"for therapeutic applications that require *in vivo* editing of cells, the challenge is greater and a solution has not been determined".

A straightforward mean to alleviate the off-target that may be detrimental in some applications is to identify/design novel nucleases with greater specificities.

Therefore, there is a need in the art to provide new fine-tuned controlled expression systems for the expression of a nucleic acid encoding a Cas nuclease, in particular a Cas9 nuclease, in an individual, in particular for safe gene therapy approaches.

## SUMMARY

In one aspect, the present disclosure refers to a nucleic acid for the controlled expression of a nucleic acid encoding a Cas nuclease in at least one target cell of an individual, comprising:

- a regulatory polynucleotide comprising a minimal promoter and from one to twenty AARE (amino acid response element) nucleic acids, said regulatory polynucleotide being activated in an individual upon consumption of a diet deficient in at least one essential amino acid; and

- a nucleic acid encoding a Cas nuclease, which is placed under the control of the said regulatory polynucleotide.

One example of the disclosure relates to a nucleic acid for the controlled expression of a nucleic acid encoding a Cas nuclease in an individual, comprising:

- a regulatory polynucleotide comprising a minimal promoter and at least one AARE (amino acid response element) nucleic acid, said regulatory polynucleotide being activated in an individual upon consumption of a diet deficient in at least one essential amino acid; and

- a nucleic acid encoding a Cas nuclease, which is placed under the control of the said regulatory polynucleotide.

Another aspect of the disclosure relates to a nucleic acid vector for the controlled expression of a nucleic acid encoding a Cas nuclease, comprising a nucleic acid, as defined herein.

A still further aspect of the disclosure relates to a delivery particle comprising a nucleic acid or a nucleic acid vector, as defined herein.

In another aspect, the disclosure also relates to a pharmaceutical composition comprising (i) a nucleic acid or a nucleic acid vector or a delivery particle, as defined herein, and (ii) a pharmaceutically acceptable vehicle.

In a further example, the disclosure relates to a host cell comprising the nucleic acid or a nucleic acid vector, as defined herein.

In yet another aspect, the present disclosure refers to a host cell comprising the nucleic acid or a nucleic acid vector as defined herein, wherein the host cell is not a germinal cell or an embryonic cell.

Another aspect of the disclosure relates to a pharmaceutical composition, as defined herein, for use as a medicament.

In yet another aspect, the present disclosure refers to a pharmaceutical composition as defined herein for use as a medicament for editing the genome into at least one target cell.

In a further example, the disclosure also relates to a pharmaceutical composition, as defined herein, for use as an active agent for editing the genome into at least one target cell.

In one example, the disclosure relates to a method for editing the genome into at least one target cell comprising at least the step of administering to an individual in need thereof the pharmaceutical composition, as defined herein.

In another example, the disclosure relates to a pharmaceutical composition, as defined herein, for use as an active agent for preventing and/or treating a disease.

An example of the disclosure also relates to a method for preventing and/or treating a disease comprising at least the step of administering to an individual in need thereof the pharmaceutical composition, as defined herein.

In yet another aspect, the present disclosure refers to use of the pharmaceutical composition as defined herein in the manufacture of a medicament for treating cancer.

Finally, in a further aspect, the disclosure concerns a kit for treating and/or preventing a disease comprising:

- a pharmaceutical composition, as defined herein, and
- a pharmaceutically active compound.

## LEGENDS OF THE FIGURES

**FIGURE 1:** Scheme illustrating the GCN2-eIF2α-ATF4 signalling pathway. In response to EAA starvation, activated GCN2 phosphorylates eIF2α, leading to an up-regulation of the transcription factor ATF4 and its recruitment to AARE sequences to induce target gene expression.

**FIGURE 2:** Scheme illustrating the depiction of the AARE-Cas nuclease construct: six copies of the AAREs from Trb3 (black spots) promoter and the Tk minimal promoter compose this construct.

**FIGURE 3:** Scheme illustrating the pTrip-2XAARE-NLS-FLAG-CAS9 plasmid. pTK indicates the position of the minimal TK promoter; 2X AARE indicates the position of the AARE nucleic acids; arrow “NLS-FLAG-CAS9” indicates the position of the nucleic acid encoding the Cas9 nuclease; arrow “AmpR” stands for the nucleic acid encoding for ampicillin resistance.

**FIGURE 4:** Scheme illustrating the pTRIP blast_U6 AAVS1_2xAARE-Cas9-Flag-RFP plasmid. The lower panel is in continuity with the upper panel. The EcoR1 restriction site on the right end of the upper panel refers to the EcoR1 restriction site on the left end of the lower panel.

**FIGURE 5:** Plots illustrating the Cas9 expression in 293T cells upon induction at T0 with either a medium deprived in Leucine (293T-C9 Leu-; plain curve) or a medium

## CLAIMS

1. A nucleic acid for the controlled expression of a nucleic acid encoding a Cas nuclease in at least one target cell of an individual, comprising:

- a regulatory polynucleotide comprising a minimal promoter and from one to twenty AARE (amino acid response element) nucleic acids, said regulatory polynucleotide being activated in an individual upon consumption of a diet deficient in at least one essential amino acid; and

- a nucleic acid encoding a Cas nuclease, which is placed under the control of the said regulatory polynucleotide.

2. The nucleic acid according to claim 1, wherein the Cas nuclease is Cas9 nuclease.

3. The nucleic acid according to any one of claims 1 or 2, wherein the amino acid response element (AARE) nucleic acid is selected in a group comprising a nucleic acid of sequence SEQ ID No: 1, SEQ ID No: 2, SEQ ID No: 3, SEQ ID No: 4 and SEQ ID No: 5.

4. The nucleic acid according to any one of claims 1 to 3, wherein the regulatory polynucleotide comprises from two to ten AARE nucleic acids.

5. The nucleic acid according to any one of claims 1 to 4, wherein the regulatory polynucleotide comprises from two to six AARE nucleic acids.

6. A nucleic acid vector for the controlled expression of a nucleic acid encoding a Cas nuclease, comprising a nucleic acid according to any one of claims 1 to 5.

7. A delivery particle comprising a nucleic acid according to any one of claims 1 to 5 or a nucleic acid vector according to claim 6.

8. The delivery particle according to claim 7, which comprises at its surface one or more ligands suitable for binding to a target receptor exposed at the membrane of a targeted cell.

9. A pharmaceutical composition comprising (i) a nucleic acid according to any one of claims 1 to 4 or a nucleic acid vector according to claim 6 or a delivery particle according to claim 7 or 8, and (ii) a pharmaceutically acceptable vehicle.

10. A host cell comprising the nucleic acid according to any one of claims 1 to 5 or a nucleic acid vector according to claim 6, wherein the host cell is not a germinal cell or an embryonic cell.

11. A pharmaceutical composition according to claim 9 for use as a medicament.

12. A pharmaceutical composition according to claim 9 for use as a medicament for editing the genome into at least one target cell.

13. The pharmaceutical composition according to claim 12, wherein the target cell has at least a genetic mutation.

14. Use of the pharmaceutical composition according to claim 9 in the manufacture of a medicament for treating cancer.

15. A kit for treating and/or preventing a disease comprising:

- a pharmaceutical composition according to claim 9, and
- a pharmaceutically active compound.