylin and eosin staining.

[000408] As shown in FIG. 18A-18B, RGLS8429 demonstrated efficacy, reducing kidney weight to body weight ratio (KW/BW) in a dose-responsive manner (Fig. 18B Left, individual KW/BW grouped by dose levels and regime; Right, individual calculated percent inhibition (CPI) of KW/BW plotted against individual kidney concentration of RG-NG-1015. Estimated kidney concentration corresponding to 50% inhibition of KW/BW, i.e. IC50 value, was shown). Kidney size and cyst number were both reduced in a dose-responsive manner following administration of RGLS8429. *See* FIG. 18A.

[000409] Wild type C57BL6 mice received a single SC dose of RGLS8429 or RGLS4326 at 0.003, 0.03, 0.1, 0.3, 1, 3, 10, 30 or 300 mg/kg. Mice were sacrificed at 7 days post dose. Kidney samples were harvested, and target engagement (displacement of miR-17 from high molecular weight polysomes) were measured by miPSA assay (Androsavich, Nucleic Acids Res. 44, e13 (2016).) Individual calculated percent inhibition (CPI) of target engagement in mouse kidney plotted against individual kidney concentrations of RGLS8429. Estimated kidney concentration corresponding to 80% inhibition of miR-17, i.e. IC80 values, were shown. *See* FIG. 18C. An AUC of ~ 12,494 h*ug/g was predicted and used as a benchmark for kidney exposure that drives max target engagement (~ 80% inhibition of miR-17).

[000410] Based on recent cohort 1 and 2 results , 1 mg/kg dose (cohort 1) and 2mg/kg dose (cohort 2) of RG-NG-1015 in ADPKD patients are predicted to yield kidney exposure of ~ 6,052 h*ug/g and ~8,900 h*ug/g, respectively (less than the predicted benchmark kidney exposure of ~12,494 h*ug/g). Based on the above results, it is expected that 3 mg/kg dose (cohort 3) would result in predicted human kidney exposure close to or slightly over ~ 12,494 h*ug/g.

[000411] Plasma to tissue modelling suggests 1mg/kg is less than half of dose response curve. The data suggested the potential to demonstrate greater urinary polycystin response at higher doses.

[000412] Based on extensive non-clinical analysis and PK/PD modeling, kidney exposure associated with peak miR-17 target engagement is anticipated to be achieved at >2.4 mg/kg in a human.

What is claimed is:

1. A method of treating polycystic kidney disease comprising administering to a subject in need thereof a modified oligonucleotide or a pharmaceutically acceptable salt thereof at a fixed dose of between about 150 mg and about 350 mg,

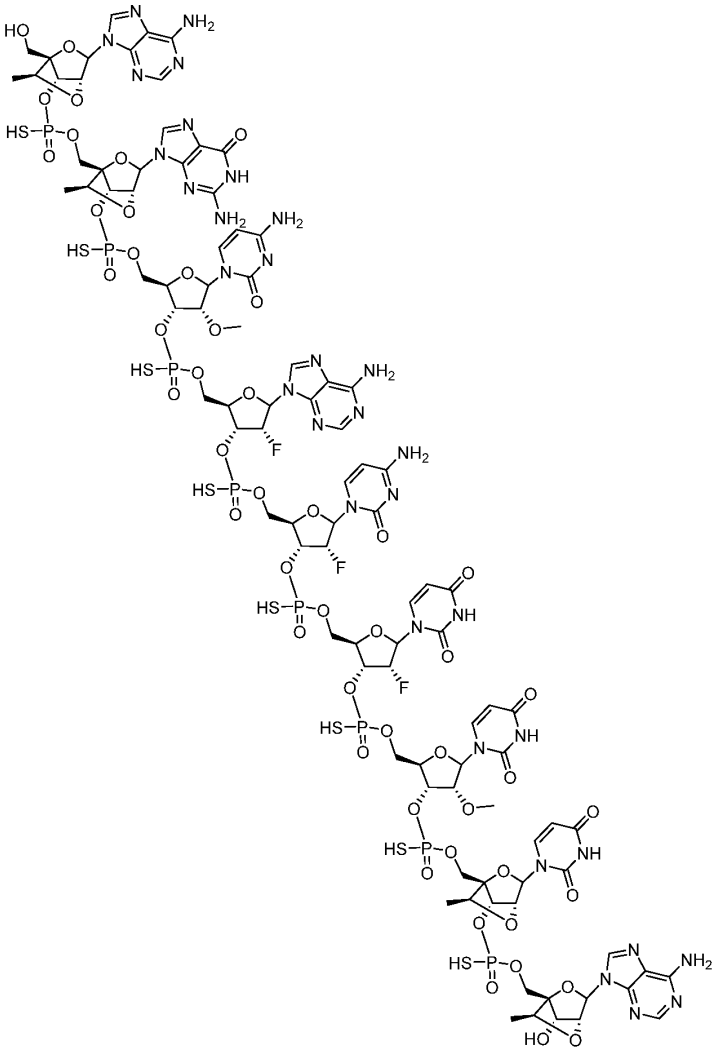
wherein the modified oligonucleotide has the structure 5'-$A_SG_SC_MA_FC_FU_FU_MU_SA_S$-3',

