cellular uptake and intracellular delivery, and a complex dependence on the magnitude and kinetics of STING signaling (Garland *et al.*, Chemical Reviews 2022 122 (6), 5977-6039).

Therefore, there is a need to develop improved agents targeting the cGAS-STING axis for safe and effective therapy, notably against cancer, neurological diseases and infectious diseases.

SUMMARY

To this end, the inventors have developed a nucleic acid molecule comprising a Nuclear Localization Signal (NLS) sequence and a sequence encoding a particular Cyclic GMP-AMP synthase (cGAS), as a vector nanoparticle, especially as a lipid-based nanoparticle, comprising it, suitable for intravenous, intramuscular, intratumoral or subcutaneous administration in patients.

Vaccines based on mRNA-containing lipid nanoparticles (LNPs) are a promising new delivery platform. LNPs can be used to deliver mRNA to cells and have led to the expression of the encoded proteins, thus providing immune-protection to the body. Other kinds of vector nanoparticles may be used such as viral nanoparticles or dendrimer nanoparticles.

In particular, the inventors demonstrate herein that subcutaneous, intramuscular, intratumoral or intravenous administration of vectors such as LNPs comprising nucleic acid molecules encoding NLS-cGAS leads to decreased tumor growth and, even more the intravenous administration LNPs leads to tumors eradication. Surprisingly, NLS-cGAS from rodents such as a mouse (*Mus musculus* or “house mouse”), or a rat (for example *Rattus Norvegicus* also known as the “brown rat”, or *Arvicanthis niloticus* also known as the “Nile rat”), strongly induces cGAMP production whereas cGAS from Homo sapiens or macaque (*Macaca mulatta*) has no significant effect.

Accordingly, the present disclosure relates to a nucleic acid comprising a sequence encoding a Nuclear Localization Signal (NLS) and a sequence encoding a Cyclic GMP-AMP synthase (cGAS) as set forth in SEQ ID NO: 1, or a variant thereof having at least about 80% or about 85% identity thereto and a R241 mutation, such as for example a R241E, R241D, R241N, or R241A substitution.

Inventors herein show that the nucleic acid of the disclosure comprising a Nuclear Localization Signal (NLS) and a sequence encoding a Cyclic GMP-AMP synthase (cGAS) may be a sequence as set forth in SEQ ID NO: 40, or a variant thereof having at least about 80% or about 85% identity thereto, for example 100% identity thereto such as SEQ ID NO: 1. Advantageously, such a (functional) variant is constitutively active, i.e., it is capable of producing cGAMP when present in a cell or subject (even in the absence of exogenous DNA).

In SEQ ID NO: 40, position 241 is herein identified as position "X". As further explained herein below, said position "X" may designate a deletion of at least one amino acid, typically the deletion of at least the amino acid "R" (designating an arginine) present in the wild-type sequence encoding cGAS of *Mus Musculus* (SEQ ID NO: 1). In another example of the disclosure, "X" (position 241 of Seq ID NO: 40) designates an insertion of at least one or two amino acids at position 241. In again another example, "X" designates an amino acid which may be any one of A, N, D, C, Q, E, G, H, I, L, K, M, F, P, O, S, U, T, W, Y, or V. Such a substitution at position 241 of SEQ ID NO: 40 is herein identified as "R241X". "X" is typically not "R".

In the present description, position "X" varies depending on the considered sequence ("parent" sequence) and is for example position 241 in reference to SEQ ID NO: 1 or 40, position 255 in reference to SEQ ID NO: 41, position 253 in reference to SEQ ID NO: 42, position 221 in reference to SEQ ID NO: 49, position 233 in reference to SEQ ID NO: 50, position 244 in reference to SEQ ID NO: 53, or position 256 in reference to SEQ ID NO: 54. In the context of the present disclosure, a mutation of position "X", typically a deletion, an addition or a substitution thereof, results in a constitutively active variant capable of producing cGAMP when present in a cell or subject, whatever the animal origin of the reference ("parent") amino acid sequence.

