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(54) Title: ANTISENSE OLIGONUCLEOTIDES FOR THE TREATMENT OF NEURODEGENERATIVE DISEASE

(57) Abstract: The present disclosure relates to guide oligonucleotides that are at least partially complementary to a portion of a target *RELN* nucleic acid sequence comprising a target nucleotide, wherein the target *RELN* nucleic acid sequence encodes a reelin protein, wherein the guide oligonucleotide is configured such that it is capable of forming a double stranded complex under physiological conditions within a cell with the portion of the target *RELN* nucleic acid sequence, and the double stranded complex is capable of recruiting a nucleic acid editing entity to perform editing of the target nucleotide to generate an edited *RELN* nucleic acid sequence comprising an edited target nucleotide; as well as compositions, vectors, and methods of use related thereto.



ANTISENSE OLIGONUCLEOTIDES FOR THE TREATMENT OF NEURODEGENERATIVE DISEASE

TECHNICAL FIELD

This disclosure relates to the field of medicine, and in particular to the field of neurodegenerative diseases such as Alzheimer's disease. The disclosure describes guide oligonucleotides that mediate nucleotide-specific editing in the *RELN* gene and/or encoded transcript to bring about changes in the encoded reelin protein that influence reelin protein activity.

BACKGROUND

The identification of effective treatments for neurodegenerative diseases such as Alzheimer's disease (AD) is an urgent and unmet medical need. AD is a progressive neurological disorder characterized by symptoms including dementia, cognitive decline, memory loss, and impaired daily functioning. AD typically starts slowly and progressively worsens with age. Thus far, extensive research efforts to identify treatments for AD have been conducted but have largely proved futile in the identification of an effective treatment.

On a molecular level, both amyloid plaques formed by aggregating Amyloid Precursor Protein (APP) and so-called neurofibrillary tangles made up of extensively phosphorylated Tau protein deposited in a patient's brain, are hallmarks of AD pathology. Tau protein is an important component of the cytoskeleton. Its normal function is that it binds to tubulin, stabilizing microtubule structures used by motor proteins in the cell to organize cellular transport. Hyperphosphorylated Tau cannot perform this function very well and Tau dysregulation is associated with disturbed neuronal migration during development and neuronal degeneration, giving rise to neurodegenerative diseases such as Parkinsonism and Frontotemporal dementia and animal models generated to express mutated Tau variants give rise to neurodegeneration. A recent study (Lopera F et al. *Nat Med.* 2023, 29:1243-1252) revealed the identification of a human subject having an amino acid mutation in the protein reelin, encoded by the *RELN* gene, which provided resilience to the onset of symptoms from autosomal dominant Alzheimer's disease (ADAD). The effectiveness of this reelin variant in protecting against AD was demonstrated in a mouse model.

Mechanistically, the protective effect of this reelin mutation can be rationalized by a model that has been postulated already in 2002 (Ohkubo N et al. *FASEB J.* 2002, 17(2):295-297). According to this model, that has later been refined by others, reelin is involved in the phosphorylation of Tau through the Apolipoprotein E receptor (APOEr)/disabled-1 (Dab1)/glycogen synthase kinase-3 β (GSK3 β) cascade. By binding of reelin protein to the

ApoE receptor and other cadherin-related neuronal receptors (CNRs) that co-operate with src family kinases as intracellular effector proteins, Dab1 is phosphorylated, activating Dab1 to inhibit two downstream kinases known to phosphorylate Tau in positions identified in neurofibrillary tangles: GSK3 β and CDK5. The result of this sequence of events being a blockade of hyperphosphorylation of Tau, with an expected improved or even normalized Tau function as outcome, lends support to the hypothesis that it is the gain-of-function aspect of the described reelin variant that leads to the observed protection against ADAD.

It is postulated here that other gain-of-function mutations in reelin can be identified and it is proposed to generate these gain-of-function variants *de novo*, using methods described herein, with the prospect of producing clinically beneficial outcomes in patients with, or prone to develop, AD and other forms of dementia, such as fronto-temporal dementia, and/or neuropsychiatric disorders, including schizophrenia, depression, autism, (temporal lobe) epilepsy and/or other neurodegenerative disorders, including Parkinsonism, Parkinson's disease, spinocerebellar ataxia and the like.

The present disclosure aims to provide guide oligonucleotides that can be used in the treatment of neurodegenerative diseases such as AD, wherein the guide oligonucleotides cause nucleic acid editing to generate an edited *RELN* nucleic acid sequence, by exploiting nucleic acid editing machinery to target and amend one or more target nucleotides in the *RELN* gene or the encoded *RELN* transcript molecules, preferably pre-mRNA and/or mRNA.

SUMMARY

The present disclosure relates to a guide oligonucleotide that is at least partially complementary to a portion of a human *RELN* nucleic acid molecule comprising a target nucleotide, wherein the *RELN* nucleic acid molecule encodes a reelin protein, wherein the guide oligonucleotide is configured such that it is capable of forming a double stranded complex under physiological conditions within a cell, preferably a brain cell, more preferably a neuron, with the portion of the *RELN* nucleic acid, and the double stranded complex is capable of recruiting a nucleic acid editing enzyme that is naturally present in the cell, to perform editing of the target nucleotide to generate an edited *RELN* nucleic acid comprising an edited target nucleotide. Preferably, editing of the target nucleotide leads to elevated activity of the encoded reelin protein. More preferably, the encoded reelin protein is provided with a gain-of-function phenotype, selected from one or more of: i) an enhanced ability to trigger signalling, preferably of the APOEr/Dab1/GSK3 β pathway; ii) an enhanced ability to increase Dab1 phosphorylation; iii) an enhanced ability to reduce Tau phosphorylation associated with neurofibrillary tangles; iv) an enhanced ability to increase tubular structure formation and/or stability and/or neuronal density; v) an enhanced resistance to degradation by proteolysis; and/or vi) enhanced binding of the reelin protein to glycosaminoglycans, preferably heparin, and/or to NRP1. In a further

preferred embodiment, editing of the target nucleotide introduces an amino acid variant at one or more of amino acid positions 3446 to 3460 of the encoded reelin protein, preferably wherein editing of the target nucleotide introduces a histidine to arginine change at amino acid position 3447 (H3447R) in the encoded reelin protein. It is noted that amino acid position 3447 relates to *RELN* isoform 203 (Ensemble transcript ENST00000428762.6; *RELN* 203), which is a transcript that is 6 nucleotides longer than the transcript of isoform 201. In isoform 201 the codon for histidine encodes amino acid 3445. For consistency, H3447R is used throughout the present disclosure, but the guide oligonucleotides disclosed herein can bring about the deamination of the target adenosine in both isoforms and provide the amino acid changes H3447R in 203 and H3445R in 201. It is noted that isoform 201 is the most abundant isoform of *RELN* present in the human iPSC forebrain neurons used in the accompanying examples, whereas isoform 203 is the second most abundant isoform present in these cells. Preferably, the target nucleotide is adenosine, and the nucleic acid editing enzyme is an Adenosine Deaminase Acting on RNA (ADAR) enzyme. Preferably, the *RELN* nucleic acid molecule is mRNA or pre-mRNA.

In a preferred embodiment, the guide oligonucleotide comprises a contiguous stretch of 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, or 33 nucleotides from SEQ ID NO:102 (5'-UG UA GAA ACI UCU GAG CCC AUG UUG UCG UGA AA-3') or SEQ ID NO:103 (5'-UG UA GAA AZI UCU GAG CCC AUG UUG UCGUGAAA-3'), and comprises at least the underlined section of nucleotides (represented by SEQ ID NO:104 (5'-UA GAA ACI UCU GAG CCC AUG UUG-3') and SEQ ID NO:105 (5'-UA GAA AZI UCU GAG CCC AUG UUG-3'), respectively), wherein Z is a cytidine analog that is a nucleotide, preferably a deoxynucleotide, comprising a 6-amino-5-nitro-3-yl-2(1H)-pyridone nucleobase, and I is a inosine, preferably deoxyinosine. In another preferred embodiment, the guide oligonucleotide as disclosed herein comprises the structure (from 5' to 3'):

$N_8N_7N_6N_5N_4N_3N_2N_1^{\theta}ZdId^{\wedge}M_2M_3M_4M_5M_6M_7M_8M_9M_{10}M_{11}M_{12}M_{13}M_{14}M_{15}M_{16}M_{17}M_{18}M_{19}M_{20}M_{21}M_{22}M_{23}M_{24}$

wherein: Zd is the orphan nucleotide at nucleotide position 0, which is a deoxynucleotide carrying a Benner's base; N_1 is Ae or Ad; N_2 is Af; N_3 and N_5 are each independently Am or Af; N_4 is Gf; N_6 is Uf; N_7 is either absent (then N_8 is also absent), Gm, or Gf; N_8 is either absent or Um; Id is deoxyinosine; M_2 is Um; M_3 is Cf; M_4 , M_{14} and M_{15} are each independently m5Ue or Um; M_5 and M_7 are Gf; M_6 is Am or Af; M_8 and M_{10} are each independently Cm or Cf; M_9 is Cf; M_{11} is Am; M_{12} is Um; M_{13} is Gm; M_{16} is Ge or Gm; M_{17} is either absent (when M_{18} to M_{24} are also absent), m5Ue, or Um; M_{18} is either absent (when M_{19} to M_{24} are also absent), Cm, or m5Ce; M_{19} is either absent (when M_{20} to M_{24} are also absent), Gm, or Ge; M_{20} is either absent (when M_{21} to M_{24} are also absent), Um, or m5Ue; M_{21} is either absent (when M_{22} to M_{24} are also absent), Gm, or Ge; M_{22} is either absent (when M_{23} and M_{24} are also absent), Am, or Ae; M_{23} is either absent (when M_{24} is also absent), or Ae; M_{24} is either absent, or Ae; θ is at

linkage position 0, and is a PO linkage or a mesyl phosphoramidate (PNms) linkage; ^ is at linkage position -2 and is an MP or a PNms linkage; all other linkages are either PO, PS, PNdmi, or PNms linkages; and wherein Gm, Am, Um, and Cm are 2'-O-methyl (2'-OMe) modified guanosine, adenosine, uridine, and cytidine, respectively; m5Ce is 2'-O-methoxyethyl (also referred to as 2'-methoxyethoxy, 2'-O-MOE, or simply 2'-MOE) modified 5-methylcytidine; Ge is 2'-MOE modified guanosine; Ae is 2'-MOE modified adenosine; m5Ue is 2'-MOE modified 5-methyluridine (also sometimes named "Te"; 2'-MOE modified thymidine); Af, Uf, Gf, and Cf are 2'-fluoro (2'-F) modified adenosine, uridine, guanosine, and cytosine, respectively.

The present disclosure also relates to a vector, preferably a viral vector, more preferably an adeno-associated virus (AAV) vector, comprising a nucleic acid molecule encoding a guide oligonucleotide as disclosed herein. The present disclosure also relates to a guide oligonucleotide as disclosed herein for use in the treatment, amelioration, or slowing down the progression of a neurodegenerative disease, preferably AD, more preferably ADAD.

The present disclosure also relates to a method of treating, ameliorating, or slowing down the progression of a neurodegenerative disease, preferably AD, more preferably ADAD, in a human subject in need thereof, the method comprising administering to said subject a guide oligonucleotide as disclosed herein, thereby editing the target *RELN* nucleic acid sequence to encode a reelin protein with the ability to delay onset of one or more symptoms of the neurodegenerative disease.

BRIEF DESCRIPTION OF THE DRAWINGS

One or more embodiments of the disclosure will now be described, by way of example only, with reference to the accompanying drawings, in which:

Fig. 1A shows a nucleotide sequence of a portion of a wild-type human *RELN* nucleic acid sequence (NCBI Reference Sequence: NM_005045.4), in the 5' to 3' direction, showing a target adenosine (in bold font) in the codon CAT (underlined) encoding histidine at position 3447 in human reelin (H3447) and the SEQ ID NO; **Fig. 1B** shows the complementary sequence of the nucleic acid sequence in **Fig. 1A** in the 3' to 5' direction, showing the position of the orphan nucleotide (the nucleotide opposite the target adenosine) in bold font, and the SEQ ID NO; **Fig. 1C** shows the antisense sequence of the nucleic acid sequence in **Fig. 1A** in the 5' to 3' direction, showing the position of the orphan nucleotide (the nucleotide opposite the target adenosine) in bold font. It is to be understood that when the target *RELN* nucleic acid is a pre-mRNA or an mRNA molecule, the thymidine residues (T) should be read as uridine residues (U). **Fig. 1D** shows the 5' to 3' transcript sequence of **Fig. 1A** and represents the same portion (SEQ ID NO:106) of the target sequence for RNA editing and displays the adenosine in bold (middle of the underlined codon). It should also be understood that the

orphan nucleotide in guide oligonucleotides (as disclosed by the present disclosure) is not a thymidine (T) as **Fig. 1B** and **Fig. 1C** may suggest, but preferably a cytidine (C), a cytidine analog, a uridine (U) or a uridine analog.

Fig. 2 shows the sequences of example guide oligonucleotides disclosed herein. The chemical modifications in the guide oligonucleotides are as follows: Gm, Am, Um, and Cm are 2'-OMe modified guanosine, adenosine, uridine, and cytidine, respectively; m5Ce is 2'-MOE modified 5-methylcytidine; Ge is 2'-MOE modified guanosine; Ae is 2'-MOE modified adenosine; m5Ue is 2'-MOE modified 5-methyluridine (also sometimes named "Te"; 2'-MOE modified thymidine); Af, Uf, Gf, and Cf are 2'-F modified adenosine, uridine, guanosine, and cytosine, respectively; Zd is the cytidine analog that is also referred to as a nucleoside carrying a 6-amino-5-nitro-3-yl-2(1H)-pyridone nucleobase (Benner's base, as further outlined herein), with a deoxy moiety (= DNA) at the 2' ribose position; Id is deoxyinosine; * refers to a PS linkage; ! refers to a PNdmi linkage; ^ refers to a MP linkage; and ^θ refers to a PO linkage.

Fig. 3 shows the sequences of a set of additional guide oligonucleotides, with their respective RM names and SEQ ID NO's. The chemical modifications are as provided in **Fig. 2**.

Fig. 4 shows the editing percentage of the target adenosine shown in **Fig. 1D**, in *RELN* target (pre-) mRNA obtained after *transfection* of the guide oligonucleotides RM116817 to RM116840, as shown below the graph, in human iPSC (WT04) derived neural progenitor cells, at day 2 after transfection. A negative (non-treated) control was taken along (Mock).

Fig. 5 shows the editing percentage of the target adenosine shown in **Fig. 1D**, in *RELN* target (pre-) mRNA obtained after *gymnotic uptake* of the guide oligonucleotides, as shown below the graph, in human iPSC (WT04) derived neural progenitor cells at day 7 after start of the gymnotic treatment with the respective guide oligonucleotides. A negative (non-treated; NT) control was taken along.

Fig. 6 shows the editing percentage of the target adenosine shown in **Fig. 1D**, in *RELN* target (pre-) mRNA obtained after *gymnotic uptake* of the guide oligonucleotides, as shown below the graph, and co-treatment with the triterpene glycoside AG1856 (*saponin*), in human iPSC (WT04) derived neural progenitor cells, at day 7 after start of the gymnotic/saponin treatment. A negative (non-treated; NT) control was taken along.

Fig. 7 shows the sequences of a further set of guide oligonucleotides, with their respective RM numbers and SEQ ID NO's. RM118550 to RM118867 are designed based on the sequence and modifications of oligonucleotide RM116835 (SEQ ID NO:58, which is also referred to as G3447-19), whereas RM118868 to RM118880 are designed based on the sequence and modifications of oligonucleotide RM116838 (SEQ ID NO:61, which is also referred to as G3447-22). The chemical modifications are as provided in **Fig. 2**, wherein # refers to a PNms linkage.

Fig. 8 shows the editing percentage of the target adenosine shown in **Fig. 1D**, in *RELN* target (pre-) mRNA obtained after *gymnotic uptake* of the guide oligonucleotides of **Fig. 7** and co-treatment with the triterpene glycoside AG1856 (*saponin*) in human iPSC (WT04) derived neural progenitor cells, at day 7 after start of the gymnotic/saponin treatment. A negative sap(onin) only control was taken along. **Fig. 8A** shows the results using a forward primer specific for the transcript sequence of isoform 201 and **Fig. 8B** shows the results using a forward primer specific for the transcript of isoform 203.

DETAILED DESCRIPTION

It is held that herein for the first time, guide oligonucleotides are disclosed that can drive editing of a target *RELN* nucleic acid sequence. Such guide oligonucleotides can find use as therapeutic agents to treat, ameliorate or slow down the progression of a neurodegenerative disease such as AD. It has been identified, for instance, that a change of only a single amino acid in the reelin protein can be sufficient to initiate or enhance a protective pathway that slows down the progression of AD. By targeting the *RELN* DNA sequence or (pre-) mRNA transcript molecule, this technique operates at the genetic level. The present disclosure therefore opens a whole new field of using specific genetic editing techniques for the treatment of neurodegenerative disease. The genetic editing technique is not particularly limited. Suitable techniques include known gene therapy techniques that utilize a guide oligonucleotide, which include DNA editing techniques such as Cas9-based techniques, as well as RNA editing techniques such as ADAR-mediated editing techniques. Both DNA editing and RNA editing technologies have advantages and disadvantages. For instance, DNA editing gene therapy can produce a permanent change in the DNA molecule and may therefore only require a single treatment for a particular disorder. In certain circumstances it may not be required or desired to have an irreversible change of the DNA. Then RNA editing has the advantage of being transient: only the RNA is edited and over time amended proteins are being produced, but when the guide oligonucleotide has been broken down by metabolic processes and new mRNA is generated, the 'old' version of the protein is again being produced. Depending on the disorder and the severity, one may select an appropriate way of altering the nucleic acids encoding the reelin protein.

Embodiments

According to a first aspect, the disclosure provides a guide oligonucleotide that is at least partially complementary to a portion of a human *RELN* nucleic acid molecule comprising a target nucleotide, wherein the *RELN* nucleic acid molecule encodes a reelin protein, wherein the guide oligonucleotide is configured such that it is capable of forming a double stranded complex under physiological conditions within a cell with the portion of the *RELN* nucleic acid,

and the double stranded complex is capable of recruiting a nucleic acid editing enzyme that is naturally (= endogenously) present in the cell, to perform editing of the target nucleotide to generate an edited *RELN* nucleic acid comprising an edited target nucleotide.

In some embodiments, editing of the target nucleotide leads to elevated activity of the encoded reelin protein, preferably selected from one or more of:

- an enhanced ability to trigger signalling, preferably of the APOEr/Dab1/GSK3 β pathway;
- an enhanced ability to increase Dab1 phosphorylation;
- an enhanced ability to reduce Tau phosphorylation associated with neurofibrillary tangles;
- an enhanced ability to increase tubular structure formation and/or stability and/or neuronal density;
- an enhanced resistance to degradation by proteolysis; and/or
- enhanced binding of the reelin protein to glycosaminoglycans, preferably heparin, and/or to NRP1.

In some embodiments, editing of the target nucleotide introduces an amino acid variant at one or more of amino acid positions 3446 to 3460 of the encoded reelin protein, preferably wherein editing of the target nucleotide introduces a histidine to arginine change at amino acid position 3447 (H3447R) in the encoded reelin protein.

In some embodiments, the cell is a brain cell, preferably a neuron.

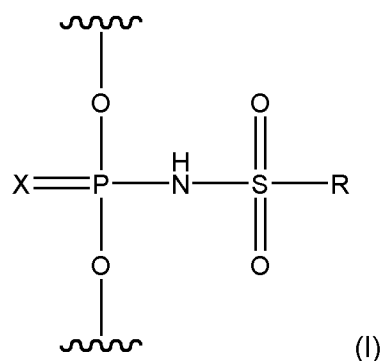
In some embodiments, the target nucleotide is adenosine, and the nucleic acid editing enzyme is an ADAR enzyme.

In some embodiments, the *RELN* nucleic acid molecule is mRNA or pre-mRNA.

In some embodiments, the orphan nucleotide is the nucleotide in the guide oligonucleotide that is opposite the target nucleotide, wherein the nucleotide numbering is such that the orphan nucleotide is number 0 and nucleotides are further positively (+) incremented towards the 5' end and negatively (-) incremented towards the 3' end, and wherein at least one nucleobase, sugar, or internucleoside linkage, has been chemically modified. In some embodiments, the orphan nucleotide is a deoxycytidine, a cytidine analog, a deoxyuridine, or a uridine analog. The cytidine analog is preferably a deoxynucleotide comprising a 6-amino-5-nitro-3-yl-2(1H)-pyridone nucleobase (also referred to herein, and elsewhere as "Benner's base", or Z). The uridine analog is preferably a deoxynucleotide comprising an iso-uracil nucleobase. In some embodiments, the guide oligonucleotide is 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, or 60 nucleotides in length.

In some embodiments, the guide oligonucleotide comprises a contiguous stretch of 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, or 33 nucleotides from SEQ ID NO:102 (5'-UG UA GAA ACI UCU GAG CCC AUG UUG UCG UGA AA-3') or SEQ ID NO:103 (5'-UG UA GAA AZI UCU GAG CCC AUG UUG UCGUGAAA-3'), comprising at least the underlined section of nucleotides (SEQ ID NO:104 (5'-UA GAA ACI UCU GAG CCC AUG UUG-3' and SEQ ID NO:105 (5'-UA GAA AZI UCU GAG CCC AUG UUG-3'), respectively), wherein Z is a nucleotide, preferably a deoxynucleotide, comprising a Benner's base, and I is inosine, preferably deoxyinosine.

In some embodiments, the guide oligonucleotide comprises one or more internucleoside linkage modifications, which are each independently selected from a phosphorothioate (PS), phosphonoacetate, phosphorodithioate, methylphosphonate (MP), sulfonylphosphoramidate, (1,3-dimethylimidazolidin-2-ylidene) phosphoramidate (PNdmi), or a linkage modification with the structure according to formula (I)



wherein: X = O or S; and R = an aryl, a substituted aryl, a heterocycle, a substituted heterocycle, an aromatic heterocycle, a substituted aromatic heterocycle, a C₁-C₆ alkoxy, a substituted C₁-C₆ alkoxy, a C₁-C₂₀ alkyl, a substituted C₁-C₂₀ alkyl, a C₁-C₆ alkenyl, a C₁-C₆ substituted alkenyl, a C₁-C₆ alkynyl, a substituted C₁-C₆ alkynyl, or a conjugate group. In a preferred embodiment, X = O and R = methyl. In that embodiment, the linkage modification is generally referred to as mesyl phosphoramidate (PNms).

In some embodiments, the internucleoside linkage numbering in the guide oligonucleotide is such that linkage number 0 is the linkage 5' from the orphan nucleotide, and the linkage positions in the oligonucleotide are positively (+) incremented towards the 5' end and negatively (-) incremented towards the 3' end, and wherein linkage position -2 is an MP or a PNms linkage.

In some embodiments, the guide oligonucleotide comprises one or more nucleotides comprising a mono- or di-substitution at the 2', 3' and/or 5' position of the ribose, each independently selected from the group consisting of: -OH; -F; substituted or unsubstituted, linear or branched lower (C₁-C₁₀) alkyl, alkenyl, alkynyl, alkaryl, allyl, or aralkyl, that may be

interrupted by one or more heteroatoms; -O-, S-, or N-alkyl; -O-, S-, or N-alkenyl; -O-, S-, or N-alkynyl; -O-, S-, or N-allyl; -O-alkyl-O-alkyl; -methoxy; -aminopropoxy; -methoxyethoxy; -dimethylamino oxyethoxy; and -dimethylaminoethoxyethoxy.

In some embodiments, the portion of the target *RELN* nucleic acid sequence comprises a contiguous stretch of 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, or 33 nucleotides from a nucleotide sequence selected from SEQ ID NO:1 and 2:

5' -... CAACATGGGCTCAGAC**A**TTTCTACAACAGAAGA ...-3' (SEQ ID NO:1); and

5' -... CAACAUGGGCUCAGAC**A**UUUCUACAACAGAAGA ...-3' (SEQ ID NO:2)

and comprises at least the section of nucleotides that is underlined, wherein the A in bold face is the target nucleotide and wherein the nucleic acid editing entity is ADAR, preferably human ADAR1 and/or human ADAR2, even more preferably wherein the ADAR enzyme is naturally expressed in the cell (= endogenous).

