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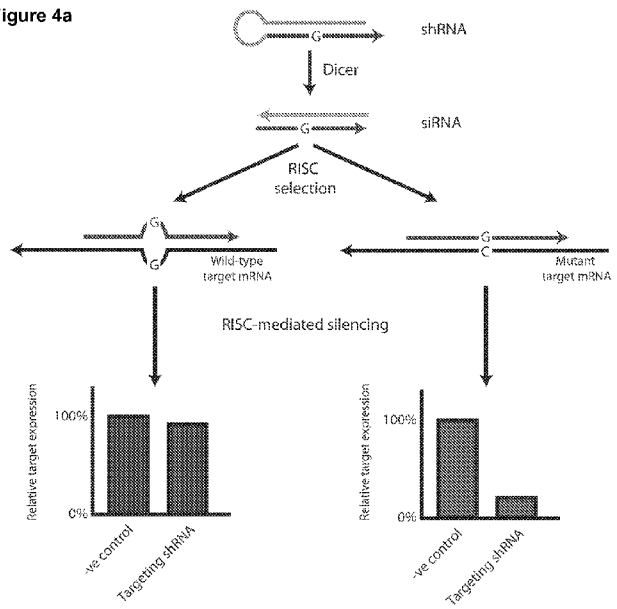
(54) **Title**: THERAPEUTIC MOLECULES FOR USE IN THE SUPPRESSION OF PARKINSON'S DISEASE

Figure 4a



(57) **Abstract**: There is provided a double stranded nucleic acid (dsNA) molecule comprising a nucleic acid sense strand and an RNA antisense strand wherein the RNA antisense strand binds to position 6176 on a target RNA nucleotide sequence that comprises the nucleotide sequence of SEQ ID NO: 10; wherein at least a portion of the RNA antisense strand and the nucleic acid sense strand together define a base-paired nucleic acid duplex wherein the nucleotides of the antisense RNA strand define consecutively numbered antisense nucleotide positions, said numbers increasing in a 5' to 3' direction on the antisense strand, with position 1 (p1) defined as the extreme 5' nucleotide present on the RNA antisense strand of the nucleic acid duplex that is base paired with a corresponding nucleotide present on the nucleic acid sense strand of the nucleic acid duplex; and wherein the RNA antisense strand has a uracil nucleotide located at any one of positions p1 to p9 that binds to an adenine nucleotide located at position 6176 of an RNA nucleotide sequence comprising the nucleotide sequence of SEQ ID NO: 10. Also provided are vectors encoding said dsNA, and therapeutic uses of the dsNA molecule for suppressing/preventing Parkinson's disease.

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Therapeutic molecules for use in the suppression of Parkinson's disease

This patent application claims priority to GB 1105137.2 filed on 28 March 2011, which is hereby incorporated by reference in its entirety.

The present invention relates to therapeutic molecules and the use of such therapeutic molecules in the suppression or prevention of Parkinson's disease.

Parkinson's disease (PD) is a progressive neurological condition that is associated with the loss of dopamine-producing neurons in the *substantia nigra* area of the brain.

PD affects approximately 120,000 people in the United Kingdom, with an average age of onset of 60 years.

Clinical symptoms of PD include bradykinesia (slowness of movement), tremor, muscle rigidity and postural instability. Due to the progressive nature of the disease, the symptoms may worsen over time and have a significant impact on the patient's quality of life. In addition, cognitive impairment may develop during the later stages of the disease. The need for high levels of support and care for PD patients can also use significant resources of health care systems.

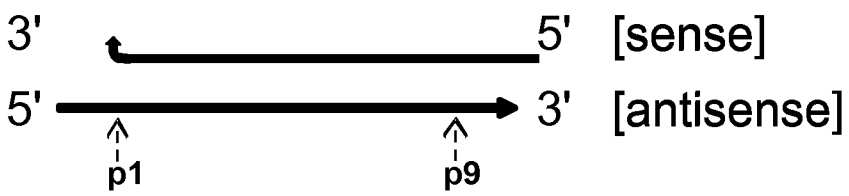
There is no known cure for PD. Current treatments focus on the relief of disease symptoms, and include the use of a variety of different pharmaceutical agents to compensate for the decreased dopamine production in the brain. However, many of these treatments are associated with undesirable side effects.

There is therefore a need for new therapies that can be used to suppress and/ or prevent Parkinson's disease.

The present invention addresses one or more of the above needs by providing double stranded nucleic acid (dsNA) molecules and nucleic acid vectors according to the present claims.

In one aspect the invention provides a double stranded nucleic acid (dsNA) molecule comprising a nucleic acid sense strand and an RNA antisense strand; wherein the RNA antisense strand binds to position 6176 on a target RNA nucleotide sequence that comprises the nucleotide sequence of SEQ ID NO: 10; wherein at least a portion of the RNA antisense strand and the nucleic acid sense strand together define a base-paired nucleic acid duplex having the structure of Formula (I):

Formula (I):



wherein the nucleotides of the antisense RNA strand define consecutively numbered antisense nucleotide positions, said numbers increasing in a 5′ to 3′ direction on the antisense strand, with position 1 (p1) defined as the extreme 5′ nucleotide present on the RNA antisense strand of the nucleic acid duplex that is base paired with a corresponding nucleotide present on the nucleic acid sense strand of the nucleic acid duplex;
and wherein the RNA antisense strand has a uracil nucleotide located at any one of positions p1 to p9 that binds to an adenine nucleotide located at position 6176 of an RNA nucleotide sequence comprising the nucleotide sequence of SEQ ID NO: 10.

While the precise causes of PD are yet to be fully elucidated, it is believed that certain cases of PD have a hereditary component. Research into the genetics of

hereditary PD has identified 16 chromosomal "PARK" loci with linkage to the disease. Subsequently, a group of ten genes have been identified which are implicated in molecular pathways leading to PD pathogenesis. In some cases, a mutant form of a gene is created by the presence of a single nucleotide polymorphism (SNP). Collectively, these hereditary cases of PD account for approximately five percent of all cases of PD and offer defined therapeutic targets for those patients bearing these genetic mutations.

One of the genes involved in hereditary PD is LRRK2. A particular mutation in this gene – the G2019S single nucleotide polymorphism – has been linked to PD.

The LRRK2-G2019S mutation is the most common PD-linked mutation at present and represents the most attractive PD-mutation for allele-specific silencing. The mutation leads to a G:A conversion in the LRRK2 mRNA. The mutant LRRK2 gene may be present in heterozygous form, such that an affected individual carries one mutant LRRK2 allele and one wildtype LRRK2 allele.

The nucleotide sequence of SEQ ID NO: 10 is an mRNA sequence encoding the product of a human LRRK2 gene that has a G2019S single nucleotide polymorphism (also referred to herein as mutant LRRK2 or LRRK2-G2019S). The G2019S SNP causes the presence in the mutant LRRK2 mRNA (SEQ ID NO: 10) of an adenine nucleotide (mutant residue) at position 6176, whereas the wildtype LRRK2 mRNA sequence (SEQ ID NO: 11) has a guanine nucleotide at position 6176.

Thus, in use the dsNA molecule of the invention targets and/or binds to the LRRK2 gene, in particular to the LRRK2-G2019S mutant.

In more detail, the present inventors have identified that it is possible to create dsNA molecules that can be used to selectively reduce the expression in a target cell of the above-described mutant LRRK2 allele, while preserving expression of

wildtype LRRK2. This effect is mediated via the mechanism of RNA interference (RNAi).

RNA interference (RNAi) has emerged as a highly credible strategy with which to sequence-specifically silence genes-of-interest by preventing the translation of targeted mRNA transcripts into proteins. The endogenous RNAi pathway is now well characterised and involves the processing of non-coding RNA sequences with characteristic stem-loop secondary structures, termed primary-microRNAs (pri-miRNAs), into short 21-23nt single-stranded mature miRNAs that are antisense to targeted transcripts – see Fig. 1. Complete complementarity of the mature miRNA sequence to the target mRNA leads to target cleavage and inhibition of protein synthesis, whereas incomplete pairing leads to translational repression either through mRNA destabilization or removal of the 5′ cap or 3′ poly-A termination signal.

Activation of the endogenous RNAi pathway may be achieved by the introduction into a cell of exogenous double stranded RNA, or by using DNA-encoded plasmids that transcribe RNA mimics of RNAi pathway precursors termed short hairpin RNAs (shRNAs) or primary-miRNA (pri-miRNA) mimics. These precursors are recognised and processed by the enzyme Dicer. The resulting short lengths of double stranded RNA are termed short interfering RNAs (siRNA). In addition to cleaving RNA, Dicer also promotes incorporation of siRNA into the RNA-induced silencing complex (RISC). Incorporation of siRNA into RISC leads to separation of the two RNA strands, termed the guide strand and the passenger strand. The guide strand binds to an exonuclease enzyme present in the RISC complex termed Argonaute, while the passenger strand is degraded. Whilst either strand of an siRNA molecule could in principle be incorporated as the guide strand, in practice the strand with the most thermodynamically unstable 5′ end is favoured. Once the guide strand is bound, Argonaute targets and cleaves mRNA molecules complementary to the guide strand. Thus, the guide strand must contain the antisense sequence of the target mRNA.

In many autosomal dominant disease settings where removal of a mutant allele is expected to be beneficial, a complete silencing of both mutant and wild-type alleles could be detrimental due to important roles of the wild-type protein. It is therefore advantageous to target the disease-causing genes involved in hereditary PD in such a way that expression of the mutant allele can be selectively limited whilst as much expression as possible of the wild-type allele is retained to carry out the endogenous function.

Thus, the dsNA molecules of the present invention reduce the expression of the mutant LRRK2 allele in preference to reducing the expression of the wildtype LRRK2 allele. This provides an advantage in that the negative effects of the mutant LRRK2 allele are reduced, while necessary wildtype LRRK2 activity is retained.

In one embodiment, the antisense strand of a dsNA molecule of the invention binds to a region of the mutant LRRK2 mRNA sequence that encodes the mutant residue defining the G2019S mutation. The antisense strand is then capable of activating the RNAi mechanisms that will lead to degradation of the mutant LRRK2 mRNA and thus decrease mutant LRRK2 expression.

In one embodiment, the antisense strand is complementary to, and binds to, a region of the mutant LRRK2 mRNA sequence that includes the mutant adenine (A) residue defining the G2019S mutation.

The wildtype LRRK2 mRNA sequence does not possess the mutant adenine (A) residue defining the G2019S SNP, and thus the antisense strand of the dsNA molecule of the invention is not complementary to the wildtype LRRK2 mRNA strand at the site of the G2019S polymorphism. Instead of an adenine (A) residue, the wildtype possesses a guanine (G) residue at the same position. Thus, the antisense strand binds more weakly to the wildtype LRRK2 mRNA

sequence than it does to the mutant LRRK2 mRNA sequence – i.e. the antisense strand has a greater affinity for the mutant LRRK2 mRNA sequence than for the wildtype LRRK2 sequence.

The antisense nucleotide located immediately 5′ to position p1 (as defined above), to the extent that any such 5′ nucleotide is present, is not base-paired with a corresponding nucleotide on the sense strand.

