

Claims

Claim 1. An agent, wherein the agent is an oligonucleotide capable of hybridising to a region of a pre-mRNA of RBM3 such as to alter splicing of the pre-mRNA such that in the resulting mature mRNA exon 3a is not incorporated, for use in treating or preventing a disease affected by RBM3 expression, wherein the treating or treatment is for:

- (a) a neurological disease and/or neonatal hypoxic ischemic encephalopathy, head injury, or stroke;
 - (b) a neurodegenerative disease optionally selected from Alzheimer's disease, Parkinson's disease, prion disease, frontotemporal dementia, a tauopathy, amyotrophic lateral sclerosis (ALS), and vascular dementia;
 - (c) neurological damage, optionally caused during cardiac surgery or induced coma;
- wherein the region of the pre-mRNA of RBM3 is selected from:
- (i) a region within exon 3a, wherein the region, corresponds to SEQ ID NO: 15 or SEQ ID NO: 27, and
 - (ii) a region spanning the 5' splice site of exon 3a and being within 50 nucleotides downstream of exon 3a.

Claim 2. The agent for use of claim 1, wherein the region within exon 3a corresponds to SEQ ID NO: 29, or wherein the region within exon 3a corresponds to SEQ ID NO: 16, 17, 19, 21, 23, or 25.

Claim 3. The agent for use of claim 1 or 2, wherein the region spanning the 5' splice site of exon 3a comprises nucleotides 257 to 269, 258 to 269, 259 to 269, 260 to 269, 261 to 269, 262 to 269, 263 to 269, 264 to 269, 265 to 269, 266 to 269, or 267 to 269 of SEQ ID NO: 6, and/or nucleotides 1 to 2, 1 to 3, 1 to 4, 1 to 5, 1 to 6, 1 to 7, 1 to 8, 1 to 9, 1 to 10, 1 to 11, or 1 to 12 of SEQ ID NO: 33.

Claim 4. The agent for use of any one of claims 1 to 3, wherein the agent is an antisense oligonucleotide (ASO), optionally wherein the ASO is 10 to 30 nucleotides long, optionally 25 nucleotides long, optionally 15, 16, 17, 18 or 19 nucleotides long, or optionally 19 nucleotides long.

Claim 5. The agent for use of claim 4, wherein

- (i) the ASO comprises or consists of one of SEQ ID NOs: 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 83, 84, 85, 86 and 87;
- (ii) the ASO comprises or consists of one of SEQ ID NOs: 51, 52, 53, 54, 55, 56 and 57; or
- (iii) the ASO comprises or consists of one of SEQ ID NOs: 34, 35, 36, 37, 38, 39, 69 and 70.

Claim 6. An antisense oligonucleotide (ASO), wherein the ASO:

- (i) comprises or consists of one of SEQ ID NOs: 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 83, 84, 85, 86 and 87,
- (ii) comprises or consists of one of SEQ ID NOs: 51, 52, 53, 54, 55, and 56, or
- (iii) comprises or consists of one of SEQ ID NOs: 34, 35, 36, 37, 38, 39, 69 and 70.

Claim 7. The agent for use of claim 4, or the ASO of claim 6 wherein the ASO comprises an LNA, RNA or DNA nucleotide, optionally

wherein the ASO comprises alternating LNA and RNA nucleotides, alternating LNA and DNA nucleotides, or alternating RNA and DNA nucleotides.

Claim 8. The agent for use of any one of claims 4, 5 or 7, or the ASO of claim 6 or 7, wherein

- (i) the ASO comprises
 - (a) a backbone modification, which is optionally a phosphorothioate linkage; and/or
 - (b) a sugar moiety modification, which is optionally 2'-O-methoxyethyl (MOE) modification; or
- (ii) the ASO comprises a phosphorodiamidate morpholino oligonucleotide.

Claim 9. The agent for use or the ASO of claim 8, wherein the ASO comprises a 2'-O-methoxyethyl (MOE) modification and a phosphorothioate linkage, optionally wherein the ASO is fully 2'-O-methoxyethyl and fully phosphorothioate modified.

Claim 10. A method of identifying an antisense oligonucleotide (ASO) capable of increasing expression of RBM3 in a cell, the method comprising

- i) identifying an ASO that targets a region of the pre-mRNA of the RBM3 gene, wherein the region is selected from: a region within exon 3a, a region spanning a splice site of exon 3a, a region located within 250 nucleotides upstream exon 3a and a region located within 250 nucleotides downstream exon 3a;
- ii) delivering the ASO identified in step i) to the cell; and
- iii) measuring the level of expression of RBM3 in the cell of step ii); optionally further comprising:
 - iv) comparing the level of expression measured in step iii) with the level of expression of RBM3 in a cell treated with a control, which is optionally an ASO or DMSO.