In some embodiments, editing of the target nucleotide leads to elevated levels of expression, elevated activity, and/or elevated stability, of the reelin protein.

In some embodiments, the nucleic acid editing entity is a nucleic acid editing enzyme, preferably a deaminase enzyme, more preferably an adenosine deaminase enzyme, such as human ADAR1 (hADAR1) and human ADAR2 (hADAR2), or a cytidine deaminase enzyme.

In some embodiments, the nucleic acid editing entity is naturally expressed in the cell (*i.e.*, endogenous to the cell).

In some embodiments, the target *RELN* nucleic acid sequence is naturally expressed within the cell.

In some embodiments, the target *RELN* nucleic acid sequence is DNA.

In some embodiments, the nucleic acid editing entity is selected from the list comprising: a Cas9 enzyme; a base editor enzyme; a dCas9-deaminase enzyme; a dCas9-adenosine deaminase enzyme; a dCas9-cytidine deaminase enzyme; a prime editing enzyme; or a Cas9 Nickase enzyme.

In some embodiments, the linkage between the most terminal two nucleotides on the 5' and/or 3' terminus of the guide oligonucleotide is a PNdmi linkage, or a PNms linkage, preferably wherein both most terminal linkages are PNms linkages.

In some embodiments, the first nucleotide 3' from the orphan nucleotide (-1) is a deoxyinosine.

In some embodiments, the guide oligonucleotide comprises a contiguous stretch of 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, or 33 nucleotides from a nucleotide sequence selected from:

5' -...TCTTCTGTTGTAGAAACGTCTGAGCCCATGTTG...-3' (SEQ ID NO:3),

5' -...TCTTCTGTTGTAGAAACITCTGAGCCCATGTTG...-3' (SEQ ID NO:4),

5' -...UCUUCUGUUGUAGAAACGUCUGAGCCCAUGUUG...-3' (SEQ ID NO:5), and

5' -...UCUUCUGUUGUAGAAACIUCUGAGCCCAUGUUG...-3' (SEQ ID NO:6)

5 and comprises at least the section of nucleotides that is underlined.

In some embodiments, the guide oligonucleotide comprises at least the underlined section of SEQ ID NO:6, wherein the orphan nucleotide (in the underlined section the middle C in bold) is a deoxynucleotide carrying a cytidine analog, preferably a Benner's base (Zd, instead of a C; see Intl. Patent Application Publication No. WO2022/252376), and wherein nucleotide position -1 is deoxyinosine (Id).

In some embodiments, the guide oligonucleotide comprises at least the underlined section of SEQ ID NO:6, wherein the orphan nucleotide (in the underlined section the middle C in bold) is a deoxyuridine, and wherein nucleotide position -1 is deoxyinosine (Id).

In some embodiments, the guide oligonucleotide comprises at least the underlined section of SEQ ID NO:6, wherein the orphan nucleotide (in the underlined section the middle C in bold) is a deoxynucleotide carrying a uridine analog, preferably an iso-uracil, and wherein nucleotide position -1 is deoxyinosine (Id).

In some embodiments, the guide oligonucleotide comprises at least the underlined section of SEQ ID NO:6, wherein the orphan nucleotide (in the underlined section the middle C in bold) is a deoxynucleotide carrying a Benner's base (Zd, instead of a C), wherein nucleotide position -1 is Id, and wherein nucleotide position +1 is deoxyadenosine (Ad) or an adenosine in which the 2' position of the ribose is substituted with 2'-O-methoxyethyl (also referred to as 2'-methoxyethoxy, 2'-O-MOE, or simply 2'-MOE) (Ae), preferably wherein the linkage position -2 is an MP or a PNms linkage, more preferably a PNms linkage.

In some embodiments, the guide oligonucleotide comprises at least the underlined section of SEQ ID NO:6, wherein the orphan nucleotide (in the underlined section the middle C in bold) is a deoxynucleotide carrying a Benner's base (Zd, instead of a C), wherein nucleotide position -1 is Id, wherein the length of the 5' part immediately adjacent to the orphan nucleotide is 6, 7, or 8 nucleotides, and wherein the length of the 3' part immediately adjacent to the orphan nucleotide is at least 16 nucleotides, more preferably 16, 17, 18, 19, 20, 21, 22, 23, or 24 nucleotides.

In some embodiments, the guide oligonucleotide comprises the structure (from 5' to 3'):

N₈N₇N₆N₅N₄N₃N₂N₁^θZdId[^]M₂M₃M₄M₅M₆M₇M₈M₉M₁₀M₁₁M₁₂M₁₃M₁₄M₁₅M₁₆M₁₇M₁₈M₁₉M₂₀M₂₁M₂₂M₂₃M₂₄

wherein:

- Zd is the orphan nucleotide at nucleotide position 0, which is a deoxynucleotide carrying a Benner's base;
- N₁ is Ae or Ad;
- 5 - N₂ is Af;
- N₃ and N₅ are each independently Am or Af;
- N₄ is Gf;
- N₆ is Uf;
- N₇ is either absent (if so, then N₈ is also absent), Gm, or Gf;
- 10 - N₈ is either absent or Um;
- Id is deoxyinosine;
- M₂ is Um;
- M₃ is Cf;
- M₄, M₁₄ and M₁₅ are each independently m5Ue or Um;
- 15 - M₅ and M₇ are Gf;
- M₆ is Am or Af;
- M₈ and M₁₀ are each independently Cm or Cf;
- M₉ is Cf;
- M₁₁ is Am;
- 20 - M₁₂ is Um;
- M₁₃ is Gm;
- M₁₆ is Ge or Gm;
- M₁₇ is either absent (if so, then M₁₈ to M₂₄ are also absent), m5Ue, or Um;
- M₁₈ is either absent (if so, then M₁₉ to M₂₄ are also absent), Cm, or m5Ce;
- 25 - M₁₉ is either absent (if so, then M₂₀ to M₂₄ are also absent), Gm, or Ge;
- M₂₀ is either absent (if so, then M₂₁ to M₂₄ are also absent), Um, or m5Ue;
- M₂₁ is either absent (if so, then M₂₂ to M₂₄ are also absent), Gm, or Ge;
- M₂₂ is either absent (if so, then M₂₃ and M₂₄ are also absent), Am, or Ae;
- M₂₃ is either absent (if so, then M₂₄ is also absent), or Ae;
- 30 - M₂₄ is either absent, or Ae;
- ^θ is at linkage position 0, and is a PO linkage or a PNms linkage;
- ^ is at linkage position -2 and is an MP or a PNms linkage;
- all other linkages are either PO, PS, PNdmi, or PNms linkages; and

wherein Gm, Am, Um, and Cm are 2'-O-methyl (2'-OMe) modified guanosine, adenosine, uridine, and cytidine, respectively; m5Ce is 2'-MOE modified 5-methylcytidine; Ge is 2'-MOE modified guanosine; Ae is 2'-MOE modified adenosine; m5Ue is 2'-MOE modified 5-

methyluridine (also sometimes named "Te"; 2'-MOE modified thymidine); Af, Uf, Gf, and Cf are 2'-F modified adenosine, uridine, guanosine, and cytosine, respectively.

In some embodiments, the guide oligonucleotide comprises or consists of the sequence of any one of SEQ ID NO:41, 44, 50, 58, 59, 60, 61, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, and 94. Preferably, the guide oligonucleotide comprises or consists of the sequence of any one of SEQ ID NO:65, 66, 90, 64, 88, 89, 69, 67, 82, 83, 84, 58, 61, 59, 60, 41, 44, 50, 68, 70, 71, 72, 73, 78, 79, 80, 81, 85, 86, and 87.

In some embodiments, the guide oligonucleotide is bound, preferably conjugated, to a triterpene glycoside, preferably AG1856.

According to a second aspect, the disclosure provides a vector, preferably a viral vector, more preferably an adeno-associated virus (AAV) vector, comprising a nucleic acid molecule encoding a guide oligonucleotide according the first aspect of the disclosure.

According to a third aspect, the disclosure provides a pharmaceutical composition comprising a guide oligonucleotide according to the first aspect of the disclosure, or a vector according to the second aspect of the disclosure, and a pharmaceutically acceptable carrier.

According to a fourth aspect, the disclosure provides a guide oligonucleotide according to the first aspect of the disclosure, or a vector according to a second aspect of the disclosure, or a pharmaceutical composition according to the third aspect of the disclosure, for use in the treatment, amelioration, or slowing down the progression of a neurodegenerative disease, preferably AD, more preferably ADAD.

According to a fifth aspect, the disclosure provides for the use of a guide oligonucleotide according to the first aspect of the disclosure, or a vector according to a second aspect of the disclosure, or a pharmaceutical composition according to the third aspect of the disclosure, for use in the manufacture of a medicament for the treatment, amelioration, or slowing down the progression of a neurodegenerative disease, preferably AD, more preferably ADAD.

According to a sixth aspect, the disclosure provides a method of treating, ameliorating, or slowing down the progression of a neurodegenerative disease, preferably AD, more preferably ADAD, in a human subject in need thereof, the method comprising administering to said subject a guide oligonucleotide according to the first aspect of the disclosure, or a vector according to a second aspect of the disclosure, or a pharmaceutical composition according to the third aspect of the disclosure, thereby editing the target *RELN* nucleic acid sequence to encode a reelin protein with the ability to delay onset of one or more symptoms of a neurodegenerative disease, preferably AD, more preferably ADAD.

According to a seventh aspect, the disclosure provides an *in vitro*, *ex vivo*, or *in vivo* method for the deamination of a target adenosine in a target *RELN* nucleic acid sequence in

a cell, the method comprising the steps of: (i) providing the cell with a guide oligonucleotide according to the first aspect of the disclosure, or a vector according to a second aspect of the disclosure, or a pharmaceutical composition according to the third aspect of the disclosure; (ii) allowing uptake by the cell of the guide oligonucleotide or vector or composition; (iii) 5 allowing annealing of the guide oligonucleotide to the target *RELN* nucleic acid sequence; and (iv) allowing a nucleic acid editing entity to edit the target.

In some embodiments, the method comprises step (v) of using a functional read-out to identify the presence of the edited target nucleotide.

In some embodiments, the method comprises the step of administering a triterpene 10 glycoside before, after or simultaneously with administering the guide oligonucleotide. In some embodiments, the triterpene glycoside is AG1856. In a preferred aspect, the triterpene glycoside, such as AG1856 is (non)covalently bound to the guide oligonucleotide to allow improved endosomal escape once the guide oligonucleotide has entered a target cell in which deamination of the target nucleotide needs to take place.

15 According to an eight aspect, the disclosure provides a method of editing a human *RELN* nucleic acid sequence in a cell, preferably a brain cell, wherein the human *RELN* nucleic acid sequence is pre-mRNA or mRNA, the method comprising contacting the target *RELN* nucleic acid sequence with a guide oligonucleotide capable of triggering an ADAR-mediated adenosine to inosine deamination, thereby editing the target *RELN* nucleic acid sequence to 20 encode a reelin protein with the ability to delay onset of one or more symptoms of a neurodegenerative disease, preferably AD, more preferably ADAD.

According to a ninth aspect, the disclosure provides a guide oligonucleotide for editing a target adenosine in a human *RELN* pre-mRNA or mRNA molecule by providing the guide oligonucleotide and allowing the guide oligonucleotide to hybridize to a human *RELN* pre- 25 mRNA or mRNA molecule and thereby to attract an ADAR enzyme to deaminate the target adenosine, wherein the target region is SEQ ID NO:106, and wherein the target adenosine is the second nucleotide of the codon encoding histidine at position 3447 of the *RELN*-encoded human reelin protein. In some embodiments of the ninth aspect, the nucleic acid molecule is selected from the group consisting of SEQ ID NO:65, 66, 90, 64, 88, 89, 69, 67, 82, 83, 84, 30 58, 61, 59, 60, 41, 44, 50, 68, 70, 71, 72, 73, 78, 79, 80, 81, 85, 86, and 87. In some embodiments of the ninth aspect, the nucleic acid molecule comprises at least one non-naturally occurring chemical modification, and/or comprising one or more additional non-naturally occurring chemical modifications in a ribose, linkage or base moiety, with the proviso that the orphan nucleotide, which is the nucleotide in the nucleic acid that is directly opposite 35 a target adenosine in the target region, is not a cytidine comprising a 2'-OMe ribose substitution.

Definitions

The guide oligonucleotides referred to herein are sometimes known or referred to as antisense oligonucleotides (AONs). They are sometimes also referred to as 'editing oligonucleotides', or 'EONs', even though the editing event is performed by the nucleic acid editing entity and the action of the oligonucleotide only triggers the editing to take place. Whenever reference is made to a guide oligonucleotide, oligonucleotide, oligo, ON, ASO, oligonucleotide composition, antisense oligonucleotide, AON, (RNA) editing oligonucleotide, EON, and RNA (antisense) oligonucleotide, both oligoribonucleotides and deoxyoligoribonucleotides are meant unless the context dictates otherwise. Potentially the oligonucleotide may completely lack RNA and DNA nucleotides (as they appear in nature) and may consist completely of modified nucleotides. Whenever reference is made to an 'oligoribonucleotide' it may comprise the bases A, G, C, U, or I. Whenever reference is made to a 'deoxyoligoribonucleotide' it may comprise the bases A, G, C, T, or I. However, a guide oligonucleotide as disclosed herein may comprise a mix of ribonucleotides and deoxyribonucleotides. When a deoxyribonucleotide is used, hence without a modification at the 2' position of the sugar, the nucleotide is often abbreviated to dA (or Ad), dC (or Cd), dG (or Gd) or T in which the 'd' represents the deoxy nature of the nucleoside, while a ribonucleoside that is either normal RNA or modified at the 2' position is often abbreviated without the 'd', and often abbreviated with their respective modifications and as explained herein.

The term 'nucleoside' refers to the nucleobase linked to the (deoxy)ribosyl sugar, without phosphate groups. A 'nucleotide' is composed of a nucleoside and one or more phosphate groups. The term 'nucleotide' thus refers to the respective nucleobase-(deoxy)ribosyl-phospholinker, as well as any chemical modifications of the ribose moiety or the phospho group. Thus, the term would include a nucleotide including a locked ribosyl moiety (comprising a 2'-4' bridge, comprising a methylene group or any other group), an unlocked nucleic acid (UNA), a threose nucleic acid (TNA), a nucleotide including a linker comprising a phosphodiester (PO), phosphonoacetate, phosphotriester, PS, phosphoro(di)thioate, MP (or MeP), methyl thiophosphonate, phosphoramidate linkages, PNdmi, and a linkage according to the structure of formula (I) as described herein. Sometimes the terms nucleobase, nucleoside and nucleotide are used interchangeably, unless the context clearly requires differently, for instance when a nucleoside is linked to a neighbouring nucleoside and the linkage between these nucleosides is modified. As stated herein, a nucleotide is a nucleoside plus one or more phosphate groups. The terms 'ribonucleoside' and 'deoxyribonucleoside', or 'ribose' and 'deoxyribose' are as used in the art.

Sometimes the terms adenosine and adenine, guanosine and guanine, cytidine and cytosine, uracil and uridine, thymine and thymidine/uridine, inosine, and hypoxanthine, are

used interchangeably to refer to the corresponding nucleobase on the one hand, and the nucleoside or nucleotide on the other. The nucleobase thymine (T) is also known as 5-methyluracil (m^5U) and is a uracil (U) derivative; thymine and 5-methyluracil can be interchanged throughout the document text. Likewise, the nucleotide thymidine is also known as 5-methyluridine and is a uridine derivative; thymidine and 5-methyluridine can be interchanged throughout the document text.

Whenever reference is made to nucleotides in the oligonucleotide, such as cytosine, 5-methylcytosine, 5-hydroxymethylcytosine, 5-formylcytosine, 5-acetylcytosine, 5-hydroxycytosine, and β -D-glucosyl-5-hydroxymethylcytosine are included. Whenever reference is made to adenine, N6-methyladenine, 8-oxo-adenine, 2,6-diaminopurine and 7-methyladenine are included. Whenever reference is made to uracil, dihydrouracil, isouracil, N3-glycosylated uracil, pseudouracil, 5-methyluracil, N1-methylpseudouracil, 4-thiouracil and 5-hydroxymethyluracil are included. Whenever reference is made to guanine, 1-methylguanine, 7-methylguanosine, N2,N2-dimethylguanosine, N2,N2,7-trimethylguanosine and N2,7-dimethylguanosine are included. Whenever reference is made to nucleosides or nucleotides, ribofuranose derivatives, such as 2'-deoxy, 2'-hydroxy, and 2'-O-substituted variants, such as 2'-OMe, are included, as well as other modifications, including 2'-4' bridged variants. Whenever reference is made to oligonucleotides, one or more linkages may be a naturally occurring phosphodiester linkage, whereas the remaining linkages between two mononucleotides may be a modified linkage. Examples of such modified linkages are phosphonoacetate, phosphotriester, PS, phosphoro(di)thioate, MP, phosphoramidate linkages, phosphoryl guanidine, thiophosphoryl guanidine, sulfono phosphoramidate, PNdmi and the linkage structure according to formula (I), further outlined in detail below.

The term 'comprising' encompasses 'including' as well as 'consisting of', e.g., a composition 'comprising X' may consist exclusively of X or may include something additional, e.g., X + Y. The term 'about' in relation to a numerical value x is optional and means, e.g., $x \pm 10\%$.

The word 'substantially' does not exclude 'completely', e.g., a composition which is 'substantially free from Y' may be completely free from Y. Where relevant, the word 'substantially' may be omitted from the definition of the disclosure or the claims.

The term 'conductive to' or 'mediate' can be used interchangeably with 'capable of facilitating'. When used in the context of a guide oligonucleotide that is conducive to ADAR editing (or can mediate ADAR editing), this means that the guide oligonucleotide, after entry into the cell, interacts with the target RNA sequence, thereby forming a double stranded structure which is recognized by the ADAR enzyme, which can then deaminate the target adenosine into an inosine. Hence, the guide oligonucleotide itself does not have the enzymatic

function (the ADAR enzyme has), but it can trigger, induce, cause, organize, mediate, provide, give, produce, facilitate, and/or result in RNA editing after binding to the target RNA molecule.

The term 'mismatch' is used herein to refer to opposing nucleotides in a double stranded RNA complex which do not form perfect base pairs according to the Watson-Crick base pairing rules. In the historical sense, mismatched nucleotides are G-A, C-A, U-C, A-A, G-G, C-C, U-U pairs. In some embodiments, guide oligonucleotides comprise fewer than four mismatches with the target sequence, for example 0, 1 or 2 mismatches. 'Wobble' base pairs are G-U, I-U, I-A, and I-C base pairs. When a U is placed opposite the target A, there is no mismatch, and the guide oligonucleotide may be 100% complementary. When a C is placed opposite the target A, there is at least 1 mismatch between the guide oligonucleotide and the target sequence. Although a G:G pairing would be considered a mismatch, that does not necessarily mean that the interaction is unstable, which means that the term 'mismatch' may be somewhat outdated based on the current disclosure where a Hoogsteen base-pairing may be seen as a mismatch based on the origin of the nucleotide but still be relatively stable. An isolated G:G pairing in duplex RNA can for instance be quite stable, but still be defined as a mismatch. Analysis of natural targets of ADAR enzymes has indicated that these generally include mismatches between the two strands that form the RNA helix edited by ADAR1 or 2. It has been suggested that these mismatches enhance the specificity of the editing reaction (Steffl et al. 2006. *Structure* 14(2):345-355; Tian et al. 2011. *Nucleic Acids Res* 39(13):5669-5681). Characterization of optimal patterns of paired/mismatched nucleotides between the guide oligonucleotides and the target RNA also appears important to the development of efficient ADAR-based AON therapy.

The term 'complementary' as used herein refers to the fact that the guide oligonucleotide hybridizes under physiological conditions to a second nucleic acid strand. Examples are (i) when the guide oligonucleotide as a first nucleic acid strand (= guide oligonucleotide) forms a heteroduplex RNA editing oligonucleotide complex with second complementary nucleic acid strand (*in vitro*), or (ii) when it forms a double stranded complex with the target RNA molecule. The term does not necessarily mean that each nucleotide in a nucleic acid strand has a perfect pairing with its opposite nucleotide in the opposite sequence. In other words, while a guide oligonucleotide may be complementary to a target sequence, there may be mismatches, wobbles and/or bulges between the guide oligonucleotide and the target sequence, while under physiological conditions that guide oligonucleotide still hybridizes to the target sequence such that the cellular RNA editing enzymes can deaminate the target adenosine to an inosine. The term 'substantially complementary' therefore also means that despite the presence of the mismatches, wobbles, and/or bulges, the guide oligonucleotide has enough matching nucleotides with the target sequence that under physiological conditions the guide oligonucleotide hybridizes to the target RNA molecule. As

shown herein, a guide oligonucleotide may be complementary, but may also comprise one or more mismatches, wobbles and/or bulges with the target sequence, if under physiological conditions the guide oligonucleotide is able to hybridize to its target.

The term 'orphan nucleotide' relates to the nucleotide in the guide oligonucleotide that is directly opposite the target adenosine, which is the adenosine that is deaminated by the deaminating enzyme. The orphan nucleotide may be a natural cytidine or deoxycytidine, or a uridine or deoxyuridine. It may also be a chemically modified nucleotide, as further described in detail below, or a known or chemically modified analog of a natural (deoxy)cytidine, such as a nucleotide carrying a Benner's base (6-amino-5-nitro-3-yl-2(1H)-pyridone), or a known or chemically modified analog of a natural (deoxy)uridine, such as iso-uridine, as further outlined in detail below.

A 'nucleotide analog' refers to an analog of a nucleic acid nucleotide. The nucleotide analog is an analog of adenosine, guanosine, cytidine, thymidine, uridine, deoxyadenosine, deoxyguanosine, deoxycytidine, deoxythymidine or deoxyuridine.

The term 'downstream' in relation to a nucleic acid sequence means further along the sequence in the 3' direction; the term 'upstream' means the converse. Thus, in any sequence encoding a polypeptide, the start codon is upstream of the stop codon in the sense strand but is downstream of the stop codon in the antisense strand. The same holds true for the guide oligonucleotides as disclosed herein. Nucleotides that are upstream of the orphan nucleotide in the antisense guide oligonucleotide are located towards the 5' terminus, and nucleotides that are downstream of the orphan nucleotide are located towards the 3' terminus.

The nucleotide 'numbering' in a guide oligonucleotide as disclosed herein is such that the orphan nucleotide is number 0 and the nucleotide 5' from the orphan nucleotide is number +1. Counting is further positively (+) incremented towards the 5' end and negatively (-) incremented towards the 3' end, wherein the first nucleotide 3' from the orphan nucleotide is number -1. The internucleoside linkage numbering in the guide oligonucleotide is such that linkage number 0 is the linkage 5' from the orphan nucleotide, and the linkage positions in the oligonucleotide are positively (+) incremented towards the 5' end and negatively (-) incremented towards the 3' end.

References to 'hybridisation' typically refer to specific hybridisation and exclude non-specific hybridisation. Specific hybridisation can occur under experimental conditions chosen, using techniques well known in the art, to ensure that most stable interactions between probe and target are where the probe and target have at least 70%, preferably at least 80%, more preferably at least 90% sequence identity.

The term 'splice mutation' relates to a mutation in a gene that encodes for a pre-mRNA, wherein the splicing machinery is dysfunctional in the sense that splicing of introns from exons is disturbed and due to the aberrant splicing, the subsequent translation is out of frame

resulting in premature termination of the encoded protein. Often such shortened proteins are degraded rapidly and do not have any functional activity.