As used herein, the term "complementary" refers to a nucleic acid molecule that forms hydrogen bonds with another nucleic acid molecule, or with itself, with Watson-Crick base pairing. Watson-Crick base pairing refers to the following hydrogen bonded nucleotide pairings: A:T and C:G (for DNA); and A:U and C:G (for RNA). For example, two or more complementary nucleic acid molecule strands can have the same number of nucleotides (i.e. have the same length and form one double-stranded region, with or without an overhang) or have a different number of nucleotides (e.g. one strand may be shorter than but fully contained within another strand or one strand may overhang the other strand).

In one embodiment, the double stranded nucleic acid (dsNA) molecule is any type of double stranded nucleic acid molecule that is able to mediate sequence-specific RNA interference against the target mutant LRRK2 gene. Thus, for example, the dsNA molecule may be a double stranded RNA, an siRNA, a short interfering nucleic acid, an shRNA, or a pri-miRNA.

One or both of the sense strand and antisense strand of the dsNA molecule may comprise additional nucleotides that do not form part of the double stranded duplex portion. For example, one or both of the sense strand and antisense strand may have a 3′ and/ or a 5' overhang region.

In one embodiment, the uracil nucleotide located at any one of positions p1 to p9 on the antisense strand is located at a position selected from p1, p2, p3, p4, p5, p6, p7 p8 or p9.

In another embodiment, the uracil nucleotide located at any one of positions p1 to p9 on the antisense strand is located at a position selected from p1, p2, p3, p4, p5, p6 or p7.

In another embodiment, the uracil nucleotide located at any one of positions p1 to p9 on the antisense strand is located at a position selected from p2, p3, p4, p5, or p6.

In another embodiment, the uracil nucleotide located at any one of positions p1 to p9 on the antisense strand is located at a position selected from p3, p4, or p5.

In another embodiment, the uracil nucleotide located at any one of positions p1 to p9 on the antisense strand is located at a position selected from p3 or p4, or from p4 or p5.

In another embodiment, the uracil nucleotide located at any one of positions p1 to p9 on the antisense strand is located at position p4.

In one embodiment, the antisense strand of the dsNA molecule comprises or consists of a nucleic acid sequence selected from any one of SEQ ID NOs: 1-9, wherein the first nucleotide position of said SEQ ID NO occupies position p1 on the antisense strand. In one embodiment, the antisense strand of the dsNA molecule comprises or consists of SEQ ID NO: 4, wherein the first nucleotide position of said SEQ ID NO occupies position p1 on the antisense strand.

In use, the underlined uracil nucleotide in said nucleic acid sequence binds to the adenine nucleotide located at position 6176 of a nucleotide sequence having the

sequence of SEQ ID NO: 10. Thus, the antisense strand binds to a mutant LRRK2 mRNA sequence at the site of the G2019S mutation.

In one embodiment, the antisense strand of the dsNA molecule comprises a nucleic acid sequence selected from any of SEQ ID NOs: 1-9, wherein the first nucleotide position of said SEQ ID NO occupies position p1 on the antisense strand, and wherein said antisense strand is up to 21 nucleotides in length (for example, 20 or 21 nucleotides).

In one embodiment, the antisense strand of the dsNA molecule comprises a nucleic acid sequence selected from any of SEQ ID NOs: 1-9, wherein the first nucleotide position of said SEQ ID NO occupies position p1 on the antisense strand, and wherein said antisense strand is up to 27 nucleotides in length (for example, 20, 21, 22, 23, 24, 25, 26 or 27 nucleotides).

In one embodiment, the antisense strand of the dsNA molecule comprises or consists of a nucleic acid sequence that differs from SEQ ID NO: 3 by a single nucleotide, wherein the first nucleotide position of said SEQ ID NO occupies position p1 on the antisense strand, and wherein said difference is that the final 3′ uracil (U) nucleotide in said SEQ ID NO has been replaced with a cytosine (C) nucleotide.

In one embodiment, the antisense strand of the dsNA molecule comprises or consists of a nucleic acid sequence that differs from SEQ ID NO: 4 by a single nucleotide, wherein the first nucleotide position of said SEQ ID NO occupies position p1 on the antisense strand, and wherein said difference is that the final 3′ uracil (U) nucleotide in said SEQ ID NO has been replaced with a cytosine (C) nucleotide.

In another embodiment, the antisense strand of the dsNA molecule comprises or consists of a nucleic acid sequence that differs (by at most 3, or 4 nucleotides)

from a nucleotide sequence selected from any one of SEQ ID NOs: 1-9, wherein the first nucleotide position of said SEQ ID NO occupies position p1 on the antisense strand, and with the proviso that said difference does not occur at the underlined uracil nucleotide identified in any of SEQ ID NOs: 1-9.

In another embodiment, the antisense strand of the dsNA molecule comprises or consists of a nucleic acid sequence that differs (by at most 1, or 2 nucleotides) from a nucleotide sequence selected from any one of SEQ ID NOs: 1-9, wherein the first nucleotide position of said SEQ ID NO occupies position p1 on the antisense strand, and with the proviso that said difference does not occur at the underlined uracil nucleotide identified in any of SEQ ID NOs: 1-9.

In one embodiment, the dsNA molecule is a dsNA molecule that is capable of being processed in the cytoplasm of a target cell by the enzyme Dicer. Dicer is an endoribonuclease enzyme present in eukaryotic cells that catalyses the breakdown of double stranded RNA molecules (including pre-microRNA molecules and short hairpin RNA molecules) into short double stranded RNA fragments approximately 20-25 nucleotides in length. The fragments produced by Dicer may be incorporated into the RNA-induced Silencing Complex (RISC).

In one embodiment, the dsNA molecule is a short hairpin RNA (shRNA). In one embodiment, the shRNA has a length of from about 40 nucleotides to about 50 nucleotides (for example, 40, 41, 42, 43, 43, 45, 46, 47, 48, 49 or 50 nucleotides). In one embodiment, the shRNA has a maximum length of 120 nucleotides.

Thus, in one embodiment the antisense strand and the sense strand comprise part of a single strand of ribonucleic acid that is folded upon itself to form a short hairpin RNA. The shRNA molecule is typically transcribed as a single length of ribonucleic acid comprising self-complementary nucleotide sequences that enable the ribonucleic acid to fold upon itself to form the short hairpin RNA that is

recognised by the RNAi pathway elements. In one embodiment, a short hairpin RNA of the invention is delivered into target cells using a nucleic acid vector as described in more detail below.

In one embodiment, the dsNA molecule comprises or consists of a nucleic acid sequence selected from any one of SEQ ID NOs: 12-20.

In one embodiment, the dsNA molecule is an shRNA comprising, or consisting of, a nucleic acid sequence that differs (by at most 1, 2, 3, 4, or 5 nucleotides) from a nucleotide sequence selected from any one of SEQ ID NOs: 12-20.

In one embodiment, the antisense strand and the sense strand comprise part of a single strand of ribonucleic acid that is folded upon itself to form a mimic of precursors of the miRNA pathway, referred to as pri-miRNAs or pre-miRNAs. The pri-miRNA or pre-miRNA molecule is typically transcribed as a single length of ribonucleic acid comprising self-complementary nucleotide sequences that enable the ribonucleic acid to fold upon itself to form the pri-miRNA or pre-miRNA that is recognised by the RNAi pathway elements. Thus, in one embodiment the dsNA molecule is a sequencing mimicking a primary microRNA (pri-miRNA) or a pre-miRNA.

In one embodiment, the dsNA molecule is a small internally segmented interfering RNA (sisiRNA), an asymmetric interfering RNA (aiRNA), or a DNA-RNA chimeric interfering RNA. These types of dsNA molecule are reviewed in Sibley *et al.*, 2010 (PMID [PubMed unique identifier]: 20087319).

In one embodiment, the dsNA molecule is an siRNA molecule. Such molecules are typically 21-23 base pairs in length, and may include 3′ and/or 5′ overhangs (typically 1-4, 1-3 or 1-2 base overhangs). siRNA molecules typically have unphosphorylated hydroxyl groups at the 2′ and 3′ positions.

In one embodiment, the dsNA molecule is a dicer substrate siRNA (D-siRNA) molecule. Thus, in one embodiment, the dsNA molecule is 25-27 base pairs in length (for example, 25, 26 or 27 base pairs). In one embodiment, the dsNA molecule that is a dicer substrate siRNA molecule includes 3′ and/or 5′ overhangs (typically 1-4, 1-3 or 1-2 base overhangs).

D-siRNAs are recognised and processed by Dicer into siRNAs of 21-23 base pairs and facilitate loading into the RNA induced silencing complex. D-siRNA molecules typically have unphosphorylated hydroxyl groups at the 2′ and 3′ positions. Thus, in one embodiment, the dsNA molecule that is a dicer substrate siRNA molecule has unphosphorylated hydroxyl groups at the 2′ and 3′ positions

The antisense strand of a dsNA molecule of the present invention may further comprise one or more nucleotides, wherein, when the antisense strand binds to position 6176 on the target RNA nucleotide sequence (e.g. SEQ ID NO: 10), said one or more nucleotides forms mismatch base-pairing with the corresponding nucleotide(s) located on the target RNA nucleotide sequence.

Mismatch pairings are formed between any two nucleotide bases that together do not form one of the hydrogen-bonded standard Watson-Crick base pairs of (in RNA) A:U and C:G (or A:T and C:G in DNA). Thus, in one embodiment, the antisense strand contains a nucleotide that does not form a standard Watson-Crick base pair when the antisense strand is bound to the target mutant LRRK2 mRNA sequence.

The present inventors have found that the deliberate incorporation of a nucleotide that introduces a mismatch base pairing with the target mutant LRRK2 mRNA sequence surprisingly improves the discrimination of dsNA molecules of the invention with regard to mutant (i.e. target) and corresponding wildtype LRRK2 mRNA. Thus, the presence of the mismatch nucleotide leads to a decrease in the

inhibition of the wildtype LRRK2 allele while preserving the inhibition of the mutant LRRK2 allele.

The antisense nucleotide that forms the mismatch pairing as described above may be any nucleotide capable of forming such a mismatch pairing.

In one embodiment, the presence of a mismatch pairing disrupts the surrounding nucleic acid duplex formed between the antisense strand of a dsNA molecule of the invention and an LRRK2 mRNA molecule.

Due to the fact that purine bases (A and G) are larger than pyrimidine bases (U and C), a purine:purine mismatch occupies more space than a standard C:G or A:U pairing and so disrupts the surrounding nucleic acid duplex to a greater extent than a pyrimidine:purine or pyrimidine:pyrimidine mismatch – see Fig. 4B & 5.

The extent of the disruption to the nucleic acid duplex surrounding the mismatch is believed to influence the ability of the antisense strand to direct cleavage (via RISC) of the LRRK2 mRNA sequence. Thus, the presence (in the antisense strand) of a nucleotide that gives rise to a mismatch pairing occupying a large amount of space (for example a purine:purine mismatch) is understood to result in a maximum decrease in the ability of said antisense strand to direct cleavage of the LRRK2 mRNA sequence.

Thus, without wishing to be bound by any theory, the present inventor believes that the presence of mismatch pairing decreases the affinity with which the antisense strand binds to both the mutant LRRK2 mRNA sequence and the wildtype LRRK2 mRNA sequence. However, because the antisense strand already has a first mismatch nucleotide for the wildtype LRRK2 mRNA sequence (i.e. the uracil (U) nucleotide that binds to the mutant G2019S SNP), the presence of a second mismatch nucleotide provides a much greater mismatch

effect between the antisense strand and the wildtype LRRK2 mRNA sequence. Thus, the effect of a second (or subsequent) mismatch on the affinity of the antisense strand for the wildtype LRRK2 sequence is more pronounced compared to LRRK2-G2019S.

