ribonucleoprotein (RNP) complexes to cells by microinjection of Cas9 protein and gRNA complexes was described by Cho [Cho et al., "Heritable gene knockout in Caenorhabditis elegans by direct injection of Cas9-sgRNA ribonucleoproteins," *Genetics* (2013) 195:1177-1180]. Delivery of Cas9 protein/gRNA complexes via electroporation was described by Kim [Kim et al., "Highly efficient RNA-guided genome editing in human cells via delivery of purified Cas9 ribonucleoproteins" *Genome Res.* (2014) 24:1012-1019]. Delivery of Cas9 protein-associated gRNA complexes via liposomes was reported by Zuris [Zuris et al., "Cationic lipid-mediated delivery of proteins enables efficient protein-based genome editing in vitro and in vivo" *Nat Biotechnol.* (2014) doi: 10.1038/nbt.3081].

SUMMARY

In one aspect, the present disclosure refers to a method of modifying a gene encoding or processed into a RNA silencing molecule to a target RNA in a eukaryotic cell isolated from its natural environment, with the proviso that said eukaryotic cell is not a plant cell, the method comprising introducing into the eukaryotic cell isolated from its natural environment a DNA editing agent which redirects a silencing specificity of said RNA silencing molecule towards a second target RNA, said target RNA and said second target RNA being distinct, thereby modifying the gene encoding the RNA silencing molecule such that the original specificity of the RNA silencing molecule is destroyed and a new specificity towards the second target RNA is gained.

In another aspect, the present disclosure refers to a non-therapeutic method of modifying a gene encoding or processed into a RNA silencing molecule to a target RNA in a eukaryotic cell, with the proviso that said eukaryotic cell is not a plant cell, the method comprising introducing into the eukaryotic cell a DNA editing agent which redirects a silencing specificity of said RNA silencing molecule towards a second target RNA, said target RNA and said second target RNA being distinct, thereby modifying the gene encoding the RNA silencing molecule such that the original specificity of the RNA silencing molecule is destroyed and a new specificity towards the second target RNA is gained.

~~In another aspect, the present disclosure refers to a method of generating a eukaryotic non-human organism, with the proviso that said organism is not a plant, wherein at least some of the cells of said organism comprise a modified gene encoding or processed into a RNA silencing molecule comprising a silencing specificity towards a target RNA , the method comprising introducing into a eukaryotic non-human cell isolated from its natural environment a DNA editing agent which redirects a silencing specificity of a gene encoding or processed into a RNA~~

~~silencing molecule to an initial target RNA towards said target RNA, said initial target RNA and said target RNA being distinct, thereby modifying the gene encoding the RNA silencing molecule such that the original specificity of the RNA silencing molecule is destroyed and a new specificity towards the target RNA is gained, thereby generating the eukaryotic non-human organism.~~

In another aspect, the present disclosure refers to a non-therapeutic method of generating a eukaryotic non-human organism, with the proviso that said organism is not a plant, wherein at least some of the cells of said organism comprise a modified gene encoding or processed into a RNA silencing molecule comprising a silencing specificity towards a target RNA , the method comprising introducing into a eukaryotic non-human cell a DNA editing agent which redirects a silencing specificity of a gene encoding or processed into a RNA silencing molecule to an initial target RNA towards said target RNA, said initial target RNA and said target RNA being distinct, thereby modifying the gene encoding the RNA silencing molecule such that the original specificity of the RNA silencing molecule is destroyed and a new specificity towards the target RNA is gained, thereby generating the eukaryotic non-human organism.

In yet another aspect, the present disclosure refers to a use of a DNA editing agent in the manufacture of a medicament for treating an infectious disease, a monogenic recessive disorder, an autoimmune disease or a cancerous disease in a subject in need thereof, wherein the use comprises that said DNA editing agent which redirects a silencing specificity of a gene encoding or processed into a RNA silencing molecule to a target RNA towards a second target RNA is to be introduced to at least one cell of the subject, the target RNA and the second target RNA being distinct, wherein the second target RNA is associated with onset or progression of said infectious disease, said monogenic recessive disorder, said autoimmune disease or said cancerous disease, wherein the original specificity of the RNA silencing molecule is destroyed and a new specificity towards the second target RNA is gained.