Whenever a 'naked' form in relation to the guide oligonucleotide as disclosed herein is referred to, it means that the guide oligonucleotide is manufactured in a laboratory or manufacturing facility, through which it is generally chemically modified to prevent it from rapid degradation after it enters the mammalian body or a tissue, or cell, upon administration. A naked form of a guide oligonucleotide is therefore different from a form in which the guide oligonucleotide is encoded (and delivered) by a viral genome or within a plasmid vector. When such viral vectors or plasmid vectors are administered, the encoded guide oligonucleotide is expressed from the viral vector genome or from the plasmid in the cell to which the viral vector or plasmid vector is delivered. Consequently, the guide oligonucleotide is then not chemically modified, and comprises solely naturally occurring nucleotides, preferably naturally occurring RNA nucleotides.

Notably, when the guide oligonucleotide comprises chemical modifications, as detailed herein, it may still be delivered through the means of a delivery vehicle. Suitable delivery vehicles are nanoparticle delivery vehicles such as polymeric nanoparticles, dendrimers, inorganic nanoparticles and nanocrystals, organic nanocrystals, and liposomes. Preferred nanoparticles are Lipid Nanoparticles (LNP's) that are nano-sized lipid vesicles that carry the guide oligonucleotide of the present disclosure and aid to the delivery of target cells. If an LNP is applied or any other similar type of carrier, the guide oligonucleotide is still considered naked because it is not transcribed from an encoding polynucleotide (such as in the case of a plasmid or a vector, in which the guide oligonucleotide is not regarded as 'naked'). So, even though a chemically modified guide oligonucleotide is encapsulated by a carrier, preferably an LNP, it is still seen as naked, as it has been manufactured as such in a laboratory setting and encapsulated thereafter in the carrier using methods known to the person skilled in the art. The disclosure also relates to a delivery vehicle, preferably an LNP, which comprises a 'naked' and chemically modified guide oligonucleotide as disclosed herein. The person skilled in the art understands that when a delivery moiety, or attachment to a guide oligonucleotide is used (such a GalNAc moiety to target hepatocytes in the liver, and/or when attached/conjugated to a saponin as discussed herein) that the guide oligonucleotide is still seen as naked as well, also when a saponin-guide is encapsulated in a delivery vehicle such as an LNP. In other words, a variety of non-limiting administration methods is feasible: i) a naked guide oligonucleotide as is; ii) a naked guide oligonucleotide encapsulated in a delivery vehicle, preferably an LNP; iii) a naked guide oligonucleotide administered together or separately from (but not bound to) a saponin such as AG1856; iv) a naked guide oligonucleotide conjugated to a saponin such as AG1856; v) a naked guide oligonucleotide conjugated to a saponin such as AG1856, and wherein the saponin-guide conjugate is encapsulated in a delivery vehicle,

preferably an LNP; or vi) through an encoding vector, such as a plasmid or a viral vector from which the guide oligonucleotide is transcribed. Depending on the disease target and the cells that need to be targeted an administration method is being selected, although such is preferably an administration in which the guide oligonucleotide is in a naked form, either or not conjugated to a delivery moiety (or endosomal release agent), or either or not encapsulated in a delivery vehicle such as an LNP.

The length of the guide oligonucleotide as disclosed herein, and when delivered in a naked form is preferably 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, or 60 nucleotides in length. However, when the guide oligonucleotide as disclosed herein is to be delivered through the expression of a viral vector, then the guide oligonucleotide may also be longer, such as 70, 80, 90, 100, 150, or 200 or more nucleotides in length.

The term 'HEON' refers to a heteroduplex double-stranded complex molecule wherein a guide oligonucleotide as disclosed herein is hybridized to a partially or fully complementary, partially or fully overlapping sense oligonucleotide. Because the guide oligonucleotide as disclosed herein often has specified chemical modifications that are different from the chemical modifications in the sense strand, the two strands form such a *heteroduplex* RNA editing oligonucleotide complex. The sense strand may be chemically modified almost in its entirety, similar or different to what is performed in the guide oligonucleotide as disclosed herein, for example by providing nucleotides with a ribose sugar moiety carrying a 2'-OMe substitution, a 2'-F substitution, or a 2'-MOE substitution. It is to be understood that the sense strand present in the HEON is a different entity in comparison to the target RNA molecule in the cell. The sense strand in an HEON is preferably 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, or 60 nucleotides in length. The HEON is often generated *in vitro* and used as a delivery tool to protect the guide oligonucleotide from degradation when administered to the cell. In other words, the HEON is preferably formed before the guide oligonucleotide is administered to the cell.

RELN nucleic acid sequence

The present disclosure relates to guide oligonucleotides that mediate editing of one or more target nucleotides present in a target *RELN* nucleic acid sequence. The *RELN* gene encodes the protein reelin. Preferably, the guide oligonucleotide mediates editing of one or more target nucleotides present in a human *RELN* nucleic acid sequence. It is particularly preferred that the human *RELN* nucleic acid sequence is present in a human cell, wherein editing occurs in the cell.

In some embodiments, the target nucleotide is any nucleotide whereby editing provides the reelin protein with one or more of: a gain-of-function phenotype; an enhanced ability to upregulate signalling pathways initiated by reelin; an enhanced ability to increase Dab1 phosphorylation, to reduce Tau phosphorylation, and/or to increase neuronal density; and/or enhanced binding of the reelin protein to glycosaminoglycans, preferably heparin, and/or to NRP1.

In some embodiments, the target nucleotide is any nucleotide whereby the edited target nucleotide produces a structural effect on any of the amino acids in the 'α-GAG binding site', 'β-GAG binding site', and/or neuropilin 1 (NRP1) binding site, of the reelin protein. The α-GAG binding site spans the six C-terminal amino acids of the human reelin protein (3455-3460). The β-GAG binding site spans amino acids 3446-3451 of the human reelin protein. The edited target nucleotide can be within a codon for an amino acid that is outside of these sites, so long as mutation of the amino acid produces an effect within one of these sites. Preferably, the target nucleotide is within a codon that encodes an amino acid within one of these sites. It is particularly preferred that the target nucleotide is within a codon that encodes one of amino acids 3446-3460 of the human reelin protein, preferably within amino acids 3446-3451 or 3455-3460 of the human reelin protein.

In a particularly preferred embodiment, the target nucleotide is within the codon for amino acid 3447 of the human reelin protein. In particular, the target *RELN* nucleic acid sequence encodes an amino acid other than arginine at position 3447 of the human reelin protein, and preferably encodes histidine at position 3447 of the human reelin protein. In these embodiments, it is preferred that the edited *RELN* nucleic acid sequence encodes arginine at position 3447 of the human reelin protein, giving rise to a changed protein variant that is herein referred to as H3447R.

In a particularly preferred embodiment, the target nucleotide is adenosine, and the nucleic acid editing entity is an adenosine deaminase. It is further preferred embodiment that the target *RELN* nucleic acid sequence comprising a target nucleotide is a *RELN* RNA transcript molecule (pre-mRNA and/or mRNA) comprising a target adenosine, and the nucleic acid editing entity is an ADAR enzyme, more preferably human ADAR1 and/or human ADAR2. Within these embodiments, it is preferred that the *RELN* RNA transcript molecule has a codon CAU encoding histidine at position 3447 of the human reelin protein, wherein the adenosine within the CAU codon is the target adenosine. In this embodiment, ADAR-mediated editing produces CIU, which the translation machinery interprets as CGU, encoding arginine. As such, in some embodiments, it is preferred that the cell is a cell of a human having DNA encoding reelin with an amino acid other than arginine, preferably histidine, at position 3447 of the human reelin protein.

In some embodiments, the edited *RELN* nucleic acid sequence encodes a reelin protein with an enhanced ability to protect against AD, and/or one or more symptoms of AD. In some embodiments, the edited *RELN* nucleic acid sequence encodes a reelin protein with an ability to upregulate or initiate a pathway that is protective against AD, and/or one or more symptoms of AD. In some embodiments, the edited *RELN* nucleic acid sequence encodes a reelin protein with an enhanced ability to bind glycosaminoglycans (GAGs), particularly at the C-terminal region of reelin. It is particularly preferred that the edited *RELN* nucleic acid sequence encodes a reelin protein with an enhanced ability to bind heparin. In some embodiments, the edited *RELN* nucleic acid sequence encodes a reelin protein with an enhanced ability to bind neuropilin 1. In some embodiments, the edited *RELN* nucleic acid sequence encodes a reelin protein with an enhanced ability to lower Tau pathology. In particular, in some embodiments, the edited *RELN* nucleic acid sequence encodes a reelin protein with enhanced ability to reduce Tau phosphorylation. In some embodiments, the edited *RELN* nucleic acid sequence encodes a reelin protein with enhanced ability to increase Dab1 phosphorylation. In some embodiments, the edited *RELN* nucleic acid sequence encodes a reelin protein with the ability to increase neuronal density.

In some embodiments, the edited *RELN* nucleic acid sequence encodes a reelin protein with a gain-of-function phenotype. Examples of gain-of-function are, in addition to the variant described in the examples, variants with: 1) an enhanced ability to trigger signalling, preferably of the APOEr/Dab1/GSK3 β pathway; 2) an enhanced ability to increase Dab1 phosphorylation; 3) an enhanced ability to reduce Tau phosphorylation found in neurofibrillary tangles; 4) an enhanced ability to increase microtubular structure formation and/or stability of microtubule structures and/or neuronal density; 5) an enhanced resistance to degradation by proteolysis; and/or enhanced binding to glycosaminoglycans, preferably heparin; and/or 6) enhanced binding to NRP1.

A preferred embodiment is one wherein the reelin protein has a gain-of-function by way of reduced inactivation by proteases. For example, ADAMTS-3 was identified as the protease that cleaves and inactivates reelin in the brain (e.g., the cerebral cortex and hippocampus). Knock down of ADAMTS-3 in mice has been shown to reduce Tau phosphorylation and dendritic branching and elongation was increased (Ogino H et al., *J Neurosci.* 2017, 37(12):3181–3191). Therefore, altering the ADAMTS-3 cleavage site in reelin, such as the Pro-Ala sequence in the N-t site (Koie M et al., *J Biol Chem.* 2014, 289:12922–12930), upregulates reelin and is a viable strategy to create a reelin with gain-of-function phenotype. The present disclosure relates to a variety of guide oligonucleotides that are aimed at deamination of a target adenosine in a target *RELN* nucleic acid sequence. However, it is not excluded that two or more adenosines may be targeted for deamination in a single treatment. Without wishing to be bound by theory, a synergistic or additive effect may be

obtained by combining guide oligonucleotides as disclosed herein for targeting a multitude of target nucleotides, such as target adenosines, and thereby a multitude of amino acids within a single reelin protein, to increase the therapeutic effect.

5 *Neurodegenerative disease*

The target nucleotide is a nucleotide whereby editing thereof produces an edited *RELN* nucleic acid sequence that encodes an edited reelin protein with beneficial therapeutic effects in comparison with the unedited reelin protein. In particular, the beneficial therapeutic effects may include the treatment, alleviation, or reduction of neurodegenerative disease, such as
10 AD, or one or more symptoms of such disease, including mild cognitive impairment, cognitive decline, and/or dementia.

In some embodiments, the guide oligonucleotides are for use in a subject that has been diagnosed with AD. In other embodiments, the guide oligonucleotides are for use prophylactically in a subject that has been identified as at risk for developing AD. In some
15 embodiments, the guide oligonucleotides are for use prophylactically in cognitively healthy subjects. In some embodiments, the guide oligonucleotides are for use in a subject having the *PSEN1*-E280A mutation (wherein *PSEN1* is the gene encoding presenilin 1), which is a mutation associated with development of mild cognitive impairment in AD. In some embodiments, the guide oligonucleotides are for use in a subject having two copies of the
20 *APOE3* Christchurch (*APOECh*) (R136S) gene variant. In some embodiments, the guide oligonucleotides are for use in a subject having ADAD.

In some embodiments, the cell is a brain cell, preferably a neuron. In some embodiments, the cell is in the medial temporal lobe, preferably the allocortex, more preferably the entorhinal cortex. In some embodiments, editing occurs in or around the endoplasmic
25 reticulum of the cell.

DNA editing

In some embodiments, the present disclosure relates to 'DNA editing'. DNA editing techniques that are compatible with what is disclosed herein include DNA editing techniques based on the CRISPR Cas9 nuclease enzyme. These techniques are well known in the art.
30 These techniques include use of CRISPR-Cas9 to introduce a double-stranded break to DNA, which can be programmed to occur at a specified site by using a guide RNA oligonucleotide with the necessary sequence to guide the CRISPR-Cas9 enzyme to the specified site. Such breaks can be used to delete, modify, and/or insert DNA sequence at the specified site in DNA. Another system derived from CRISPR-Cas9 is the use of a Base Editor (BE) system,
35 which uses a catalytically dead Cas9 (dCas9) that has been fused to a functional enzyme such as a DNA deaminase. The dCas9 does not introduce double-stranded breaks to DNA, but

instead locates the DNA deaminase such that it results in deamination of the target DNA nucleotide, again guided to the specific site by a guide oligonucleotide. The DNA deaminase can be a cytidine deaminase (to induce C to T substitutions) or an adenine deaminase (to induce A to G substitutions). dCas9 fused to a cytidine deaminase enzyme is also known as a Cytidine Base Editor (CBE), and dCas9 fused to an adenine deaminase enzyme is also known as an Adenine Base Editor (ABE). A further development is known as Prime Editing. Prime Editing uses a Cas9 nickase fused to a reverse transcriptase enzyme. Prime Editing again uses a guide oligonucleotide to guide the enzyme to a specific site in the DNA. Prime Editing also uses an oligonucleotide that comprises a prime editing guide RNA (pegRNA) which comprises a primer binding site sequence and a sequence containing the desired edit. This can be part of the same molecule as the guide oligonucleotide. Initially, the Cas9 nickase, guided by the guide oligonucleotide, creates a cut at the specified section of one strand of the DNA. Next, the reverse transcriptase then uses the pegRNA as a template for reverse transcription to provide the edited sequence into the DNA.

RNA editing

In some embodiments, the present disclosure relates to 'RNA editing'. RNA editing is a natural process through which eukaryotic cells alter the sequence of their RNA molecules, often in a site-specific and precise way, thereby increasing the repertoire of genome-encoded RNAs by several orders of magnitude. RNA editing enzymes have been described for eukaryotic species throughout the animal and plant kingdoms, and these processes play an important role in managing cellular homeostasis in metazoans from the simplest life forms (such as *Caenorhabditis elegans*) to humans. Examples of RNA editing are adenosine to inosine (A to I) conversions and cytidine to uridine conversions, which occur through enzymes called Adenosine Deaminases acting on RNA (ADAR) and APOBEC/AID (cytidine deaminases that act on RNA), respectively.

ADAR is a multi-domain protein, comprising of a catalytic domain and two to three double-stranded RNA recognition domains, depending on the enzyme in question. Each recognition domain recognizes a specific double-stranded RNA (dsRNA) sequence and/or conformation. The catalytic domain does also play a role in recognizing and binding a part of the dsRNA helix, although the key function of the catalytic domain is to convert an A into an I in a nearby, predefined, position in the target RNA, by deamination of the nucleobase. Inosine is read as guanosine by the translational machinery of the cell, meaning that, if an edited adenosine is in a coding region of an mRNA or pre-mRNA, it can recode the protein sequence. Interestingly, A to I conversions may also occur in 5' non-coding sequences of a target mRNA, creating new translational start sites upstream of the original start site, which gives rise to N-terminally extended proteins, or in the 3' untranslated region (UTR) or other non-coding parts

of the transcript, which may affect the processing and/or stability of the RNA. In addition, A to I conversions may take place in splice elements in introns or exons in pre-mRNAs, thereby altering the pattern of splicing. As a result, exons may be included or skipped. The enzymes catalysing adenosine deamination are within an enzyme family of ADARs, which include human deaminases hADAR1 and hADAR2, as well as hADAR3. However, for hADAR3 no deaminase activity has been demonstrated.

The use of guide oligonucleotides (or antisense oligonucleotides; AONs or EONs) to edit a target RNA, applying adenosine deaminase, has been described (*e.g.*, Woolf *et al. Proc Natl Acad Sci USA*. 1995, 92:8298-8302; Montiel-Gonzalez *et al. Proc Natl Acad Sci USA*. 2013, 110(45):18285–18290; Vogel *et al. Angewandte Chemie* 2014, Int Ed 53:267-271). A disadvantage of the method described by Montiel-Gonzalez *et al.* (2013) is the need for a fusion protein consisting of the boxB recognition domain of bacteriophage Lambda N-protein, fused to the adenosine deaminase domain of a truncated natural ADAR protein. It requires target cells to be either transduced with the fusion protein, which is a major hurdle, or that target cells are transfected with a nucleic acid construct encoding the engineered adenosine deaminase fusion protein for expression. The system described by Vogel *et al.* (2014) suffers from similar drawbacks, in that it is not clear how to apply the system without having to genetically modify the ADAR first and subsequently transfect or transform the cells harboring the target RNA, to provide the cells with this genetically engineered protein. US 9,650,627 describes a similar system. The oligonucleotides of Woolf *et al.* (1995) that were 100% complementary to the target RNA sequences, suffered from severe lack of specificity: nearly all adenosines in the target RNA strand that were complementary to the guide oligonucleotide were edited.

It is known that ADAR may act on any dsRNA. Through a process sometimes referred to as 'promiscuous editing', the enzyme will edit multiple A's in the dsRNA. Hence, there was a need for methods and means that circumvent such promiscuous editing and only target specific adenosines in a target RNA molecule to become therapeutically applicable. Vogel *et al.* (2014) showed that such off-target editing can be suppressed by using 2'-OMe modified nucleosides in the guide oligonucleotide at positions opposite to adenosines that should not be edited and used a non-modified nucleoside directly opposite to the specifically targeted adenosine on the target RNA. However, the specific editing effect at the target nucleotide has not been shown to take place without the use of recombinant ADAR enzymes having covalent bonds with the guide oligonucleotides. Several publications have now shown that the recruitment of endogenous ADAR (hence without the need for an exogenous and/or recombinant source) is feasible while maintaining a specificity in which a single adenosine within a target RNA molecule can be targeted and deaminated to an inosine. Intl. Patent Application Publication No. WO2016/097212 discloses AONs for the targeted editing of RNA,

wherein the AONs are characterized by a sequence that is complementary to a target RNA sequence (therein referred to as the 'targeting portion') and by the presence of a stem-loop / hairpin structure (therein referred to as the 'recruitment portion'), which is preferably non-complementary to the target RNA. Such oligonucleotides are referred to as 'self-looping AONs'. The recruitment portion acts in recruiting a natural ADAR enzyme present in the cell (that is, endogenously present) to the dsRNA formed by hybridization of the target sequence with the targeting portion. Due to the recruitment portion, there is no need for conjugated entities or presence of modified recombinant ADAR enzymes. Intl. Patent Application Publication No. WO2016/097212 describes the recruitment portion as being a stem-loop structure mimicking either a natural substrate (e.g., the GluB receptor) or a Z-DNA structure known to be recognized by the dsRNA binding domains, or Z-DNA binding domains, of ADAR enzymes. A stem-loop structure can be an intermolecular stem-loop structure, formed by two separate nucleic acid strands, or an intramolecular stem loop structure, formed within a single nucleic acid strand. The stem-loop structure of the recruitment portion as described is an intramolecular stem-loop structure, formed within the AON itself, and are thought to attract (endogenous) ADAR. Similar stem-loop structure-comprising systems for RNA editing have since then been described in Intl. Patent Application Publication Nos. WO2017/050306, WO2020/001793, WO2017/010556, US11,390,865, WO2020/246560, and WO2022/078995.

Intl. Patent Application Publication Nos. WO2017/220751 and WO2018/041973 describe a next generation type of AONs that do not comprise such a stem-loop structure but that are (almost fully) complementary to the targeted area, and that appeared still capable of attracting endogenous ADAR enzymes. In one embodiment, one or more mismatching nucleotides, wobbles, or bulges exist between the oligonucleotide and the target sequence. A sole mismatch may be at the site of the nucleoside opposite the target adenosine, but in other embodiments AONs were described with multiple bulges and/or wobbles when attached to the target sequence area. It appeared possible to achieve *in vitro*, *ex vivo* and *in vivo* RNA editing with AONs lacking a stem-loop structure and with endogenous ADAR enzymes when the sequence of the AON was carefully selected such that it could attract/recruit ADAR. The 'orphan nucleoside', which is defined as the nucleoside in the guide oligonucleotide (or AON) that is positioned directly opposite the target adenosine in the target RNA molecule, was a nucleotide with an unmodified cytosine nucleobase and that did not carry a 2'-OMe modification. The orphan nucleoside can be a deoxyribonucleoside (DNA), wherein the remainder of the guide oligonucleotide could still carry 2'-O-alkyl modifications at the sugar entity (such as 2'-OMe), or the nucleotides directly surrounding the orphan nucleoside contained chemical modifications (such as DNA in comparison to RNA) that further improved the RNA editing efficiency and/or increased the resistance against nucleases. Such effects could even be further improved by using sense oligonucleotides (SONs) that 'protected' the

AONs against breakdown upon delivery to the cells (described in Intl. Patent Application Publication Nos. WO2018/134301 and US11,274,300).

The use of chemical modifications and particular structures in oligonucleotides that could be used in ADAR-mediated editing of specific adenosines in a target RNA have been the subject of numerous disclosures in the field, such as Intl. Patent Application Publication Nos. WO2019/111957, WO2019/158475, WO2020/165077, WO2020/201406, WO2020/211780, WO2021/008447, WO2021/020550, WO2021/060527, WO2021/117729, WO2021/136408, WO2021/182474, WO2021/216853, WO2021/242778, WO2021/242870, WO2021/242889, WO2022/007803, WO2022/018207, WO2022/026928, and WO2022/124345. The use of specific sugar moieties has been disclosed in for instance Intl. Patent Application Publication Nos. WO2020/154342, WO2020/154343, WO2020/154344, WO2022/103839, and WO2022/103852, whereas the use of stereo-defined linker moieties (in general for oligonucleotides that for instance can be used for exon skipping, in gapmers, in siRNA, or specifically for RNA-editing oligonucleotides, related to a wide variety of target sequences) has been described in Intl. Patent Application Publication Nos. WO2011/005761, WO2014/010250, WO2014/012081, WO2015/107425, WO2017/015575 (HTT), WO2017/062862, WO2017/160741, WO2017/192664, WO2017/192679 (DMD), WO2017/198775, WO2017/210647, WO2018/067973, WO2018/098264, WO2018/223056 (PNPLA3), WO2018/223073 (APOC3), WO2018/223081 (PNPLA3), WO2018/237194, WO2019/032607 (C9orf72), WO2019/055951, WO2019/075357 (SMA/ALS), WO2019/200185 (DM1), WO2019/217784 (DM1), WO2019/219581, WO2020/118246 (DM1), WO2020/160336 (HTT), WO2020/191252, WO2020/196662, WO2020/219981 (USH2A), WO2020/219983 (RHO), WO2020/227691 (C9orf72), WO2021/071788 (C9orf72), WO2021/071858, WO2021/178237 (MAPT), WO2021/234459, WO2021/237223, WO2022/099159, WO2021/030778, WO2022/174053, and WO2023/278589.

Next to these disclosures, an extensive number of publications relate to the targeting of specific RNA target molecules, or specific adenosines within such RNA target molecules, be it to repair a mutation that resulted in a premature stop codon, or other mutation causing disease. Examples of such disclosures in which adenosines are targeted within specified target RNA molecules are Intl. Patent Application Publication Nos. WO2020/157008 and WO2021/136404 (USH2A); WO2021/113270 (APP); WO2021/113390 (CMT1A); WO2021/209010 (IDUA, Hurler syndrome); WO2021/231673 and WO2021/242903 (LRRK2); WO2021/231675 (ASS1); WO2021/231679 (GJB2); WO2019/071274 and WO2021/231680 (MECP2); WO2021/231685 and WO2021/231692 (OTOF, autosomal recessive non-syndromic hearing loss); WO2021/231691 (XLR5); WO2021/231698 (argininosuccinate lyase deficiency); WO2021/130313 and WO2021/231830 (ABCA4); and WO2021/243023 (SERPINA1).

It is particularly preferred that the ADAR1 and/or ADAR2 are endogenously present in the cell. Such guide oligonucleotide can mediate RNA editing of a target adenosine present in a target RNA molecule after it is bound to the target RNA molecule, since the deaminating enzymes are recruited to the double-stranded oligonucleotide/target RNA molecule complex and subsequently deaminate the target adenosine into an inosine.