wherein nucleosides followed by subscript "M" are 2'-O-methyl nucleosides; nucleosides followed by subscript "F" are 2'-fluoro nucleosides; and nucleosides followed by subscript "S" are S-cEt nucleosides, and wherein each cytosine is a non-methylated cytosine.

2. The method of claim 1, wherein the modified oligonucleotide or a pharmaceutically acceptable salt thereof is administered at a fixed dose of 150 mg, 160 mg, 170 mg, 180 mg, 190 mg, 200 mg, 210 mg, 220 mg, 230 mg, 240 mg, 250 mg, 260 mg, 270 mg, 280 mg, 290 mg, 300 mg, 310 mg, 320 mg, 330 mg, 340 mg, or 350 mg.

3. The method of claim 1 or 2, wherein the pharmaceutically acceptable salt is a sodium salt.

4. A method of treating polycystic kidney disease comprising administering to a subject in need thereof a modified oligonucleotide at a fixed dose of between about 150 mg and about 350 mg, wherein the modified oligonucleotide has the structure:



or a pharmaceutically acceptable salt thereof.

5. The method of claim 4, wherein the modified oligonucleotide is administered at a fixed dose of 150 mg, 160 mg, 170 mg, 180 mg,190 mg, 200 mg, 210 mg, 220 mg, 230 mg, 240 mg, 250 mg, 260 mg, 270 mg, 280 mg, 290 mg, 300 mg, 310 mg, 320 mg, 330 mg, 340 mg, or 350 mg.

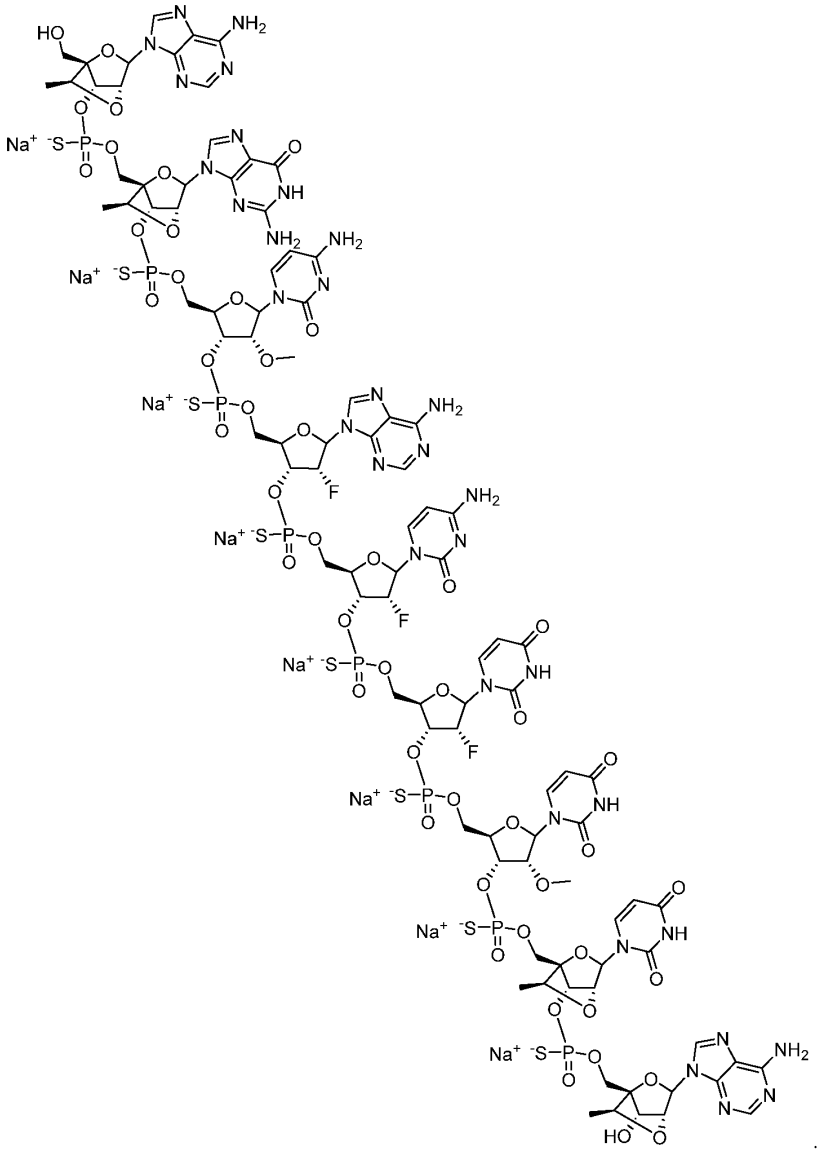
6. The method of claim 4 or 5, wherein pharmaceutically acceptable salt is a sodium salt.