A hierarchy of mismatch pairs showing the extent to which the formation of a given mismatch increases the ability of the antisense strand to discriminate between target LRRK2-G2019S and wildtype LRRK2 is shown in Figure 5.

In one embodiment, the above-described mismatch is provided at a position adjacent to the uracil nucleotide on the antisense strand that binds to position 6176 on SEQ ID NO: 10.

Thus, in one embodiment, the mismatch nucleotide is located immediately 5′ and/or 3′ to the uracil nucleotide (i.e. the uracil that binds to position 6176 on SEQ ID NO: 10).

In another embodiment, the mismatch nucleotide may be located 2, 3, 4, 5 or 6 nucleotide positions 3′ and/or 5′ away from the uracil nucleotide (i.e. the uracil that binds to position 6176 on SEQ ID NO: 10).

In another embodiment, the mismatch nucleotide may be located up to 18 nucleotide positions (for example, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or 18 nucleotide positions) 3′ and/or 5′ away from the uracil nucleotide (i.e. the uracil that binds to position 6176 on SEQ ID NO: 10).

In one embodiment, the antisense strand of a dsNA molecule of the invention comprises a variant sequence of any one of SEQ ID NOs: 1-9. Thus, in one embodiment, the antisense strand of a dsNA molecule of the invention comprises or consists of a variant of any one of SEQ ID NOs: 1-9, wherein one or more (e.g. at most 1, at most 2, at most 3, at most 4, or at most 5) mismatch nucleotides

other than the underlined uracil nucleotide is replaced by a different nucleotide. This may include inclusion of cognate ribonucleotide bases of A, C, G or U, or synthetic ribonucleotide analogues such as, but not exclusively, locked nucleic acids (LNA) or peptide nucleic acids (PNA). In this embodiment, the variant retains the underlined uracil nucleotide as depicted in SEQ ID NOs: 1-9 as a uracil nucleotide.

In one embodiment, the one or more mismatch nucleotide(s) forms a mismatch pairing with the corresponding nucleotide located on the target mRNA, wherein said mismatch pairing is selected from a purine:purine, a purine:pyrimidine, or a pyrimidine:pyrimidine mismatch pairing.

In one embodiment at least one strand of the dsNA molecule of the invention may comprise one or more chemical modifications. Said chemical modification may be introduced to the antisense strand and/ or to the sense strand.

In one embodiment, the at least one chemical modification improves the stability of the dsNA molecule. Thus, in one embodiment, a chemical modification increases the half-life of a dsNA molecule of the invention (e.g. in an aqueous solution). In one embodiment, a chemical modification increases the half-life of a dsNA molecule of the invention when introduced into a target cell.

The chemical modification may comprise a substitution or modification in which the substitution or modification may be in a phosphate backbone bond, a sugar, a base, or a nucleoside. Such nucleoside substitutions can include natural non-standard nucleosides (e.g., 5-methyluridine or 5-methylcytidine or a 2-thioribothymidine), and such backbone, sugar, or nucleoside modifications can include an alkyl or heteroatom substitution or addition, such as a methyl, alkoxyalkyl, halogen, nitrogen or sulphur, or other modifications known in the art.

Reference to nucleic acid(s) and/or nucleotide(s) embraces modified nucleic acid(s). For example a nucleic acid or nucleotide may be modified to increase or decrease the stability of said nucleic acid or nucleotide. In one embodiment, a modified nucleic acid comprises a locked nucleotide (LNA). In more detail, the ribose moiety of an LNA nucleotide is modified with an extra bridge connecting the 2′ oxygen and 4′ carbon. The bridge "locks" the ribose in the 3′-endo (North) conformation, which is often found in the A-form duplexes. LNA nucleotides can be mixed with DNA or RNA residues in the oligonucleotide whenever desired. Such oligomers are commercially available. The locked ribose conformation enhances base stacking and backbone pre-organization. This significantly increases the hybridization properties (melting temperature) of oligonucleotides.

Reference to modified nucleic acid(s) and/or to modified nucleotide(s) also embraces nucleic acid analogues. Nucleic acid analogues are composed of three parts: a phosphate backbone, a pucker-shaped pentose sugar, either ribose or deoxyribose, and one of four nucleobases. An analogue may have any of these altered. Typically the analogue nucleobases confer, among other things, different base pairing and base stacking proprieties. Examples include universal bases, which can pair with all four canon bases, and phosphate-sugar backbone analogues such as PNA, which affect the properties of the chain (PNA can even form a triple helix). Artificial nucleic acids include peptide nucleic acid (PNA), Morpholino and LNA, as well as glycol nucleic acid (GNA) and threose nucleic acid (TNA). Each of these is distinguished from naturally-occurring DNA or RNA by changes to the backbone of the molecule.

The dsNA molecules of the present invention may be made using any suitable process known in the art. Thus, the dsNA molecules of the present invention may be made using chemical synthesis techniques. Alternatively, the dsNA molecules of the present invention may be made using molecular biology techniques (for example, as reported in Yu *et al*. 2002, PMID: 11972060, which is hereby

incorporated by reference in its entirety). Alternatively, the dsNA molecules of the present invention may be synthesized by a commercial supplier.

In one aspect, the invention provides a nucleic acid vector comprising a nucleic acid sequence encoding a dsNA molecule as described herein. In this scenario, the resultant RNA may be produced *in vivo*.

The dsNA molecules of the present invention may be made by conventional expression of a nucleic acid vector encoding said dsNA molecule, followed by conventional RNA recovery. Thus, in this scenario, the RNA is produced *in vitro*.

In one embodiment the nucleic acid vector is a plasmid.

In one embodiment the nucleic acid vector is a viral vector. Thus, in one embodiment the dsNA molecules of the present invention may be delivered into a target cell using a viral vector. The viral vector may be any virus which can serve as a viral vector. Suitable viruses are those which infect the target cells, can be propagated *in vitro*, and can be modified by recombinant nucleotide technology known in the art.

In one embodiment, the viral vector is selected from the group consisting of: an adenovirus vector; an adeno-associated virus vector; a pox virus vector, such as a fowlpox virus vector; an alpha virus vector; a bacloviral vector; a herpes virus vector; a retrovirus vector, such as a lentivirus vector; a Modified Vaccinia virus Ankara vector; a Ross River virus vector; a Sindbis virus vector; a Semliki Forest virus vector; and a Venezuelan Equine Encephalitis virus vector.

In one aspect, the invention provides a method for reducing the expression in a cell of a human LRRK2 gene having the G2019S SNP, comprising administering a therapeutically effective amount of a dsNA molecule as described above to a patient in need thereof, wherein the antisense strand of the dsNA molecule is

capable of binding to position 6176 on an RNA nucleotide sequence having the sequence of SEQ ID NO: 10

In one aspect, the invention provides a dsNA molecule as described herein, or a nucleic acid vector as described herein, for use in the treatment of Parkinson's disease. In one embodiment, the treatment of Parkinson's disease comprises the treatment of a human subject having Parkinson's disease.

In one aspect, the invention provides a therapeutic and/or prophylactic formulation, comprising a dsNA molecule as described above. In one embodiment, the therapeutic and/or prophylactic formulation may be used in the therapeutic and/or prophylactic treatment of Parkinson's Disease.

In one aspect, the invention provides a pharmaceutical composition, comprising a dsNA molecule as described above; and a pharmaceutically acceptable carrier.

The dsNA molecule of the invention may be formulated into a pharmaceutical composition as neutral or salt forms. Pharmaceutically acceptable salts include acid addition salts formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or with organic acids such as acetic, oxalic, tartaric, maleic, and the like. Salts formed with the free carboxyl groups may also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

Administration of pharmaceutical compositions is generally by conventional routes e.g. intravenous, subcutaneous, intraperitoneal, or mucosal routes. The administration may be by parenteral administration; for example, a subcutaneous or intramuscular injection.

Accordingly, the pharmaceutical compositions of the invention may be prepared as injectables, either as liquid solutions or suspensions. Solid forms suitable for solution in, or suspension in, liquid prior to injection may alternatively be prepared. The preparation may also be emulsified, or the peptide encapsulated in liposomes or microcapsules.

The active ingredients are often mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, the pharmaceutical compositions may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, and/or pH buffering agents.

Non-limiting examples of pharmaceutically acceptable carriers include water, saline, and phosphate-buffered saline. In some embodiments, however, the composition is in lyophilized form, in which case it may include a stabilizer, such as bovine serum albumin (BSA). In some embodiments, it may be desirable to formulate the composition with a preservative, such as thiomersal or sodium azide, to facilitate long term storage.

Examples of buffering agents include, but are not limited to, sodium succinate (pH 6.5), and phosphate buffered saline (PBS; pH 6.5 and 7.5).

Additional formulations which are suitable for other modes of administration include suppositories and, in some cases, oral formulations or formulations suitable for distribution as aerosols. For suppositories, traditional binders and carriers may include, for example, polyalkylene glycols or triglycerides; such suppositories may be formed from mixtures containing the active ingredient in the range of 0.5% to 10%, preferably 1%-2%.

Oral formulations include such normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, and the like. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders.

It may be desired to direct the dsNA molecules of the present invention (as described above) to the respiratory system of a subject. Efficient transmission of a therapeutic/prophylactic formulation or medicament to the site of infection in the lungs may be achieved by oral or intra-nasal administration.

Formulations for intranasal administration may be in the form of nasal droplets or a nasal spray. An intranasal formulation may comprise droplets having approximate diameters in the range of 100-5000 μm, such as 500-4000 μm, 1000-3000 μm or 100-1000 μm. Alternatively, in terms of volume, the droplets may be in the range of about 0.001-100 μl, such as 0.1-50 μl or 1.0-25 μl, or such as 0.001-1 μl.

Alternatively, the therapeutic/prophylactic formulation or medicament may be an aerosol formulation. The aerosol formulation may take the form of a powder, suspension or solution. The size of aerosol particles is relevant to the delivery capability of an aerosol. Smaller particles may travel further down the respiratory airway towards the alveoli than would larger particles. In one embodiment, the aerosol particles have a diameter distribution to facilitate delivery along the entire length of the bronchi, bronchioles, and alveoli. Alternatively, the particle size distribution may be selected to target a particular section of the respiratory airway, for example the alveoli. In the case of aerosol delivery of the medicament, the particles may have diameters in the approximate range of 0.1-50 μm, preferably 1-25 μm, more preferably 1-5 μm.

Aerosol particles may be for delivery using a nebulizer (e.g. via the mouth) or nasal spray. An aerosol formulation may optionally contain a propellant and/or surfactant.

By controlling the size of the droplets/particles to within the defined range of the present invention, it is possible to avoid (or minimize) inadvertent medicament delivery to the alveoli and thus avoid alveoli-associated pathological problems such as inflammation and fibrotic scarring of the lungs.

In one embodiment, the therapeutic formulations and pharmaceutical compositions of the invention comprise a pharmaceutically acceptable carrier, and optionally one or more of a salt, excipient, diluent and/ or adjuvant.

