

NANOPARTICLES COMPRISING NUCLEIC ACID SEQUENCES ENCODING CYCLIC GMP-AMP SYNTHASE**FIELD OF THE INVENTION**

The invention pertains to the field of medicine. The invention relates to nucleic acid(s) or nanoparticle
5 vectors (such as a lipid-based nanoparticle) comprising said nucleic acid molecule(s), and use thereof to
treat conditions such as cancers and infections.

BACKGROUND OF THE INVENTION

Cellular recognition of exogenous DNA is an evolutionarily conserved mechanism in mammals by which
10 the innate immune system detects pathogens.

Cyclic GMP-AMP synthase (cGAS) and its downstream effector, stimulator of interferon genes (STING), are
involved in mediating fundamental innate antimicrobial immunity by promoting the release of type I
interferons (IFNs) and other inflammatory cytokines.

STING signaling has also been identified as a therapeutic target in autoinflammatory disorders and
15 neurological diseases with its role in neuroinflammation being increasingly recognized. The cGAS-STING
pathway is nowadays investigated as to be implicated in the progression of neuroinflammation in host of
central nervous system (CNS) pathologies including Alzheimer's disease, traumatic brain injury and
amyotrophic lateral sclerosis (ALS) (Fryer *et al.*, Front. Neurosci., 2021 Volume 15).

Finally, the activation of the cGAS-STING axis is also critical for antitumor immunity. The downstream
20 cytokines regulated by cGAS-STING, especially type I IFNs, serve as bridges connecting innate immunity
with adaptive immunity. Accordingly, a growing number of studies have focused on the synthesis and
screening of STING pathway agonists. To date, various kinds of STING agonists have been discovered, and
they are mainly divided into: cyclic dinucleotides and their derivatives, flavone-8-acetic acid derivative 5,6-
dimethylxanthenone-4-acetic acid (DMXAA) and its analogs, and small molecular agonists (Zheng *et al.*,
25 Mol Cancer. 2020 Aug 27;19(1):133).

However, emerging evidence suggest the pro-tumor roles of the cGAS-STING pathway, from tumor
initiation and development to metastasis (Jiang *et al.*, J Hematol Oncol 13, 81 (2020)), so that the
application of STING agonists in the clinic remains a major challenge. The efficacy of STING pathway
agonists is also limited by many drug delivery and pharmacological challenges. Depending on the class of
30 STING agonist and the desired administration route, these may include poor drug stability, immunocellular
toxicity, immune-related adverse events, limited tumor or lymph node targeting and/or retention, low

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.

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SUMMARY OF THE INVENTION

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 invention 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 invention 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).

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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 aspect of the invention, "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 aspect, "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 invention, 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 aspect, 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).

Thus, an object of the invention 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 aspect, 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 aspect, 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 aspect, 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 aspect, 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 aspect, the constitutively active variant is or comprises SEQ ID NO: 51 or 52.

5 In another particular aspect, 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 aspect, the constitutively active variant is or comprises SEQ ID NO: 55 or 56.

10 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 aspect, the nucleic acid
15 comprises a sequence as set forth in SEQ ID NO: 5.

The present invention 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.
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The present invention 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 addition, the present invention relates to a pharmaceutical composition comprising the nucleic acid,
25 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
30 suitable for subcutaneous, intramuscular, intratumoral or intravenous injection, preferably intravenous injection.

The present invention 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 invention relates to a method for treating a disease in a subject
- 5 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.
- 10 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,
- 15 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
- 20 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
- 25 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
- 30 Frontotemporal Lobar Degeneration.

Finally, the present invention 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:

(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 encoding a Nuclear Localization Signal (NLS) and a Cyclic GMP-AMP synthase (cGAS) as set forth in SEQ ID NO: 40, or any nucleic acid sequence of interest as herein described such as SEQ ID NO: 1, or a variant thereof having at least 85% identity thereto and a R241 mutation in reference to SEQ ID NO: 40 or 1, for example a R241E, R241D, R241N, or R241A substitution, preferably mRNA molecules; and

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

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DETAILED DESCRIPTION OF THE INVENTION

Definition

In order that the present invention may be more readily understood, certain terms are defined hereafter. Additional definitions are set forth throughout the detailed description.

15 Unless otherwise defined, all terms of art, notations and other scientific terminology used herein are intended to have the meanings commonly understood by those of ordinary skill in the art to which this invention pertains.