7. The method of any one of claims 1-6, wherein the modified oligonucleotide is present in a pharmaceutical composition comprising a pharmaceutically acceptable diluent.

8. The method of claim 7, wherein the pharmaceutically acceptable diluent is a sterile aqueous solution.

9. The method of claim 8, wherein the sterile aqueous solution is a saline solution.

10. A method of treating polycystic kidney disease comprising administering to a subject in need thereof a modified oligonucleotide at a fixed dose of between about 150 mg and about 350 mg, wherein the modified oligonucleotide has the structure:



11. The method of claim 10, wherein the modified oligonucleotide is administered at a fixed dose of 150 mg, 160 mg, 170 mg, 180 mg, 190 mg, 200 mg, 210 mg, 220 mg, 230 mg, 240 mg, 250 mg, 260 mg, 270 mg, 280 mg, 290 mg, 300 mg, 310 mg, 320 mg, 330 mg, 340 mg, or 350 mg.

12. The method of claim 10 or 11, wherein the modified oligonucleotide is present in a pharmaceutical composition comprising a pharmaceutically acceptable diluent.

13. The method of claim 12, wherein the pharmaceutically acceptable diluent is a sterile aqueous solution.

14. The method of claim 13, wherein the sterile aqueous solution is a saline solution.

15. The method of any one of claims 1-14, wherein the subject has polycystic kidney disease.

16. The method of any one of claims 1-15, wherein the subject has been diagnosed as having polycystic kidney disease using clinical, histopathologic, and/or genetic criteria.

17. The method of any one of claims 1-16, wherein the polycystic kidney disease is autosomal dominant polycystic kidney disease (ADPKD).

18. The method of claim 17, wherein the subject has Mayo Imaging Classification 1C, 1D, or 1E of ADPKD.

19. The method of any one of claims 1-18, wherein the subject, prior to administration of the modified oligonucleotide, has an estimated glomerular filtration rate (eGFR) between 30-90 mL/min/1.73 m^2.

20. The method of any one of claims 1-19, wherein the subject, prior to administration of the modified oligonucleotide, was determined to have a decreased level of polycystin-1 (PC1) and/or polycystin-2 (PC2) in the kidney, urine or blood of the subject.

21. The method of any one of claims 1-20, wherein the subject has a mutation selected from a mutation in the *PKD1* gene or a mutation in the *PKD2* gene.

22. The method of any one of claims 1-21, wherein the subject has increased total kidney volume.

23. The method of any one of claims 1-22, wherein the subject has hypertension.

24. The method of any one of claims 1-23, wherein the subject has impaired kidney function.

25. The method of any one of claims 1-24, wherein the method comprises administering the modified oligonucleotide at a fixed dose of 150 mg.

26. The method of any one of claims 1-24, wherein the method comprises administering the modified oligonucleotide at a fixed dose of 160 mg.

27. The method of any one of claims 1-24, wherein the method comprises administering the modified oligonucleotide at a fixed dose of 170 mg.

28. The method of any one of claims 1-24, wherein the method comprises administering the modified oligonucleotide at a fixed dose of 180 mg.

29. The method of any one of claims 1-24, wherein the method comprises administering the modified oligonucleotide at a fixed dose of 190 mg.

30. The method of any one of claims 1-24, wherein the method comprises administering the modified oligonucleotide at a fixed dose of 200 mg.

31. The method of any one of claims 1-24, wherein the method comprises administering the modified oligonucleotide at a fixed dose of 210 mg.

32. The method of any one of claims 1-24, wherein the method comprises administering the modified oligonucleotide at a fixed dose of 220 mg.

33. The method of any one of claims 1-24, wherein the method comprises administering the modified oligonucleotide at a fixed dose of 230 mg.

34. The method of any one of claims 1-24, wherein the method comprises administering the modified oligonucleotide at a fixed dose of 240 mg.

