

COMPOSITIONS FOR TREATMENT OF CONDITIONS AND DISEASES ASSOCIATED WITH POLYCYSTIN EXPRESSION

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 63/145,288, filed February 3, 2021, which is incorporated herein by reference in its entirety.

BACKGROUND

[0002] Alternative splicing events in genes can lead to non-productive mRNA transcripts which in turn can lead to aberrant protein expression, and therapeutic agents which can target the alternative splicing events in genes can modulate the expression level of functional proteins in patients and/or inhibit aberrant protein expression. Such therapeutic agents can be used to treat a condition or disease caused by protein deficiency.

SUMMARY

[0002a] Described herein, in certain embodiments, is an in-vitro method of increasing expression of polycystin-2 in a cell, the method comprising contacting the cell a composition that comprises an agent or a vector encoding the agent, wherein the agent is an antisense oligomer (ASO) that is the reverse complement sequence of a target sequence of a pre-mRNA, whereby binding of the agent to the target sequence modulates splicing of a non-sense mediated RNA decay-inducing exon (NMD exon) from the pre-mRNA, thereby increasing the level of a processed mRNA that is processed from the pre-mRNA, and increasing the expression of polycystin-2 in the cell, wherein the polycystin-2 is encoded by a PKD2 gene, wherein the agent modulates splicing by:

- i. excluding from the processed mRNA an NMD exon defined by genomic sites GRCh38/ hg38: chr4:88031085-88031140; or
- ii. excluding from the processed mRNA an alternative exon defined by genomic sites GRCh38/ hg38: chr4:88036220-88036480 and including in the processed mRNA the canonical exon defined by genomic sites GRCh38/ hg38: chr4:88036220-88036353.

[0002b] Described herein, in certain embodiments, is a composition comprising an agent or a vector encoding the agent, wherein the agent is an antisense oligomer (ASO) that is the reverse complement sequence of a target sequence of a pre-mRNA, wherein the agent (a) binds to a target sequence of the pre-mRNA that is transcribed from a target gene and that comprises a non-sense mediated RNA decay-inducing exon (NMD exon), and (b) modulates splicing of the NMD exon from the pre-mRNA, thereby increasing the level of a processed mRNA that is processed from the pre-mRNA, and increasing expression of a target protein in a cell having the pre-mRNA, wherein the pre-mRNA is encoded by a PKD2 gene; and wherein the agent modulates splicing by:

- i. excluding from the processed mRNA an NMD exon defined by genomic sites GRCh38/ hg38: chr4:88031085-88031140; or
- ii. excluding from the processed mRNA an alternative exon defined by genomic sites GRCh38/ hg38: chr4:88036220-88036480 and including in the processed mRNA the canonical exon defined by genomic sites GRCh38/ hg38: chr4:88036220-88036353.

[0002c] Described herein, in certain embodiments, is the composition as described herein for use in therapy.

[0002d] Described herein, in certain embodiments, is a use of the composition as described herein, in manufacturing a medicament for treating a disease or condition in a subject in need thereof, wherein the disease or condition is associated with a deficient amount or activity of polycystin 2 in the subject.

[0003] Described herein, in certain embodiments, is a method of modulating expression of a target protein in a cell having a pre-mRNA that is transcribed from a target gene and that comprises a non-sense mediated RNA decay-inducing exon (NMD exon), the method comprising: contacting an agent or a vector encoding the agent to the cell, whereby the agent modulates splicing of the NMD exon from the pre-mRNA, thereby modulating the level of a processed mRNA that is processed from the pre-mRNA, and modulating the expression of the target protein in the cell, wherein the target protein is encoded by a PKD2 gene.

[0004] Described herein, in certain embodiments, is a method of treating or reducing the likelihood of developing a disease or condition in a subject in need thereof by modulating expression of a target protein in a cell of the subject, comprising: contacting an agent or a vector encoding the agent to the cell of the subject, whereby the agent modulates splicing of a non-sense mediated mRNA decay-inducing exon (NMD exon) from a pre-mRNA that is transcribed from a target gene and that comprises the NMD exon, thereby modulating the level of a processed mRNA that is processed from the pre-mRNA, and modulating the expression of the target protein in the cell of the subject, wherein the target protein encoded by a *PKD2* gene.

[0005] In some embodiments, the target protein is polycystin 2.