In one embodiment, the therapeutic formulations and pharmaceutical compositions of the invention may comprise one or more immunoregulatory agents selected from, for example, immunoglobulins, antibiotics, interleukins (e.g. IL-2, IL-12), and/or cytokines (e.g. IFNγ).

In one embodiment, the therapeutic formulations and pharmaceutical compositions of the invention may comprise one or more antimicrobial compounds, (for example, conventional anti-tuberculosis drugs such as rifampicin, isoniazid, ethambutol or pyrizinamide).

The therapeutic formulations and pharmaceutical compositions of the invention may be given in a single dose schedule (i.e. the full dose is given at substantially one time). Alternatively, the immunogenic compositions, therapeutic formulations, medicaments, pharmaceutical compositions, and prophylactic formulations of the invention may be given in a multiple dose schedule.

A multiple dose schedule is one in which a primary course of treatment (e.g. vaccination) may be with 1-6 separate doses, followed by other doses given at

subsequent time intervals required to maintain and or reinforce the immune response, for example (for human subjects), at 1-4 months for a second dose, and if needed, a subsequent dose(s) after a further 1-4 months.

The dosage regimen will be determined, at least in part, by the need of the individual and be dependent upon the judgment of the practitioner (e.g. doctor)

Simultaneous administration means administration at (substantially) the same time.

Sequential administration of two or more compositions/therapeutic agents means that the compositions/therapeutic agents are administered at (substantially) different times, one after the other.

The therapeutic formulations and pharmaceutical compositions may contain 5% to 95% of active ingredient, such as at least 10% or 25% of active ingredient, or at least 40% of active ingredient or at least 50, 55, 60, 70 or 75% active ingredient.

The therapeutic formulations and pharmaceutical compositions are administered in a manner compatible with the dosage formulation, and in such amount as will be prophylactically and/or therapeutically effective.

In this regard, as used herein, an “effective amount” is a dosage or amount that is sufficient to achieve a desired biological outcome. As used herein, a “therapeutically effective amount” is an amount which is effective, upon single or multiple dose administration to a subject (e.g. a human) for treating, preventing, curing, delaying, reducing the severity of, ameliorating at least one symptom of a disorder or recurring disorder, or prolonging the survival of the subject beyond that expected in the absence of such treatment.

Accordingly, the quantity of active ingredient to be administered depends on the subject to be treated, capacity of the subject's immune system to generate a protective immune response, and the degree of protection desired. Precise amounts of active ingredient required to be administered may depend on the judgment of the practitioner and may be particular to each subject.

Key to SEQ ID NOs.

All nucleotide sequences are written in the direction 5′ – 3′.

SEQ ID NOs 1-9	Nucleotide sequences of an RNA antisense strand complementary to LRRK2-G2019S wherein the uracil nucleotide at position p1-9 (underlined), respectively, binds to the site of the G2019S SNP on the LRRK2-G2019S mRNA.
SEQ ID NO: 10	mRNA sequence of LRRK2 gene having G2019S SNP.
SEQ ID NO: 11	mRNA sequence of wildtype LRRK2 gene.
SEQ ID NO: 12-20	shRNA molecules directed against LRRK2-G2019S, having an antisense strand corresponding to SEQ ID NOs 1-9, respectively (i.e. wherein the nucleotide that binds the G2019S SNP location on the target mRNA is located on the shRNA antisense strand at positions p1-p9, respectively (as defined above)).

Sequences

SEQ ID NO: 1 UGUAGUCAGCAAUCUUUGC

SEQ ID NO: 2 CUGUAGUCAGCAAUCUUUG

SEQ ID NO: 3 GCUGUAGUCAGCAAUCUUU

SEQ ID NO: 4 UGCUGUAGUCAGCAAUCUU

SEQ ID NO: 5 AUGCUGUAGUCAGCAAUCU

SEQ ID NO: 6 AAUGCUGUAGUCAGCAAUC

SEQ ID NO: 7 CAAUGCUGUAGUCAGCAAU

SEQ ID NO: 8 GCAAUGCUGUAGUCAGCAA

SEQ ID NO: 9 AGCAAUGCUGUAGUCAGCA

SEQ ID NO: 10

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SEQ ID NO: 13

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SEQ ID NO: 14
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SEQ ID NO: 19
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SEQ ID NO: 20
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Description of figures

Figure 1

Mechanisms of RNA interference that reduce expression of a target gene.

Figure 2

Figures 2a-b show a comparison of multiple shRNA molecules directed against the LRRK2-G2019S mRNA. The position of the nucleotide in the shRNA antisense strand that binds the G2019S SNP location on the target mRNA varies from positions p1 to p9 (wherein the positions are defined as above). Changes to the sense strand in order to bias correct processing of the shRNA are indicated in bold.

Figure 3

Figures 3a-c show the effect on LRRK2 mutant and wildtype expression of the shRNA molecules depicted in Figure 2.

Figure 4

Figures 4a-b show examples of how the presence of a mismatch nucleotide decreases targeting of non-target wildtype sequence.

Figure 5

A hierarchy of mismatch pairs showing the extent to which the formation of a given mismatch increases the ability of the antisense strand to discriminate between a given target nucleotide is shown. The mismatch of a G:U that results between an antisense strand targeting the LRRK2-G2019S RNA and the wildtype LRRK2 RNA sequence is shown with a box.

Figure 6

Screening of LRRK2 G2019S-targeting siRNAs against dual-luciferase targets.
(A) Dual-luciferase assays at 48 hrs with stated siRNAs targeting the G2019S

LRRK2 mutant following co-transfection with wild-type (dark grey lines – left hand bar of each pair) or mutant (light grey lines – right hand bar of each pair) luciferase targets.

(B) Dual luciferase assays at 48 hrs with siRNA p4 targeting the G2019S LRRK2 mutant at stated concentration following co-transfection with wild-type (dark grey – upper line) or mutant (light grey – lower line) luciferase targets. Values represent mean ratios of Renilla:Firefly luciferase +/2 S.D. from n = 6. Values are normalized to cells transfected with non-specific shRNA and respective luciferase target. * = P,0.05 relative to respective normalising control.

Example 1

Dual-luciferase screening reveals shRNAs that can discriminate the LRRK2-G2019S mutant allele from the wild-type allele

Kinetic studies on RNAi suggest that alignments in the 5' region of the antisense species could lead to improved discrimination of the G2019S mutation. Introduction of G:U wobbles, which the targeting shRNA will create with the wild-type allele, have been reported to strongly interfere with pairings of antisense species to mRNA when placed either 5' or centrally in the RNAi effector. To test this hypothesis, shRNAs were designed with mutation at sequential positions from p1-9 of the antisense arm (Figure 2a-b).

Screening of shRNAs against partial-length LRRK2 dual-luciferase targets revealed sequences that displayed significant allele-specific discrimination (Fig 3a-c) at 24hrs post-transfection. Alignment of the G2019S mutation at p4, displayed 1.88 ($p<0.005$) fold discrimination. Analysis at different points post-transfection revealed that all shRNAs led to increased levels of silencing of both the mutant and wild-type alleles over time (Fig 3a-c). At 72hrs, three shRNAs displayed significant discrimination between the mutant and wild-type alleles; p2, p4 and p5. Construct p4 displayed the greatest 3.7-fold ($p<0.001$) discrimination between mutant and wild-type alleles respectively.

Example 2

In order to verify the sequence alignment of the G2019S mutation in the generated antisense species of the p4 construct, siRNAs with alignment of the mutations at p3, p4 and p5 were screened against the luciferase targets. At 48 hrs post-transfection, siRNA p4 displayed a 7.7-fold (p,0.001) discrimination that was improved upon that seen with shRNA p4 at this time point (Figure 6a). siRNAs p3 and p5 displayed less discrimination between the two alleles, agreeing with the trends from previous shRNA data which showed alignment at p4 to be superior to these two constructs. Further, discrimination by siRNA p4 was evident using siRNA concentrations as low as 0.1 nM, and was increased to

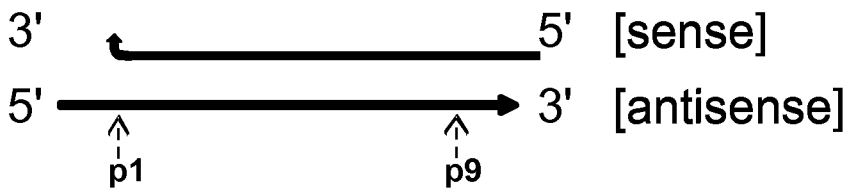
.8-fold by concentrations of siRNA greater than 1 nM in separate experiments (Figure 6b). The greatest discrimination was 10.8-fold (p,0.001) when using 10 nM siRNA, and at this concentration a 96% silencing of the mutant target was seen which was accompanied by a modest 58% silencing of the wild-type target. However, the greatest difference in target silencing was the 61% difference between the 92% silencing of the mutant target and 31% silencing of the wild-type target when using 1 nM siRNA. Collectively this data strongly suggests that the alignment of the G2019S mutation in shRNA p4 is as stated, whilst additionally demonstrating that siRNA p4 has impressive and potent discriminating ability that makes it useful in future preclinical models of G2019S associated pathology.

Claims

1. A double stranded nucleic acid (dsNA) molecule comprising a nucleic acid sense strand and an RNA antisense strand;

wherein the RNA antisense strand binds to position 6176 on a target RNA nucleotide sequence that comprises the nucleotide sequence of SEQ ID NO: 10;

wherein at least a portion of the RNA antisense strand and the nucleic acid sense strand together define a base-paired nucleic acid duplex having the structure of Formula (I):



wherein the nucleotides of the antisense RNA strand define consecutively numbered antisense nucleotide positions, said numbers increasing in a 5′ to 3′ direction on the antisense strand, with position 1 (p1) defined as the extreme 5′ nucleotide present on the RNA antisense strand of the nucleic acid duplex that is base paired with a corresponding nucleotide present on the nucleic acid sense strand of the nucleic acid duplex;

and wherein the RNA antisense strand has a uracil nucleotide located at any one of positions p1 to p9 that binds to an adenine nucleotide located at position 6176 of an RNA nucleotide sequence comprising the nucleotide sequence of SEQ ID NO: 10.

2. The dsNA molecule of claim 1 wherein the uracil nucleotide located at any one of positions p1 to p9 is located at position selected from p3, p4 or p5.

3. The dsNA molecule of claim 1 wherein the uracil nucleotide located at any one of positions p1 to p9 is located at position p4.

4. The dsNA molecule of claim 1 wherein the antisense strand comprises a nucleic acid sequence selected from SEQ ID NOs: 1-9.

5. The dsNA molecule of claim 4 wherein the antisense strand comprises SEQ ID NO: 4.

6. The dsNA molecule of any preceding claim wherein the dsRNA molecule is selected from a short hairpin RNA (shRNA), a pri-miRNA, a pre-miRNA, and an siRNA molecule.

7. The dsNA molecule of any preceding claim wherein the antisense strand comprises one or more nucleotides, wherein, when the antisense strand binds to position 6176 on an RNA nucleotide sequence having the sequence of SEQ ID NO: 10, said one or more nucleotides forms mismatch base-pairing with the corresponding nucleotide(s) located on the target RNA sequence.

8. The dsNA molecule of claim 7 wherein the one or more nucleotides on the antisense strand that forms the mismatch base-pairing is located at a position adjacent to the uracil nucleotide that binds to position 6176 on the target RNA sequence.