There is a constant need for improving the pharmacokinetic properties of the guide oligonucleotides without negatively affecting the efficiency in which the target adenosine is edited in the target RNA, and/or without negatively affecting the stability of the guide oligonucleotide itself, which is constantly prone to breakdown because of nucleases present in a natural cell. Many chemical modifications are available for the generation of oligonucleotides (and many have been applied in the art). However, many of these properties are not always compatible with the desire of achieving efficient RNA editing. In the search for better pharmacokinetic properties, it was found earlier that a 2'-MOE modification of the ribose of some, but not all, nucleotides surprisingly appeared compatible with efficient ADAR engagement and editing (Intl. Patent Application Publication No. WO2019/158475). In a similar fashion, it was found earlier that a PS linkage at some, but not all, internucleoside linkages surprisingly appeared compatible with efficient ADAR engagement and editing (Intl. Patent Application Publication No. WO2019/219581). Also, it was found earlier that phosphonoacetate linkage modifications and/or UNA ribose modifications of some, but not all, positions in the guide oligonucleotide appeared compatible with efficient engagement of an enzyme with nucleotide deamination activity and with subsequent deamination (Intl. Patent Application Publication No. WO2020/165077). Whereas the properties of phosphonoacetate and UNA modifications were known as such, the compatibility thereof with engagement of enzymes with nucleotide deamination activity and with the deamination reaction was not known.

In some embodiments, the present disclosure provides guide oligonucleotides that can provide (mediate, cause, or trigger) RNA editing of a target adenosine in a target transcript molecule, such as pre-mRNA and/or mRNA. The target transcript molecule may be encoded by a mutated gene, wherein the mutation is the cause of a disease and wherein the editing can reverse the mutation to give rise to a wildtype protein, or a protein with a wildtype function (for instance when the mutated amino acid is changed to an amino acid that does not cause the disease, or that provides an improved phenotype). As disclosed in more detail herein, the target transcript molecule may also be encoded by a wildtype gene, such as in a preferred aspect of the present disclosure, wherein the target *RELN* nucleic acid molecule is a transcript from a wildtype human *RELN* gene as shown in the present disclosure. In particular, the RNA editing encodes a modified reelin protein that improves the disease state of the treated subject. In particular, the target *RELN* nucleic acid sequence is the sequence as naturally present in a

subject. In other words, the target *RELN* nucleic acid sequence is the sequence prior to treatment with guide oligonucleotides according to the disclosure.

Non-limiting examples of transcript molecules that are targeted using RNA editing for a variety of treatments are *SERPINA1* (for the treatment of alpha1-antitrypsin (A1AT) deficiency; see e.g., Intl. Patent Application Publication Nos. WO2016/097212, WO2017/220751, WO2018/041973, and WO2021/243023), *IDUA* (for the treatment of Hurler syndrome; see e.g., Intl. Patent Application Publication Nos. WO2017/220751, WO2018/041973, and WO2021/209010), *LRRK2* (for the treatment of Parkinson's disease; see e.g., Intl. Patent Application Publication Nos. WO2016/097212, WO2017/220751, WO2018/041973, WO2021/231673 and WO2021/242903), *ABCA4* (for the treatment of Stargardt disease; see e.g., Intl. Patent Application Publication Nos. WO2021/130313 and WO2021/231830), *USH2A* (for the treatment of Usher syndrome; see e.g., Intl. Patent Application Publication Nos. WO2020/157008, WO2020/219981 and WO2021/136404), *APP* (see e.g., Intl. Patent Application Publication No. WO2021/113270), *CMT1A* (see e.g., Intl. Patent Application Publication No. WO2021/113390), *ASS1* (see e.g., Intl. Patent Application Publication No. WO2021/231675), *GJB2* (see e.g., Intl. Patent Application Publication No. WO2021/231679), *MECP2* (for the treatment of Rett syndrome; see e.g., Intl. Patent Application Publication Nos. WO2019/071274 and WO2021/231680), *OTOF* (for the treatment of autosomal recessive non-syndromic hearing loss; see e.g., Intl. Patent Application Publication Nos. WO2021/231685 and WO2021/231692), *XLRS* (see e.g., Intl. Patent Application Publication No. WO2021/231691), *PCSK9* (for the treatment of hypercholesterolemia; see e.g., Intl. Patent Application Publication No. WO2023/152371), and *HFE* (for the treatment of hemochromatosis / iron overload; see e.g., Intl. Patent Application Publication No. WO2024/110565).

Chemical modifications

Various chemistries and modifications are known in the field of oligonucleotides that can be readily used in accordance with the disclosure. The chemical modifications listed herein may be used with guide oligonucleotides intended for DNA editing or RNA editing, as appropriate, and/or unless otherwise noted. All chemical modifications listed herein that may be used in the guide oligonucleotide as disclosed herein may also be used for a sense strand that is complementary to the guide oligonucleotide, when the guide oligonucleotide and the complementary strand form a HEON complex, such as described in Intl. Patent Application Publication No. WO2024/084048 and as disclosed above, except that the opposite sense strand does not have an orphan nucleotide. Hence, the modification related to the orphan nucleotide relate only to the guide oligonucleotide as disclosed herein, but all other modifications relate to the guide oligonucleotide as disclosed herein and any (protecting)

sense oligonucleotide that may be used together with the guide oligonucleotide in a pharmaceutical product. This includes the use of hydrophobic moieties (such as tocopherol and cholesterol) which may either be bound to the guide oligonucleotide or its opposite strand, or both.

5 The skilled person knows that an oligonucleotide, such as a guide oligonucleotide as outlined herein, generally consists of repeating monomers. Such a monomer is most often a nucleotide or a chemically modified nucleotide. The most common naturally occurring nucleotides in RNA are adenosine monophosphate (A), cytidine monophosphate (C), guanosine monophosphate (G), and uridine monophosphate (U). These consist of a pentose
10 sugar, a ribose, a 5'-linked phosphate group which is linked via a phosphate ester, and a 1'-linked base. The sugar connects the base and the phosphate and is therefore often referred to as the "scaffold" of the nucleotide. A modification in the pentose sugar is therefore often referred to as a 'scaffold modification'. The original pentose sugar may be replaced in its
15 entirety by another moiety that similarly connects the base and the phosphate. It is therefore understood that while a pentose sugar is often a scaffold, a scaffold is not necessarily a pentose sugar. Examples of scaffold modifications that may be applied in the monomers of the guide oligonucleotides as disclosed herein are disclosed in Intl. Patent Application Publication Nos. WO2020/154342, WO2020/154343, and WO2020/154344.

 A nucleoside in the guide oligonucleotides as disclosed herein may be a natural
20 nucleoside (deoxyribonucleoside or ribonucleoside) or a non-natural nucleoside. It is noted that for RNA editing, in which double-stranded RNA is generally the substrate for enzymes with deamination activity (such as ADARs), ribonucleosides are considered 'natural', while deoxyribonucleosides may then be, for the sake of argument, considered as non-natural, or modified, simply because DNA is not present in the RNA-RNA double stranded (natural)
25 substrate configurations. The skilled person appreciates that when the nucleotide has a natural ribose moiety, it may still be non-naturally modified in the base and/or the linkage.

 It is recognized in the art that common limiting factors in oligonucleotide-based therapies are the oligonucleotide's ability to be taken up by the cell (when delivered per se, or 'naked' without applying a delivery vehicle such as a viral vector or plasmid), the biodistribution
30 and the resistance to nuclease-mediated breakdown. The skilled person is aware, and it has been described in detail in the art, that a variety of chemical modifications can assist in overcoming such limitations. Examples of such now commonly used chemical modifications are the 2'-OMe, 2'-F and 2'-MOE modifications of the sugar and the use of PS linkages between nucleosides, as described herein.

Ribose modifications

The ribose 2' groups in all nucleotides of the guide oligonucleotides as disclosed herein, except for the ribose sugar moiety of the orphan nucleotide that has certain limitations in respect of compatibility with RNA editing, can be independently selected from 2'-H (i.e., DNA), 2'-OH (i.e., RNA), 2'-OMe, 2'-MOE, 2'-F, or 2'-4'-linked (for instance a locked nucleic acid (LNA)), or other ribosyl 1'-substitutions, 2' substitutions, 3' substitutions, 4' substitutions or 5' substitutions. The orphan nucleotide in the guide oligonucleotide that comprises no other chemical modifications to the ribose sugar, the base, or the linkage preferably does not carry a 2'-OMe or 2'-MOE substitution when the nucleobase is a naturally occurring cytosine, but may carry a 2'-F, a 2',2'-difluoro (diF), or 2'-ara-F (FANA) substitution or may be DNA. Intl. Patent Application Publication No. WO2024/013360 describes the modification of the 2' position of the ribose sugar moiety of the orphan nucleotide by a 2',2'-disubstituted substitution such as diF, which is also applicable to what is disclosed here. The 2'-4' linkage can be selected from many linkers known in the art, such as a methylene linker, amide linker, or constrained ethyl linker (cEt).

A guide oligonucleotide as disclosed herein may comprise one or more nucleotides carrying a 2'-MOE ribose modification. Also, a guide oligonucleotide as disclosed herein may comprise one or more nucleotides not carrying a 2'-MOE ribose modification, or wherein the 2'-MOE ribose modifications are at positions that do not prevent the enzyme with adenosine deaminase activity from deaminating the target adenosine. A guide oligonucleotide as disclosed herein may comprise a 2'-OMe ribose modification at a position that does not comprise a 2'-MOE ribose modification. A guide oligonucleotide as disclosed herein may comprise deoxynucleotides at positions that do not comprise a 2'-MOE or a 2'-OMe ribose modification, or other 2' ribose substitution. A guide oligonucleotide as disclosed herein may comprise one or more nucleotides comprising a 2' substitution comprising a 2'-MOE, 2'-OMe, 2'-OH, 2'-deoxy, TNA, 2'-fluoro (2'-F), 2',2'-difluoro (diF) modification, 2'-fluoro-2'-C-methyl modification, or a 2'-4'-linkage (i.e., a bridged nucleic acid such as an LNA, or examples mentioned in e.g. Intl. Patent Application Publication No. WO2018/007475). Other nucleic acid monomers that may be used in a guide oligonucleotide as disclosed herein are arabinonucleic acids and 2'-deoxy-2'-fluoroarabinonucleic acid (FANA), for instance for improved affinity purposes. The 2'-4' linkage can be selected from linkers known in the art, such as a methylene linker or constrained ethyl linker. A wide variety of 2' modifications that may present in a guide oligonucleotide as disclosed herein are known in the art, including but not limited to the modifications outlined in detail in Intl. Patent Application Publication Nos. WO2016/097212, WO2017/220751, WO2018/041973, WO2018/134301, WO2019/219581, WO2019/158475, and WO2022/099159. In all cases, the modifications should be compatible with RNA editing such that the guide oligonucleotide fulfils its role as an oligonucleotide that can form a double

stranded complex with the target RNA and by generating this double-stranded nucleic acid complex, recruit a deaminating enzyme, which can subsequently deaminate the target adenosine. Where a monomer in a guide oligonucleotide as disclosed herein comprises an UNA ribose modification, that monomer can have a 2' position comprising the same modifications discussed above, such as a 2'-MOE, a 2'-OMe, a 2'-OH, a 2'-deoxy, a 2'-F, a 2',2'-diF, a 2'-fluoro-2'-C-methyl, an arabinonucleic acid, a FANA, or a 2'-4'-linkage (i.e., a bridged nucleic acids such as an LNA). In an aspect, the guide oligonucleotide as disclosed herein comprises at least one nucleotide comprising a threose nucleic acid (TNA) ribose modification. In one aspect, the guide oligonucleotide as disclosed herein comprises at least one nucleotide with a sugar moiety that comprises a 2'-F modification. A preferred position for the nucleotide that carries a 2'-F modification is position -3 in the guide oligonucleotide, which may be present together with an identical 2' modification in the orphan nucleotide as discussed above.

Base modifications

A base, sometimes called a nucleobase, is generally adenine, cytosine, guanine, thymine or uracil, or a derivative thereof. A nucleobase is defined as a moiety that can bond to another nucleobase through H-bonds, polarized bonds (such as through CF moieties) or aromatic electronic interactions. Cytosine, thymine, and uracil are pyrimidine bases, and are generally linked to the scaffold through their 1-nitrogen. Adenine and guanine are purine bases and are generally linked to the scaffold through their 9-nitrogen. The terms 'adenine', 'guanine', 'cytosine', 'thymine', 'uracil' and 'hypoxanthine' as used herein refer to the nucleobases as such. The terms 'adenosine', 'guanosine', 'cytidine', 'thymidine', 'uridine' and 'inosine' refer to the nucleobases linked to the (deoxy)ribosyl sugar. The nucleobases in a guide oligonucleotide as disclosed herein can be adenine, cytosine, guanine, thymine, or uracil or any other moiety able to interact with another nucleobase through H-bonds, polarized bonds (such as CF) or aromatic electronic interactions. The nucleobases at any position in the guide oligonucleotide as disclosed herein can be a modified form of adenine, cytosine, guanine, or uracil, such as hypoxanthine (the nucleobase in inosine), pseudouracil, pseudocytosine, isouracil, N3-glycosylated uracil, 1-methylpseudouracil, orotic acid, agmatidine, lysidine, 2-thiouracil, 2-thiothymine, 5-substituted pyrimidine (e.g., 5-halouracil, 5-halomethyluracil, 5-trifluoromethyluracil, 5-propynyluracil, 5-propynylcytosine, 5-aminomethyluracil, 5-hydroxymethyluracil, 5-formyluracil, 5-aminomethylcytosine, 5-formylcytosine), 5-hydroxymethylcytosine, 7-deazaguanine, 7-deazaadenine, 7-deaza-2,6-diaminopurine, 8-aza-7-deazaguanine, 8-aza-7-deazaadenine, 8-aza-7-deaza-2,6-diaminopurine, 8-oxo-adenine, 3-deazapurine (such as a 3-deaza-adenosine), pseudoisocytosine, N4-ethylcytosine, N2-cyclopentylguanine, N2-cyclopentyl-2-aminopurine, N2-propyl-2-

aminopurine, 2,6-diaminopurine, 2-aminopurine, G-clamp and its derivatives, Super A, Super T, Super G, amino-modified nucleobases or derivatives thereof; and degenerate or universal bases, like 2,6-difluorotoluene, or absent like abasic sites (e.g. 1-deoxyribose, 1,2-dideoxyribose, 1-deoxy-2-O-methylribose, azaribose). Modified bases comprise synthetic and natural bases such as inosine, xanthine, hypoxanthine and other -aza, deaza, -hydroxy, -halo, -thio, thiol, -alkyl, -alkenyl, -alkynyl, thioalkyl derivatives of pyrimidine and purine bases that are or will be known in the art. Purine nucleobases and/or pyrimidine nucleobases may be modified to alter their properties, for example by amination or deamination of the heterocyclic rings. The exact chemistries and formats may vary from oligonucleotide construct to oligonucleotide construct and from application to application, and may be worked out in accordance with the wishes and preferences of those of skill in the art.

A scaffold modification indicates the presence of a modified version of the ribosyl moiety as naturally occurring in RNA (i.e., the pentose moiety), such as bicyclic sugars, tetrahydropyrans, hexoses, morpholinos, 2'-modified sugars, 4'-modified sugar, 5'-modified sugars and 4'-substituted sugars. Examples of suitable modifications include, but are not limited to 2'-O-modified RNA monomers, such as 2'-O-alkyl or 2'-O-(substituted)alkyl such as 2'-OMe, 2'-O-(2-cyanoethyl), 2'-MOE, 2'-O-(2-thiomethyl)ethyl, 2'-O-butyryl, 2'-O-propargyl, 2'-O-allyl, 2'-O-(2-aminopropyl), 2'-O-(2-(dimethylamino)propyl), 2'-O-(2-amino)ethyl, 2'-O-(2-(dimethylamino)ethyl); 2'-deoxy (DNA); 2'-O-(haloalkyl)methyl such as 2'-O-(2-chloroethoxy)methyl (MCEM), 2'-O-(2,2-dichloroethoxy)methyl (DCEM); 2'-O-alkoxycarbonyl such as 2'-O-[2-(methoxycarbonyl)ethyl] (MOCE), 2'-O-[2-N-methylcarbamoyl]ethyl] (MCE), 2'-O-[2-(N,N-dimethylcarbamoyl)ethyl] (DCME); 2'-halo e.g. 2'-F, FANA; 2'-O-[2-(methylamino)-2-oxoethyl] (NMA); a bicyclic or bridged nucleic acid (BNA) scaffold modification such as a conformationally restricted nucleotide (CRN) monomer, an LNA monomer, a *xy/o*-LNA monomer, an α -LNA monomer, an α -I-LNA monomer, a β -d-LNA monomer, a 2'-amino-LNA monomer, a 2'-(alkylamino)-LNA monomer, a 2'-(acylamino)-LNA monomer, a 2'-N-substituted 2'-amino-LNA monomer, a 2'-thio-LNA monomer, a (2'-O,4'-C) constrained ethyl (cEt) BNA monomer, a (2'-O,4'-C) constrained methoxyethyl (cMOE) BNA monomer, a 2',4'-BNA^{NC}(NH) monomer, a 2',4'-BNA^{NC}(NMe) monomer, a 2',4'-BNA^{NC}(NBn) monomer, an ethylene-bridged nucleic acid (ENA) monomer, a carba-LNA (cLNA) monomer, a 3,4-dihydro-2H-pyran nucleic acid (DpNA) monomer, a 2'-C-bridged bicyclic nucleotide (CBBN) monomer, an oxo-CBBN monomer, a heterocyclic-bridged BNA monomer (such as triazolyl or tetrazolyl-linked), an amido-bridged BNA monomer (such as AmNA), an urea-bridged BNA monomer, a sulfonamide-bridged BNA monomer, a bicyclic carbocyclic nucleotide monomer, a TriNA monomer, an α -I-TriNA monomer, a bicyclo DNA (bcDNA) monomer, an F-bcDNA monomer, a tricyclo DNA (tcDNA) monomer, an F-tcDNA monomer, an alpha anomeric bicyclo DNA (abcDNA) monomer, an oxetane nucleotide monomer, a

locked PMO monomer derived from 2'-amino LNA, a guanidine-bridged nucleic acid (GuNA) monomer, a spirocyclopropylene-bridged nucleic acid (scpBNA) monomer, and derivatives thereof; cyclohexenyl nucleic acid (CeNA) monomer, alatriol nucleic acid (ANA) monomer, hexitol nucleic acid (HNA) monomer, fluorinated HNA (F-HNA) monomer, pyranosyl-RNA (p-RNA) monomer, 3'-deoxypyranosyl DNA (p-DNA), UNA; an inverted version of any of the monomers above. All these modifications are known to the person skilled in the art.

The orphan nucleotide

Mutagenesis studies of human ADAR2 revealed that a single mutation at residue 488 from glutamate to glutamine (E488Q), gave an increase in the rate constant of deamination by 60-fold when compared to the wild-type enzyme (Kuttan and Bass. *Proc Natl Acad Sci USA*. 2012, 109(48):3295-3304). During the deamination reaction, ADAR flips the edited base out of its RNA duplex, and into the enzyme active site (Matthews et al. *Nat Struct Mol Biol* 2016. 23(5):426-433). When ADAR2 edits adenosines in the preferred context (an A:C mismatch) the nucleotide opposite the target adenosine is often referred to as the 'orphan nucleotide' (or 'orphan cytidine' as the case may be), as indicated above. The crystal structure of ADAR2 E488Q bound to double stranded RNA (dsRNA) revealed that the glutamine (Gln; Q) side chain at position 488 can donate an H-bond to the N3 position of the orphan cytidine, which leads to the increased catalytic rate of ADAR2 E488Q. In the wild-type enzyme, wherein a glutamate (or glutamic acid; Glu; E) is present at position 488 instead of a glutamine (Gln) the amide group of the glutamine is absent and is instead a carboxylic acid. To obtain the same contact of the orphan cytidine with the E488Q mutant would then, for the wild-type situation, require protonation for this contact to occur. To make use of endogenously expressed ADAR2 to correct disease relevant mutations, it is essential to maximize the editing efficiency of the wild type ADAR2 enzyme present in the cell. Intl. Patent Application Publication No. WO2020/252376 discloses the use of guide oligonucleotides with modified RNA bases, especially at the position of the orphan cytidine to mimic the hydrogen-bonding pattern observed by the E488Q ADAR2 mutant. By replacing the nucleotide opposite the target adenosine in the guide oligonucleotide with cytidine analogs that serve as H-bond donors at N3, it was envisioned that it would be possible to stabilize the same contact that is believed to provide the increase in catalytic rate for the mutant enzyme. Two cytidine analogs were of particular interest: pseudoisocytidine (also referred to as 'piC'; Lu et al. *J Org Chem*. 2009, 74(21):8021-8030; Burchenal et al. *Cancer Res*. 1976, 36:1520-1523) and Benner's base Z (also referred to as 'dZ'; Yang et al. *Nucl Acid Res*. 2006, 34(21):6095-6101) that were initially selected because they offer hydrogen-bond donation at N3 with minimal perturbation to the shape of the nucleobase. Benner's base is also referred to with its chemical name 6-amino-5-nitro-3-yl-2(1H)-pyridone. The presence of the cytidine analog in the guide

oligonucleotide may exist in addition to modifications to the ribose 2' group. The ribose 2' groups in the orphan nucleotide can be independently selected from 2'-H (i.e., DNA), 2'-OH (i.e., RNA), 2'-OMe, 2'-MOE, 2'-F, or 2'-4'-linked (i.e., a bridged nucleic acid such as an LNA), or other 2' substitutions. The 2'-4' linkage can be selected from linkers known in the art, such as a methylene linker or constrained ethyl linker.

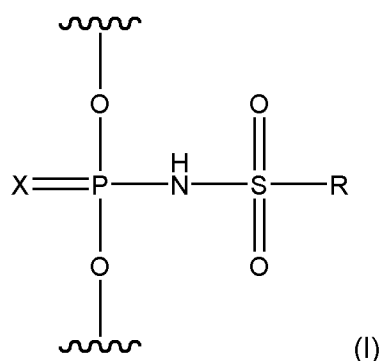
The orphan nucleotide in the guide oligonucleotide as disclosed herein is preferably a cytidine or analog thereof (such as a nucleotide carrying a Benner's base) or a uridine or analog thereof (such as iso-uridine). The orphan nucleotide, whether it is a cytidine or analog thereof, or a uridine or analog thereof, preferably comprises a deoxyribose (2'-H; = DNA) but may also comprise a diF modification at the 2' position of the sugar. In an embodiment at least one and in another embodiment both the neighbouring (directly adjacent) nucleotides flanking the orphan nucleotide do not comprise a 2'-OMe modification. Complete modification wherein all nucleotides of the oligonucleotide hold a 2'-OMe modification (including the orphan nucleotide), with natural bases, results in a non-functional oligonucleotide as far as RNA editing goes (known in the art), presumably because it hinders the ADAR activity at the targeted position. In general, an adenosine in a target RNA can be protected from editing by providing an opposing nucleotide with a 2'-OMe group (at least when there are no other chemical substitutions or modifications within the nucleotide), or by providing a guanine or adenine as opposing base, as these two nucleobases are also able to reduce editing of the opposing adenosine.

Linkage modifications

A nucleoside is generally connected to neighboring nucleosides through condensation of its 5'-phosphate moiety to the 3'-hydroxyl moiety of the neighboring nucleotide monomer. Similarly, its 3'-hydroxyl moiety is generally connected to the 5'-phosphate of a neighboring nucleotide monomer. This forms PO bonds. The PO and the scaffold form an alternating copolymer. The bases are grafted on this copolymer, namely to the scaffold moieties. Because of this characteristic, the alternating copolymer formed by linked scaffolds of an oligonucleotide is often called the 'backbone' of the oligonucleotide. Because PO bonds connect neighboring monomers together, they are often referred to as 'backbone linkages'. It is understood that when a phosphate group is modified so that it is instead an analogous moiety such as a PS, such a moiety is still referred to as the backbone linkage of the monomer. This is referred to as a 'backbone linkage modification'. In general terms, the backbone of an oligonucleotide comprises alternating scaffolds and backbone linkages.