[0006] In some embodiments, the disease or condition is a disease or condition associated with a deficiency in amount or activity of polycystin 2. In some embodiments, the disease or condition is a disease or condition associated with a deficiency in amount or activity of polycystin 1.

CLAIMS

What is claimed is:

1. An in-vitro method of increasing expression of polycystin-2 in a cell, the method comprising contacting the cell a composition that comprises an agent or a vector encoding the agent, wherein the agent is an antisense oligomer (ASO) that is the reverse complement sequence of a target sequence of a pre-mRNA, whereby binding of the agent to the target sequence modulates splicing of a non-sense mediated RNA decay-inducing exon (NMD exon) from the pre-mRNA, thereby increasing the level of a processed mRNA that is processed from the pre-mRNA, and increasing the expression of polycystin 2 in the cell, wherein the polycystin 2 is encoded by a *PKD2* gene, wherein the agent modulates splicing by:
 - i. excluding from the processed mRNA an NMD exon defined by genomic sites GRCh38/ hg38: chr4:88031085-88031140; or
 - ii. excluding from the processed mRNA an alternative exon defined by genomic sites GRCh38/ hg38: chr4:88036220-88036480 and including in the processed mRNA the canonical exon defined by genomic sites GRCh38/ hg38: chr4:88036220-88036353.
2. A composition comprising an agent or a vector encoding the agent, wherein the agent is an antisense oligomer (ASO) that is the reverse complement sequence of a target sequence of a pre-mRNA, wherein the agent (a) binds to a target sequence of the pre-mRNA that is transcribed from a target gene and that comprises a non-sense mediated RNA decay-inducing exon (NMD exon), and (b) modulates splicing of the NMD exon from the pre-mRNA, thereby increasing the level of a processed mRNA that is processed from the pre-mRNA, and increasing expression of a target protein in a cell having the pre-mRNA, wherein the pre-mRNA is encoded by a *PKD2* gene; and wherein the agent modulates splicing by:
 - i. excluding from the processed mRNA an NMD exon defined by genomic sites GRCh38/ hg38: chr4:88031085-88031140; or
 - ii. excluding from the processed mRNA an alternative exon defined by genomic sites GRCh38/ hg38: chr4:88036220-88036480 and including in the processed mRNA the canonical exon defined by genomic sites GRCh38/ hg38: chr4:88036220-88036353.
3. The method according to claim 1, or the composition of claim 2, wherein the target sequence of the pre-mRNA comprises a sequence with 8 contiguous nucleic acids of a sequence selected from the group consisting of premrna ENST00000237596.7, premrna ENST00000502363.1, premrna ENST00000506367.1, premrna ENST00000506727.1, premrna ENST00000508588.5, premrna ENST00000511337.5, premrna ENST00000512858.1, transcript ENST00000237596.7, transcript ENST00000502363.1, transcript ENST00000506367.1, transcript ENST00000506727.1, transcript ENST00000508588.5, transcript ENST00000511337.5, transcript ENST00000512858.1, and PKD2 5' UTR sequence (ENST00000237596.7).
4. The method according to claim 1, or the composition of claim 2, wherein the target sequence of the pre-mRNA overlaps with the NMD exon.