Claim 11. The method of claim 10, wherein the level of expression is measured by RT-qPCR or by western blotting.

Claim 12. An expression construct, which is optionally a vector, which is optionally a viral vector, encoding the ASO defined in any one of claims 4 to 9.

Claim 13. A host cell comprising the expression construct of claim 12.

Claim 14. A first guide RNA and a second guide RNA for removing exon 3a from the gene of RBM3, wherein the first guide RNA is capable of hybridising to the genomic sequence of RBM3 upstream of exon 3a and the second guide RNA is capable of hybridising to the genomic sequence of RBM3 downstream of exon 3a,
wherein the first guide RNA comprises SEQ ID NO: 11 or SEQ ID NO: 12 and the second guide RNA comprises SEQ ID NO: 13.

Claim 15. A CRISPR/Cas-based base editing system for altering an RNA splice site encoded in the genomic DNA of a subject,
the CRISPR/Cas-based base editing system comprising a fusion protein and at least one guide RNA (gRNA),
wherein the fusion protein comprises a Cas protein and a base-editing domain,

wherein the at least one gRNA is capable of hybridising to a region in the gene of RBM3,
and

wherein the region comprises:

- (i) nucleotides 136 to 147, 137 to 147, 138 to 147, 139 to 147, 140 to 147, 141 to 147, 142 to 147, 143 to 147, 144 to 147, 145 to 147, or 146 to 147 of SEQ ID NO: 32, or a complement thereof, such that the splice site located at nucleotides 146-147 of SEQ ID NO: 32 is altered, optionally wherein the region further comprises nucleotides 1 and 2, 1 to 3, 1 to 4, 1 to 5, 1 to 6, 1 to 7, 1 to 8, 1 to 9, 1 to 10, 1 to 11, 1 to 12, 1 to 13, 1 to 14, or 1 to 15 of SEQ ID NO: 6, or a complement thereof;
- (ii) SEQ ID NO: 6, or a complement thereof, such that the splice site located at nucleotides 106-107 of SEQ ID NO: 6 is altered; or
- (iii) nucleotides 1 to 2, 1 to 3, 1 to 4, 1 to 5, 1 to 6, 1 to 7, 1 to 8, 1 to 9, 1 to 10, 1 to 11, or 1 to 12 of SEQ ID NO: 33, or a complement thereof, such that the splice site located at the first two nucleotides of SEQ ID NO: 33 is altered, optionally wherein the region further comprises nucleotides 254 to 269, 255 to 269, 256 to 269, 257 to 269, 258 to 269, 259 to 269, 260 to 269, 261 to 269, 262 to 269, 263 to 269, 264 to 269, 265 to 269, 266 to 269, or 267 to 269 of SEQ ID NO: 6, optionally wherein the gRNA, or the targeting domain of the gRNA which hybridises to the target region, is 16, 17, 18, 19, 20, or 21 nucleotides long.

Claim 16. The CRISPR/Cas-based base editing system of claim 15, wherein the region spans nucleotides 106-17 of SEQ ID NO: 6 and comprises 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleotides, such that the splice site located at nucleotides 106-107 of SEQ ID NO: 6 is altered.

Claim 17. The CRISPR/Cas-based base editing system of claim 15 or 16, wherein the target region is selected from SEQ ID NOs: 90, 91, and 92, and optionally the at least one gRNA comprises or consists of a sequence which is the RNA equivalent of SEQ ID NOs: 90, 91, and 92 or a complement thereof.

Claim 18. The CRISPR/Cas-based base editing system of any one of claims 15 to 17, wherein altering the RNA splice site encoded in the genomic DNA results in non-incorporation of exon 3a in the resulting mature mRNA.

Claim 19. The CRISPR/Cas-based base editing system of any one of claims 15 to 18, wherein the Cas protein comprises Cas9, optionally wherein the Cas protein comprises a Cas9 nickase; and/or

wherein the base-editing domain comprises a cytidine deaminase domain or an adenosine deaminase domain.

Claim 20. A CRISPR/Cas-based base editing system of any one of claims 15-19 for use in treating or preventing a disease affected by RBM3 expression, wherein the treating or treatment is for:

- (i) a neurological disease and/or neonatal hypoxic ischemic encephalopathy, head injury, or stroke;
- (ii) a neurodegenerative disease optionally selected from Alzheimer's disease, Parkinson's disease, prion disease, frontotemporal dementia, a tauopathy, amyotrophic lateral sclerosis (ALS), and vascular dementia; or

- (iii) neurological damage, optionally caused during cardiac surgery or induced coma;.