As outlined in detail herein, naked guide oligonucleotides as disclosed herein comprise at least one, preferably multiple linkage modifications. It is generally more preferred that the guide oligonucleotide as disclosed herein comprises linkage modifications at most, and

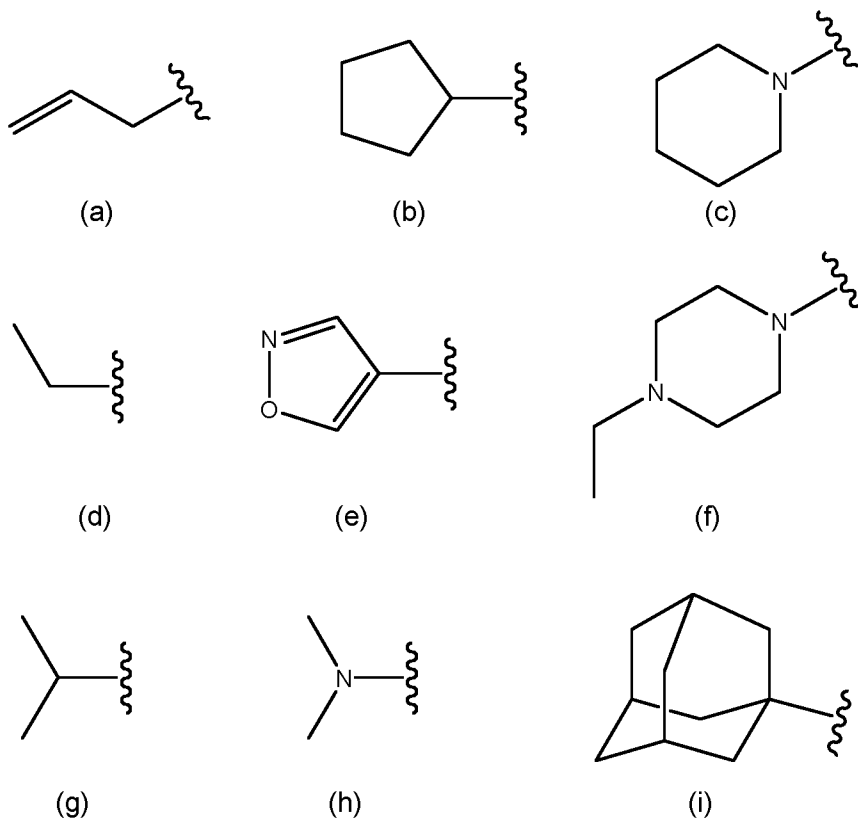
potentially all positions if the guide oligonucleotide is capable of mediating editing. A linkage modification can be, but is not limited to, a modified version of the PO present in RNA, such as PS, chirally pure PS, (*R*)-PS, (*S*)-PS, MP, chirally pure MP, (*R*)-MP, (*S*)-MP, phosphoryl guanidine (such as PNdmi), chirally pure phosphoryl guanidine, (*R*)-phosphoryl guanidine, (*S*)-phosphoryl guanidine, phosphorodithioate (PS₂), phosphonacetate (PACE), phosphonoacetamide (PACA), thiophosphonoacetate, thiophosphonoacetamide, methyl phosphorohioate, methyl thiophosphonate, PS prodrug, alkylated PS, H-phosphonate, ethyl phosphate, ethyl PS, boranophosphate, borano PS, metyl boranophosphate, methyl borano PS, methyl boranophosphonate, methyl boranophosphothioate, phosphate, phosphotriester, aminoalkylphosphotriester, and their derivatives. Another modification includes phosphoramidite, phosphoramidate, N3'→P5' phosphoramidate, phosphorodiamidate, phosphorothiodiamidate, sulfamate, diethylenesulfoxide, amide, sulfonate, siloxane, sulfide, sulfone, formacetyl, alkenyl, methylenehydrazino, sulfonamide, triazole, oxalyl, carbamate, methyleneimino (MMI), and thioacetamide nucleic acid (TANA); and their derivatives. Various salts, mixed salts, deprotonated, protonated, tautomeric, and free acid forms are also included, as well as 3'→3' and 2'→5' linkages. A guide oligonucleotide as disclosed herein may also comprise one or more linkage modifications according to the structure of formula (I):



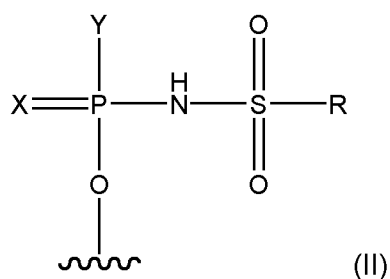
wherein:

X = O or S; and

R = an aryl, a substituted aryl, a heterocycle, a substituted heterocycle, an aromatic heterocycle, a substituted aromatic heterocycle, a C₁-C₆ alkoxy, a substituted C₁-C₆ alkoxy, a C₁-C₂₀ alkyl, a substituted C₁-C₂₀ alkyl, a C₁-C₆ alkenyl, a C₁-C₆ substituted alkenyl, a C₁-C₆ alkynyl, a substituted C₁-C₆ alkynyl, or a conjugate group. In a preferred embodiment, X = O and R = methyl, in which the linkage modification is referred to as MsPA or PNms. In other preferred aspects, R equals one of the following structures (a), (b), (c), (d), (e), (f), (g), (h), or (i):



Disclosed herein is also a guide oligonucleotide that is able to mediate adenosine deamination by recruitment of a deaminating enzyme in a cell after the guide oligonucleotide has formed a double-stranded complex with a region of a target RNA nucleic acid molecule in a cell, wherein the region comprises a target adenosine, wherein the deaminating enzyme can deaminate the target adenosine into an inosine, and wherein the guide oligonucleotide comprises a moiety at one and/or both termini with a structure according to formula (II):



wherein: X = O or S ;

10 Y = O⁻ or S⁻; and

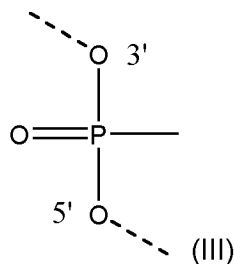
R = an aryl, a substituted aryl, a heterocycle, a substituted heterocycle, an aromatic heterocycle, a substituted aromatic heterocycle, a C₁-C₆ alkoxy, a substituted C₁-C₆ alkoxy, a C₁-C₂₀ alkyl, a substituted C₁-C₂₀ alkyl, a C₁-C₆ alkenyl, a C₁-C₆ substituted alkenyl, a C₁-C₆

alkynyl, a substituted C₁-C₆ alkynyl, or a conjugate group. In a preferred embodiment, X = O and R = methyl.

A guide oligonucleotide as disclosed herein may comprise a substitution of one of the non-bridging oxygens in the PO linkage. This modification slightly destabilizes base pairing but adds significant resistance to nuclease degradation. A preferred nucleotide analogue or equivalent comprises PS, phosphonoacetate, phosphorodithioate, phosphotriester, aminoalkylphosphotriester, H-phosphonate, methyl and other alkyl phosphonate including 3'-alkylene phosphonate, 5'-alkylene phosphonate and chiral phosphonate, phosphinate, phosphoramidate including 3'-amino phosphoramidate and aminoalkylphosphoramidate, thionophosphoramidate, thionoalkylphosphonate, thionoalkylphosphotriester, selenophosphate or boranophosphate. Particularly preferred are internucleoside linkages that are modified to contain a PS. Particularly preferred are internucleoside linkages that are modified to contain a PNms. Particularly preferred are internucleoside linkages that are modified to contain a PNdmi. The regular internucleosidic linkages between the nucleotides may be altered by mono- or di-thioation of the PO bonds to yield PS esters or phosphorodithioate esters, respectively. Other modifications of the internucleosidic linkages are possible, including amidation and peptide linkers. The skilled person can determine for what target nucleic acid sequence the guide oligonucleotide comprises a certain linkage modification at each linkage position of the guide oligonucleotide as disclosed herein to generate the most effective and most stable oligonucleotide compound.

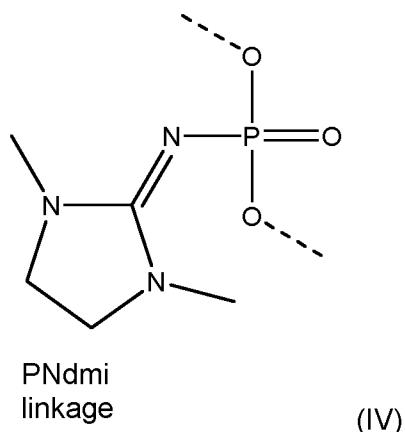
Many of the non-naturally occurring modification of the linkage, such as PS, are chiral. This means that there are Rp and Sp configurations, known to the person skilled in the art. In one embodiment, the chirality of the PS linkages is controlled, which means that each of the linkages is either in the Rp or in the Sp configuration, whichever is preferred. The choice of an Rp or Sp configuration at a specified linkage position may depend on the target sequence and the efficiency of binding and induction of causing editing of the target adenosine. However, if such is not specifically desired, a composition may comprise guide oligonucleotides as active compounds with both Rp and Sp configurations at a certain specified linkage position. Mixtures of such guide oligonucleotides are also feasible, wherein certain positions have preferably either one of the configurations, while for other positions such does not matter. In one aspect, the guide oligonucleotide as disclosed herein comprises one or more (chirally pure or chirally mixed) PS linkages. In one aspect, the guide oligonucleotide as disclosed herein comprises one or more (chirally pure or chirally mixed) phosphoramidate (PN) linkages. In one aspect, the guide oligonucleotide as disclosed herein comprises one or more (chirally pure or chirally mixed) PNms linkages. In one aspect, a PN linkage connects the terminal two nucleotides on each end of the guide oligonucleotide. Guide oligonucleotides as disclosed herein may also comprise linkage modifications at all positions that are not chirally controlled. The guide

oligonucleotide as disclosed herein may also comprise one or more naturally occurring internucleoside linkages. The choice and number of modified linkages may depend on the specific target, the sequence, the length, and the stability of the guide oligonucleotide observed in a particular cell type of interest, which can be assessed by methods known to the person skilled in the art. In one aspect, at least one, at least two, at least three, or at least four internucleoside linkages between the 5' and/or the 3' terminal two, three, four, or five nucleosides respectively of the guide oligonucleotide as disclosed herein are modified internucleoside linkages. In one aspect, the guide oligonucleotide as disclosed herein comprises at least one MP internucleoside linkage according to the structure of formula (III):



As was noted in the art, a preferred position for an MP linkage in a guide oligonucleotide is linkage position -2, thereby connecting the nucleoside at position -1 with the nucleoside at position -2. In a preferred embodiment, this position, in a guide oligonucleotide as disclosed herein, comprises a linkage modification according to the structure of formula (I), instead of an MP linkage. Intl. Patent Application Publication No. WO2020/201406 discloses the use of MP linkage modifications at certain positions surrounding the orphan nucleotide in the first nucleic acid strand. Although the presence of MP linkages is compatible with RNA editing by human ADAR enzymes, introducing MP linkages during the manufacturing of oligonucleotides is challenging in view of additional manufacturing (purification) steps in the coupling and decoupling process. In one aspect, the guide oligonucleotide does not comprise an MP linkage.

In one aspect, the guide oligonucleotide as disclosed herein comprises at least one PNdmi linkage, preferably linking the most terminal two nucleosides at the 5' and/or 3' end of the guide oligonucleotide. A PNdmi linkage as preferably used in the guide oligonucleotides as disclosed herein has the structure of formula (IV):



Other internucleoside linkages that may be used in the guide oligonucleotides as disclosed herein are those that are disclosed in Intl. Patent Application Publication No. WO2023/278589. In one aspect, the guide oligonucleotide as disclosed herein comprises at least one phosphonoacetate and/or at least one phosphonoacetamide internucleoside linkage.

Conjugate chemistries

In one aspect, the guide oligonucleotide is covalently or non-covalently, directly or through a linker, bound to a triterpene glycoside, preferably AG1856. AG1856 is also referred to as a 'saponin' (see Intl. Patent Application No. PCT/EP2024/051278, unpublished; and Intl. Patent Application Publication No. WO2021/122998). The saponin allows for improved endosomal release of the guide oligonucleotide once taken up by the target cell and may provide more efficient editing during treatment. The saponin, preferably AG1856, may be co-administered (or administered before/after administration of the guide oligonucleotide), but is preferably conjugated to the guide oligonucleotide, either directly or indirectly through one or more linking moieties. The skilled person is, based on these teachings, capable of to find the best format of such conjugates to serve the purpose of targeting the *RELN* pre-mRNA and/or mRNA and to direct the guide oligonucleotide to the target cell of choice.

In one aspect, the guide oligonucleotide as disclosed herein, or the sense strand to which it may be annealed before entering a target cell (in an HEON as disclosed herein), is bound to a hydrophobic moiety, such as palmityl or an analog thereof, cholesterol or analog thereof, or tocopherol or analog thereof. It is preferably bound to the 5' terminus. In case a hydrophobic moiety is bound to the 5' terminus as well as to the 3' terminus, such hydrophobic moieties may be the same or different. The hydrophobic moiety bound to the oligonucleotide may be bound directly, or indirectly mediated by another substance. When the hydrophobic moiety is bound directly, it is sufficient if the moiety is bound via a covalent bond, an ionic bond, a hydrogen bond, or the like. When the hydrophobic moiety is bound indirectly, it may be bound

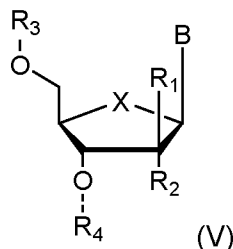
via a linking group (a linker). The linker may be a cleavable or an uncleavable linker. A cleavable linker refers to a linker that can be cleaved under physiological conditions, for example, in a cell or an animal body (e.g., a human body). A cleavable linker is selectively cleaved by an endogenous enzyme such as a nuclease, or by physiological circumstances specific to parts of the body or cell, such as pH or reducing environment (such as glutathione concentrations). Examples of a cleavable linker comprise, but is not limited to, an amide, an ester, one or both esters of a PO, a phosphoester, a carbamate, and a disulfide bond, as well as a natural DNA linker. Cleavable linkers also include self-immolative linkers. An uncleavable linker refers to a linker that is not cleaved under physiological conditions, or very slowly compared to a cleavable linker, for example, in a PS linkage, modified or unmodified deoxyribonucleosides linked by a PS linkage, a spacer connected through a PS bond and a linker consisting of modified or unmodified ribonucleosides. There is no restriction on the chain length, when a linker is a nucleic acid such as DNA, or an oligonucleotide. However, it may be usually from 2 to 20 bases in length, from 3 to 10 bases in length, or from 4 to 6 bases in length. There is no restriction on the length or composition of a spacer that connects the ligand and the oligonucleotide, and may include for example ethylene glycol, triethylene glycol (TEG), HEG, alkyl chains, propyl, 6-aminohexyl, or dodecyl. One or more other types of molecules may be bound to the guide oligonucleotide through one or more linkers, including peptides, sugars, vitamins, polymers, aptamers, (fragments of) antibodies, small molecules, and the like.

General

In addition to the specific preferred chemical modifications at certain positions in compounds as disclosed herein, guide oligonucleotides as disclosed herein may comprise one or more (additional) modifications to the nucleobase, scaffold and/or backbone linkage, which may or may not be present in the same monomer, for instance at the 3' and/or 5' position. In one aspect, the guide oligonucleotide as disclosed herein comprises at least one internucleoside linkage according to the structure of formula (I), and/or the guide oligonucleotide further comprises at least one nucleotide with a sugar moiety that comprises a 2'-OMe modification, and/or the guide oligonucleotide comprises at least one nucleotide with a sugar moiety that comprises a 2'-MOE modification, and/or the guide oligonucleotide comprises at least one nucleotide with a sugar moiety that comprises a 2'-F modification, and/or the guide oligonucleotide comprises an orphan nucleotide that carries a 2'-H in the sugar moiety and is therefore referred to as a DNA nucleotide, even though additional modifications may exist in its base and/or linkage to its neighbouring nucleosides. In one aspect, the orphan nucleotide carries a 2'-F in the sugar moiety. In one aspect, the orphan nucleotide carries a diF substitution in the sugar moiety. In one aspect, the orphan nucleotide

carries a 2'-F and a 2'-C-methyl in the sugar moiety. In one aspect, the orphan nucleotide comprises a 2'-F in the arabinose configuration (FANA) in the sugar moiety.

In one aspect, the guide oligonucleotide is an antisense oligonucleotide that can form a double stranded nucleic acid complex with portion of a target *RELN* nucleic acid sequence comprising a target adenosine, wherein the double stranded nucleic acid complex can recruit an adenosine deaminating enzyme for deamination of the target adenosine in the target RNA molecule, wherein the nucleotide in the guide oligonucleotide that is opposite the target adenosine is the orphan nucleotide, and wherein the orphan nucleotide has the structure of formula (V):



wherein: X is O, NH, OCH₂, CH₂, Se, or S; B is a nitrogenous base selected from the group consisting of: cytosine, uracil, isouracil, N3-glycosylated uracil, pseudoisocytosine, 8-oxo-adenine, and 6-amino-5-nitro-3-yl-2(1H)-pyridone; R₁ and R₂ are both selected, independently, from H, OH, F or CH₃; R₃ is the part of the guide oligonucleotide that is 5' of the orphan nucleotide, consisting of 6, 7 or 8 nucleotides; and R₄ is the part of the guide oligonucleotide that is 3' of the orphan nucleotide, consisting of 16, 17, 18, 19, 20, 21, 22, 23, or 24 nucleotides. The nucleotide 3' and/or 5' from the orphan nucleotide may be DNA, more preferably the nucleotide at the 3' (position -1).

Other chemical modifications of the guide oligonucleotide as disclosed herein include the substitution of one or more than one of any of the hydrogen atoms with deuterium or tritium, examples of which can be found in e.g., Intl. Patent Application Publication Nos. WO2014/022566 or WO2015/011694. Again, in all cases, the modifications should be compatible with editing such that the guide oligonucleotide fulfils its role as an oligonucleotide that can, after binding to its target sequence, recruit an adenosine deaminase enzyme because of the double-stranded nucleic acid entity that arises. In all aspects of the disclosure, the enzyme with adenosine deaminase activity is preferably ADAR1, ADAR2, or ADAT.

Guide oligonucleotides as disclosed herein preferably do not include a 5'-terminal O6-benzylguanosine or a 5'-terminal amino modification and preferably are not covalently linked to a SNAP-tag domain (an engineered O6-alkylguanosine-DNA-alkyl transferase). A guide oligonucleotide as disclosed herein preferably does not comprise a boxB RNA hairpin sequence. In one aspect, a guide oligonucleotide as disclosed herein comprises 0, 1, 2 or 3 wobble base pairs with the target sequence, and/or 0, 1, 2, 3, 4, 5, 6, 7, or 8 mismatching base

pairs with the target RNA sequence. No mismatch exists when the orphan nucleotide is uridine, which may be defined differently when the orphan nucleotide is a uridine analog or derivative. One alternative for uridine is positioning an iso-uridine opposite the target adenosine, which likely does not pair like G pairs with U. Preferably, the target adenosine in the target sequence forms a mismatch base pair with the nucleoside in the guide oligonucleotide that is directly opposite the target adenosine.

As outlined above, a guide oligonucleotide as disclosed herein makes use of specific nucleotide modifications at predefined spots to ensure stability as well as proper ADAR binding and activity. These changes may vary and may include modifications in the backbone of the guide oligonucleotide, in the sugar moiety of the nucleotides as well as in the nucleobases or the PO linkages, as outlined in detail herein. They may also be variably distributed throughout the sequence of the guide oligonucleotide. Specific modifications may be needed to support interactions of different amino acid residues within the RNA-binding domains of ADAR enzymes, as well as those in the deaminase domain. For example, PS linkages between nucleotides or 2'-OMe or 2'-MOE modifications may be tolerated in some parts of the guide oligonucleotide, while in other parts they should be avoided so as not to disrupt crucial interactions of the enzyme with the phosphate and 2'-OH groups. Specific nucleotide modifications may also be necessary to enhance the editing activity on substrate RNAs where the target sequence is not optimal for ADAR editing. Previous work has established that certain sequence contexts are more amenable to editing. For example, a target sequence 5'-UAG-3' (with the target A in the middle) contains the most preferred nearest-neighbor nucleotides for ADAR2, whereas a 5'-CAA-3' target sequence is disfavored (Schneider et al. *Nucleic Acids Res.* 2014, 42(10):e87). The structural analysis of ADAR2 deaminase domain hints at the possibility of enhancing editing by careful selection of the nucleotides that are opposite to the target trinucleotide. For example, the 5'-CAA-3' target sequence, paired to a 3'-GCU-5' sequence on the opposing strand (with the A-C mismatch formed in the middle), is disfavored because the guanosine base sterically clashes with an amino acid side chain of ADAR2. The guanosine opposite the C in such circumstances is preferably replaced by an inosine (hence, at the -1 position within the guide oligonucleotide), more preferably an Id, as further outlined in the present disclosure.

The guide oligonucleotide as disclosed herein, in contrast to what has been described for siRNA, or gapmers and their relation towards RNase breakdown and the use of such gapmers in double-stranded complexes (see for instance EP 3954395 A1), does not comprise a stretch of DNA nucleotides which would make a target sequence (or a sense nucleic acid strand) a target for RNase-mediated breakdown. It is not desired that the target transcript molecule is degraded through the binding of the guide oligonucleotide to the transcript molecule. In one embodiment, the guide oligonucleotide does not comprise four or more

consecutive DNA nucleotides anywhere within its sequence. In an embodiment, the guide oligonucleotide is composed of as much (chemically) modified nucleotides as possible to enhance the resistance towards RNase-mediated breakdown, while at the same time being as efficient as possible in producing an RNA editing effect. This means that the orphan nucleotide and several other nucleotides within the guide oligonucleotide may be DNA, but also that there is no stretch of four or more consecutive DNA nucleotides within the guide oligonucleotide. Hence, the guide oligonucleotide as disclosed herein is not a gapmer. A gapmer reduces the expression of a target transcript but does not produce RNA editing of a specified adenosine within the target transcript. A gapmer is in principle a single-stranded nucleic acid consisting of a central region (DNA gap region with at least four consecutive deoxyribonucleotides) and wing regions positioned directly at the 5' end (5' wing region) and the 3' end (3' wing region) thereof. In contrast, the guide oligonucleotide as disclosed herein may be any oligonucleotide that produces an RNA editing effect in which a target adenosine in a target RNA molecule is deaminated to an inosine, and accordingly is resistant to RNase-mediated breakdown as much as possible to yield this effect and to allow the mRNA transcript being translated into a protein.

The guide oligonucleotides as disclosed herein may also be administered in the context of aids that will increase the entry of the guide oligonucleotide into the target cell and/or its endosomal escape as soon as it is in the cell. Moieties that can be applied for such applications are for example a set of chemical compounds (generally purified from nature) referred to as "saponins" or "triterpene glycosides". A preferred saponin that can be used in the methods as disclosed herein is AG1856, disclosed in Intl. Patent Application Publication No. WO2021/122998 and further described for use with RNA editing producing oligonucleotides in Intl. Patent Application No. PCT/EP2024/051278 (unpublished).

Compositions and methods

Disclosed herein is also a pharmaceutical composition comprising the guide oligonucleotide as disclosed herein, and further comprising a pharmaceutically acceptable carrier, solvent, diluent, and/or other additive (such as a saponin or triterpene glycoside like AG1856 (as discussed above), which in fact may also be administered separately from the guide oligonucleotide) and may be dissolved in a pharmaceutically acceptable organic solvent, or the like. Dosage forms in which the guide oligonucleotide or the pharmaceutical composition are administered may depend on the disorder to be treated and the tissue that needs to be targeted and can be selected according to common procedures in the art. The pharmaceutical compositions may be administered by a single-dose administration or by multiple dose administration. It may be administered daily or at appropriate time intervals, which may be

determined using common general knowledge in the field and may be adjusted based on the disorder and the efficacy of the active ingredient.