9. The dsNA molecule of any preceding claim, wherein the antisense strand comprises or consists of a variant sequence of any one of reference SEQ ID NOs: 1-9, wherein said variant sequence comprises at most 1, at most 2, at most 3, or at most 4 nucleotide changes compared with the nucleotide sequence of the reference SEQ ID NO, though with the proviso that the underlined uracil nucleotide is retained as uracil.

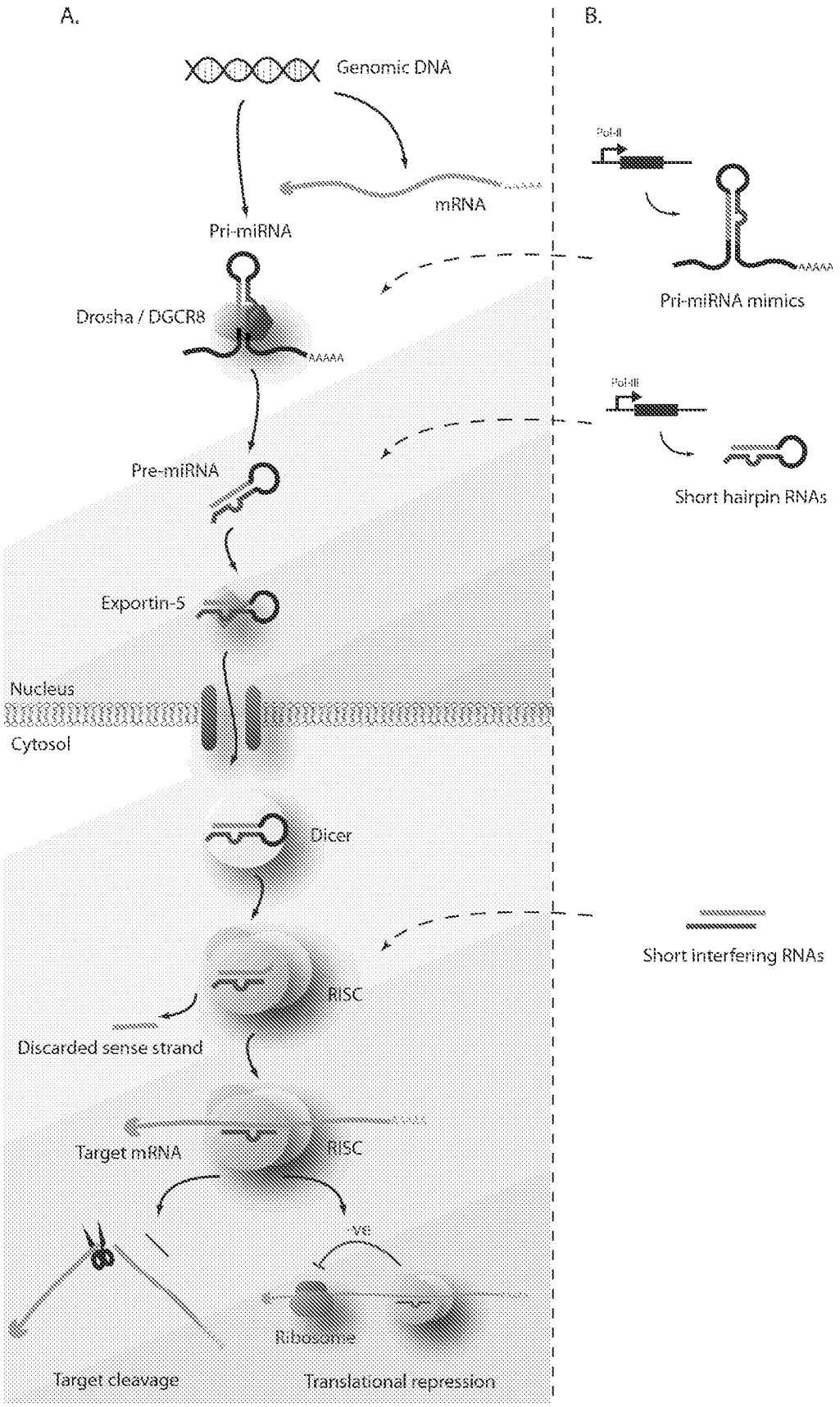
10. The dsNA molecule of claim 1, wherein the dsNA molecule comprises or consists of any one of SEQ ID NOs: 12-20.

11. A nucleic acid vector comprising a nucleic acid sequence wherein the nucleic acid sequence encodes a dsNA molecule according to any one of claims 1 to 10.

12. The nucleic acid vector of claim 11, wherein the vector is a plasmid or a viral vector.

13. The dsNA molecule of any one of claims 1 to 10, or the nucleic acid vector of claim 11 or claim 12, for use in preventing or suppressing Parkinson's disease.

Figure 1


A.
B.
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mRNA
Pri-miRNA
Drosha / DGCR8
Pol-II
Pri-miRNA mimics
Pol-III
Short hairpin RNAs
Pre-miRNA
Exportin-5
Nucleus
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RISC
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Target mRNA
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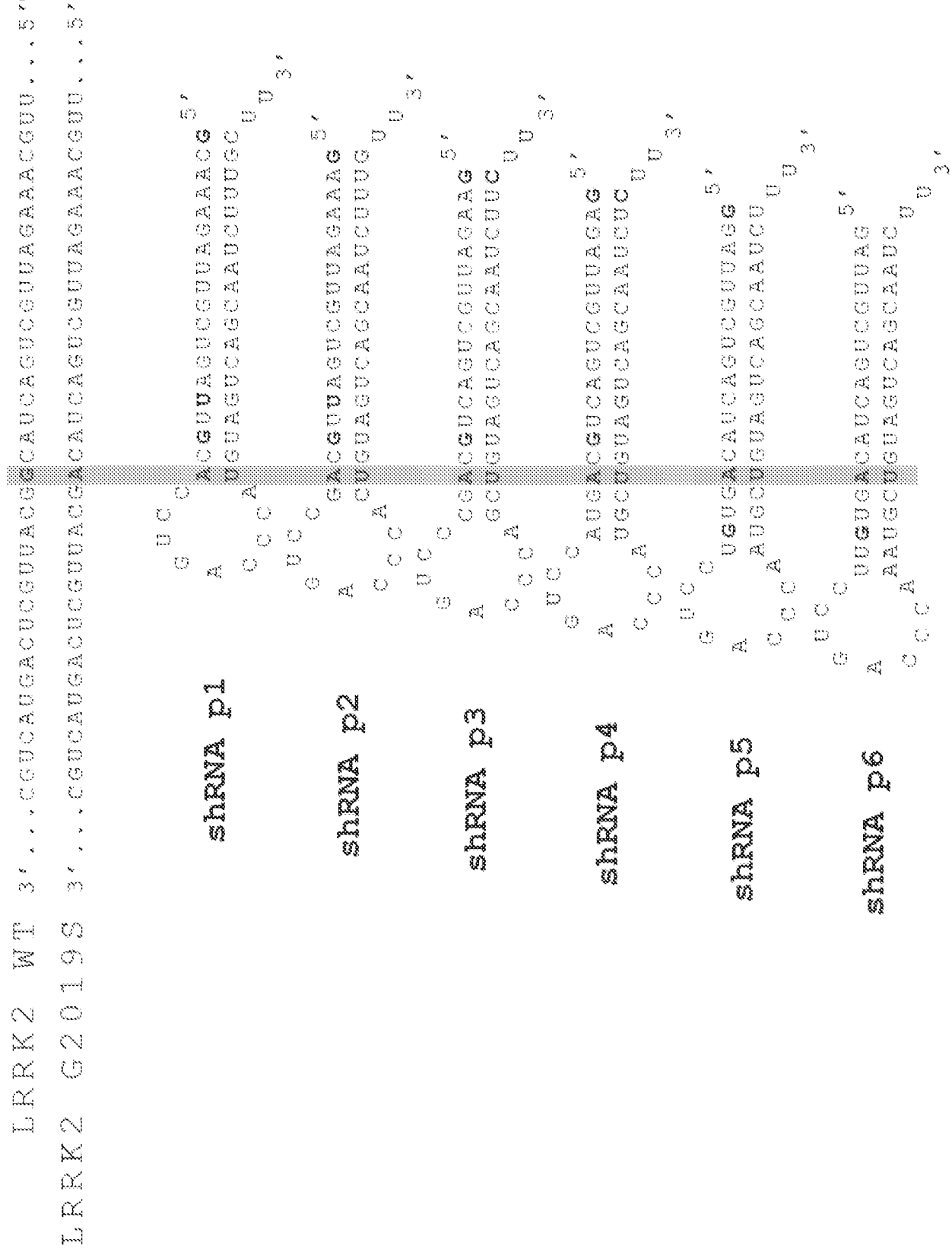
Ribosome
Target cleavage
Translational repression

Figure 2a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LRRK2 G2019S 3'...CGUCAUGACUCGUUACGUCAUCAGUCGUUAGAAACGUU...5'
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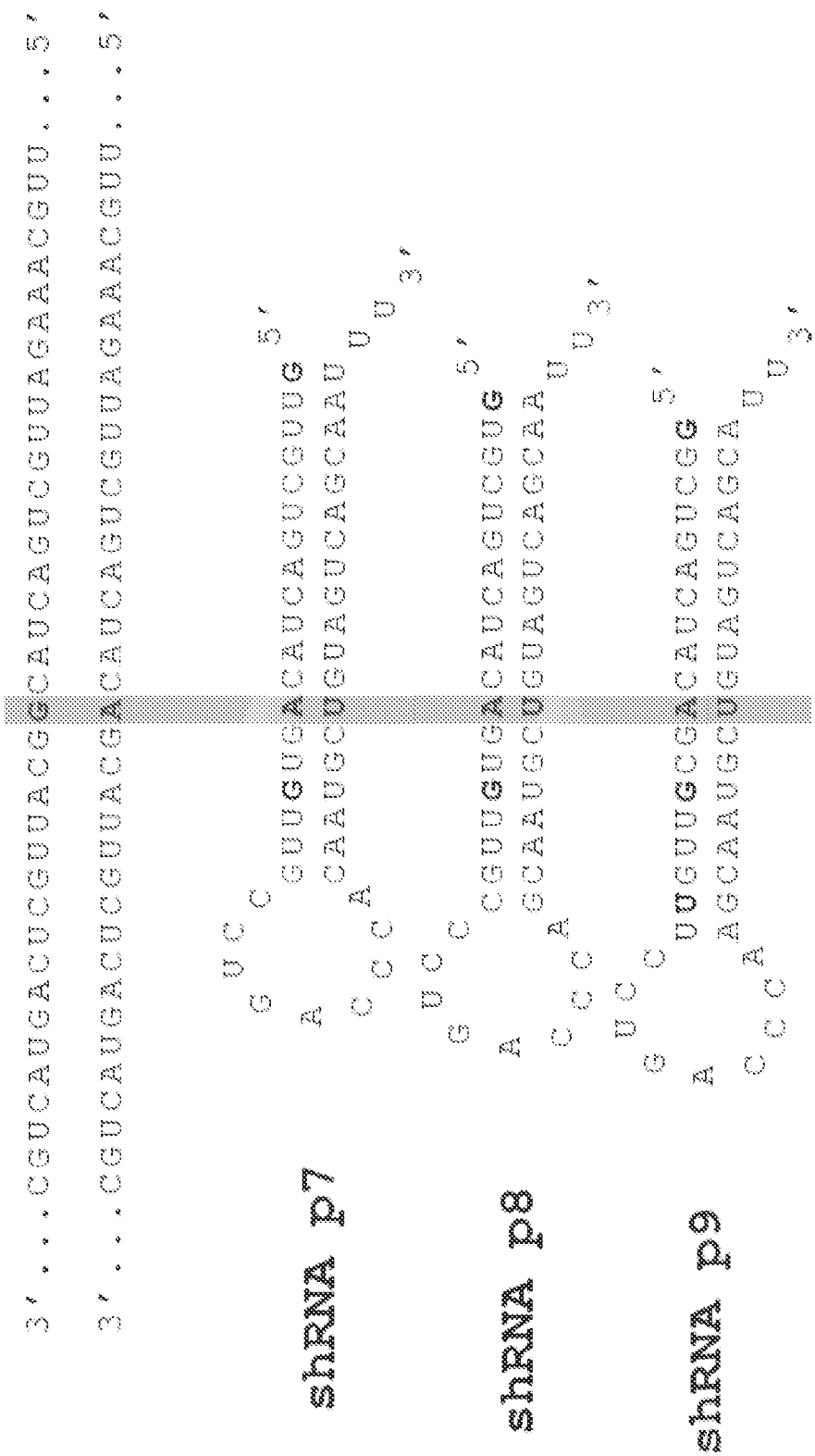
shRNA p3
shRNA p4
shRNA p5
shRNA p6