35. The method of any one of claims 1-24, wherein the method comprises administering the modified oligonucleotide at a fixed dose of 250 mg.

36. The method of any one of claims 1-24, wherein the method comprises administering the modified oligonucleotide at a fixed dose of 260 mg.

37. The method of any one of claims 1-24, wherein the method comprises administering the modified oligonucleotide at a fixed dose of 270 mg.

38. The method of any one of claims 1-24, wherein the method comprises administering the modified oligonucleotide at a fixed dose of 280 mg.

39. The method of any one of claims 1-24, wherein the method comprises administering the modified oligonucleotide at a fixed dose of 290 mg.

40. The method of any one of claims 1-24, wherein the method comprises administering the modified oligonucleotide at a fixed dose of 300 mg.

41. The method of any one of claims 1-24, wherein the method comprises administering the modified oligonucleotide at a fixed dose of 310 mg.

42. The method of any one of claims 1-24, wherein the method comprises administering the modified oligonucleotide at a fixed dose of 320 mg.

43. The method of any one of claims 1-24, wherein the method comprises administering the modified oligonucleotide at a fixed dose of 330 mg.

44. The method of any one of claims 1-24, wherein the method comprises administering the modified oligonucleotide at a fixed dose of 340 mg.

45. The method of any one of claims 1-24, wherein the method comprises administering the modified oligonucleotide at a fixed dose of 350 mg.

46. The method of any one of claims 1-45, wherein the method comprises administering the modified oligonucleotide once every 2 weeks.

47. The method of any one of claims 1-46, wherein the method comprises administering the modified oligonucleotide at least 7 times.

48. The method of any one of claims 1-47, wherein the modified oligonucleotide is administered subcutaneously.

49. The method of any one of claims 1-48, wherein the treatment reduces total kidney volume in the subject.

50. The method of any one of claims 1-49, wherein the treatment slows the rate of increase of total kidney volume in the subject.

51. The method of claim 49 or 50, wherein the total kidney volume is height-adjusted total kidney volume (htTKV).

52. The method of any one of claims 1-51, wherein the treatment slows the rate of decline of glomerular filtration rate in the subject.

53. The method of any one of claims 1-52, wherein the treatment increases glomerular filtration rate in the subject.

54. The method of claim 52 or 53, wherein the glomerular filtration rate is estimated glomerular filtration rate.

55. The method of any one of claims 1-54, wherein the treatment inhibits or slows the increase in the growth of cysts in the kidney and/or liver of the subject.

56. The method of claim 55, wherein the treatment inhibits or slows the increase in total cyst volume, number and/or size distribution.

57. The method of any one of claims 1-56 wherein the treatment:

a) improves or slows the rate of decrease of creatinine clearance in the subject;

b) reduces or slows the rate of increase of albumin:creatinine ratio in the subject;

c) reduces or slows the rate of increase of blood urine nitrogen (BUN) level in the subject;

d) reduces or slows the rate of increase of serum creatinine (SCr) level in the subject;

e) increases polycystin-1 (PC1) in the urine of the subject;

f) increases polycystin-2 (PC2) in the urine of the subject;

g) reduces or slows the rate of increase of neutrophil gelatinase-associated lipocalin (NGAL) protein in the urine of the subject; and/or

h) reduces or slows the rate of increase of kidney injury molecule-1 (KIM-1) protein in the urine of the subject.

58. The method of any one of claims 1-57 wherein the administering:

a) reduces or slows the rate of increase of monocyte chemoattractant protein-1 (MCP-1) in the urine of the subject;

b) reduces or slows the rate of increase of beta-2 microglobulin (B2M) in the urine of the subject;

c) reduces or slows the rate of increase of complement split products C3a and/or Bb in the plasma of the subject;

d) reduces or slows the rate of increase of serum insulin-like growth factor binding protein acid labile subunit (IGFALS) in the subject;

e) reduces or slows the rate of increase of serum copeptin (CT-proAVP) in the subject;

f) reduces or slows the rate of increase of serum N-acetyl-1-methylhistidine in the subject; and/or

g) reduces or slows the rate of increase of acute phase proteins in the subject.

59. The method of any one of claims 1-58, wherein the treatment results in little to no CNS impairment in the subject.

60. The method of claim 59, wherein the treatment results in little to no change in the Scale for the Assessment and Rating of Ataxia (SARA) test score for the subject.