As used herein, the "sequence identity" between two sequences is described by the parameter "sequence identity", "sequence similarity" or "sequence homology". For purposes of the present invention, the "percentage identity" between two sequences (A) and (B) is determined by comparing the two sequences aligned in an optimal manner, through a window of comparison. The percent identity between the two sequences is particularly a function of the number of identical positions shared by the sequences (i.e., % identity = number of identical positions/total number of positions x 100), taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. Said alignment of sequences can be carried out by well-known methods in the art, for example, using the algorithm for global alignment of Needleman-Wunsch. Protein analysis software matches similar sequences using measures of similarity assigned to various substitutions, deletions, and other modifications, including conservative amino acid substitutions. Once the total alignment is obtained, the percentage of identity can be obtained by dividing the full number of identical amino acid residues aligned by the full number of residues contained in the longest sequence between the sequence (A) and (B). Sequence identity is typically determined using sequence analysis software. For comparing two amino acid sequences, one can use, for example, the tool "Emboss needle" for pairwise sequence alignment of proteins providing by EMBL-EBI and available on:

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www.ebi.ac.uk/Tools/services/web/toolform.ebi?tool=emboss_needle&context=protein, for example using default settings: (i) Matrix: BLOSUM62, (ii) Gap open: 10, (iii) gap extend: 0.5, (iv) output format: pair, (v) end gap penalty: false, (vi) end gap open: 10, (vii) end gap extend: 0.5.

5 The percent identity between two amino acid sequences or nucleotide sequences can also be determined using the algorithm of E. Meyers and W. Miller (Comput. Appl. Biosci., 4:11-17, 1988) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. Alternatively, the percent identity between two amino acid sequences or nucleotide sequences can be determined using the Needleman and Wunsch (J. Mol. Biol. 48:444-453, 1970) algorithm which has been incorporated into the GAP program in the GCG software package
10 (available at <http://www.gcg.com>), using either a Blossom 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. The percent identity between two nucleotide or amino acid sequences may also be determined using for example algorithms such as the BLASTN program for nucleic acid or amino acid sequences using as defaults a word length (W) of 11, an expectation (E) of 10, M=5, N=4, and a comparison of both strands.

15 Alternatively, sequence identity can also be typically determined using sequence analysis software Clustal Omega using the HAlign algorithm and its default settings as its core alignment engine. The algorithm is described in Söding, J. (2005) 'Protein homology detection by HMM-HMM comparison'. Bioinformatics 21, 951-960, with the default settings.

By "amino acid change", "amino acid modification" or "amino acid mutation" is meant herein a change in
20 the amino acid sequence of a polypeptide. "Amino acid modifications" include substitution, insertion and/or deletion in a polypeptide sequence. By "amino acid substitution" or "substitution" herein is meant the replacement of an amino acid at a particular position in a parent polypeptide sequence with another amino acid. By "amino acid insertion" or "insertion" is meant the addition of at least one or two amino acid(s) at a particular position in a parent polypeptide sequence. By "amino acid deletion" or "deletion"
25 is meant the removal of an amino acid at a particular position in a parent polypeptide sequence. The amino acid substitutions may be conservative. A conservative substitution is the replacement of a given amino acid residue by another residue having a side chain ("R-group") with similar chemical properties (e.g., charge, bulk and/or hydrophobicity). As used herein, "amino acid position" or "amino acid position number" are used interchangeably and refer to the position of a particular amino acid in an amino acids
30 sequence, generally specified with the one letter codes for the amino acids. The first amino acid in the amino acids sequence (i.e., starting from the N terminus), for example a methionine ("M"), should be considered as having position 1.

A conservative substitution is the replacement of a given amino acid residue by another residue having a side chain ("R-group") with similar chemical properties (e.g., charge, bulk and/or hydrophobicity). In

general, a conservative amino acid substitution will not substantially change the functional properties of a protein. Conservative substitutions and the corresponding rules are well-described in the state of the art. For instance, conservative substitutions can be defined by substitutions within the groups of amino acids reflected in the following tables:

Amino Acid groups	Amino Acid Residues
Acidic Residues	ASP and GLU
Basic Residues	LYS, ARG, and HIS
Hydrophilic Uncharged Residues	SER, THR, ASN, and GLN
Aliphatic Uncharged Residues	GLY, ALA, VAL, LEU, and ILE
Non-polar Uncharged Residues	CYS, MET, and PRO
Aromatic Residues	PHE, TYR, and TRP

5 **Table A – Amino Acid Residue**

1	Alanine (A)	Serine (S)	Threonine (T)
2	Aspartic acid (D)	Glutamic acid (E)	
3	Asparagine (N)	Glutamine (Q)	
4	Arginine (R)	Lysine (K)	
5	Isoleucine (I)	Leucine (L)	Methionine (M)
6	Phenylalanine (F)	Tyrosine (Y)	Tryptophan (W)
7	Cysteine (C)	Glycine (G)	Histidine (H)
8	Proline (P)	Valine (V)	Selenocysteine (U)
9	Pyrrolysine (O)		