5. The method according to claim 1, or the composition of claim 2, wherein the target sequence of the pre-mRNA overlaps with an intron upstream or downstream of the NMD exon.
6. The method according to claim 1, or the composition of claim 2, wherein the target sequence of the pre-mRNA comprises 5' NMD exon–intron junction or 3' NMD exon-intron junction.
7. The method according to claim 1, or the composition of claim 2, wherein the target sequence of the pre-mRNA is within the NMD exon.
8. The method according to claim 1, or the composition of claim 2, wherein the NMD exon is located in an intronic region between two canonical exonic regions of the pre-mRNA, and wherein the intronic region contains the NMD exon.
9. The method according to claim 1 or 3-8, or the composition according to any one of claims 2-8, wherein the pre-mRNA comprises an alternative 5' splice-site downstream of a canonical 5' splice-site, wherein a processed mRNA that is produced by splicing of the pre-mRNA at the alternative 5' splice-site undergoes non-sense mediated RNA decay, wherein the NMD exon comprises a canonical exon and an exon region extended by the splicing at the alternative 5' splice-site, wherein the agent modulates processing of the pre-mRNA by modulating splicing at the alternative 5' splice-site, thereby increasing expression of the target protein.
10. The method according to claim 1 or 3-9, or the composition according to any one of claims 2-9, wherein the processed mRNA that is produced by splicing of the pre-mRNA at the alternative 5' splice-site comprises a premature termination codon (PTC).
11. The method according to claim 1 or 3-10, or the composition according to any one of claims 2-10, wherein the NMD exon is defined by genomic sites GRCh38/ hg38: chr4:88031085-88031140.
12. The method according to claim 1 or 3-11, or the composition according to any one of claims 2-11, wherein the target sequence of the pre-mRNA is closer than 1500 nucleotides, 1000 nucleotides, 800 nucleotides, 700 nucleotides, 600 nucleotides, 500 nucleotides, 400 nucleotides, 300 nucleotides, 200 nucleotides, 100 nucleotides, 80 nucleotides, 70 nucleotides, 60 nucleotides, 50 nucleotides upstream of genomic site of GRCh38/ hg38: chr4:88031085.
13. The method according to claim 1 or 3-12, or the composition according to any one of claims 2-12, wherein the target sequence of the pre-mRNA is closer than 1500 nucleotides, 1000 nucleotides, 800 nucleotides, 700 nucleotides, 600 nucleotides, 500 nucleotides, 400 nucleotides, 300 nucleotides, 200 nucleotides, 100 nucleotides, 80 nucleotides, 70 nucleotides, 60 nucleotides, 50 nucleotides downstream of genomic site of GRCh38/ hg38: chr4:88031085.
14. The method according to claim 1 or 3-13, or the composition according to any one of claims 2-13, wherein the target sequence of the pre-mRNA is closer than 1500 nucleotides, 1000 nucleotides, 800 nucleotides, 700 nucleotides, 600 nucleotides, 500 nucleotides, 400 nucleotides, 300 nucleotides, 200 nucleotides, 100 nucleotides, 80 nucleotides, 70 nucleotides, 60 nucleotides, 50 nucleotides upstream of genomic site of GRCh38/ hg38: chr4:88031140.

15. The method according to claim 1 or 3-14, or the composition according to any one of claims 2-14, wherein the target sequence of the pre-mRNA is closer than 1500 nucleotides, 1000 nucleotides, 800 nucleotides, 700 nucleotides, 600 nucleotides, 500 nucleotides, 400 nucleotides, 300 nucleotides, 200 nucleotides, 100 nucleotides, 80 nucleotides, 70 nucleotides, 60 nucleotides, 50 nucleotides downstream of genomic site of GRCh38/ hg38: chr4:88031140.
16. The method according to claim 1 or 3-10, or the composition according to any one of claims 2-10, wherein the agent is a non-sense mediated RNA decay alternative splice site (NSASS) modulating agent and the NMD exon is defined by genomic sites GRCh38/ hg38: chr4: 88036220-88036480 .
17. The method according to claim 16, or the composition according to claim 16, wherein the target sequence of the pre-mRNA is closer than 1500 nucleotides, 1000 nucleotides, 800 nucleotides, 700 nucleotides, 600 nucleotides, 500 nucleotides, 400 nucleotides, 300 nucleotides, 200 nucleotides, 100 nucleotides, 80 nucleotides, 70 nucleotides, 60 nucleotides, 50 nucleotides upstream of genomic site of GRCh38/ hg38: chr4 88036480.
18. The method according to claim 16, or the composition according to claim 16, wherein the target sequence of the pre-mRNA is closer than 1500 nucleotides, 1000 nucleotides, 800 nucleotides, 700 nucleotides, 600 nucleotides, 500 nucleotides, 400 nucleotides, 300 nucleotides, 200 nucleotides, 100 nucleotides, 80 nucleotides, 70 nucleotides, about 60 nucleotides, about 50 nucleotides downstream of genomic site of GRCh38/ hg38: chr4:88036480.
19. The method according to claim 1 or 3-18, or the composition according to any one of claims 2-19, wherein the ASO comprises 8, 9, 10, 11, 12, 13, 14, 16, 16, 17 or 18 contiguous nucleic acids of a sequence selected from the group consisting of SEQ ID NOs: 1-226.
20. The method according to claim 1 or 3-19, or the composition according to any one of claims 2-20, wherein the ASO comprises a backbone modification comprising a phosphorothioate linkage or a phosphorodiamidate linkage.
21. The method according to claim 1 or 3-20, or the composition according to any one of claims 2-21, wherein the ASO comprises a phosphorodiamidate morpholino, a locked nucleic acid, a peptide nucleic acid, a 2'-O-methyl, a 2'-Fluoro, or a 2'-O-methoxyethyl moiety.
22. The method according to claim 1 or 3-21, or the composition according to any one of claims 2-22, wherein the ASO comprises a modified sugar moiety.
23. The method according to claim 1 or 3-22, or the composition according to any one of claims 2-23, wherein each sugar moiety of the ASO is a modified sugar moiety.
24. The method according to claim 1 or 3-24, or the composition according to any one of claims 2-24, wherein the ASO is from 8 to 50 nucleobases, 8 to 40 nucleobases, 8 to 35 nucleobases, 8 to 30 nucleobases, 8 to 25 nucleobases, 8 to 20 nucleobases, 8 to 15 nucleobases, 9 to 50 nucleobases, 9 to 40 nucleobases, 9 to 35 nucleobases, 9 to 30 nucleobases, 9 to 25 nucleobases, 9 to 20 nucleobases, 9 to 15 nucleobases, 10 to 50 nucleobases, 10 to 40 nucleobases, 10 to 35 nucleobases, 10 to 30 nucleobases, 10 to 25 nucleobases, 10 to 20 nucleobases, 10 to 15 nucleobases, 11 to 50 nucleobases, 11 to 40