In a particular example, the (constitutively active) variant of SEQ ID NO: 40, for example the (constitutively active) variant of SEQ ID NO: 1, has a mutation at position R241 and said mutation is a substitution of arginine ("R"), a deletion, or an insertion of one amino acid which is not a R or of several amino acids (the first amino acid of said added amino acid being not a R).

In one aspect, the present disclosure provides a nucleic acid comprising a sequence encoding a Nuclear Localization Signal (NLS) and a sequence encoding a Cyclic GMP-AMP synthase (cGAS) as set forth in SEQ ID NO: 40 or a constitutively active variant thereof having at least about 85% identity thereto. Thus, an object of the disclosure is a nucleic acid comprising a sequence encoding a Nuclear Localization Signal (NLS) and a sequence encoding a Cyclic GMP-AMP synthase (cGAS) as set forth in SEQ ID NO: 40 or a constitutively active variant thereof having at least about 80% or about 85% identity thereto.

In a preferred example, the constitutively active variant of SEQ ID NO: 40 has a mutation at position X, said position X being position 241 in SEQ ID NO: 40 and said mutation being a substitution, a deletion or an insertion.

In a particular example, the constitutively active variant of SEQ ID NO: 40 is a mouse cGAS variant comprising or consisting of SEQ ID NO: 1, SEQ ID NO:1 having a R241E mutation.

In another particular example, the constitutively active variant of SEQ ID NO: 40 is a mouse cGAS variant comprising or consisting of anyone of SEQ ID NO: 4, 41, 42, 43, 44, 45, 46, 47 or 48, with SEQ ID NO: 41 having no amino acid or at least one amino acid which is not R at position (X=) 255, or with any one of SEQ ID NO: 42-48 having no amino acid or at least one amino acid which is not R at position (X=) 253.

In another particular example, the constitutively active variant of SEQ ID NO: 40 is a rat cGAS variant comprising or consisting of SEQ ID NO: 49 or SEQ ID NO: 50, with SEQ ID NO: 49 having no amino acid or

at least one amino acid which is not R at position (X=) 221, or with SEQ ID NO: 50 having no amino acid or at least one amino acid which is not R at position (X=) 233. In a particular example, the constitutively active variant is or comprises SEQ ID NO: 51 or 52.

In another particular example, the constitutively active variant of SEQ ID NO: 40 is a rat cGAS variant comprising or consisting of SEQ ID NO: 53 or 54, with SEQ ID NO: 53 having no amino acid or at least one amino acid which is not R at position (X=) 244 or with SEQ ID NO: 54 having no amino acid or at least one amino acid which is not R at position (X=) 256. In a particular example, the constitutively active variant is or comprises SEQ ID NO: 55 or 56.

Optionally, the NLS sequence is a classical nuclear localization signal (cNLS) or a non-classical nuclear localization signal (ncNLS), preferably a NLS sequence as set forth in anyone of SEQ ID NO: 9, 13 to 20 or 39.

Preferably, the nucleic acid is a mRNA. Optionally, the mRNA comprises a flanking region, a 5'-cap structure, a chain terminating nucleotide, a stem loop, a 3'-poly-A tail sequence and/or a polyadenylation signal, preferably a 5' cap structure and a poly-A tail sequence. In a very particular example, the nucleic acid comprises a sequence as set forth in SEQ ID NO: 5.

In another aspect, the present disclosure provides a vector comprising a nucleic acid as disclosed herein, the vector being a nanoparticle, for example a lipid-based nanoparticle, a viral nanoparticle or a dendrimer nanoparticle. The present disclosure further relates to a vector nanoparticle, in particular a lipid-based nanoparticle (LNP) comprising a nucleic acid as disclosed herein.

Optionally, the lipid-based nanoparticle comprises a lipid mixture of an ionizable cationic lipid, a helper lipid, a sterol and a polyethylene glycol-lipid, preferably a lipid mixture of an ionizable cationic lipid, 1,2-distearoyl-sn-glycero-3-phosphocholine, cholesterol and a polyethylene glycol-lipid.