Table B - Alternative Conservative Amino Acid Residue Substitution Groups

Alcohol group-containing residues	S and T
Aliphatic residues	I, L, V, and M
Cycloalkenyl-associated residues	F, H, W, and Y
Hydrophobic residues	A, C, F, G, H, I, L, M, R, T, V, W, and Y
Negatively charged residues	D and E
Polar residues	C, D, E, H, K, N, Q, R, S, and T
Small residues	A, C, D, G, N, P, S, T, and V
Very small residues	A, G, and S
Residues involved in turn formation	A, C, D, E, G, H, K, N, Q, R, S, P, and T
Flexible residues	E, Q, T, K, S, G, P, D, E, and R

Table C – Further Alternative Physical and Functional Classifications of Amino Acid Residues

The term “and/or” as used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. For example, “A and/or B” is to be taken as specific disclosure of each of (i) A, (ii) B and (iii) A and B, just as if each is set out individually.

The term “a” or “an” can refer to one of or a plurality of the elements it modifies (e.g., “a reagent” can mean one or more reagents) unless it is contextually clear either one of the elements or more than one of the elements is described.

The term “at least one” means “one or more” or “one or several”. For instance, it refers to one, two, three or more.

The term “about” as used herein in connection with any and all values (including lower and upper ends of numerical ranges) means any value having an acceptable range of deviation of up to +/- 10% (e.g., +/- 0.5%, +/- 1 %, +/- 1.5%, +/- 2%, +/- 2.5%, +/- 3%, +/- 3.5%, +/- 4%, +/- 4.5%, +/- 5%, +/- 5.5%, +/- 6%, +/- 6.5%, +/- 7%, +/- 7.5%, +/- 8%, +/- 8.5%, +/- 9%, +/- 9.5%). The use of the term “about” at the beginning of a string of values modifies each of the values (i.e., “about 1, 2 and 3” refers to about 1, about 2 and about 3). Further, when a listing of values is described herein (e.g., about 50%, 60%, 70%, 80%, 85% or 86%) the listing includes all intermediate and fractional values thereof (e.g., 54%, 85.4%).

10 The methods of the invention as disclosed below may be *in vivo*, *ex vivo* or *in vitro* methods, preferably *in vitro* or *ex vivo* methods.

The term “comprise” or “comprising” can be replaced by “consist” or “consisting” or by “essentially consist” or “essentially consisting” in any aspects or embodiments of the present application. The term “essentially” as used herein in connection with any given biological sequence means said biological sequence varies from the reference (“parent”) sequence contained in the sequence listing by up to 10% of the biological sequence length. In particular, by “consists essentially of” is intended that the biological sequence consists of that sequence, but it may also include 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 substitutions, additions, deletions or a mixture thereof, preferably 1, 2, 3, 4, or 5 substitutions, additions, deletions or a mixture thereof, with the proviso that said biological sequence varies from the reference sequence contained in the sequence listing by up to 10% of the biological sequence length.

Nucleic acid molecules

The present invention relates to nucleic acid molecule(s) comprising a Nuclear Localization Signal (NLS) and a sequence encoding a *Mus musculus* Cyclic GMP-AMP synthase (cGAS) comprising an activating mutation.

Any of the below aspects for nucleic acid molecule(s) applies to all of the vector nanoparticles, for example lipid-based nanoparticles, viral-based nanoparticles or dendrimer nanoparticles disclosed below.

The term “nucleic acid”, “nucleic acid construct” or “nucleic acid molecule” means a nucleic acid molecule, either single- or double-stranded, which is modified to contain segments of nucleic acids in a manner that would not otherwise exist in nature or which is synthetic, which comprises one or more control sequences. The nucleic acid molecule(s) can be DNA molecule(s) and/or RNA molecule(s).

Preferably, the nucleic acid is an mRNA encoding a polypeptide of interest, capable of being expressed and translated in a targeted cell of interest.

In a particular aspect, the nucleic acid comprises a mRNA polynucleotide or a set of mRNA polynucleotides. The technology of mRNA polynucleotide is now well-known by the person skilled in the art, as illustrated in WO21159130, the disclosure thereof being incorporated herein by reference.

In one aspect, the mRNA molecule particularly comprises structural elements that allows its encapsulation, in particular into the vector nanoparticle, for example into the lipid-based nanoparticle, the viral nanoparticle or the dendrimer nanoparticle, and/or its expression into the targeted cell.

Preferably, the mRNA molecule contains stabilizing elements, including, but not limited to untranslated regions (UTR) at their 5'-end (5'-UTR) and/or at their 3'-end (3'-UTR), in addition to other structural features, such as a 5'-cap structure and/or a 3'-poly-A tail.

In some aspects, the mRNA of the invention includes 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.