Although in a preferred embodiment, the guide oligonucleotide as disclosed herein is a single-stranded oligonucleotide comprising an orphan nucleotide opposite the target nucleotide, wherein the orphan nucleotide is chemically modified as disclosed herein, and wherein the remainder of the oligonucleotide is chemically modified to prevent it from nuclease breakdown also as disclosed herein, in another embodiment, disclosed is any kind of oligonucleotide or heteroduplex oligonucleotide complex, that may or may not be bound to hairpin structures (internally or at the terminal end(s)), that may be bound to nucleic acid editing entities or catalytic domains thereof, or wherein the oligonucleotide is in a circular format. In a preferred aspect, the guide oligonucleotide as disclosed herein is a 'naked' oligonucleotide, comprising a variety of chemical modifications in the ribose sugar and/or the base of one or more of the nucleotides within the sequence, that preferably comprises at least one linkage according to the structure of formula (I) as disclosed herein, that can hybridize to the target nucleic acid sequence or a part thereof that includes the target adenosine, and can recruit endogenous (naturally present) nucleic acid editing entity in the target cell for the editing of the target nucleotide. In another aspect, the guide oligonucleotide as disclosed herein, that is delivered in a 'naked' form, does not comprise a stem-loop structure for recruitment of the deaminating enzyme, which allows for a shorter guide oligonucleotide and improved cellular delivery and trafficking.

It is known in the art that RNA editing entities (such as human ADAR enzymes) edit dsRNA structures with varying specificity, depending on several factors. One important factor is the degree of complementarity of the two strands making up the dsRNA sequence. Perfect complementarity of the two strands usually causes the catalytic domain of human ADAR to deaminate adenosines in a non-discriminative manner, reacting with any adenosine it encounters. The specificity of hADAR1 and 2 can be increased by introducing chemical modifications and/or ensuring several mismatches in the dsRNA, which presumably helps to position the dsRNA binding domains in a way that has not been clearly defined yet. Additionally, the deamination reaction itself can be enhanced by providing an oligonucleotide that comprises a mismatch opposite the adenosine to be edited. Following the instructions in the present application, those of skill in the art will be capable of designing the complementary portion of the oligonucleotide according to their needs.

It will be understood by a person having ordinary skill in the art that the extent to which the editing entities, such as editing enzymes, inside the cell are redirected to other target sites may be regulated by varying the affinity of the first nucleic acid strand for the recognition domain of the editing entity. The exact modification may be determined through some trial and error and/or through computational methods based on structural interactions between the

guide oligonucleotide and the recognition domain of the editing enzyme. In addition, or alternatively, the degree of recruiting and redirecting the editing enzyme resident in the cell may be regulated by the dosing and the dosing regimen of the guide oligonucleotide. This is something to be determined by the experimenter (*in vitro*) or the clinician, usually in phase I and/or II clinical trials.

Disclosed herein is the site-specific editing of target adenosines in RNA sequences in eukaryotic, preferably metazoan, more preferably mammalian, more preferably neuronal cells, more preferably human neuronal cells, and most preferably human cells from the central nervous system. The target cell can be located *in vitro*, *ex vivo* or *in vivo*. One advantage of the guide oligonucleotide as disclosed herein is that it can be used with cells *in situ* in a living organism, but it can also be used with cells in culture. In some embodiments cells are treated *ex vivo* and are then introduced into a living organism (e.g., re-introduced into an organism from whom they were originally derived). The guide oligonucleotide as disclosed herein can also be used to edit target RNA sequences in cells from a transplant or within a so-called organoid, e.g., a brain tissue organoid. Organoids can be thought of as three-dimensional *in vitro*-derived tissues but are driven using specific conditions to generate individual, isolated tissues. In a therapeutic setting they are useful because they can be derived *in vitro* from a patient's cells, and the organoids can then be re-introduced to the patient as autologous material which is less likely to be rejected than a normal transplant.

Without wishing to be bound by theory, the RNA editing through human ADAR2 for example is thought to take place on primary transcripts in the nucleus, during transcription or splicing, or in the cytoplasm, where e.g., mature mRNA, miRNA or ncRNA can be edited. Generally spoken, RNA editing may be used to create RNA sequences with different properties. Such properties may be coding properties (creating proteins with different sequences or length, leading to altered protein properties or functions), or binding properties (causing inhibition or over-expression of the RNA itself or a target or binding partner; entire expression pathways may be altered by recoding miRNAs or their cognate sequences on target RNAs). Protein function or localization may be changed at will, by functional domains or recognition motifs, including but not limited to signal sequences, targeting or localization signals, recognition sites for proteolytic cleavage or co- or post-translational modification, catalytic sites of enzymes, binding sites for binding partners, signals for degradation or activation and so on. These and other forms of RNA and protein "engineering", whether to prevent, delay or treat disease or for any other purpose, in medicine or biotechnology, as diagnostic, prophylactic, therapeutic, research tool or otherwise, are encompassed by the present disclosure.

The amount of guide oligonucleotide to be administered, the dosage and the dosing regimen can vary from cell type to cell type, the disease to be treated, the target population,

the mode of administration (e.g., systemic versus local), the severity of disease and the acceptable level of side activity, but these can and should be assessed by trial and error during *in vitro* research, in pre-clinical and clinical trials. The trials are particularly straightforward when the modified sequence leads to an easily detected phenotypic change, or a change in (the level of, or activity of) a specified biomarker (such as plasma levels of bile acids for example). It is possible that higher doses of guide oligonucleotides could compete for binding to an ADAR enzyme within a cell, thereby depleting the amount of the enzyme, which is free to take part in RNA editing, but routine dosing trials will reveal any such effects for a given guide oligonucleotide and a given target.

One suitable trial technique involves delivering the guide oligonucleotide to cell lines, or a test organism and then taking biopsy samples at various time points thereafter. The sequence of the target RNA can be assessed in the biopsy sample and the proportion of cells having the modification can easily be followed. As mentioned above, plasma level concentrations of bile acids in a sample from a treated subject is a proper biomarker for assessing the function of certain proteins in the subject, before and after treatment, or with or without treating the subject with a guide oligonucleotide as disclosed herein. After this trial has been performed once then the knowledge can be retained, and future delivery can be performed without needing to take biopsy samples. A method as disclosed herein can thus include a step of identifying the presence of the desired change in the cell's target RNA sequence, thereby verifying that the target RNA sequence has been modified. This step will typically involve sequencing of the relevant part of the target RNA, or a cDNA copy thereof (or a cDNA copy of a splicing product thereof, in case the target RNA is a pre-mRNA), as discussed above, and the sequence change can thus be easily verified. Alternatively, as indicated above, the change may be assessed on the function of the protein before, during, and/or after treatment or assessing any other potential marker, which measurements are preferably performed *in vitro* on samples obtained from the treated subject.

After RNA editing has occurred in a cell, the modified RNA can become diluted over time, for example due to cell division, limited half-life of the edited RNAs, etc. Thus, in practical therapeutic terms a method as disclosed herein may involve repeated delivery of a guide oligonucleotide until enough target RNAs have been modified to provide a tangible benefit to the patient and/or to maintain the benefits over time.

Guide oligonucleotides as disclosed herein are particularly suitable for therapeutic use, and so disclosed is also a pharmaceutical composition comprising a guide oligonucleotide as disclosed herein and a pharmaceutically acceptable carrier, solvent, or diluent. In some embodiments the pharmaceutically acceptable carrier can simply be a saline solution. This can usefully be isotonic or hypotonic, particularly for pulmonary delivery. The guide oligonucleotide as disclosed herein is suitably administered in aqueous solution, e.g. saline,

or in suspension, optionally comprising additives, excipients and other ingredients, compatible with pharmaceutical use, at concentrations ranging from 1 ng/ml to 1 g/ml, preferably from 10 ng/ml to 500 mg/ml, more preferably from 100 ng/ml to 100 mg/ml. Dosage may suitably range from between about 1 µg/kg to about 100 mg/kg, preferably from about 10 µg/kg to about 10 mg/kg, more preferably from about 100 µg/kg to about 1 mg/kg. Administration may be by inhalation (e.g., through nebulization), intranasally, orally, by injection or infusion, intravenously, subcutaneously, intradermally, intramuscularly, intra-tracheally, intra-peritoneally, intrarectally, intrathecally, intracerebroventricularly (e.g. in the intra-cisterna magna), parenterally, and the like. Administration may be in solid form, in the form of a powder, a pill, a gel, a solution, a slow-release formulation, or in any other form compatible with pharmaceutical use in humans.

In one embodiment, relating to the deamination effect of A to I conversion, the identification step of whether the editing has taken place, comprises the following steps: sequencing the target nucleic acid sequence; assessing the presence or absence of a non-, or less-functional protein; assessing whether splicing of pre-mRNA was altered by the deamination of a target adenosine in RNA; or using a functional read-out, because the target nucleic acid after the deamination should encode a protein with a lower or absent functionality, or on the other hand, an increased, regained or newly gained functionality. The identification of the deamination into inosine may be a functional read-out using a suitable biomarker. A functional assessment will generally be according to methods known to the skilled person. A suitable manner to identify the presence of an inosine after deamination of a target adenosine is dPCR or sequencing, using methods that are well-known to the person skilled in the art. However, the person skilled in the art of neurodegenerative disease will preferably apply tests to monitor certain biomarkers related to neurological function(s).

In one embodiment, a method as disclosed herein comprises the steps of administering to the subject a guide oligonucleotide or vector capable of expressing it, as disclosed herein, allowing the formation of a double stranded nucleic acid complex of the guide oligonucleotide with the target nucleic acid sequence in a cell in the subject; allowing the engagement of a nucleotide editing entity, such as an endogenously present adenosine deaminating enzyme, such as ADAR 1 or ADAR2; and allowing the entity to edit the target nucleotide in the target nucleic acid sequence, thereby alleviating, treating, ameliorating, or slowing down progression of the disease.

Nucleotide editing entities present in a cell will usually be proteinaceous in nature, such as the ADAR enzymes found in metazoans, including mammals. Particularly preferred are the human ADARs, hADAR1 and hADAR2, including any isoforms thereof. RNA editing enzymes known in the art, for which oligonucleotide constructs as disclosed herein may conveniently be designed, include the adenosine deaminases acting on RNA (ADARs), such as hADAR1

and hADAR2 in humans or human cells and cytidine deaminases. It is known that hADAR1 exists in two isoforms; a long 150 kDa interferon inducible version and a shorter, 110 kDa version, that is produced through alternative splicing from a common pre-mRNA. Consequently, the level of the 150 kDa isoform available in the cell may be influenced by interferon, particularly interferon-gamma (IFN- γ). hADAR1 is also inducible by TNF- α . This provides an opportunity to develop combination therapy, whereby IFN- γ or TNF- α and guide oligonucleotides as disclosed herein are administered to a patient either as a combination product, or as separate products, either simultaneously or subsequently, in any order. Certain disease conditions may already coincide with increased IFN- γ or TNF- α levels in certain tissues of a patient, creating further opportunities to make editing more specific for diseased tissues. It will be understood by a person having ordinary skill in the art that the extent to which the editing entities inside the cell are redirected to other target sites may be regulated by varying the affinity of the first nucleic acid strand for the recognition domain of the editing molecule.

In some embodiments, a guide oligonucleotide as disclosed herein can utilise endogenous cellular pathways and naturally available ADAR enzymes to specifically edit a target adenosine in the target RNA sequence. Certain guide oligonucleotides as disclosed herein are capable of recruiting ADAR and complexing with it, which then facilitates the deamination of a (single) specific target adenosine nucleotide in a target RNA sequence to which it is bound. In some embodiments, only one adenosine is deaminated. A guide oligonucleotide as disclosed herein, when complexed to ADAR, preferably brings about the deamination of a single target adenosine.

A guide oligonucleotide as disclosed herein, especially when it is in a naked form, is normally longer than 16 nucleotides. In one aspect the guide oligonucleotide as disclosed herein is longer than 20 nucleotides. The guide oligonucleotide as disclosed herein is preferably shorter than 100 nucleotides, still more preferably shorter than 60 nucleotides, still more preferably shorter than 50 nucleotides. In a preferred aspect, the guide oligonucleotide as disclosed herein comprises 18 to 70 nucleotides, more preferably comprises 18 to 60 nucleotides, and even more preferably comprises 18 to 50 nucleotides. Hence, in a particularly preferred aspect, the guide oligonucleotide as disclosed herein comprises 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, or 60 nucleotides. In one particularly preferred embodiment, the guide oligonucleotide as disclosed herein is 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, or 33 nucleotides in length. Preferably, the guide oligonucleotide, when it targets pre-mRNA and/or mRNA of the human *RELN* gene, has an a-symmetrical design, as shown in the accompanying examples. Preferably the length of the 5' part – seen from the orphan position that is position 0 – is 6, 7, or 8 nucleotides. Preferably the length of the 3' part

– seen from the orphan position that is position 0 – is 16, 17, 18, 19, 20, 21, 22, 23, or 24 nucleotides.

In an embodiment, the guide oligonucleotides are not for use with editing systems that do not require a guide oligonucleotide. In an embodiment, the guide oligonucleotides are not for use in generating a *Reln*-H3448R mutation in mice by homologous recombination. In an embodiment, the guide oligonucleotides are not for use in generating a mouse *Reln*-H3448R mutation and/or a human *RELN*-H3447R mutation by homologous recombination. In an embodiment, the guide oligonucleotides are not for use in generating a *Reln*-H3448R-Tg knock in mouse model carrying the *Reln*-COLBOS variant via homologous recombination.

In an embodiment, the nucleic acid editing enzyme does not edit using homologous recombination. In an embodiment, the nucleic acid editing enzyme does not generate a mouse *Reln*-H3448R mutation and/or a human *RELN*-H3447R mutation by homologous recombination. In an embodiment, the nucleic acid editing entity does not generate a *Reln*-H3448R-Tg knock in mouse model carrying the *Reln*-COLBOS variant by homologous recombination.

EXAMPLES

Example 1. RNA editing of a *RELN* transcript using different guide oligonucleotides.

A variety of guide oligonucleotides was designed for targeting the adenosine in the CAU codon (in the human *RELN* transcript), which codon encodes histidine (H) at amino acid position 3447 in the human reelin (H3447) protein and wherein the deamination from adenosine to inosine (CIU), would provide a codon that would translate to arginine (R) at this position. Hence, the amino acid change is herein generally referred to as H3447R. The sequence of the target codon as well its surrounding sequence in the human *RELN* DNA is provided in **Fig. 1A**. Clearly, the target sequence for deamination using the guide oligonucleotides of the present disclosure and the endogenous ADAR enzyme takes place on the transcript that is transcribed from the DNA and therefore the real target sequence comprises uridines (U's) instead of thymidine residues (T's). Hence the sequence of **Fig. 1A** represents that target transcript where T's are replaced by U's. The sequences, designs, and chemical modifications of the guide oligonucleotides are provided in **Figs. 2, 3, and 7**. The chemical modifications are discussed in the brief description of the drawings.

First, human iPSC (WT04) derived neural progenitor cells were differentiated into mature cortical neurons using neural progenitor medium and generally according to protocols known to the person skilled in the art. For the initial screen of the guide oligonucleotides and to determine RNA editing efficiency, the following was performed. At day 0, the mature neurons were plated in 12-well plates in a concentration of 2.0×10^5 cells per well and allowed to expand until day 11. Treatment with guide oligonucleotides was carried out with three different protocols, as follows:

- 1) Cells were transfected on day 11 in with 200 nM guide oligonucleotides (N = 2 or 3 biological replicates per guide oligonucleotide), using Lipofectamine® RNAiMAX Reagent, following the manufacturer's instructions. Plates were incubated at 37 °C, 5% CO₂ for 48 hr until harvest. On day 2 (48 hr post-transfection) supernatants were discarded, and cells were processed for RNA isolation as described below.
- 2) Cells were treated by gymnotic uptake (gymnosis, without transfection aids) on day 11 with 5 µM guide oligonucleotides (N=2 biological replicates per guide oligonucleotide) that was dissolved in the medium and plates were incubated at 37 °C, 5% CO₂ for 7 days until harvest. On day 2 and 4 after start of the guide oligonucleotide treatment, cells were refreshed with 50% fresh medium without guide oligonucleotides. On day 7 after the start of the treatment, supernatants were discarded, and cells were processed for RNA isolation as described below.

- 3) Cells were treated by gymnotic uptake on day 11 with 5 μ M guide nucleotides (N = 1 or 2 biological replicates per guide oligonucleotide), that was dissolved in the medium that also contained 1 μ M triterpene glycoside AG1856 (saponin). Subsequently, plates were incubated at 37 °C, 5% CO₂ for 7 days until harvest. On day 2 and 4 after start of the guide oligonucleotide + saponin treatment, cells were refreshed with 50% fresh medium without guide oligonucleotides or saponin. On day 7 after start of the treatment, supernatants were discarded, and cells were processed for RNA isolation as described below.

Cells were collected and used for RNA isolation using the ReliaPrep™ RNA Cell Miniprep System (Promega-Z612) according to the manufacturer's instructions. The total RNA was reverse-transcribed using the Maxima Reverse Transcriptase (Thermo-EP0742) kit with oligo-dT primer, random Hexamer Primer, and dNTP Mix (10 mM each). A quantitative PCR was then performed with the Digital PCR System (QIAGEN, QIAcuity dPCR system) in 12 μ l aliquots of reaction mixtures containing cDNA, appropriate pairs of primers and probes and dPCR mastermix (QIAGEN - QIAcuity Mastermix (4x)). Priming of the plate was performed by the QIAcuity system. The forward and reverse primers given in **Table 1** were used with a PCR program that was as follows: 2 min at 95 °C; 40 cycles for 15 sec at 95 °C and 30 sec at 63 °C. Imaging of the channels (HEX, FAM and CY5, see **Table 1** for the probe sequences) was done for 500, 500 and 400 ms respectively with a gain of 6, 6, and 8 respectively.

Table 1. Primers and probes for the dPCR of H3447R editing in human RELN transcripts. The "+" symbol represents a Locked Nucleic Acid (LNA) at the 3' side of the symbol. The SEQ ID NO is given between brackets.

Name	Sequence 5'-3'	Item
hRELN_201_e63-64 fw 1	ATGTGGAGGTCGTCCTCACTCGC (95)	Fw primer 1
hRELN_e65 rv 2	TGGGTATCGCCTAAGTGACC (96)	Rv primer 1
hRELN_e65_G_ FAM 4	/56-FAM/ATGGGCTCAG+A+C+G+TTTC+TACAACAG/3IABkFQ/ (97)	Fam probe
hRELN_e65_A_ HEX 4	/5HEX/ATGGGCTCAG+A+C+A+TT+TC+TACAACAG/3IABkFQ/ (98)	Hex probe
hRELN_e54_fw 1	CACTGTGAGACAAGCGGTTACAC (99)	Fw primer 2
hRELN_e55 rv 1	GATGGGACGATGGCAAGAGG (100)	Rv primer 2
hRELN_e54-55_Con_Cy5 1	/5Cy5/TCTTCGAGGTGC+AAA+GTT+CCTGCA/3IAbRQSp/ (101)	Cy5 probe

The editing percentage was calculated by pooling the biological replicates for each transfection for all A and G counts and then scored according to the formula:

$$\text{score} = \text{SUM}(G) / (\text{SUM}(A+G)) * 100$$

The results of the transfection experiment are shown in **Fig. 4**. The results of the experiment with gymnotic uptake (without saponin) in which RNA editing could be readily detected are shown in **Fig. 5**. The results of the experiment with gymnotic uptake (with saponin co-treatment) in which RNA editing could be readily detected are shown in **Fig. 6**. The transfection data showed that particularly asymmetrical guide oligonucleotides, with relatively short 5' parts of approximately 8 nucleotides and relatively long 3' parts of approximately 16 to 22 nucleotides, generated the highest editing with around 10 – 12% editing efficiency (see RM116835, RM116836, and RM116837). These experiments indicate that RM116835, RM116836, RM116837, RM116838 (with the typical asymmetric design) as well as RM116818 and RM116827 are preferred embodiments of what is disclosed herein. It also shows that an asymmetrical design – in which the 5' part of the guide oligonucleotide, calculated from the orphan position, is relatively short, and in which the 3' part of the guide oligonucleotide, calculated from the orphan position, is relatively long – is a preferred embodiment of the present disclosure.

Not unexpectedly, the RNA efficiencies after gymnotic uptake were lower than observed with transfection. Although overall editing efficiency was below 3%, RM116827, and some asymmetrical guide oligonucleotides (RM116836, RM116837, and RM116838) performed well. When using gymnotic uptake in the context of endosomal release factor AG1856 (the triterpene glycoside, or saponin), it was found that the asymmetrical designed guide oligonucleotides RM116835, RM116836, RM116837, and RM116838, together with RM116821 and RM116827 performed particularly well, reaching around 9.5% editing.

Example 2. RNA editing of a RELN transcript using an additional set of guide oligonucleotides.

Based on the results obtained in Example 1, the design of RM116835 and RM116838 was taken as the basis for further designing additional guide oligonucleotides to test. These further 31 designs are depicted in **Fig. 7**, in which RM118850 to RM118867 are based on RM116835 (SEQ ID NO:58), and RM118868 to RM118880 are based on RM116838 (SEQ ID NO:61). Together with newly synthesized RM116835 and RM116838 oligonucleotides, these 31 new guide oligonucleotides were tested in a gymnotic uptake experiment, in the context of saponin as described above in experimental setup 3), which was then followed by an RNA isolation, cDNA generation and dPCR detection setup as described in Example 1. RNA editing of the target adenosine was measured as described above. Notably, there are different *RELN* isoforms, known as 201 and 203, in which isoform 210 lacks exon 64 that is present in isoform

203. Exon 64 is only 6 nucleotides long and its sequence happens to coincide with the forward primer “hRELN 201_e63-64 fw 1” (Table 1) used for cDNA generation in the experimental setup outlined above. To distinguish between the effects of the guide oligonucleotides on isoform 201 and isoform 203 (and to distinguish mainly for the cDNA generation of both isoforms), a further forward primer, “hRELN_e63-64-65_fw 2”, was generated with the sequence 5'-ATG TGG AGG TCG TCC TAG TAA GC-3' (SEQ ID NO:107). The effect on isoform 201 and 203 was assessed separately, using these two different forward primers in two different cDNA generations, and these were then subsequently assessed for RNA editing separately.

The editing percentage results are provided in **Fig. 8**, wherein **Fig. 8A** shows the editing percentage using the forward primer for the 201 isoform, and **Fig. 8B** shows the editing percentage using the forward primer for the 203 isoform. The editing percentage pattern between the two isoforms appeared to be similar. The two originating guide oligonucleotides performed slightly better in this new experiment in comparison to the data shown in **Fig. 6**, with RM116835 showing an editing percentage of 13.9 % (201) and 18.3 % (203) and RM116838 showing an editing percentage of 11.3 % (201) and 13.2 % (203). Interestingly, it proved to be possible to design further improved guide oligonucleotides that showed an important increase in editing efficiencies, with the best performers being:

RM118851 (SEQ ID NO:65) – 22.3 % (201) and 23.2 % (203)
 RM118852 (SEQ ID NO:66) – 20.4 % (201) and 24.2 % (203)
 RM118850 (SEQ ID NO:64) – 18.4 % (201) and 20.3 % (203)
 RM118874 (SEQ ID NO:88) – 20.0 % (201) and 21.1 % (203)
 RM118876 (SEQ ID NO:90) – 19.1 % (201) and 25.4 % (203)
 RM118875 (SEQ ID NO:89) – 17.2 % (201) and 17.1 % (203)
 RM118855 (SEQ ID NO:69) – 16.4 % (201) and 18.1 % (203)
 RM118853 (SEQ ID NO:67) – 16.3 % (201) and 17.2 % (203)
 RM118868 (SEQ ID NO:82) – 16.2 % (201) and 16.6 % (203)
 RM118869 (SEQ ID NO:83) – 14.2 % (201) and 17.4 % (203)
 RM118870 (SEQ ID NO:84) – 16.0 % (201) and 17.6 % (203)

In a further experiment, these guide oligonucleotides, and new to be designed guide oligonucleotides – based on the designs of these best performers – are tested in *in vivo* experiments. For this, it is preferred to conjugate a triterpene glycoside (preferably saponin AG1856 as was used in the gymnotic/saponin experiment outlined above) to the guide oligonucleotide to provide very efficient endosomal escape once the guide oligonucleotide has entered the target cell in which it needs to edit the *RELN* transcript molecule. Conjugation of

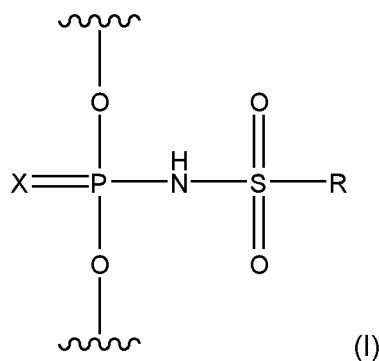
AG1856 to the guide oligonucleotide is performed generally as described in Intl. Patent Application PCT/EP2024/051278 (not published). In an alternative experimental setup, the guide oligonucleotides are delivered *in vivo* using LNP's.