In yet another aspect, the present disclosure refers to a use of a donor oligonucleotide in the manufacture of a medicament for treating an infectious disease, a monogenic recessive disorder, an autoimmune disease or a cancerous disease in a subject in need thereof, wherein the use comprises that said donor oligonucleotide is to be introduced to at least one cell of the subject, together with a DNA editing agent which redirects a silencing specificity of a gene encoding or processed into a RNA silencing molecule to a target RNA towards a second target RNA, the target RNA and the second target RNA being distinct, wherein the second target RNA is associated with onset or progression of said infectious disease, said monogenic recessive disorder, said autoimmune disease

WHAT IS CLAIMED IS:

1. A method of modifying a gene encoding or processed into a RNA silencing molecule to a target RNA in a eukaryotic cell isolated from its natural environment, with the proviso that said eukaryotic cell is not a plant cell, the method comprising introducing into the eukaryotic cell isolated from its natural environment a DNA editing agent which redirects a silencing specificity of said RNA silencing molecule towards a second target RNA, said target RNA and said second target RNA being distinct, thereby modifying the gene encoding the RNA silencing molecule such that the original specificity of the RNA silencing molecule is destroyed and a new specificity towards the second target RNA is gained.

2. A non-therapeutic method of modifying a gene encoding or processed into a RNA silencing molecule to a target RNA in a eukaryotic cell, with the proviso that said eukaryotic cell is not a plant cell, the method comprising introducing into the eukaryotic cell a DNA editing agent which redirects a silencing specificity of said RNA silencing molecule towards a second target RNA, said target RNA and said second target RNA being distinct, thereby modifying the gene encoding the RNA silencing molecule such that the original specificity of the RNA silencing molecule is destroyed and a new specificity towards the second target RNA is gained.

3. The method of claim 1 or claim 2, wherein the gene encoding the RNA silencing molecule is endogenous to the eukaryotic cell.

4. The method of any one of claims 1-3, wherein said modifying said gene encoding said RNA silencing molecule comprises imparting said RNA silencing molecule with at least 45 % complementarity towards said second target RNA.

5. The method of any one of claims 1-4, wherein said silencing specificity of said RNA silencing molecule is determined by measuring a RNA or protein level of said second target RNA, and/or is determined phenotypically and/or is determined genotypically.

6. The method of any one of claims 1-5, wherein said RNA silencing molecule or said

non-coding RNA molecule is processed from a precursor,

optionally wherein said non-coding RNA molecule or said RNA silencing molecule is a RNA interference (RNAi) molecule,

optionally wherein said RNAi molecule is selected from the group consisting of a small interfering RNA (siRNA), a short hairpin RNA (shRNA), a microRNA (miRNA), a Piwi-interacting RNA (piRNA) and trans-acting siRNA (tasiRNA);

optionally wherein said RNAi molecule is modified to preserve secondary RNA structure and to be recognized by cellular RNAi factors.

7. The method of any one of claims 1-6, wherein said modifying said gene is affected by a modification selected from the group consisting of a deletion, an insertion, a point mutation and a combination thereof,

optionally wherein said modification is in a stem region of said non-coding RNA molecule or said RNA silencing molecule, is in a loop region of said RNA silencing molecule, is in a non-structured region of said RNA silencing molecule, is in a stem region and a loop region of said RNA silencing molecule, or is in a stem region and a loop region and in non-structured region of said RNA silencing molecule,

optionally wherein said modification comprises a modification of at most 200 nucleotides.

8. The method of claim 7, wherein said method further comprises introducing into said eukaryotic cell a donor oligonucleotide sequence.

9. The method of any one of claims 1-8, wherein said DNA editing agent comprises an endonuclease,

optionally wherein the DNA editing agent comprises an endonuclease and a DNA targeting molecule,

optionally wherein the DNA targeting molecule is gRNA,

optionally wherein said endonuclease comprises Cas9.

10. The method of any one of claims 1-8, wherein said DNA editing agent is applied to the cell as DNA, RNA or RNP, wherein the DNA editing agent is introduced into the eukaryotic cell using DNA delivery methods or DNA-free methods,

optionally wherein the DNA editing agent is introduced into the eukaryotic cell using expression vectors,

optionally wherein said DNA editing agent comprises at least one gRNA operatively linked to a cis-acting regulatory element active in eukaryotic cells.