The present disclosure further relates to other vector nanoparticles, in particular dendrimer nanoparticle or a viral ("-based") nanoparticle comprising a nucleic acid as disclosed herein, such as a retroviral ("-based") nanoparticle for example a lentiviral nanoparticle, or an AAV ("-based") nanoparticle.

In another aspect, the present disclosure provides a pharmaceutical composition comprising the nucleic acid as disclosed herein, the lipid based nanoparticle as disclosed herein, the viral nanoparticle as disclosed herein or the dendrimer nanoparticle as disclosed herein, and a pharmaceutical acceptable carrier, or a combination comprising a) the nucleic acid as disclosed herein, the lipid-based nanoparticle as disclosed herein, the viral nanoparticle as disclosed herein or the dendrimer nanoparticle as disclosed herein, and b) a distinct therapeutic agent, preferably an anti-cancer or antiviral agent.

In addition, the present disclosure relates to a pharmaceutical composition comprising the nucleic acid, the vector nanoparticle, for example the LNP, the viral nanoparticle or the dendrimer nanoparticle, as disclosed herein and a pharmaceutical acceptable carrier. It also relates to a combination comprising a) the nucleic acid, the vector nanoparticle, for example the lipid-based nanoparticle, the viral nanoparticle or the dendrimer nanoparticle, as disclosed herein; and b) a distinct therapeutic agent, preferably an anti-cancer or antiviral agent. Optionally, the pharmaceutical composition or combination is formulated to be suitable for subcutaneous, intramuscular, intratumoral or intravenous injection, preferably intravenous injection.

In another aspect, the present disclosure provides a use of the nucleic acid as disclosed herein, the lipid-based nanoparticle as disclosed herein, the viral nanoparticle as disclosed herein, the dendrimer nanoparticle as disclosed herein, or the pharmaceutical composition as disclosed herein in the manufacture of a medicament for treating a disease selected from the group consisting of cancer, an infectious disease and a neurological disease.

In another aspect, the present disclosure provides a use of the nucleic acid as disclosed herein, the lipid-based nanoparticle as disclosed herein, the viral nanoparticle as disclosed herein, the dendrimer nanoparticle as disclosed herein, or the pharmaceutical composition as disclosed herein and an anticancer or antiviral therapeutic agent in the manufacture of a medicament for treating a disease selected from the group consisting of cancer, an infectious disease and a neurological disease.

In another aspect, the present disclosure provides a use of the nucleic acid as disclosed herein, the lipid-based nanoparticle as disclosed herein, the viral nanoparticle as disclosed herein, the dendrimer nanoparticle as disclosed herein, or the pharmaceutical composition as disclosed herein in the manufacture of a first medicament and use of an anticancer or antiviral therapeutic agent in the manufacture of a second medicament, wherein the first and second medicaments are to be used in combination therapy for treating a disease selected from the group consisting of cancer, an infectious disease and a neurological disease.

In another aspect, the present disclosure provides a use of the nucleic acid as disclosed herein, the lipid-based nanoparticle as disclosed herein, the viral nanoparticle as disclosed herein, the dendrimer nanoparticle as disclosed herein, or the pharmaceutical composition as disclosed herein in the manufacture of a medicament for use in combinatorial therapy for treating a disease selected from the group consisting of cancer, an infectious disease and a neurological disease, wherein said medicament is to be administered in combination with an anticancer or antiviral therapeutic agent.

The present disclosure relates to a nucleic acid, a vector nanoparticle, for example a LNP, a viral nanoparticle, or a dendrimer nanoparticle, a pharmaceutical composition or a combination as disclosed

herein for use as a medicament; and to the use of nucleic acid, vector, for example LNP, viral nanoparticle or dendrimer nanoparticle, pharmaceutical composition or combination as disclosed herein for the manufacture of a medicament. Optionally, the medicament is for use in combination with an anticancer or antiviral therapeutic agent. The present disclosure relates to a method for treating a disease in a subject in need thereof, typically an animal, preferably a mammal, in particular a domestic animal or a primate, preferably a human, comprising administering a therapeutically effective amount of a nucleic acid, vector, for example LNP, viral nanoparticle or dendrimer nanoparticle, pharmaceutical composition or combination as disclosed herein; and optionally further administering a therapeutically effective amount of an anticancer or antiviral therapeutic agent.