Next to this, the amount and/or rate of phosphorylated Dab1 protein is determined using western blot and immunofluorescent staining in relevant mammalian (primary) cell cultures.

CLAIMS

1. A guide oligonucleotide that is at least partially complementary to a portion of a human *RELN* nucleic acid molecule comprising a target nucleotide, wherein the *RELN* nucleic acid molecule encodes a reelin protein, wherein the guide oligonucleotide is configured such that it is capable of forming a double stranded complex under physiological conditions within a cell with the portion of the *RELN* nucleic acid, and the double stranded complex is capable of recruiting a nucleic acid editing enzyme that is naturally present in the cell, to perform editing of the target nucleotide to generate an edited *RELN* nucleic acid comprising an edited target nucleotide.
2. A guide oligonucleotide according to claim 1, wherein editing of the target nucleotide leads to elevated activity of the encoded reelin protein.
3. A guide oligonucleotide according to claim 2, wherein the encoded reelin protein is provided with a gain-of-function phenotype, selected from one or more of:
 - (i) an enhanced ability to trigger signalling, preferably of the APOEr/Dab1/GSK3 β pathway;
 - (ii) an enhanced ability to increase Dab1 phosphorylation;
 - (iii) an enhanced ability to reduce Tau phosphorylation associated with neurofibrillary tangles;
 - (iv) an enhanced ability to increase tubular structure formation and/or stability and/or neuronal density;
 - (v) an enhanced resistance to degradation by proteolysis; and/or
 - (vi) enhanced binding of the reelin protein to glycosaminoglycans, preferably heparin, and/or to NRP1.
4. A guide oligonucleotide according to any one of claims 1 to 3, wherein editing of the target nucleotide introduces an amino acid variant at one or more of amino acid positions 3446 to 3460 of the encoded reelin protein, preferably wherein editing of the target nucleotide introduces a histidine to arginine change at amino acid position 3447 (H3447R) in the encoded reelin protein.
5. A guide oligonucleotide according to any one of claims 1 to 4, wherein the cell is a brain cell, preferably a neuron.

6. A guide oligonucleotide according to any one of claims 1 to 5, wherein the target nucleotide is adenosine, and the nucleic acid editing enzyme is an adenosine deaminase acting on RNA (ADAR) enzyme.
7. A guide oligonucleotide according to any one of claims 1 to 6, wherein the *RELN* nucleic acid molecule is mRNA or pre-mRNA.
8. A guide oligonucleotide according to any one of claims 1 to 7, wherein the orphan nucleotide is the nucleotide in the guide oligonucleotide that is opposite the target nucleotide, wherein the nucleotide numbering is such that the orphan nucleotide is number 0 and nucleotides are further positively (+) incremented towards the 5' end and negatively (-) incremented towards the 3' end, and wherein at least one nucleobase, sugar, or internucleoside linkage, has been chemically modified.
9. A guide oligonucleotide according to claim 8, wherein the orphan nucleotide is a deoxycytidine, a cytidine analog, a deoxyuridine, or a uridine analog.
10. A guide oligonucleotide according to any one of claims 1 to 9, wherein the guide oligonucleotide is 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, or 60 nucleotides in length.
11. A guide oligonucleotide according to any one of claims 1 to 10, wherein the guide oligonucleotide comprises a contiguous stretch of 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, or 33 nucleotides from SEQ ID NO:102 (5'-UG UA GAA ACI UCU GAG CCC AUG UUG UCG UGA AA-3') or SEQ ID NO:103 (5'-UG UA GAA AZI UCU GAG CCC AUG UUG UCGUGAAA-3'), comprising at least the underlined section of nucleotides (SEQ ID NO:104 (5'-UA GAA ACI UCU GAG CCC AUG UUG-3' and SEQ ID NO:105 (5'-UA GAA AZI UCU GAG CCC AUG UUG-3'), respectively), wherein Z is a nucleotide comprising a 6-amino-5-nitro-3-yl-2(1H)-pyridone nucleobase, and I is inosine.
12. A guide oligonucleotide according to any one of claims 1 to 11, wherein the guide oligonucleotide comprises one or more internucleoside linkage modifications, which are each independently selected from a phosphorothioate (PS), phosphonoacetate, phosphorodithioate, methylphosphonate (MP), sulfonylphosphoramidate, (1,3-dimethylimidazolidin-2-ylidene) phosphoramidate (PNdmi), or a linkage modification with the structure according to formula (I)



wherein: X = O or S; and

R = an aryl, a substituted aryl, a heterocycle, a substituted heterocycle, an aromatic heterocycle, a substituted aromatic heterocycle, a C₁-C₆ alkoxy, a substituted C₁-C₆ alkoxy, a C₁-C₂₀ alkyl, a substituted C₁-C₂₀ alkyl, a C₁-C₆ alkenyl, a C₁-C₆ substituted alkenyl, a C₁-C₆ alkynyl, a substituted C₁-C₆ alkynyl, or a conjugate group;

preferably wherein X = O and R = methyl and the linkage modification is mesyl phosphoramidate (PNms).

13. A guide oligonucleotide according to any one of claims 1 to 12, wherein the internucleoside linkage numbering in the guide oligonucleotide is such that linkage number 0 is the linkage 5' from the orphan nucleotide, and the linkage positions in the oligonucleotide are positively (+) incremented towards the 5' end and negatively (-) incremented towards the 3' end, and wherein linkage position -2 is an MP or a PNms linkage.

14. A guide oligonucleotide according to any one of claims 1 to 13, wherein the guide oligonucleotide comprises one or more nucleotides comprising a mono- or di-substitution at the 2', 3' and/or 5' position of the ribose, each independently selected from the group consisting of: -OH; -F; substituted or unsubstituted, linear or branched lower (C₁-C₁₀) alkyl, alkenyl, alkynyl, alkaryl, allyl, or aralkyl, that may be interrupted by one or more heteroatoms; -O-, S-, or N-alkyl; -O-, S-, or N-alkenyl; -O-, S-, or N-alkynyl; -O-, S-, or N-allyl; -O-alkyl-O-alkyl; -methoxy; -aminopropoxy; -methoxyethoxy; -dimethylamino oxyethoxy; and -dimethylaminoethoxyethoxy.

15. A guide oligonucleotide according to any one of claims 1 to 14, comprising the structure (from 5' to 3'):

N₈N₇N₆N₅N₄N₃N₂N₁⁰ZdId[^]M₂M₃M₄M₅M₆M₇M₈M₉M₁₀M₁₁M₁₂M₁₃M₁₄M₁₅M₁₆M₁₇M₁₈M₁₉M₂₀M₂₁M₂₂M₂₃M₂₄

wherein:

- Zd is the orphan nucleotide at nucleotide position 0, which is a deoxynucleotide carrying a Benner's base;
- N₁ is Ae or Ad;
- N₂ is Af;
- N₃ and N₅ are each independently Am or Af;
- N₄ is Gf;
- N₆ is Uf;
- N₇ is either absent (when N₈ and N₉ are also absent), Gm, or Gf;
- N₈ is either absent (when N₉ is also absent) or Um;
- Id is deoxyinosine;
- M₂ is Um;
- M₃ is Cf;
- M₄, M₁₄ and M₁₅ are each independently m5Ue or Um;
- M₅ and M₇ are Gf;
- M₆ is Am or Af;
- M₈ and M₁₀ are each independently Cm or Cf;
- M₉ is Cf;
- M₁₁ is Am;
- M₁₂ is Um;
- M₁₃ is Gm;
- M₁₆ is Ge or Gm;
- M₁₇ is either absent (when M₁₈ to M₂₄ are also absent), m5Ue, or Um;
- M₁₈ is either absent (when M₁₉ to M₂₄ are also absent), Cm, or m5Ce;
- M₁₉ is either absent (when M₂₀ to M₂₄ are also absent), Gm, or Ge;
- M₂₀ is either absent (when M₂₁ to M₂₄ are also absent), Um, or m5Ue;
- M₂₁ is either absent (when M₂₂ to M₂₄ are also absent), Gm, or Ge;
- M₂₂ is either absent (when M₂₃ and M₂₄ are also absent), Am, or Ae;
- M₂₃ is either absent (when M₂₄ is also absent), or Ae;
- M₂₄ is either absent, or Ae;
- ^θ is at linkage position 0, and is a PO linkage or a PNms linkage;
- [^] is at linkage position -2 and is an MP or a PNms linkage;
- all other linkages are either PO, PS, PNdmi, or PNms linkages; and

wherein Gm, Am, Um, and Cm are 2'-O-methyl (2'-OMe) modified guanosine, adenosine, uridine, and cytidine, respectively; m5Ce is 2'-MOE modified 5-methylcytidine; Ge is 2'-MOE modified guanosine; Ae is 2'-MOE modified adenosine; m5Ue is 2'-MOE modified 5-

methyluridine (also sometimes named “Te”; 2'-MOE modified thymidine); Af, Uf, Gf, and Cf are 2'-F modified adenosine, uridine, guanosine, and cytosine, respectively.

16. A guide oligonucleotide according to any one of claims 1 to 15, wherein the guide oligonucleotide comprises or consists of the sequence of any one of SEQ ID NO:65, 66, 90, 64, 88, 89, 69, 67, 82, 83, 84, 58, 61, 59, 60, 41, 44, 50, 68, 70, 71, 72, 73, 78, 79, 80, 81, 85, 86, and 87.

17. A guide oligonucleotide according to any one of claims 1 to 16, wherein the guide oligonucleotide is bound, preferably conjugated, to a triterpene glycoside, preferably AG1856.

18. A vector, preferably a viral vector, more preferably an adeno-associated virus (AAV) vector, comprising a nucleic acid molecule encoding a guide oligonucleotide according to any one of claims 1 to 7.

19. A pharmaceutical composition comprising a guide oligonucleotide according to any one of claims 1 to 17, or a vector according to claim 18, and a pharmaceutically acceptable carrier.

20. A guide oligonucleotide according to any one of claims 1 to 17 for use in the treatment, amelioration, or slowing down the progression of a neurodegenerative disease, preferably Alzheimer's Disease, more preferably Autosomal Dominant Alzheimer's Disease.

21. Use of a guide oligonucleotide according to any one of claims 1 to 17 for use in the manufacture of a medicament for the treatment, amelioration, or slowing down the progression of a neurodegenerative disease, preferably Alzheimer's Disease, more preferably Autosomal Dominant Alzheimer's Disease.

22. A method of treating, ameliorating, or slowing down the progression of a neurodegenerative disease, preferably Alzheimer's disease, more preferably Autosomal Dominant Alzheimer's Disease, in a human subject in need thereof, the method comprising administering to said subject a guide oligonucleotide according to any one of claims 1 to 17, or a vector according to claim 18, thereby editing the target *RELN* nucleic acid sequence to encode a reelin protein with the ability to delay onset of one or more symptoms of the neurodegenerative disease.

23. An *in vitro*, *ex vivo*, or *in vivo* method for the deamination of a target adenosine in a target *RELN* nucleic acid sequence in a brain cell, preferably a neuron, the method comprising the steps of:

- (i) providing the cell with a guide oligonucleotide according to any one of claims 1 to 17, or a vector according to claim 18;
- (ii) allowing uptake by the cell of the guide oligonucleotide or vector;
- (iii) allowing annealing of the guide oligonucleotide to the target *RELN* nucleic acid sequence; and
- (iv) allowing a nucleic acid editing entity to edit the target.

24. A method according to claim 23, further comprising the step of administering a triterpene glycoside, preferably AG1856, before, after or simultaneously with administering the guide oligonucleotide.

25. A method of editing a human *RELN* nucleic acid sequence in a cell, preferably a brain cell, wherein the human *RELN* nucleic acid sequence is pre-mRNA or mRNA, the method comprising contacting the target *RELN* nucleic acid sequence with a guide oligonucleotide capable of triggering an ADAR-mediated adenosine to inosine deamination, thereby editing the target *RELN* nucleic acid sequence to encode a reelin protein with the ability to delay onset of one or more symptoms of a neurodegenerative disease, preferably Alzheimer's Disease, more preferably Autosomal Dominant Alzheimer's Disease.

26. A method according to claim 25, wherein the guide nucleotide is according to any one of claims 1 to 17.

27. A nucleic acid molecule for editing a target adenosine in a human *RELN* pre-mRNA or mRNA molecule, wherein the target region is SEQ ID NO:106, and wherein the target adenosine is the second nucleotide of the codon encoding histidine at position 3447 of the *RELN*-encoded human reelin protein.

28. The nucleic acid molecule of claim 27, wherein the nucleic acid molecule is selected from the group consisting of SEQ ID NOS:41, 44, 50, 58, 59, 60, 61, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, and 94.

29. The nucleic acid molecule of claim 27 or 28, further comprising at least one non-naturally occurring chemical modification, and/or comprising one or more additional non-naturally occurring chemical modifications in a ribose, linkage or base moiety, with the proviso that the orphan nucleotide, which is the nucleotide in the nucleic acid that is directly opposite a target adenosine in the target region, is not a cytidine comprising a 2'-OMe ribose substitution.

Fig. 1A

TCGCAAAACAAAATTACATGATGAATTTTTCACGACAACATGGGCTCAGACATTTTCTACAACAGAAGACGAAGGTCACTTAGCGGATACCCATGAAGAATCA
(SEQ ID NO: 37)

Fig. 1B

AGCGTTTGTTTTAATGTACTACTTAAAAAGTGCTGTTGTACCCGAGTCTGTAAAGATGTTGTCTTCTGCTTCCAGTGAATCCGCTATGGGTACTTCTTAGT
(SEQ ID NO: 38)

Fig. 1C

TGATTCTTCATGGGTATCGCCTAAGTGACCTTCGTCTTCTGTTGTAGAAATGTCTGAGCCCATGTTGTCTGTAATAATTCATCATGTAATTTTGTTCGGA
(SEQ ID NO: 39)

Fig. 1D

UCGCAAAACAAAUAUACAUGAUAUUUUUUCACGACAACAUGGGCUCAGACAUUUUCACAACAGAAGACGAAGGUCACUAGGCGAUACCCCAUGAAGAAUCA
(SEQ ID NO: 106)

Fig. 2

SEQ ID NO:	Desc	Sequence
07	RELN1-01	m5Ce!Ge°m5Ue°m5Ce°m5Ue°Um°Cm°Um°Gm°Um°Gf°Uf°Af°Gf°Af°Af°Ae°Zd°Id°Um°Cf°m5Ue°Gf°Af°Gf°Cf°Cf°Am°Um!Gm
08	RELN1-02	m5Ce!Ge°m5Ue°m5Ce°m5Ue°Um°Cm°Um°Gm°Um°Gm°Uf°Am°Gf°Am°Af°Ae°Zd°Id°Um°Cf°m5Ue°Gf°Am°Gf°Cm°Cf°Am°Um!Gm
09	RELN1-03	m5Ce!Ge°m5Ue°m5Ce°m5Ue°Um°Cm°Um°Gm°Um°Gm°Uf°Af°Gf°Af°Af°Ae°Zd°Id°Um°Cf°m5Ue°Gf°Af°Gf°Cf°Cf°Am°Um!Gm
10	RELN1-04	m5Ce!Ge°m5Ue°m5Ce°m5Ue°Um°Cm°Um°Gm°Um°Gm°Uf°Am°Gf°Am°Af°Ae°Zd°Id°Um°Cf°m5Ue°Gf°Am°Gf°Cm°Cf°Am°Um!Gm
11	RELN1-05	m5Ue!m5Ce°m5Ue°m5Ue°m5Ce°Um°Gm°Um°Um°Gm°Uf°Af°Gf°Af°Af°Ae°Zd°Id°Um°Cf°m5Ue°Gf°Af°Gf°Cf°Cf°Am°Um!Gm
12	RELN1-06	m5Ue!m5Ce°m5Ue°m5Ue°m5Ce°Um°Gm°Um°Um°Gm°Uf°Am°Gf°Am°Af°Ae°Zd°Id°Um°Cf°m5Ue°Gf°Am°Gf°Cm°Cf°Am°Um!Gm
13	RELN1-07	m5Ue!m5Ce°m5Ue°m5Ue°m5Ce°Um°Gm°Um°Um°Gm°Uf°Af°Gf°Af°Af°Ae°Zd°Id°Um°Cf°m5Ue°Gf°Af°Gf°Cf°Cf°Am°Um!Gm
14	RELN1-08	m5Ue!m5Ce°m5Ue°m5Ue°m5Ce°Um°Gm°Um°Um°Gm°Uf°Am°Gf°Am°Af°Ae°Zd°Id°Um°Cf°m5Ue°Gf°Am°Gf°Cm°Cf°Am°Um!Gm
15	RELN1-09	m5Ue!m5Ce°m5Ue°m5Ue°m5Ce°Um°Gm°Um°Um°Gf°Uf°Af°Gf°Af°Af°Ae°Zd°Id°Um°Cf°m5Ue°Gf°Af°Gf°Cf°Cf°Am°Um!Gm
16	RELN1-10	m5Ue!m5Ue°m5Ce°m5Ue°m5Ue°Um°Gm°Um°Um°Gm°Uf°Am°Gf°Am°Af°Ae°Zd°Id°Um°Cf°m5Ue°Gf°Am°Gf°Cm°Cf°Am°Um!Gm
17	RELN1-11	m5Ue!m5Ue°m5Ce°m5Ue°m5Ue°Um°Gm°Um°Um°Gf°Uf°Af°Gf°Af°Af°Ae°Zd°Id°Um°Cf°m5Ue°Gf°Af°Gf°Cf°Cf°Am°Um!Gm
18	RELN1-12	m5Ue!m5Ue°m5Ce°m5Ue°m5Ue°Um°Gm°Um°Um°Gm°Uf°Am°Gf°Am°Af°Ae°Zd°Id°Um°Cf°m5Ue°Gf°Am°Gf°Cm°Cf°Am°Um!Gm
19	RELN1-13	m5Ce!m5Ue°m5Ue°Um°Gf°Uf°Af°Gf°Af°Af°Ae°Zd°Id°Um°Cf°m5Ue°Gf°Af°Gf°Cf°Cf°Am°Um!Gm
20	RELN1-14	m5Ce!m5Ue°m5Ue°Um°Gm°Uf°Am°Gf°Am°Af°Ae°Zd°Id°Um°Cf°m5Ue°Gf°Am°Gf°Cm°Cf°Am°Um!Gm
21	RELN1-15	m5Ue°m5Ue°Um°Gf°Uf°Af°Gf°Af°Af°Ae°Zd°Id°Um°Cf°m5Ue°Gf°Af°Gf°Cf°Cf°Am°Um!Gm
22	RELN1-16	m5Ce!m5Ue°m5Ue°Um°Gm°Uf°Am°Gf°Am°Af°Ae°Zd°Id°Um°Cf°m5Ue°Gf°Am°Gf°Cm°Cf°Am°Um!Gm
23	RELN1-17	m5Ce!Ge°m5Ue°m5Ce°m5Ue°Um°Cm°Um°Gm°Um°Gf°Uf°Af°Gf°Af°Af°Ae°Zd°Id°Um°Cf°m5Ue°Gf°Af°Gf°Cf°Cf°Am
24	RELN1-18	m5Ce!Ge°m5Ue°m5Ce°m5Ue°Um°Cm°Um°Gm°Um°Gm°Uf°Am°Gf°Am°Af°Ae°Zd°Id°Um°Cf°m5Ue°Gf°Am°Gf°Cm°Cf°Am
25	RELN1-19	m5Ce!Ge°m5Ue°m5Ce°m5Ue°Um°Cm°Um°Gm°Um°Gm°Uf°Af°Gf°Af°Af°Ae°Zd°Id°Um°Cf°m5Ue°Gf°Af°Gf°Cf°Cf°Am
26	RELN1-20	m5Ce!Ge°m5Ue°m5Ce°m5Ue°Um°Cm°Um°Gm°Um°Gm°Uf°Am°Gf°Am°Af°Ae°Zd°Id°Um°Cf°m5Ue°Gf°Am°Gf°Cm°Cf°Am
27	RELN1-21	m5Ce!Ge°m5Ue°m5Ce°m5Ue°Um°Cm°Um°Gm°Um°Gm°Uf°Am°Gf°Uf°Af°Gf°Af°Af°Ae°Zd°Id°Um°Cf°m5Ue°Gf°Af°Gf°Cf°Cf
28	RELN1-22	m5Ce!Ge°m5Ue°m5Ce°m5Ue°Um°Cm°Um°Gm°Um°Gm°Uf°Am°Gf°Am°Af°Ae°Zd°Id°Um°Cf°m5Ue°Gf°Am°Gf°Cm°Cf
29	RELN1-23	m5Ce!Ge°m5Ue°m5Ce°m5Ue°Um°Cm°Um°Gm°Um°Gm°Uf°Af°Gf°Af°Af°Ae°Zd°Id°Um°Cf°m5Ue°Gf°Af°Gf°Cf°Cf
30	RELN1-24	m5Ce!Ge°m5Ue°m5Ce°m5Ue°Um°Cm°Um°Gm°Um°Gm°Uf°Am°Gf°Am°Af°Ae°Zd°Id°Um°Cf°m5Ue°Gf°Am°Gf°Cm°Cf
31	RELN1-25	Am!Cm°Cm°Um°Uf°Cf°Gm°Um°Cf°Uf°Um°Cm°Uf°Gf°Um°Um°Gf°Uf°Af°Gf°Af°Af°Ae°Zd°Id°Um°Cf°m5Ue°Gm!Am
32	RELN1-26	Am!Cm°Cm°Uf°Uf°Cm°Gm°Uf°Cf°Cf°Um°Uf!Cf°Uf°Gf!Uf°Um°Gm°Uf°Af°Gm°Am°Af°Ae°Zd°Id°Um°Cf°m5Ue°Gm!Am
33	RELN1-27	Am!Cm°Cm°Uf°Uf°Cf°Gm°Uf°Cf°Cf°Um°Uf!Cm°Uf°Gf!Uf°Um°Gm°Uf°Af°Gf°Am°Af°Ae°Zd°Id°Um°Cf°m5Ue°Gm!Am
34	RELN1-28	Am!Cm°Cm°Uf°Uf°m5Ce°Ge°Uf°Cf°Um°Um!Cm°Uf°Gf!Uf°m5Ue°Gf°Um°Af!Gm°Ae°Af°Ae°Zd°Id°Um°Cf°m5Ue°Gm!Am
35	RELN1-29	Am!Cm°Cm°Uf°Uf°m5Ce°Ge°Uf°Cf°m5Ue°Uf!Cm°Uf°Gf!Uf°m5Ue°Gf°Uf°Af!Ge°Ae°Af°Ae°Zd°Id°Um°Cf°m5Ue°Gm!Am
36	RELN1-30	m5Ce°m5Ue°Um!m5Ce°Uf°Gf!Um°m5Ue°Ge°Um°Am°Ge°Ae°Af°Ae°Zd°Id°Um°Cf°m5Ue°Gf°Am°Gf°Cm°Cf°Am