In some aspects, the mRNA of the invention comprises a flanking region. A 5'-UTR or a 3'-UTR may be provided as a flanking region to the mRNA of the invention. A 5'-UTR may be homologous or heterologous to the coding region of the mRNA. Multiple 5'-UTRs or 3'-UTRs may be included in the flanking region and may be of the same or of different sequences. Any portion of the flanking regions, including none, may be codon optimized and any may independently contain one or more different structural or chemical alterations, before and/or after codon optimization. Variants of the 5'-UTRs and/or 3'-UTRs may be utilized wherein one or more nucleotides are added or removed to the termini, including A, T, C or G. 5'-UTRs and/or 3'-UTRs may also be codon-optimized, or altered in any manner known to the man skilled in the art. Preferably, the 5'-UTR comprises Tobacco Etch Virus (TEV) leader sequence, for example such as described under SEQ ID NO: 11.

In some aspects, the mRNA comprises an Internal Ribosome Entry Site (IRES) or a Kozak sequence in the 5'-UTR region. The Kozak consensus sequence (Kozak consensus or Kozak sequence) is a nucleic acid motif that functions as the protein translation initiation site. An internal ribosome entry site (IRES) is an RNA element that allows for translation initiation in a cap-independent manner.

In some aspects, the mRNA of the invention comprises a 5'-capping region or structure. The 5'-cap structure of a polynucleotide is involved in nuclear export and increases polynucleotide stability since it binds the mRNA Cap Binding Protein (CBP). The 5'-cap structure is responsible for polynucleotide stability in the cell and translation competency through the association of CBP with poly-A binding protein to form the mature cyclic mRNA species. Alterations to polynucleotides may generate a non-hydrolysable cap structure preventing de-capping and thus increasing polynucleotide half-life. Multiple distinct 5'-cap

structures can be used to generate the 5'-cap of an mRNA molecule. For example, recombinant Vaccinia Virus Capping Enzyme or a Faustovirus Capping Enzyme and recombinant 2'-O-methyltransferase enzyme can create a canonical 5'-5'-triphosphate linkage between the 5'-terminal nucleotide of a polynucleotide and a guanosine cap nucleotide wherein the cap guanosine contains an N7-methylation and the 5'-terminal nucleotide of the polynucleotide contains a 2'-O-methyl. Such a structure is termed the Cap1 structure. This cap results in a higher translational-competency, cellular stability, and a reduced activation of cellular pro-inflammatory cytokines, as compared, e.g., to other 5'-cap analog structures known in the art. Other exemplary cap structures include 7mG(5')ppp(5')N,pN2p (Cap 0), 7mG(5')ppp(5')NImpNp (Cap 1), 7mG(5')-ppp(5')NImpN2mp (Cap 2), and m(7)Gpppm(3)(6,6,2')Apm(2')Apm(2')Cpm(2')(3,2')Up (Cap 4). In particular, trinucleotide cap1 analog and m1Ψ-5'-triphosphate can be used, such as provided by Cleancap technology (TriLink).

In some aspects, the mRNA includes a chain terminating nucleoside, for example chain-terminating nucleoside analogs (CTNAs) in particular that lack the 3'-hydroxy group or chain-terminating dideoxynucleoside triphosphates (Prober *et al.* Science 238, 336-41 (1987)).

In some aspects, the mRNA includes a 3'UTR comprising a poly-A sequence and/or polyadenylation signal. A poly-A sequence may be comprised entirely or mostly of adenine nucleotides or analogs or derivatives thereof. A poly-A sequence may be a tail located adjacent to a 3' untranslated region of a nucleic acid. The length of a poly-A region of the present disclosure is of 20, 40, 80, 100, 110, 120, 140 or 160 nucleotides in length on an mRNA molecule described herein. Preferably, the poly-A region is of between 50 and 150, between 60 and 140, between 70 and 130, between 80 and 120, between 90 and 110 or between 95 and 105 nucleotides in length. Even more preferably, the poly-A region is of between 95 and 105 nucleotides in length, preferably of 101 nucleotides in length. Preferably, the 3'UTR comprises the human beta globin sequence and 101 nucleotides long poly(A) tail, such as described under SEQ ID NO: 12.

In some aspects, the mRNA molecule of the invention may include one or more naturally occurring components, including any of the canonical nucleotides A (adenosine), G (guanosine), C (cytosine), U (uridine), or T (thymidine). Any one of the regions of a nucleic acid may include one or more alternative components (e.g., an alternative nucleoside) which impart useful properties including increased stability and/or the lack of a substantial induction of the innate immune response of a cell into which the polynucleotide is introduced. Non-limiting examples of such non-naturally occurring modified nucleotides and nucleosides can be found, inter alia, in published patent application Nos. WO2013052523; WO2014093924; WO2015051173; WO2015051169; WO2015089511; WO2015196130; WO2015196118; WO2015196128; or WO2017153936 all of which are incorporated by reference herein.