Optionally, the disease to be treated is selected from the group consisting of cancer, an infectious disease and a neurological disease. Optionally, the disease to be treated is cancer, preferably a cancer of the brain, lung, bone, pancreas, skin, head, neck, uterus, ovaries, stomach, colon, breast, esophagus, small intestine, bowel, endocrine system, thyroid gland, parathyroid gland, adrenal gland, urethra, prostate, penis, testes, ureter, bladder, kidney or liver; rectal cancer; cancer of the anal region; carcinomas of the fallopian tubes, endometrium, cervix, vagina, vulva, renal pelvis, renal cell; sarcoma of soft tissue; myxoma; rhabdomyoma; fibroma; lipoma; teratoma; cholangiocarcinoma; hepatoblastoma; angiosarcoma; hemangioma; hepatoma; fibrosarcoma; chondrosarcoma; myeloma; chronic or acute leukemia; lymphocytic lymphomas; primary CNS lymphoma; neoplasms of the CNS; spinal axis tumors; squamous cell carcinomas; synovial sarcoma; malignant pleural mesothelioma; brain stem glioma; pituitary adenoma; bronchial adenoma; chondromatous hamartoma; mesothelioma; Hodgkin’s Disease or a combination of one or more of the foregoing cancers. Optionally, the disease to be treated is an infectious disease, in particular a viral infection caused by a virus selected from the group consisting of Retrovirus, Anellovirus, Circovirus, Herpesvirus, Varicella zoster virus, Cytomegalovirus, Epstein-Barr virus, Polyomavirus, Adeno-associated virus, Herpes simplex, Adenovirus, Influenza virus, Corona virus, Dengue virus, Kaposi's sarcoma herpesvirus , Hepatitis B virus, Hepatitis C virus, Hepatitis D virus, Papilloma virus, Human immunodeficiency virus, Human T cell leukemia virus type 1, Rubella virus, German measles, Parvovirus B19, Measles virus and Coxsackie virus. Optionally, the disease to be treated is a neurological disease, preferably a neurological disease selected from the group consisting of multiple sclerosis, Amyotrophic Lateral Sclerosis, Parkinson's disease, Alzheimer's disease, Huntington's disease and Frontotemporal Lobar Degeneration.

In another aspect, the present disclosure provides an *in vitro* method for producing a LNP as disclosed herein, said method comprising: (a) mixing a first solution with a second solution in a mixing device so as to generate lipid-based nanoparticles, wherein: (i) the first solution is an ethanolic solution and comprises a lipid mixture of an ionizable cationic lipid, a helper lipid, a sterol and a polyethylene glycol-lipid; (ii) the second solution is an acidic aqueous solution and comprises nucleic acid molecules as disclosed herein; and (b) recovering the lipid-based nanoparticles comprising the nucleic acid molecules.

Finally, the present disclosure relates to an *in vitro* method for producing a LNP as herein described, said method comprising:

(a) mixing a first solution with a second solution in a mixing device so as to generate lipid-based nanoparticles, wherein:

CLAIMS

1. A nucleic acid comprising a sequence encoding a Nuclear Localization Signal (NLS) and a sequence encoding a Cyclic GMP-AMP synthase (cGAS) as set forth in SEQ ID NO: 40 or a constitutively active variant thereof having at least about 85% identity thereto.

2. The nucleic acid of claim 1, wherein the constitutively active variant of SEQ ID NO: 40 has a mutation at position 241 and said mutation is a substitution, deletion or insertion.

3. The nucleic acid of claim 1 or 2, wherein the constitutively active variant of SEQ ID NO: 40 is a mouse cGAS variant consisting of SEQ ID NO: 1, SEQ ID NO:1 having a R241E mutation.

4. The nucleic acid of claim 1 or 2, wherein the constitutively active variant of SEQ ID NO: 40 is a mouse cGAS variant consisting of any one of SEQ ID NO: 4, 41, 42, 43, 44, 45, 46, 47 or 48, with SEQ ID NO: 41 having no amino acid or at least one amino acid which is not R at position 255, or with any one of SEQ ID NO: 42-48 having no amino acid or at least one amino acid which is not R at position 253.