Figure 2b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shRNA p7
shRNA p8
shRNA p9

Figure 3a

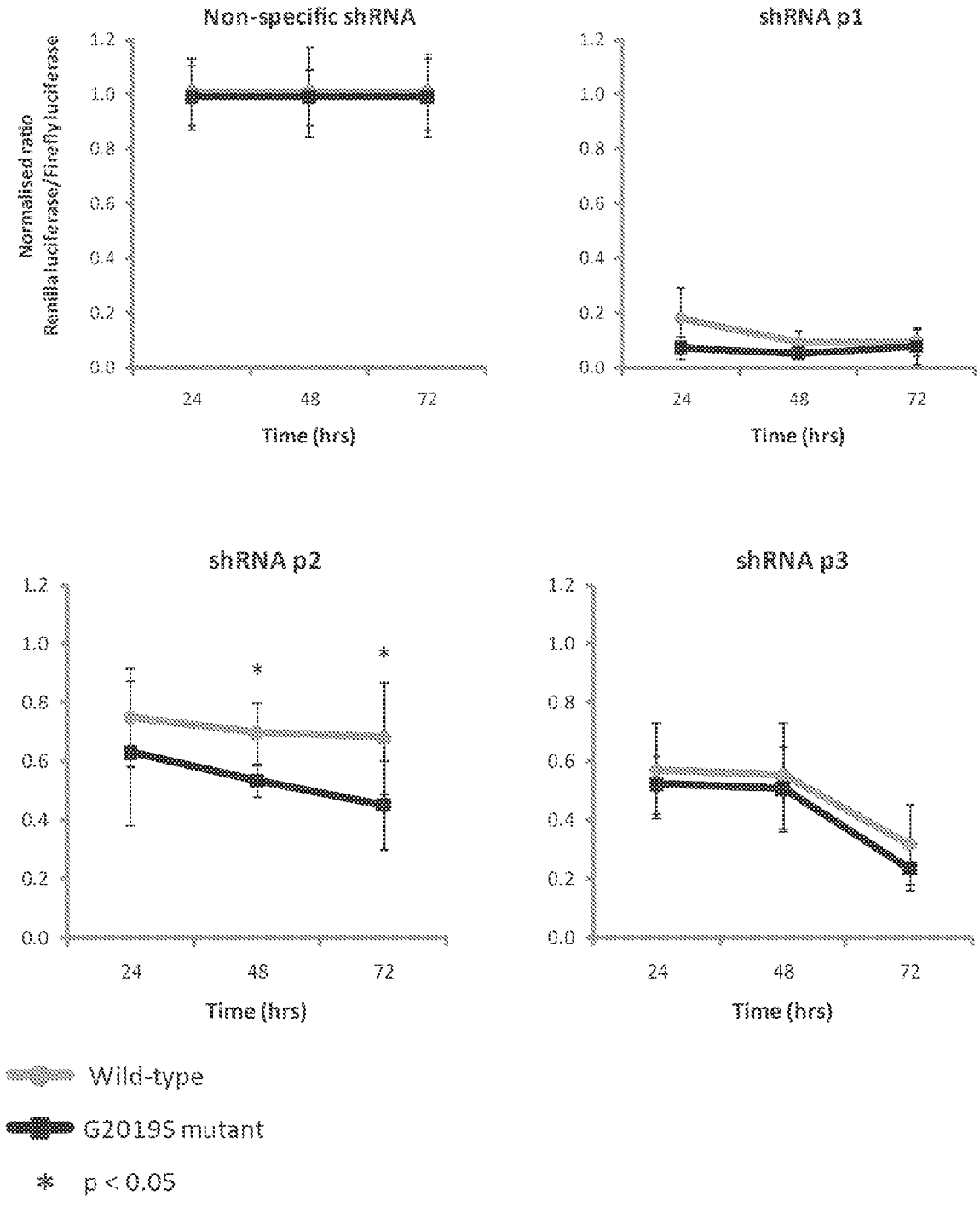

Non-specific shRNA
shRNA p1
shRNA p2
shRNA p3
Normalised ratio
Renilla luciferase/Firefly luciferase
1.2
1.0
0.8
0.6
0.4
0.2
0.0
24
48
72
Time (hrs)
Wild-type
G2019S mutant
* p < 0.05

Figure 3b

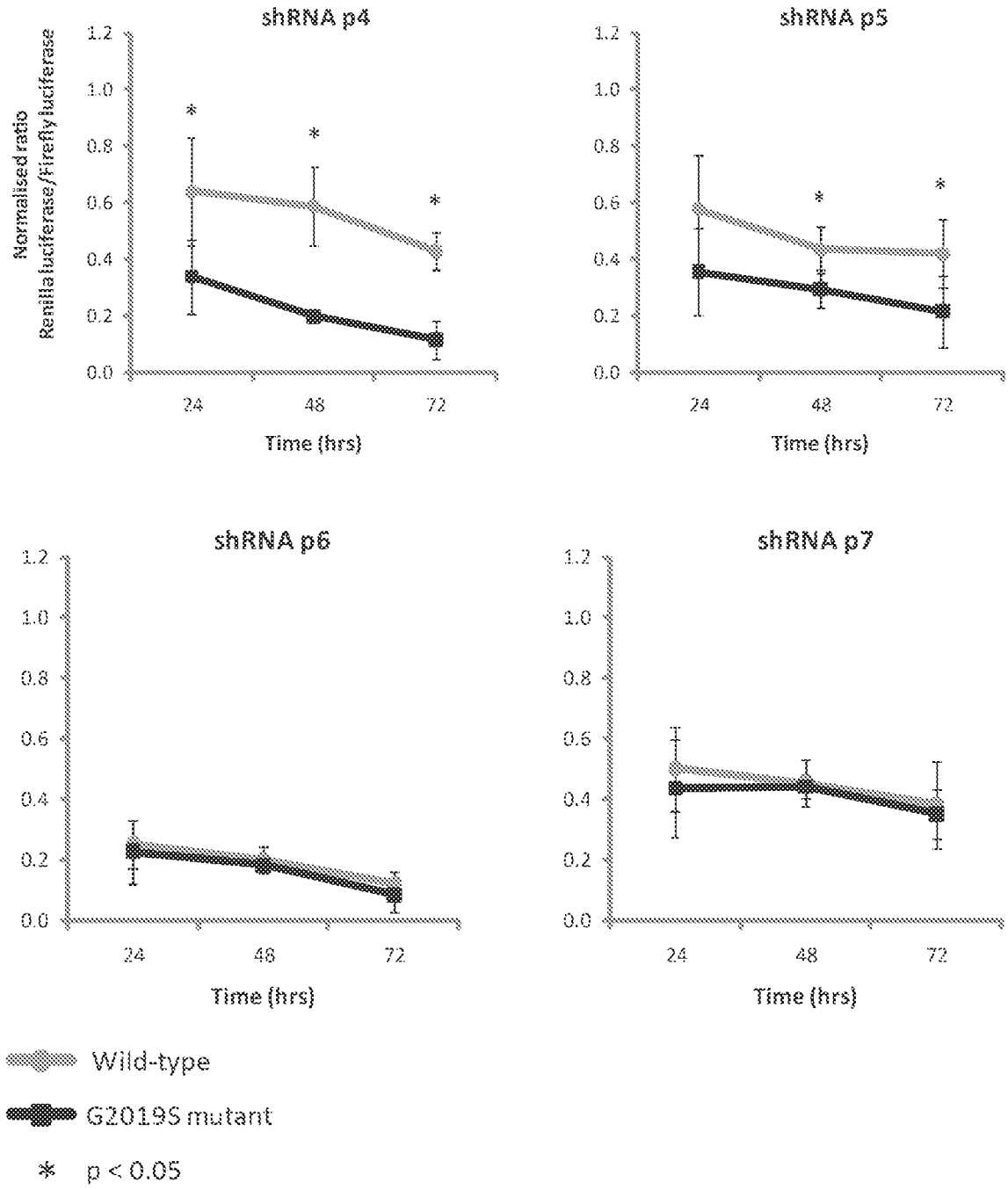

shRNA p4
shRNA p5
shRNA p6
shRNA p7
Normalised ratio
Renilla luciferase/Firefly luciferase
1.2
1.0
0.8
0.6
0.4
0.2
0.0
24
48
72
Time (hrs)
Wild-type
G2019S mutant
* p < 0.05