In some aspects, the mRNA molecule of the invention may include optimized codons, in particular for expression in a eukaryotic cell. Codon-optimization describes gene engineering approaches that use

synonymous codon changes notably to increase protein production. Most optimization strategies use codons with host bias to replace less frequently occurring codons. Another strategy is to adjust the original codon sequence to match the natural distribution of the host codons. Several softwares are available for the codon optimization. For instance, the codons are optimized by Lasergene software package .

- 5 In some aspects, the mRNA molecule of the invention may include at least 5%, 10 %, 15%, 20%, 25%, 30%, 35%, 40%, or 50% of optimized codons.

The nucleic acid molecule of the invention encodes (i) a Nuclear Localization Signal (NLS) sequence and (ii) a Cyclic GMP-AMP synthase (cGAS). Each of these two components is further described here below. Any of the specific aspect can be combined to arrive at the nucleic acid of the invention.

- 10 The terms “nuclear localization sequence” and “NLS” are used herein interchangeably to indicate a peptide that directs the transport of a protein or peptide with which it is associated from the cytoplasm of a cell across the nuclear envelope barrier. The term “NLS” is intended to encompass not only the nuclear localization sequence of a particular peptide or polypeptide, but also derivatives thereof that are capable of directing translocation of a cytoplasmic polypeptide across the nuclear envelope barrier.

- 15 NLS are capable of directing nuclear translocation of a polypeptide when attached to the N-terminus, the C-terminus, or both the N- and C-termini of the polypeptide. Preferably, the NLS is attached to the N-terminus of the sequence encoding cGAS.

Preferably, the NLS is a classical nuclear localization signal (cNLS) or a non-classical nuclear localization signal (ncNLS), for example such as described in Lu *et al.*, Cell Communication and Signaling volume 19,

- 20 Article number: 60 (2021), incorporated herein by reference.

The cNLS encompass two categories, termed “monopartite” (MP) and “bipartite” (BP). MP NLS are a single cluster composed of 4–8 basic amino acids, which generally contains 4 or more positively charged residues, that is, arginine (R) or lysine (K). The characteristic motif of MP NLS is usually defined as K (K/R) X (K/R), where X can be any residue.

- 25 Examples of classical NLS include those from the SV40 large T antigen (PPKKRKRK; SEQ ID NO: 13), adenovirus E1A (SCKRPRP; SEQ ID NO: 14), human lamin A (SVTKKRKL; SEQ ID NO: 15); polyoma large T antigen (PPKKARED; SEQ ID NO: 16), polyoma large T antigen (VSRKRPRP; SEQ ID NO: 17), human c-myc (PAAKRVKLD; SEQ ID NO: 18), rat glucocorticoid receptor (RKTKKKIK; SEQ ID NO: 19), human estrogen receptor (IRKDRRG; SEQ ID NO: 20), and human MYC gene (GGGGSPAARKRVKLD, SEQ ID NO: 39).

- 30 ncNLSs are for example PY-NLS that is characterized by 20–30 amino acids that assume a disordered structure, consisting of N-terminal hydrophobic or basic motifs and C-terminal R/K/H(X)2-5PY motifs (where X2-5 is any sequence of 2–5 residues). The PY-NLS consensus particularly corresponds to [basic/hydrophobic]-Xn- [R/H/K]-(X)2–5-PY, where X can be any residue.

Examples of nonclassical nuclear localization signals include, but are not limited to, M9 of Heterogeneous nuclear ribonucleoprotein 1 (hnRNP A1), the K nuclear shuttling domain (KNS) of the heterogeneous nuclear ribonucleoprotein K (hnRNP K), and the HuR Nucleocytoplasmic Shuttling (HNS) motif of the Human antigen R protein HuR.

- 5 For instance, alternative NLS can be selected in the following group: PKKKRKV (SEQ ID NO: 38), PKLKRQ (SEQ ID NO: 21), RPRK (SEQ ID NO: 22), RRARRPRG (SEQ ID NO: 23), GKRKLITSEERSPAKRGRKS (SEQ ID NO: 24), KGKKGRTQKEKKAARARSKGKN (SEQ ID NO: 25), RKRCAAGVGGGPAGCPAGSTPLKKPRR (SEQ ID NO: 26), RKPVTAQERQREEREKRRRRQERAKEREKRRQERER (SEQ ID NO: 27), RSGGNHRRNNGRGGGGYNNRRNNGYHPY (SEQ ID NO: 28), TLLRETMNNLGVSDHAVLSRKTQPYPY (SEQ ID NO: 29), PGKMDKGHRQERRDRPY (SEQ ID NO: 30), GKKKKGKPGKRREQRKKKRRT (SEQ ID NO: 31), SANKVTKNKSNSSPYLNKRKGKPGPDS (SEQ ID NO: 32), VSHKKKKIPTSPTFTTPKTLRRQPKYPRKSAPRRNKLDHY (SEQ ID NO: 33), RKHKTNRKPR (SEQ ID NO: 34), NRRAKAKR (SEQ ID NO: 35), RNKKKK (SEQ ID NO: 36), RKVIK (SEQ ID NO: 37) and GGGGSPAARKVKLD (SEQ ID NO: 39).