61. The method of one of claims 1-60, comprising:

a) measuring height-adjusted total kidney volume (HtTKV) in the subject;

b) measuring polycystin-1 (PC1) in the urine of the subject;

c) measuring polycystin-2 (PC2) in the urine of the subject;

d) measuring blood urea nitrogen (BUN) level in the subject;

e) measuring serum creatinine (SCr) level in the subject;

f) measuring creatinine clearance in the subject;

g) measuring urine albumin:creatinine ratio (UACR) in the subject;

h) measuring estimated glomerular filtration rate (eGFR) in the subject;

i) measuring neutrophil gelatinase-associated lipocalin (NGAL) protein in the urine of the subject;

j) measuring kidney injury molecule-1 (KIM-1) protein in the urine of the subject;

k) measuring monocyte chemoattractant protein-1 (MCP-1) in the urine of the subject;

l) measuring beta-2 microglobulin (B2M) in the urine of the subject;

m) measuring serum insulin-like growth factor binding protein acid labile subunit (IGFALS) in the subject;

n) measuring serum copeptin (CT-proAVP) in the subject;

o) measuring serum N-acetyl-1-methylhistidine in the subject;

p) measuring complement split products C3a and/or Bb in the plasma of the subject; and/or

q) measuring total cyst volume, number and/or size distribution in the subject; and/or

r) measuring SARA test score for the subject.

62. The method of any one of claims 1-61, wherein the subject is a human subject.

63. The method of any one of claims 1-62, which has an acceptable safety and tolerability profile.

64. A modified oligonucleotide or a pharmaceutically acceptable salt thereof for use in treating polycystic kidney disease, wherein the modified oligonucleotide has the structure 5'-$A_SG_SC_MA_FC_FU_FU_MU_SA_S$-3',

wherein nucleosides followed by subscript "M" are 2'-O-methyl nucleosides; nucleosides followed by subscript "F" are 2'-fluoro nucleosides; and nucleosides followed by subscript "S" are S-cEt nucleosides, and wherein each cytosine is a non-methylated cytosine;

and wherein the modified oligonucleotide is administered at a fixed dose of between about 150 mg and about 350 mg.

65. The modified oligonucleotide for use of claim 64, wherein the modified oligonucleotide is administered at a fixed dose of 150 mg, 160 mg, 170 mg, 180 mg, 190 mg, 200 mg, 210 mg, 220 mg, 230 mg, 240 mg, 250 mg, 260 mg, 270 mg, 280 mg, 290 mg, 300 mg, 310 mg, 320 mg, 330 mg, 340 mg, or 350 mg.

66. The modified oligonucleotide for use of claim 64 or 65, wherein the pharmaceutically acceptable salt is a sodium salt.

67. The modified oligonucleotide for use of any one of claims 64-66, wherein the modified oligonucleotide is present in a pharmaceutical composition comprising a sterile saline solution.

68. The modified oligonucleotide for use of any one of claims 64-67, wherein the polycystic kidney disease is autosomal dominant polycystic kidney disease (ADPKD).

69. The modified oligonucleotide for use of any one of claims 64-68, wherein the modified oligonucleotide is administered once every two weeks.

70. The modified oligonucleotide for use of any one of claims 64-69, wherein the modified oligonucleotide is administered at least seven times.

71. Use of a modified oligonucleotide or a pharmaceutically acceptable salt thereof for the preparation of a medicament for treating polycystic kidney disease, wherein the modified oligonucleotide has the structure 5'-$A_SG_SC_MA_FC_FU_FU_MU_SA_S$-3',

wherein nucleosides followed by subscript "M" are 2'-O-methyl nucleosides; nucleosides followed by subscript "F" are 2'-fluoro nucleosides; and nucleosides followed by subscript "S" are S-cEt nucleosides, and wherein each cytosine is a non-methylated cytosine;

wherein the modified oligonucleotide is formulated for administration at a fixed dose of between about 150 mg and about 350 mg.

72. The use of claim 71, wherein the modified oligonucleotide is administered at a fixed dose of 150 mg, 160 mg, 170 mg, 180 mg, 190 mg, 200 mg, 210 mg, 220 mg, 230 mg, 240 mg, 250 mg, 260 mg, 270 mg, 280 mg, 290 mg, 300 mg, 310 mg, 320 mg, 330 mg, 340 mg, 350 mg.

73. The use of claim 71 or 72, wherein the pharmaceutically acceptable salt is a sodium salt.

74. The use of any one of claims 71-73, wherein the modified oligonucleotide is present in a pharmaceutical composition comprising a sterile saline solution.

75. The use of any one of claims 71-74, wherein the polycystic kidney disease is autosomal dominant polycystic kidney disease (ADPKD).

76. The use of any one of claims 71-75, wherein the modified oligonucleotide is administered at least once every two weeks.

77. The use of any one of claims 71-76, wherein the modified oligonucleotide is administered at least seven times.