Figure 3c

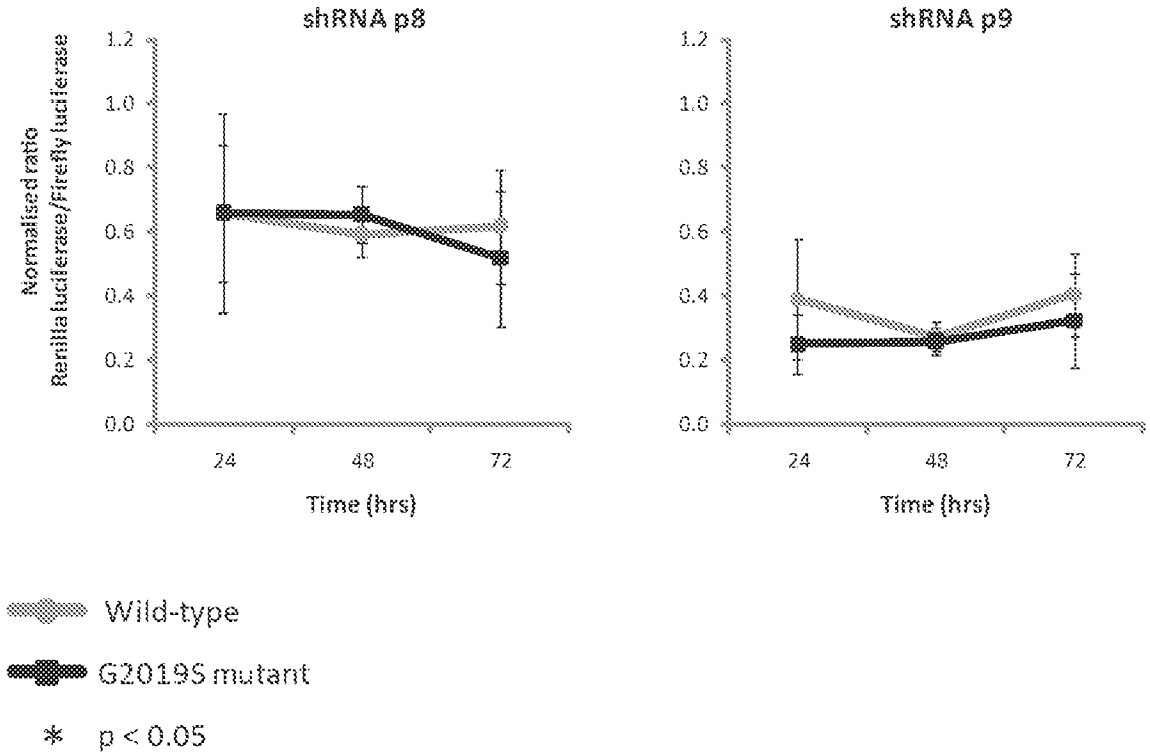

shRNA p8
shRNA p9
Normalised ratio
Renilla luciferase/Firefly luciferase
1.2
1.0
0.8
0.6
0.4
0.2
0.0
24
48
72
Time (hrs)
Wild-type
G2019S mutant
* p < 0.05

Figure 4a

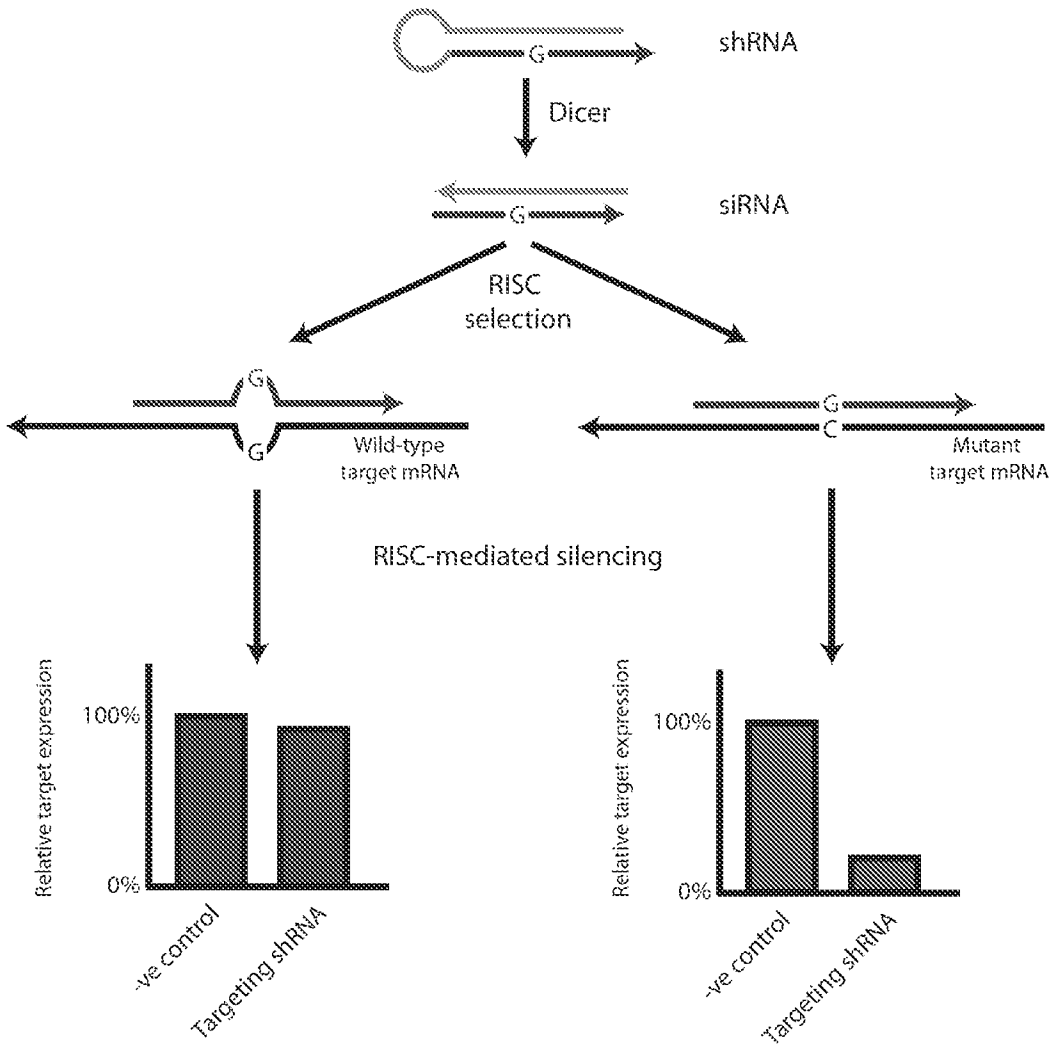

shRNA
G
Dicer
siRNA
G
RISC
selection
G
G
Wild-type
target mRNA
G
C
Mutant
target mRNA
RISC-mediated silencing
Relative target expression
100%
0%
-ve control
Targeting shRNA
Relative target expression
100%
0%
-ve control
Targeting shRNA

Figure 4b

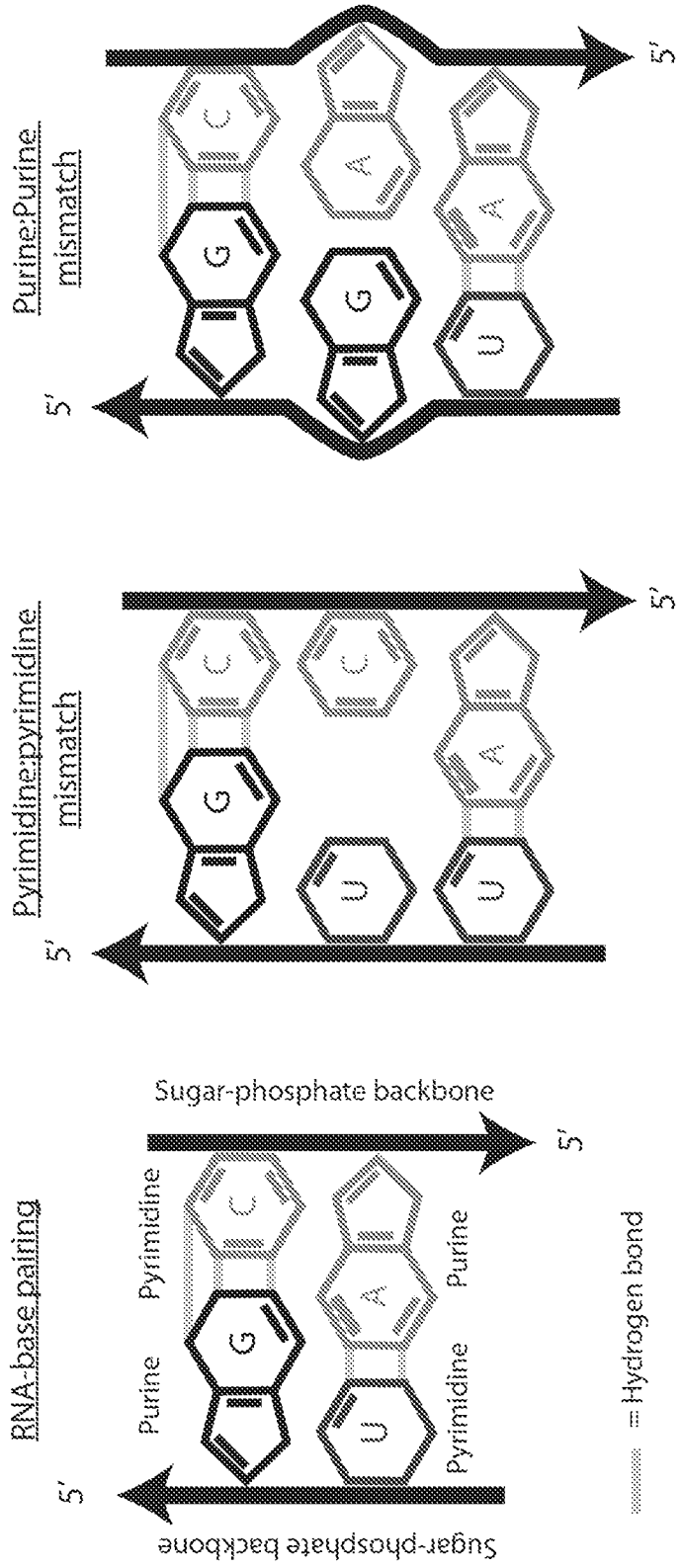

Purine:Purine mismatch
5'
C
G
A
G
A
U
5'
Pyrimidine:pyrimidine mismatch
5'
C
G
C
U
A
U
5'
RNA-base pairing
Sugar-phosphate backbone
5'
Purine
Pyrimidine
Pyrimidine
Purine
5'
Sugar-phosphate backbone
= Hydrogen bond