- 15 Preferably the NLS sequence encoded by the nucleic acid molecule of the invention is as set forth in SEQ ID NO: 9 or 37 (SEQ ID NO: 9 being NLS of SEQ ID NO: 37 with a spacer) or a variant having at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

Preferably, the nucleic acid sequence of the NLS is as set forth in SEQ ID NO: 10 or a variant having at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

- 20 In a particular aspect, the nucleic acid sequence of the NLS is as set forth in SEQ ID NO: 39 or a variant having at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

The nucleic acid molecule of the invention also comprises a sequence encoding a Cyclic GMP-AMP synthase (cGAS). The sequence encoding cGAS or a variant thereof is operably linked to a NLS sequence such as described above.

- 25 The term “operably linked” means a configuration in which a control sequence is placed at an appropriate position relative to a coding sequence, in such a way that the control sequence directs expression of the coding sequence.

Accordingly, the NLS and the cGAS sequences form a fusion protein. As used herein, the term “Fusion protein” refers to a polypeptide including at least two segments, these segments being not included in a single peptide in the nature.

30

Cyclic GMP-AMP synthase (cGAS) is a cytosolic DNA sensor that signals by catalyzing the synthesis of a second messenger, cGAMP. cGAS binds double-stranded DNA in a sequence non-specific manner and this induces a conformational change in its enzymatic site allowing for cyclic GMP-AMP (cGAMP) synthesis (Wu

et al., 2012, *Science*, 339, 826-830; Sun *et al.*, 2012, *Science*, 339, 786-791; WO2014/099824; Ablasser *et al.*, 2013, *Nature*, 498, 380-384).

Several members of this family have been recently identified and characterized, in particular murine cGAS and human cGAS (Wu *et al.*, 2012, *Science*, 339, 826-830; Sun *et al.*, 2012, *Science*, 339, 786-791).

- 5 Human cGAS is referenced in UniprotKB ID No Q8N884. The reference sequences are disclosed in NCBI RefSeq as NP_612450.2 for the amino acid sequence and as NM_138441.2 for the mRNA sequence.

Murine (*Mus Musculus*) cGAS is referenced in UniprotKB ID No Q8C6L5. The reference sequences are disclosed in NCBI RefSeq as NP_775562.2 for the amino acid sequence and as NM_173386.4 for the mRNA sequence.

- 10 Brown rat cGAS is referenced in UniprotKB ID No A0A0G2JVC4. The reference sequences are disclosed in NCBI RefSeq as XP_038938458.1 for the amino acid sequence, and as XM_039082530.2 for the mRNA sequence (incorporated herein by reference).

Nile rat cGAS is referenced in UniParc ID No UPI00148669AF. The reference sequences are disclosed in NCBI RefSeq as XP_034340251.1 for the amino acid sequence, and as XM_034484360.1 for the mRNA

- 15 sequence (incorporated herein by reference).

Macaque cGAS is referenced in UniprotKB ID No A0A1D5QFG4. The reference sequences are disclosed in NCBI RefSeq as XP_001109400.2 for the amino acid sequence, and as XM_001109400.4 for the mRNA sequence (incorporated herein by reference).

- cGAS have also been well characterized in Bovine, pig and *Vibrio cholera* serotype O1 (respectively, see
20 UniprotKB ID Nos E1BGN7, I3LM39 and Q9KVG7) and can be also found in *Drosophila* (e.g., *D. melanogaster*), zebrafish (*D. rerio*), *A. carolinensis*, *A. melanoleuca*, *A. mellifera*, *B. floridae*, *C. lupus familiaris*, *E. caballus*, *F. catus*, *G. gallus*, *G. gorilla*, *H. magnipapillata*, *I. scapularis*, *M. brevicollis*, *M. domestica*, *M. gallopavo*, *M. mulatta*, *N. vectensis*, *N. vitrioennis*, *O. anatinus*, *O. aries*, *O. cuniculus*, *O. latipes*, *P. abelii*, *P. anubis*, *P. paniscus*, *P. troglodytes*, *R. norvegicus*, *S. harrisii*, *T. castaneum*, *T. guttata* and
25 *X. tropicalis* or *laevis* (Wu *et al.*, 2014, *Nucleic Acids Research*, 42, 8243-8257; the disclosure of which is incorporated by reference).