5. The nucleic acid of claim 1 or 2, wherein the constitutively active variant of SEQ ID NO: 40 is a rat cGAS variant consisting of SEQ ID NO: 49 or SEQ ID NO: 50, with SEQ ID NO: 49 having no amino acid or at least one amino acid which is not R at position 221, or with SEQ ID NO: 50 having no amino acid or at least one amino acid which is not R at position 233.

6. The nucleic acid of claim 5, wherein the constitutively active variant is SEQ ID NO: 51 or 52.

7. The nucleic acid of claim 1 or 2, wherein the constitutively active variant of SEQ ID NO: 40 is a rat cGAS variant consisting of SEQ ID NO: 53 or 54, with SEQ ID NO: 53 having no amino acid or at least one amino acid which is not R at position 244 or with SEQ ID NO: 54 having no amino acid or at least one amino acid which is not R at position 256.

8. The nucleic acid of claim 7, wherein the constitutively active variant is SEQ ID NO: 55 or 56.

9. The nucleic acid of anyone of claims 1 to 8, wherein the NLS sequence is a classical nuclear localization signal (cNLS) or a non-classical nuclear localization signal (ncNLS), preferably a NLS sequence as set forth in anyone of SEQ ID NO: 9, 13 to 20 or 39.

10. The nucleic acid of any one of claims 1 to 9, wherein the nucleic acid is a mRNA.

11. The nucleic acid of claim 10, wherein the mRNA comprises a flanking region, a 5'-cap structure, a chain terminating nucleotide, a stem loop, a 3’-poly-A tail sequence and/or a polyadenylation signal, preferably a 5’ cap structure and a poly-A tail sequence.

12. The nucleic acid of claim 10 or 11, wherein the nucleic acid comprises a sequence as set forth in SEQ ID NO: 5.

13. A vector comprising a nucleic acid according to any one of claims 1 to 12, the vector being a nanoparticle, for example a lipid-based nanoparticle, a viral nanoparticle or a dendrimer nanoparticle.

14. The vector of claim 13, wherein the vector is a lipid-based nanoparticle comprising a nucleic acid according to claims 1 to 12.

15. The lipid-based nanoparticle of claim 14, wherein the lipid-based nanoparticle comprises a lipid mixture of an ionizable cationic lipid, a helper lipid, a sterol and a polyethylene glycol-lipid, preferably a lipid mixture of an ionizable cationic lipid, 1,2-distearoyl-sn-glycero-3-phosphocholine, cholesterol and a polyethylene glycol-lipid.

16. The vector of claim 13, wherein the vector is a viral nanoparticle comprising a nucleic acid according to any one of claims 1 to 12.

17. The vector of claim 13, wherein the vector is a dendrimer nanoparticle comprising a nucleic acid according to any one of claims 1 to 12.

18. A pharmaceutical composition comprising the nucleic acid of any one of claims 1-12, the lipid based nanoparticle of claim 14 or 15, the viral nanoparticle of claim 16 or the dendrimer nanoparticle of claim 17, and a pharmaceutical acceptable carrier, or a combination comprising a) the nucleic acid of any one of claims 1-12, the lipid-based nanoparticle of claim 14 or 15, the viral nanoparticle of claim 16 or the dendrimer nanoparticle of claim 17, and b) a distinct therapeutic agent, preferably an anti-cancer or antiviral agent.

19. The pharmaceutical composition or the combination of claim 18, formulated so as to be suitable for subcutaneous, intramuscular, intratumoral or intravenous injection, preferably intravenous injection.

20. Use of the nucleic acid of any one of claims 1-12, the lipid-based nanoparticle according to claim 14 or 15, the viral nanoparticle of claim 16, the dendrimer nanoparticle of claim 17, or the pharmaceutical composition according to claim 18 or 19 in the manufacture of a medicament for treating a disease selected from the group consisting of cancer, an infectious disease and a neurological disease.