Figure 5

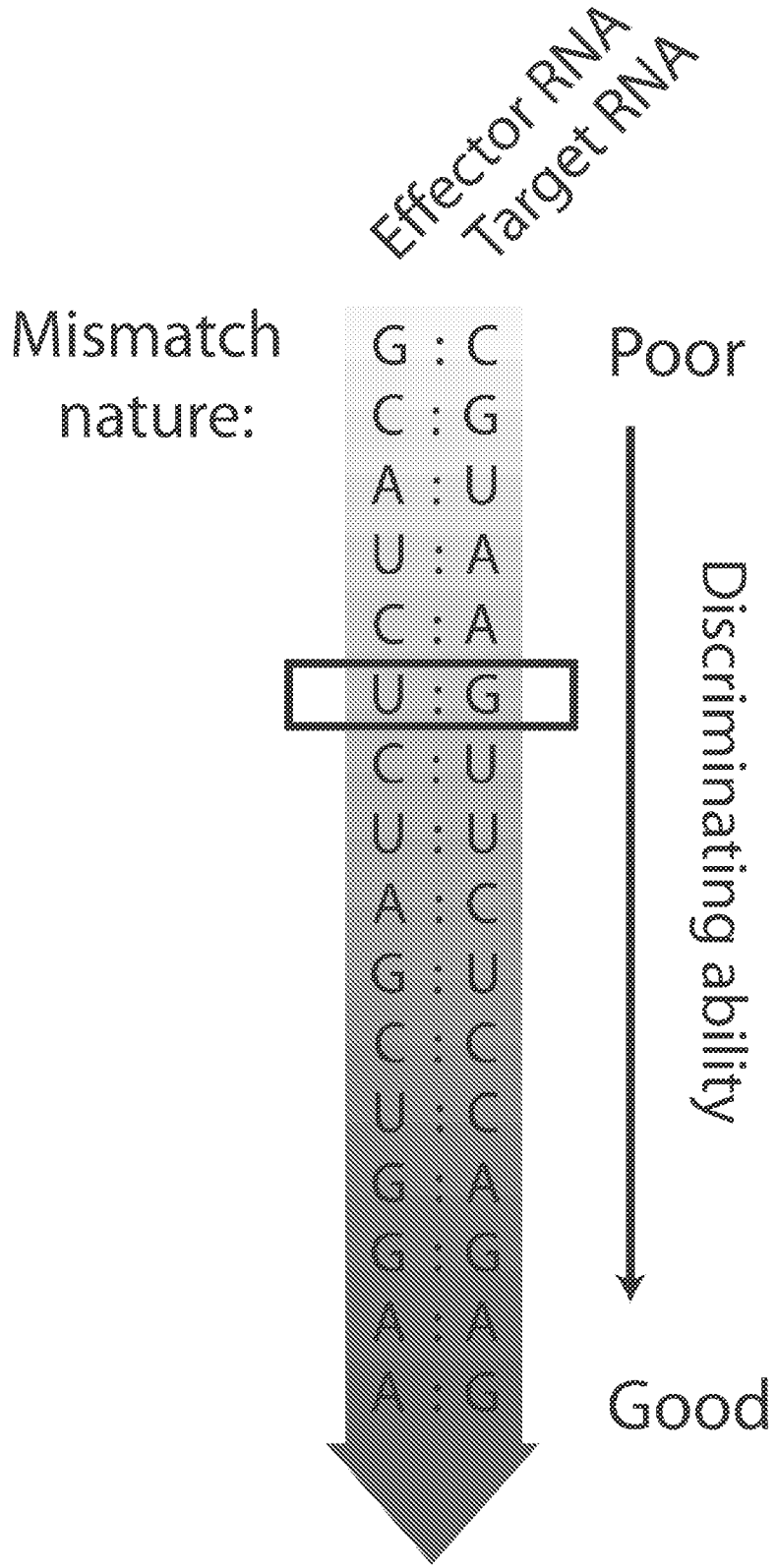
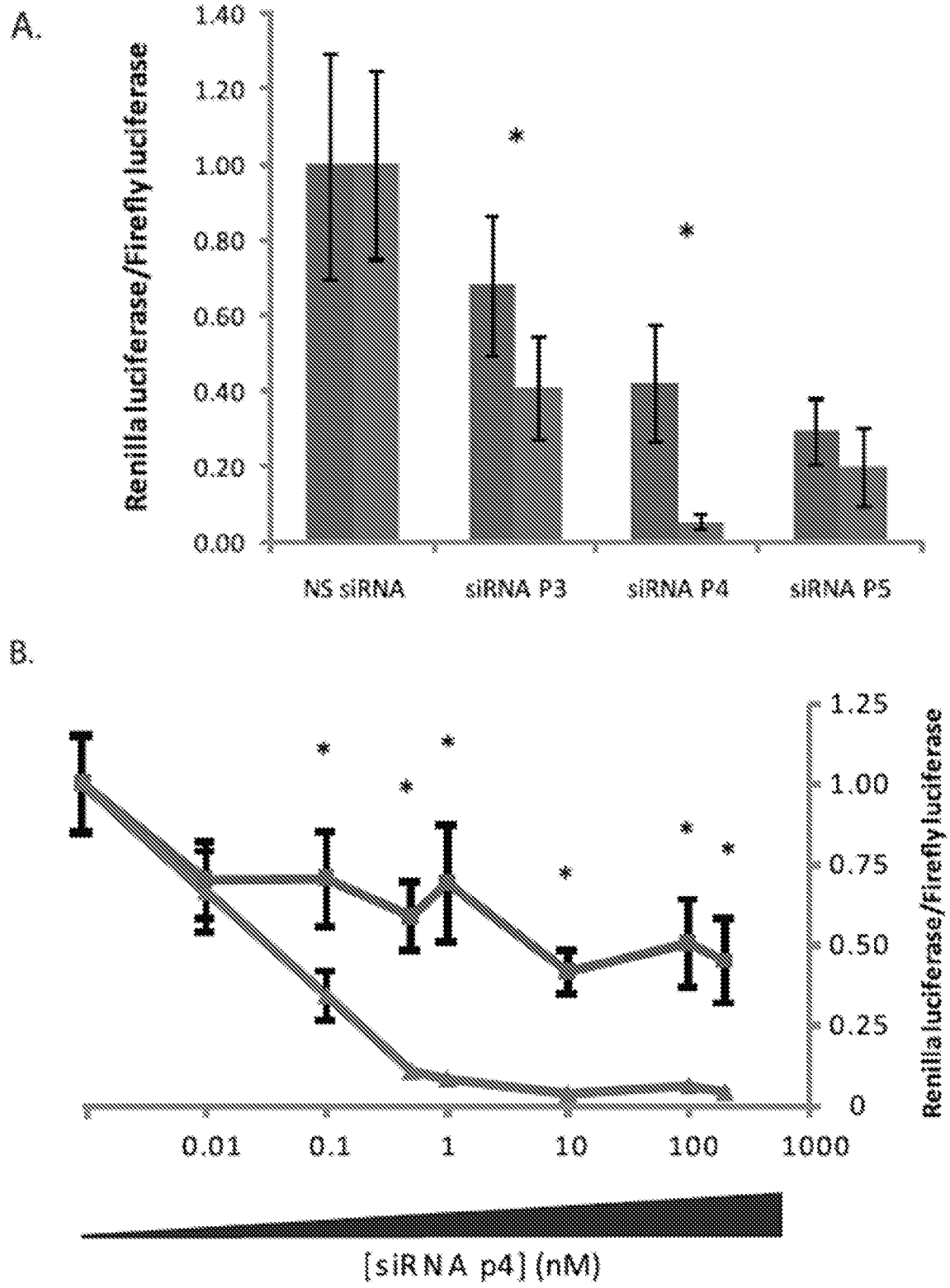

Effector RNA
Target RNA
Mismatch nature:
G : C
C : G
A : U
U : A
C : A
U : G
C : U
U : U
A : C
G : U
C : C
U : C
G : A
G : G
A : A
A : G
Poor
Discriminating ability
Good

Figure 6


A.
Renilla luciferase/Firefly luciferase
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
NS siRNA
siRNA P3
siRNA P4
siRNA P5
B.
1.25
1.00
0.75
0.50
0.25
0
Renilla luciferase/Firefly luciferase
0.01
0.1
1
10
100
1000
[siRNA p4] (nM)

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2012/050692

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/113 A61K31/713
ADD.

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, BIOSIS, EMBASE, WPI Data, EMBL, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ANONYMOUS: "LRRK2 Cohort Workshop", The Michael J. Fox Foundation for Parkinson's Research, New York, US , 6 May 2010 (2010-05-06), pages 1-29, XP002678904, Retrieved from the Internet: URL:www.pdonlineresearch.org/sites/default/files/Meeting%20Booklet%20-%20Final.doc [retrieved on 2012-06-29] page 25	1-13

Y	WO 2007/044362 A2 (UNIV MASSACHUSETTS [US]; XU ZUOSHANG [US]) 19 April 2007 (2007-04-19) claims 37,39	1-13

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[X] Further documents are listed in the continuation of Box C.

[X] 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	Date of mailing of the international search report
9 July 2012	18/07/2012

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Bucka, Alexander

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2012/050692

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SAPRU M K ET AL: "Silencing of human alpha-synuclein in vitro and in rat brain using lentiviral-mediated RNAi", EXPERIMENTAL NEUROLOGY, ACADEMIC PRESS, NEW YORK, NY, US, vol. 198, no. 2, 1 April 2006 (2006-04-01) , pages 382-390, XP024945762, ISSN: 0014-4886, DOI: 10.1016/J.EXPNEUROL.2005.12.024 [retrieved on 2006-04-01] the whole document	1-13
Y	MILLER V M ET AL: "Allele-specific silencing of dominant disease genes", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, NATIONAL ACADEMY OF SCIENCES, US, vol. 100, no. 12, 10 June 2003 (2003-06-10), pages 7195-7200, XP002278730, ISSN: 0027-8424, DOI: 10.1073/PNAS.1231012100 the whole document	1-13
A	JEREMY NICHOLS R ET AL: "Substrate specificity and inhibitors of LRRK2, a protein kinase mutated in Parkinson's disease", BIOCHEMICAL JOURNAL, THE BIOCHEMICAL SOCIETY, LONDON, GB, vol. 424, 9 September 2009 (2009-09-09), pages 47-60, XP002654035, ISSN: 0264-6021, DOI: 10.1042/BJ20091035 the whole document	1-13
A	J. ALEGRE-ABARRATEGUI ET AL: "LRRK2 regulates autophagic activity and localizes to specific membrane microdomains in a novel human genomic reporter cellular model", HUMAN MOLECULAR GENETICS, vol. 18, no. 21, 29 July 2009 (2009-07-29) , pages 4022-4034, XP55004708, ISSN: 0964-6906, DOI: 10.1093/hmg/ddp346 the whole document	1-13
A	WO 2004/042027 A2 (UNIV MASSACHUSETTS [US]; XU ZUOSHANG [US]; ZAMORE PHILLIP D [US]) 21 May 2004 (2004-05-21) the whole document	1-13

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INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2012/050692

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ABDELGANY A ET AL: "Allele-specific silencing of a pathogenic mutant acetylcholine receptor subunit by RNA interference", HUMAN MOLECULAR GENETICS, OXFORD UNIVERSITY PRESS, SURREY, vol. 12, no. 20, 15 October 2003 (2003-10-15), pages 2637-2644, XP002354611, ISSN: 0964-6906, DOI: 10.1093/HMG/DDG280 the whole document	1-13
A	FENG X ET AL: "Allele-specific silencing of Alzheimer's disease genes", GENE, ELSEVIER, AMSTERDAM, NL, vol. 371, no. 1, 12 April 2006 (2006-04-12), pages 68-74, XP024934331, ISSN: 0378-1119, DOI: 10.1016/J.GENE.2005.11.006 [retrieved on 2006-04-12] the whole document	1-13
A	A DESSY ET AL: "The Emerging Therapeutic Role of RNA Interference in Disorders of the Central Nervous System", CLINICAL PHARMACOLOGY & THERAPEUTICS, vol. 89, no. 3, 19 January 2011 (2011-01-19), pages 450-454, XP55031277, ISSN: 0009-9236, DOI: 10.1038/clpt.2010.312 page 453, left-hand column	1-13
A	SCHOLEFIELD J ET AL: "Therapeutic gene silencing strategies for polyglutamine disorders", TRENDS IN GENETICS, ELSEVIER SCIENCE PUBLISHERS B.V. AMSTERDAM, NL, vol. 26, no. 1, 1 January 2010 (2010-01-01), pages 29-38, XP026816557, ISSN: 0168-9525 [retrieved on 2009-12-03] the whole document	1-13
A	WO 2007/124096 A2 (UNIV COLUMBIA [US]; ABELIOVICH ASA [US]) 1 November 2007 (2007-11-01) example 1	1-13

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INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2012/050692

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y,P	LAURA DE YÑIGO-MOJADO ET AL: "Efficient Allele-Specific Targeting of LRRK2 R1441 Mutations Mediated by RNAi", PLOS ONE, vol. 6, no. 6, 17 June 2011 (2011-06-17), page E21352, XP55031179, ISSN: 1932-6203, DOI: 10.1371/journal.pone.0021352 the whole document	1-13

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/GB2012/050692

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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