- Mutations in cGAS have been described in the art, for example in Volkman *et al.*, *Elife* . 2019 Dec 6;8:e47491, which is incorporated herein by reference. Mutations R255E (in human cGAS, for example such as described under SEQ ID NO: 7) or R241E (in mouse cGAS for example such as described under SEQ
30 ID NO: 1 or in relation with SEQ ID NO: 40) detach cGAS from histone and allow its activation by chromatin. These mutations lead to a constitutive activation of cGAS in absence of (exogenous) DNA.

Accordingly, the present invention 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: 40 or a constitutively active variant thereof having at least about 80% or about 85% identity thereto.

5 In a preferred aspect, 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 aspect, 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.

10 In another particular aspect, 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.

15 A particular nucleic acid molecule of the invention encodes for a murine cGAS bearing a R241 mutation, for example i) the R241E mutation, in particular such as described in SEQ ID NO: 1 or 43, ii) the R241D mutation, in particular such as described in SEQ ID NO: 45, iii) the R241N mutation, in particular such as described in SEQ ID NO: 44, or iv) the R241A mutation, in particular such as described in SEQ ID NO: 46. Preferably, the murine cGAS bearing the R241E mutation has a nucleic acid sequence such as described in SEQ ID NO : 2 or SEQ ID NO: 3, preferably SEQ ID NO: 3.

20 Preferably, the murine cGAS bearing the R241D mutation has a nucleic acid sequence such as described in SEQ ID NO : 45. In SEQ ID NO : 45, position "X" is position 253 and the mutation may be identified as R253D.

Preferably, the murine cGAS bearing the R241A mutation has a nucleic acid sequence such as described in SEQ ID NO : 46. In SEQ ID NO : 46, position "X" is position 253 and the mutation may be identified as R253A.

25 In a particular aspect, a nucleic acid molecule of the invention, such as SEQ ID NO: 41, encodes for a murine cGAS bearing a mutation (deletion, addition or substitution) at position 255 which is considered as equivalent to R241X in reference to SEQ ID NO: 40, and results in a constitutively active variant capable of producing cGAMP when present in a cell or subject.

30 In another particular aspect, a nucleic acid molecule of the invention, such as SEQ ID NO: 42, encodes for a murine cGAS bearing a mutation (deletion, addition or substitution) at position 253 which is considered as equivalent to R241X in reference to SEQ ID NO: 40 and results in a constitutively active variant capable of producing cGAMP when present in a cell or subject.

An example of a nucleic acid molecule of the invention encoding for a murine cGAS bearing a deletion at position "X" is SEQ ID NO: 47. In SEQ ID NO: 47, position "X" is position 253.

5 An example of a nucleic acid molecule of the invention encoding for a murine cGAS bearing an addition at position "X" is SEQ ID NO: 48. In SEQ ID NO: 48, "X" designates "AAA", the "AAA" addition occupying position 253-255.

A particularly preferred nucleic acid molecule of the invention, such as SEQ ID NO: 44 or a nucleic acid molecule comprising such a sequence, encodes for a murine cGAS bearing a R241N mutation in reference to SEQ ID NO: 40 or, for example, R253N in reference to SEQ ID NO: 44.

10 The murine cGAS envisioned herein also includes variants of SEQ ID NO: 40 or SEQ ID NO: 1, i.e., variants having additional distinct mutations in SEQ ID NO: 40 or 1 except in amino acid position 241 (position 241 referring to amino acid position 241 in "parent" sequences SEQ ID NO: 40 or 1, said position number being likely to change depending on the reference or "parent" sequence) so that mutation R241X, for example substitution R241E, R241D, R241N or R241A, is conserved among the variants. Such variants may vary by 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acids substitutions, deletions and/or additions. Preferably, the variants
15 have at least 75, 80, 85, 90, 95 or 99 % of identity with the murine cGAS bearing the R241X mutation, for example the R241E mutation. The variant may be obtained by various techniques well known in the art. In particular, examples of techniques for altering the DNA sequence encoding the wild-type protein, include, but are not limited to, site-directed mutagenesis, random mutagenesis and synthetic oligonucleotide construction.

20 In another particular aspect, the nucleic acid molecule of the invention encodes for a mutated rat cGAS such as the mutated rat cGAS encoded by SEQ ID NO: 49-52, in particular SEQ ID NO: 49.

In SEQ ID NO: 49, position "X" is position 221, and may be any kind of mutation, i.e., a deletion, addition or substitution, similarly to position "X" in SEQ ID NO: 40.

25 Thus, in a particular aspect, 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 aspect, the constitutively active variant is or comprises SEQ ID NO: 51 or 52.