Fig. 4

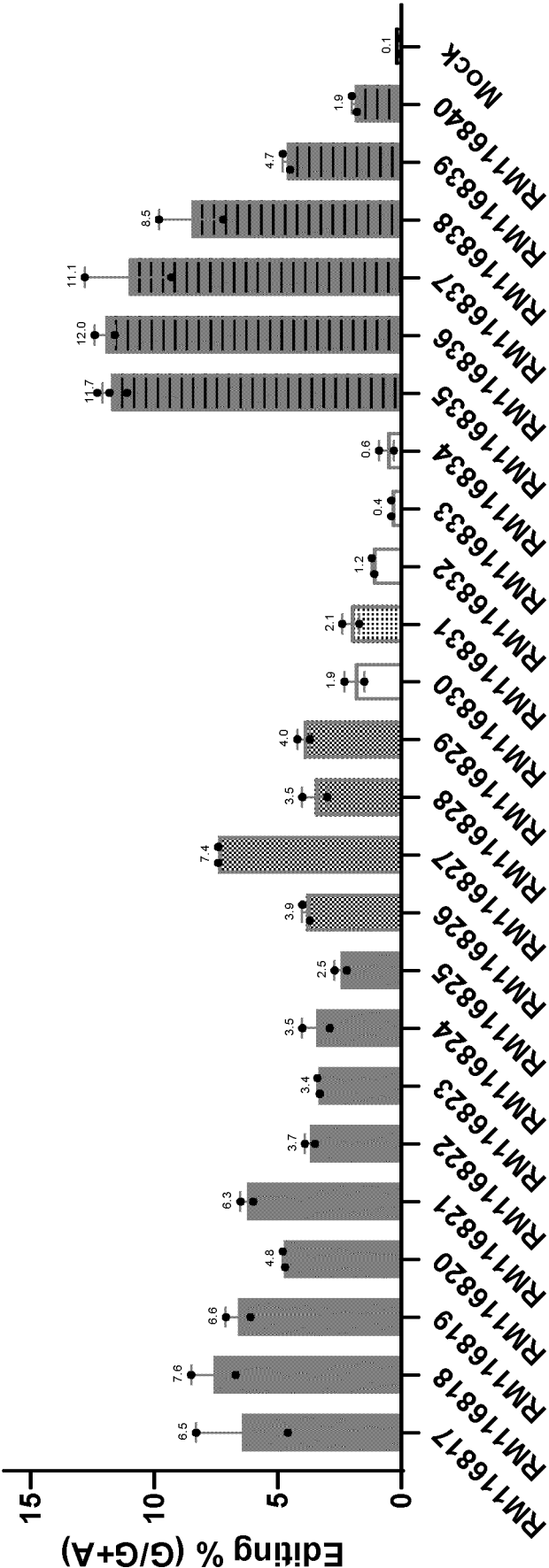


Fig. 5

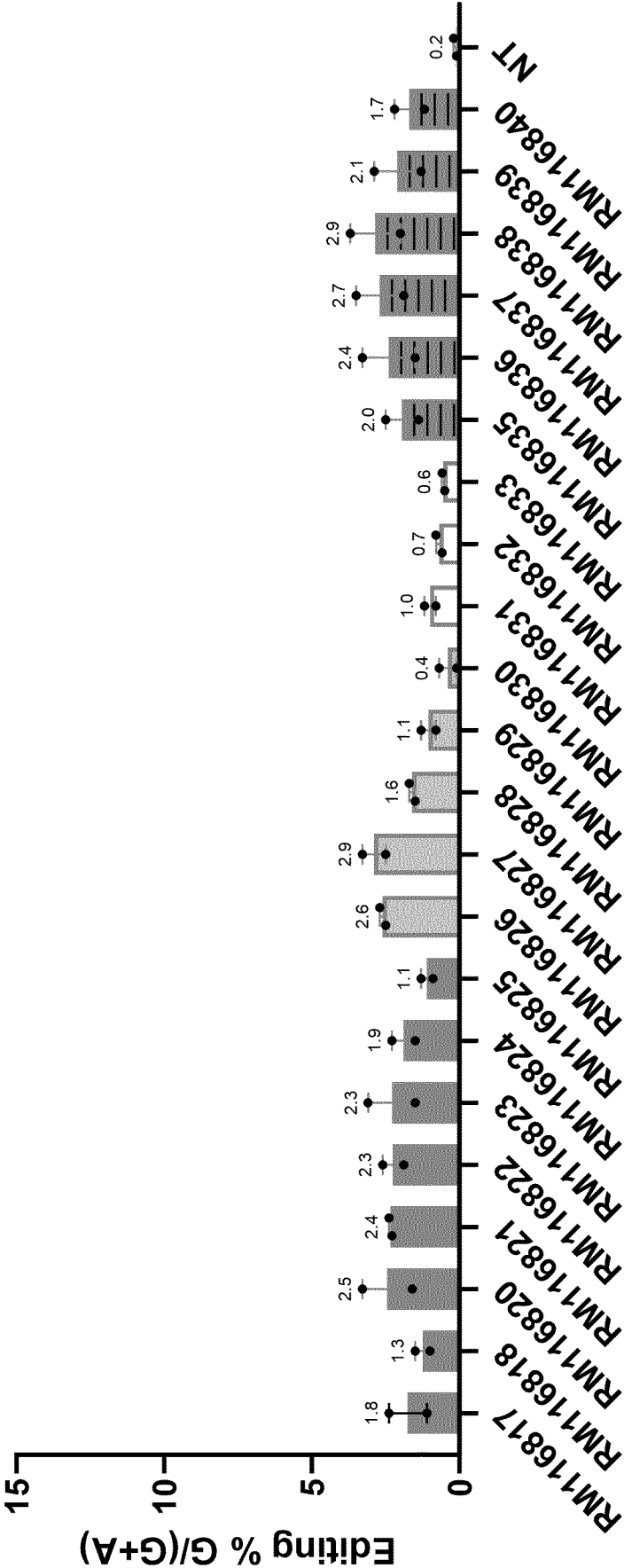


Fig. 6

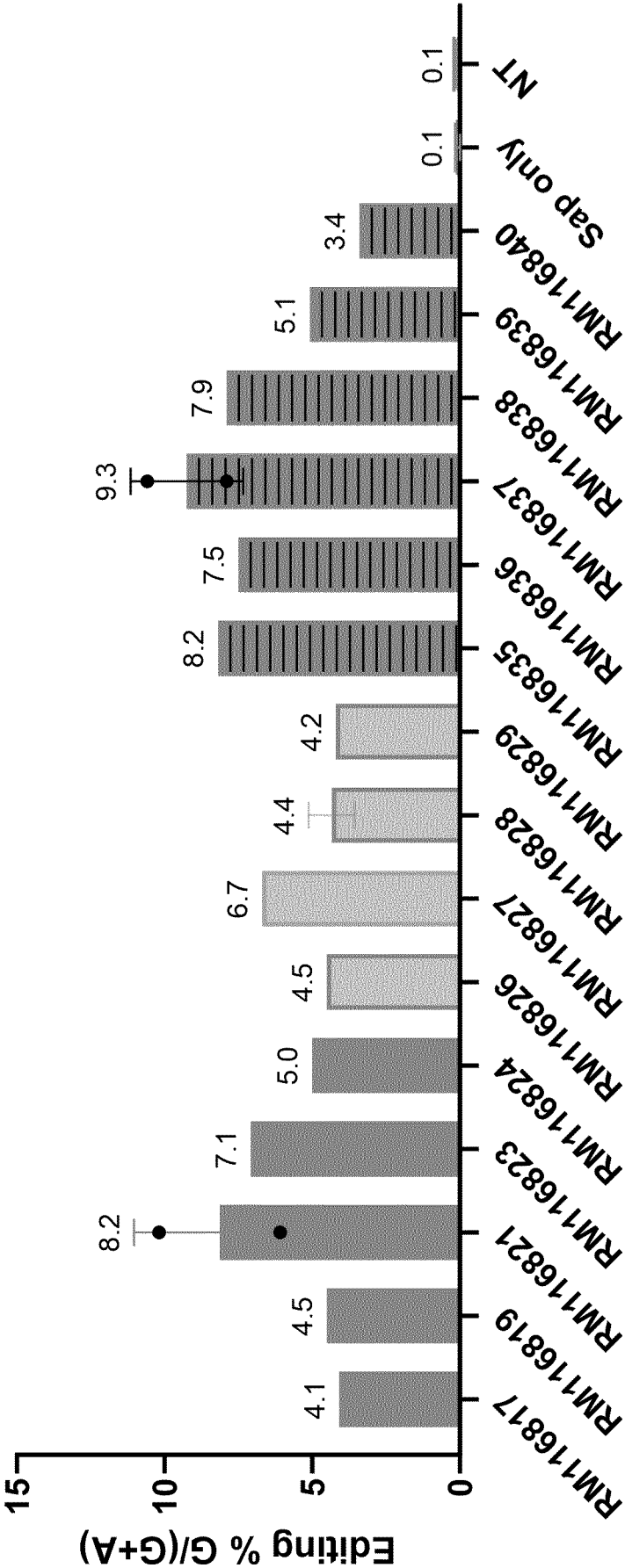


Fig. 7

Name	SEQ ID NO	sequence (5' to 3')
RM1118850	64	Um*Gm*Uf*Am*Gf*Am*Af*Ad*Zd*Id^Um^Cf*m5Ue*Gf*Am*Gf*Cm*Am*Um*Gm^m5Ue^m5Ue^m5Ce^Ge^m5Ue^Ge^Ae
RM1118851	65	Um*Gm*Uf*Am*Gf*Am*Af*Ad#Zd*Id^Um^Cf*m5Ue*Gf*Am*Gf*Cm*Am*Um*Gm^m5Ue^m5Ue^m5Ce^Ge^m5Ue^Ge^Ae
RM1118852	66	Um*Gm*Uf*Am*Gf*Am*Af*Ad*Zd#Id^Um^Cf*m5Ue*Gf*Am*Gf*Cm*Am*Um*Gm^m5Ue^m5Ue^m5Ce^Ge^m5Ue^Ge^Ae
RM1118853	67	Um*Gm*Uf*Am*Gf*Am*Af*Ad*Zd*Id#Um^Cf*m5Ue*Gf*Am*Gf*Cm*Am*Um*Gm^m5Ue^m5Ue^m5Ce^Ge^m5Ue^Ge^Ae
RM1118854	68	Um*Gm*Uf*Af*Gf*Am*Af*Ad*Zd*Id^Um^Cf*m5Ue*Gf*Am*Gf*Af*Am*Gf*Am*Um*Gm^m5Ue^m5Ue^m5Ce^Ge^m5Ue^Ge^Ae
RM1118855	69	Um*Gm*Uf*Af*Gf*Am*Af*Ad*Zd*Id^Um^Cf*m5Ue*Gf*Am*Gf*Af*Am*Gf*Am*Um*Gm^m5Ue^m5Ue^m5Ce^Ge^m5Ue^Ge^Ae
RM1118856	70	Um!Gm*Uf*Am*Gf*Am*Af*Ad*Zd*Id^Um^Cf*m5Ue*Gf*Am*Gf*Am*Gf*Am*Um*Gm^m5Ue^m5Ue^m5Ce^Ge^m5Ue^Ge!Ae
RM1118857	71	Um!Gf*Uf*Af*Gf*Am*Af*Ad*Zd*Id^Um^Cf*m5Ue*Gf*Am*Gf*Am*Gf*Am*Um*Gm^m5Ue^m5Ue^m5Ce^Ge^m5Ue^Ge!Ae
RM1118858	72	Um!Gm*Uf*Am*Gf*Am*Af*Ad*Zd*Id^Um^Cf*m5Ue*Gf*Am*Gf*Am*Gf*Am*Um*Gm^m5Ue^m5Ue^m5Ce^Ge^m5Ue^Ge!Ae
RM1118859	73	Um!Gm*Uf*Am*Gf*Am*Af*Ad*Zd*Id^Um^Cf*m5Ue*Gf*Am*Gf*Am*Gf*Am*Um*Gm^m5Ue^m5Ue^m5Ce^Ge^m5Ue^Ge!Ae
RM1118860	74	Gm!Uf*Am*Gf*Am*Af*Ad*Zd*Id^Um^Cf*m5Ue*Gf*Am*Gf*Am*Gf*Am*Um*Gm^m5Ue^m5Ue^m5Ce^Ge^m5Ue^Ge!Ae
RM1118861	75	Gm!Uf*Am*Gf*Am*Af*Ad*Zd*Id^Um^Cf*m5Ue*Gf*Am*Gf*Am*Gf*Am*Um*Gm^m5Ue^m5Ue^m5Ce^Ge^m5Ue^Ge!Ae
RM1118862	76	Uf!Am*Gf*Am*Af*Ad*Zd*Id^Um^Cf*m5Ue*Gf*Am*Gf*Am*Gf*Am*Um*Gm^m5Ue^m5Ue^m5Ce^Ge^m5Ue^Ge!Ae
RM1118863	77	Uf!Am*Gf*Am*Af*Ad*Zd*Id^Um^Cf*m5Ue*Gf*Am*Gf*Am*Gf*Am*Um*Gm^m5Ue^m5Ue^m5Ce^Ge^m5Ue^Ge!Ae
RM1118864	78	Um!Gm*Uf*Am*Gf*Am*Af*Ad*Zd*Id^Um^Cf*m5Ue*Gf*Am*Gf*Am*Gf*Am*Um*Gm^m5Ue^m5Ue^m5Ce^Ge^m5Ue^Ge^Ae!Ae
RM1118865	79	Um!Gm*Uf*Am*Gf*Am*Af*Ad*Zd*Id^Um^Cf*m5Ue*Gf*Am*Gf*Am*Gf*Am*Um*Gm^m5Ue^m5Ue^m5Ce^Ge^m5Ue^Ge!Ae
RM1118866	80	Um!Gm*Uf*Am*Gf*Am*Af*Ad*Zd*Id^Um^Cf*m5Ue*Gf*Am*Gf*Am*Gf*Am*Um*Gm^m5Ue^m5Ue^m5Ce^Ge^m5Ue^Ge!Ae
RM1118867	81	Um!Gm*Uf*Am*Gf*Am*Af*Ad*Zd*Id^Um^Cf*m5Ue*Gf*Am*Gf*Am*Gf*Am*Um*Gm^m5Ue^m5Ue^m5Ce^Ge^m5Ue^Ge!Am
RM1118868	82	Um*Gm*Uf*Am*Gf*Am*Af*Ad*Zd*Id^Um^Cf*m5Ue*Gf*Am*Gf*Am*Gf*Am*Um*Gm^m5Ue^m5Ue^m5Ue^Ge
RM1118869	83	Um*Gm*Uf*Am*Gf*Am*Af*Ad#Zd*Id^Um^Cf*m5Ue*Gf*Am*Gf*Am*Gf*Am*Um*Gm^m5Ue^m5Ue^m5Ue^Ge
RM1118870	84	Um*Gm*Uf*Am*Gf*Am*Af*Ad*Zd#Id^Um^Cf*m5Ue*Gf*Am*Gf*Am*Gf*Am*Um*Gm^m5Ue^m5Ue^m5Ue^Ge
RM1118871	85	Um*Gm*Uf*Am*Gf*Am*Af*Ad*Zd*Id#Um^Cf*m5Ue*Gf*Am*Gf*Am*Gf*Am*Um*Gm^m5Ue^m5Ue^m5Ue^Ge
RM1118872	86	Um*Gm*Uf*Af*Gf*Am*Af*Ad*Zd*Id^Um^Cf*m5Ue*Gf*Am*Gf*Am*Gf*Am*Um*Gm^m5Ue^m5Ue^m5Ue^Ge
RM1118873	87	Um*Gm*Uf*Af*Gf*Am*Af*Ad*Zd*Id^Um^Cf*m5Ue*Gf*Am*Gf*Am*Gf*Am*Um*Gm^m5Ue^m5Ue^m5Ue^Ge
RM1118874	88	Um!Gm*Uf*Am*Gf*Am*Af*Ad*Zd*Id^Um^Cf*m5Ue*Gf*Am*Gf*Am*Gf*Am*Um*Gm^m5Ue^m5Ue^m5Ue!Ge
RM1118875	89	Um!Gf*Uf*Af*Gf*Am*Af*Ad*Zd*Id^Um^Cf*m5Ue*Gf*Am*Gf*Am*Gf*Am*Um*Gm^m5Ue^m5Ue^m5Ue!Ge
RM1118876	90	Um!Gm*Uf*Am*Gf*Am*Af*Ad*Zd*Id^Um^Cf*m5Ue*Gf*Am*Gf*Am*Gf*Am*Um*Gm^m5Ue^m5Ue^m5Ue!Ge

Fig. 7
continued

RM118877	91	Um#Gf*Um*Af*Gm*Ae*Af*Ae°Zd#Id*Um°Cf*m5Ue*Gm*Af*Gf*Cm*Cm*Cf*Af*Um*Gm*Uf*Uf*Gm*Um*Cm*Gm*Um*Gm#Am
RM118878	92	Um#Gf*Uf*Af*Gf*Ae*Af*Ae°Zd#Id*Um°Cf*m5Ue*Gm*Af*Gm*Cm*Cf*Cm*Af*Um*Gm*Um*Gm*Um*Gm*Um*Gf#Am
RM118879	93	Um*Gf*Um*Af*Gm*Af*Am*Ae°Zd*Id^Um°Cf*m5Ue*Gf*Am*Gf*Cm*Cf*Am*Um*Gm°m5Ue°m5Ue°Ge
RM118880	94	Um*Gm*Uf*Am*Gf*Am*Af*Ae°Zd*Id^Um°Cf*m5Ue*Gm*Af*Gm*Cf*Cm*Am*Um*Gm°m5Ue°m5Ue°Ge

Fig. 8A

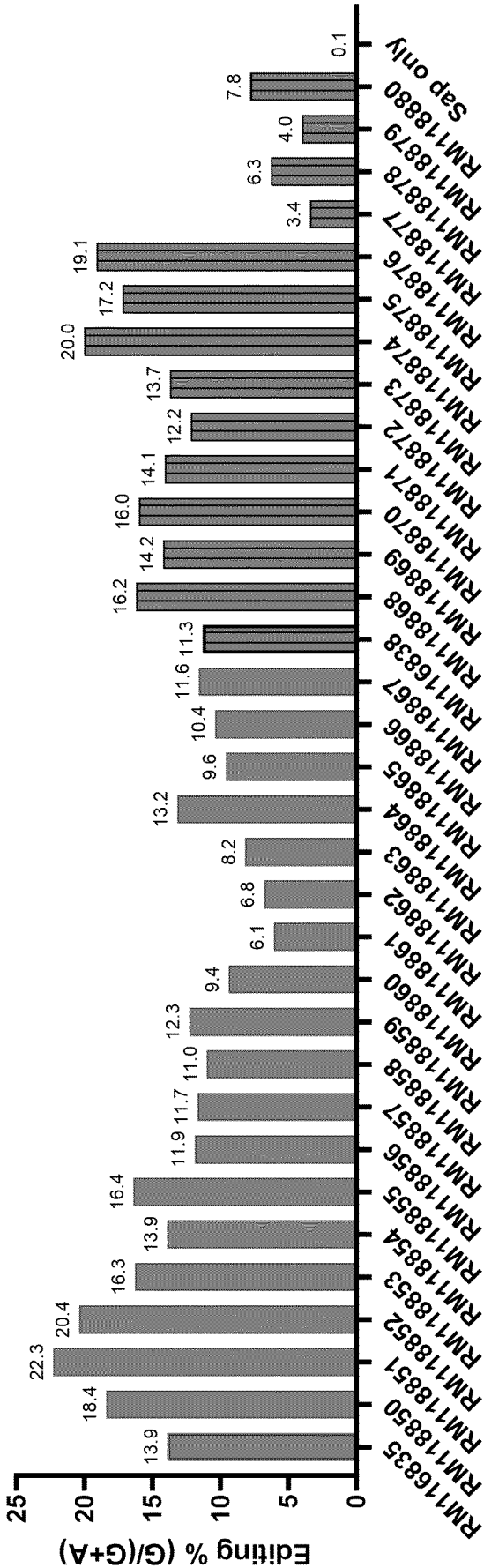
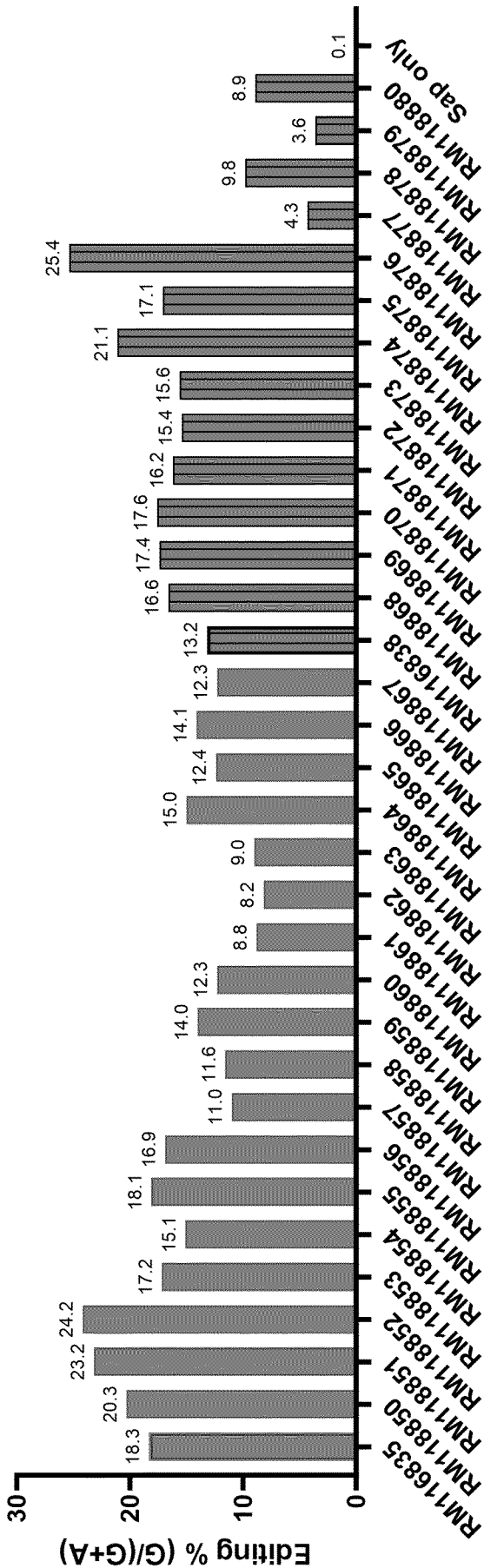


Fig. 8B



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2024/066520

A. CLASSIFICATION OF SUBJECT MATTER INV. C12N15/113 ADD. A61K31/7088 According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) C12N A61K Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) EPO-Internal, WPI Data, BIOSIS, CHEM ABS Data, COMPENDEX, EMBASE, FSTA		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LOPERA FRANCISCO ET AL: "Resilience to autosomal dominant Alzheimer's disease in a Reelin-COLBOS heterozygous man", NATURE MEDICINE, vol. 29, no. 5, 1 May 2023 (2023-05-01), pages 1243-1252, XP093194404, New York ISSN: 1078-8956, DOI: 10.1038/s41591-023-02318-3 Retrieved from the Internet: URL:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10202812/pdf/41591_2023_Article_2318.pdf> the whole document ----- - / - -	1-10, 12-14, 17-26
☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 23 September 2024		Date of mailing of the international search report 02/10/2024
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer Piret, Bernard

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2024/066520

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2010/091399 A2 (UNIV SOUTH FLORIDA [US]; UNIV VANDERBILT [US] ET AL.) 12 August 2010 (2010-08-12) claim 1 -----	1 - 10, 12 - 14, 17 - 26
X,P	WO 2024/081934 A1 (MASSACHUSETTS GEN HOSPITAL [US] ET AL.) 18 April 2024 (2024-04-18) the whole document -----	1 - 29

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP2024/066520

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☐ forming part of the international application as filed.
 - b. ☒ furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).
☒ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.
2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2024/066520

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2010091399	A2	12-08-2010	US 2012058109 A1 08-03-2012
			US 2016271213 A1 22-09-2016
			US 2018236028 A1 23-08-2018
			WO 2010091399 A2 12-08-2010

WO 2024081934	A1	18-04-2024	NONE