nucleobases, 11 to 35 nucleobases, 11 to 30 nucleobases, 11 to 25 nucleobases, 11 to 20 nucleobases, 11 to 15 nucleobases, 12 to 50 nucleobases, 12 to 40 nucleobases, 12 to 35 nucleobases, 12 to 30 nucleobases, 12 to 25 nucleobases, 12 to 20 nucleobases, or 12 to 15 nucleobases in length.

25. The method according to claim 1 or 3-24, or the composition according to any one of claims 2-25, wherein the composition comprises a vector encoding the agent, and wherein the vector is a viral vector.
26. The method according to claim 1 or 3-25, or the composition according to any one of claims 2-26, wherein viral vector is an adenovirus-associated viral vector.
27. The method according to claim 1 or 3-26, or the composition according to any one of claims 2-27, wherein the agent is a polynucleotide, wherein the polynucleotide further comprises a modified snRNA.
28. The method according to claim 1 or 3-27, or the composition according to any one of claims 2-28, wherein the modified human snRNA is a modified U1 snRNA or a modified U7 snRNA.
29. The composition according to any one of claims 2-28, for use in therapy.
30. Use of the composition according to any one of claims 2-28, in manufacturing a medicament for treating a disease or condition in a subject in need thereof, wherein the disease or condition is associated with a deficient amount or activity of polycystin 2 in the subject.
31. The use according to claim 30, wherein the disease or condition is associated with a loss-of-function mutation in a polycystin 2 gene or a polycystin 2 protein.
32. The use according to claim 30, wherein the disease or condition is associated with haploinsufficiency of a *PKD2* gene, and wherein the subject has a first allele encoding functional polycystin 2, and a second allele from which polycystin 2 is not produced, or a second allele encoding nonfunctional polycystin 2.
33. The use according to claim 30, wherein one or both alleles are hypomorphs.
34. The use according to claim 30, wherein the disease or condition is associated with a mutation of a *PKD2* gene.
35. The use according to claim 34, wherein the mutation is a hypomorphic mutation.
36. The use according to claim 30, wherein the disease or condition is selected from the group consisting of: polycystic kidney disease with or without polycystic liver disease, autosomal dominant polycystic kidney disease, and intracranial aneurysm.
37. The use according to claim 30, wherein the subject is a human.
38. The use according to claim 30, wherein the subject is a non-human animal.
39. The use according to claim 30, wherein the subject is a fetus, an embryo, or a child.
40. The use according to claim 30, wherein the cells are ex vivo.
41. The use according to claim 30, wherein the agent is to be administered by intrathecal injection, intracerebroventricular injection, intraperitoneal injection, intramuscular injection, subcutaneous injection, intravitreal, or intravenous injection of the subject.