11. The method of any one of claims 1-8, wherein said DNA editing agent is of a DNA editing system selected from the group consisting of a meganuclease, a zinc finger nucleases (ZFN), a transcription-activator like effector nuclease (TALEN) and CRISPR.

12. The method of any one of claims 1-11, wherein said second target RNA is endogenous or exogenous to said eukaryotic cell.

13. The method of claim 12, wherein said second target RNA is a product of a gene associated with a cancer or with an infectious disease.

14. The method of any one of claims 1-13, wherein said eukaryotic cell was obtained from a eukaryotic organism selected from the group consisting of a mammal, such as a cattle or a pig, an insect, a nematode, a bird, a reptile, a fish, a crustacean, such as a shrimp, a fungi and an algae.

15. The method of any one of claims 1-14, wherein said eukaryotic cell is a mammalian cell, optionally a non-human mammalian cell.

~~16. A method of generating a eukaryotic non-human organism, with the proviso that said organism is not a plant, wherein at least some of the cells of said organism comprise a modified gene encoding or processed into a RNA silencing molecule comprising a silencing specificity towards a target RNA , the method comprising introducing into a eukaryotic non-human cell isolated from its natural environment a DNA editing agent which redirects a silencing~~

~~specificity of a gene encoding or processed into a RNA silencing molecule to an initial target RNA towards said target RNA, said initial target RNA and said target RNA being distinct, thereby modifying the gene encoding the RNA silencing molecule such that the original specificity of the RNA silencing molecule is destroyed and a new specificity towards the target RNA is gained, thereby generating the eukaryotic non-human organism.~~

~~17~~16. A non-therapeutic method of generating a eukaryotic non-human organism, with the proviso that said organism is not a plant, wherein at least some of the cells of said organism comprise a modified gene encoding or processed into a RNA silencing molecule comprising a silencing specificity towards a target RNA , the method comprising introducing into a eukaryotic non-human cell a DNA editing agent which redirects a silencing specificity of a gene encoding or processed into a RNA silencing molecule to an initial target RNA towards said target RNA, said initial target RNA and said target RNA being distinct, thereby modifying the gene encoding the RNA silencing molecule such that the original specificity of the RNA silencing molecule is destroyed and a new specificity towards the target RNA is gained, thereby generating the eukaryotic non-human organism.

~~18~~17. The method of claim 16~~ or 17~~, wherein said eukaryotic non-human organism is a mammal, such as a cattle or a pig, an insect, a nematode, a bird, a reptile, a fish, a crustacean, such as a shrimp, a fungi or an algae.

~~19~~18. Use of a DNA editing agent in the manufacture of a medicament for treating an infectious disease, a monogenic recessive disorder, an autoimmune disease or a cancerous disease in a subject in need thereof, wherein the use comprises that said DNA editing agent which redirects a silencing specificity of a gene encoding or processed into a RNA silencing molecule to a target RNA towards a second target RNA is to be introduced to at least one cell of the subject, the target RNA and the second target RNA being distinct,

wherein the second target RNA is associated with onset or progression of said infectious disease, said monogenic recessive disorder, said autoimmune disease or said cancerous disease,

wherein the original specificity of the RNA silencing molecule is destroyed and a new specificity towards the second target RNA is gained.

~~20~~19. Use of a donor oligonucleotide in the manufacture of a medicament for treating an infectious disease, a monogenic recessive disorder, an autoimmune disease or a cancerous disease in a subject in need thereof, wherein the use comprises that said donor oligonucleotide is to be introduced to at least one cell of the subject, together with a DNA editing agent which redirects a silencing specificity of a gene encoding or processed into a RNA silencing molecule to a target RNA towards a second target RNA, the target RNA and the second target RNA being distinct,

wherein the second target RNA is associated with onset or progression of said infectious disease, said monogenic recessive disorder, said autoimmune disease or said cancerous disease,

wherein the original specificity of the RNA silencing molecule is destroyed and a new specificity towards the second target RNA is gained.

~~21~~20. The method of claim 16~~,~~ or 17 ~~or 18~~, the use of claim ~~19 or 20~~18 or 19, wherein said target RNA is endogenous or exogenous to the eukaryotic non-human cell.