21. Use of the nucleic acid of any one of claims 1-12, the lipid-based nanoparticle according to claim 14 or 15, the viral nanoparticle of claim 16, the dendrimer nanoparticle of claim 17, or the pharmaceutical composition according to claim 18 or 19 and an anticancer or antiviral therapeutic

agent in the manufacture of a medicament for treating a disease selected from the group consisting of cancer, an infectious disease and a neurological disease.

22. Use of the nucleic acid of any one of claims 1-12, the lipid-based nanoparticle according to claim 14 or 15, the viral nanoparticle of claim 16, the dendrimer nanoparticle of claim 17, or the pharmaceutical composition according to claim 18 or 19 in the manufacture of a first medicament and use of an anticancer or antiviral therapeutic agent in the manufacture of a second medicament, wherein the first and second medicaments are to be used in combination therapy for treating a disease selected from the group consisting of cancer, an infectious disease and a neurological disease.

23. Use of the nucleic acid of any one of claims 1-12, the lipid-based nanoparticle according to claim 14 or 15, the viral nanoparticle of claim 16, the dendrimer nanoparticle of claim 17, or the pharmaceutical composition according to claim 18 or 19 in the manufacture of a medicament for use in combinatorial therapy for treating a disease selected from the group consisting of cancer, an infectious disease and a neurological disease, wherein said medicament is to be administered in combination with an anticancer or antiviral therapeutic agent.

24. The use of any one of claims 20-23, wherein the disease is cancer, preferably a cancer of the brain, lung, bone, pancreas, skin, head, neck, uterus, ovaries, stomach, colon, breast, esophagus, small intestine, bowel, endocrine system, thyroid gland, parathyroid gland, adrenal gland, urethra, prostate, penis, testes, ureter, bladder, kidney or liver; rectal cancer; cancer of the anal region; carcinomas of the fallopian tubes, endometrium, cervix, vagina, vulva, renal pelvis, renal cell; sarcoma of soft tissue; myxoma; rhabdomyoma; fibroma; lipoma; teratoma; cholangiocarcinoma; hepatoblastoma; angiosarcoma; hemangioma; hepatoma; fibrosarcoma; chondrosarcoma; myeloma; chronic or acute leukemia; lymphocytic lymphomas; primary CNS lymphoma; neoplasms of the CNS; spinal axis tumors; squamous cell carcinomas; synovial sarcoma; malignant pleural mesothelioma; brain stem glioma; pituitary adenoma; bronchial adenoma; chondromatous hamartoma; mesothelioma; Hodgkin’s Disease or a combination of one or more of the foregoing cancers.

25. The use of any one of claims 20-23, wherein the disease is an infectious disease, in particular a viral infection caused by a virus selected from the group consisting of Retrovirus, Anellovirus, Circovirus, Herpesvirus, Varicella zoster virus, Cytomegalovirus, Epstein-Barr virus, Polyomavirus, Adeno-associated virus, Herpes simplex, Adenovirus, Influenza virus, Corona virus, Dengue virus, Kaposi's sarcoma herpesvirus , Hepatitis B virus, Hepatitis C virus , Hepatitis D virus, Papilloma virus, Human immunodeficiency virus, Human T cell leukemia virus type 1, Rubella virus, German measles, Parvovirus B19, Measles virus and Coxsackie virus.

26. The use of any one of claims 20-23, wherein the disease is a neurological disease, preferably a neurological disease selected from the group consisting of multiple sclerosis, Amyotrophic Lateral Sclerosis, Parkinson's disease, Alzheimer's disease, Huntington's disease and Frontotemporal Lobar Degeneration.

27. An *in vitro* method for producing a LNP of claim 14 or 15, said method comprising:

(a) mixing a first solution with a second solution in a mixing device so as to generate lipid-based nanoparticles, wherein:

(i) the first solution is an ethanolic solution and comprises a lipid mixture of an ionizable cationic lipid, a helper lipid, a sterol and a polyethylene glycol-lipid;

(ii) the second solution is an acidic aqueous solution and comprises nucleic acid molecules according to any one of claims 1-12; and

(b) recovering the lipid-based nanoparticles comprising the nucleic acid molecules.