30 In another particular aspect, the nucleic acid construct envisioned herein encodes for a fusion protein such as described under SEQ ID NO: 50 (i.e., NLS and rat cGAS R221X) or a variant thereof having at least about 85, 90, 95 or 99 % of sequence identity thereto and bearing a R221X mutation, for example a R221E mutation such as for example SEQ ID NO: 51, or a R221 deletion such as for example SEQ ID NO: 52.

In again another particular aspect, the nucleic acid molecule of the invention encodes for a mutated rat cGAS such as the mutated brown rat cGAS encoded by SEQ ID NO: 53-56, in particular SEQ ID NO: 53.

In SEQ ID NO: 53, position "X" is position 244, and may be any kind of mutation, i.e., a deletion, addition or substitution, similarly to position "X" in SEQ ID NO: 40.

- 5 Thus, in a particular aspect, 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 aspect, the constitutively active variant is or comprises SEQ ID NO: 55 or 56.

- 10 In another particular aspect, the nucleic acid construct envisioned herein encodes for a fusion protein such as described under SEQ ID NO: 54 (i.e., NLS and rat cGAS R244X) or a variant thereof having at least about 85, 90, 95 or 99 % of sequence identity thereto and bearing a R244X mutation, for example a R244E mutation such as for example SEQ ID NO: 55, or a R244 deletion such as for example SEQ ID NO: 56.

- 15 In another particular aspect, the nucleic acid molecule of the invention encodes for a mutated cGAS which is not a mutated primate cGAS, in particular a macaque or a human cGAS including the R255E substitution (in reference to the human cGAS sequence).

In another aspect, the nucleic acid molecule of the invention encodes for a mutated cGAS and does not include any mutated cGAS sequence of primate, in particular of human origin, or of macaque origin such as SEQ ID NO: 57.

- 20 The present disclosure relates preferably to nucleic acid constructs comprising a sequence encoding a Nuclear Localization Signal (NLS) sequence, preferably as set forth in SEQ ID NO :9 or SEQ ID NO: 39, and a sequence encoding a Cyclic GMP-AMP synthase (cGAS) as set forth in SEQ ID NO: 40 or 1 or a variant thereof having at least 85% identity thereto and a mutation at position 241, for example a R241X mutation such as a deletion, an addition or a substitution selected from R241E, R241D, R241N or R241A (in reference
25 to SEQ ID NO: 40 or 1). In other words, the variant has a sequence having at least about 80% or about 85% identity with SEQ ID NO: 40 or 1 and has no amino acid, a distinct amino acid (for example an amino acid E, D, N or A), or additional amino acid(s) in position 241.

- The present disclosure relates to a nucleic acid construct comprising a sequence encoding a Nuclear Localization Signal (NLS) sequence, preferably as set forth in SEQ ID NO :9 or SEQ ID NO: 39, and a sequence
30 encoding a Cyclic GMP-AMP synthase (cGAS) as set forth for example in anyone of SEQ ID NO: 41, 42, 43, 44, 45, 46, 47, 48, 50, 51, 52, 54, 55 or 56.

In a particular aspect, the nucleic acid construct envisioned herein encodes for a fusion protein such as described under SEQ ID NO: 4 (i.e., NLS and murine cGAS R241E) or a variant thereof having at least about

85, 90, 95 or 99 % of sequence identity thereto and bearing a R241X mutation, for example the R241E mutation, such as SEQ ID NO: 43.

In another example, the nucleic acid construct envisioned herein encodes for a fusion protein such as described under SEQ ID NO: 44 (i.e., NLS and mouse cGAS R241N) or a variant thereof having at least about
5 85, 90, 95 or 99 % of sequence identity thereto and bearing an identical or different NLS and a R241X mutation, for example the R241N mutation (in reference to SEQ ID NO: 40).

In another example, the nucleic acid construct envisioned herein encodes for a fusion protein such as described under SEQ ID NO: 45 (i.e., NLS and mouse cGAS R241D) or a variant thereof having at least about
10 85, 90, 95 or 99 % of sequence identity thereto and bearing an identical or different NLS and a R241X mutation, for example the R241D mutation (in reference to SEQ ID NO: 40).

In another example, the nucleic acid construct envisioned herein encodes for a fusion protein such as described under SEQ ID NO: 46 (i.e., NLS and mouse cGAS R241A) or a variant thereof having at least about
85, 90, 95 or 99 % of sequence identity thereto and bearing an identical or different NLS and a R241X mutation, for example the R241A mutation (in reference to SEQ ID NO: 40).

15 In an alternative aspect, the present invention also relates to a nucleic acid comprising a sequence encoding a NLS and a sequence encoding a Cyclic GMP-AMP synthase (cGAS) as set forth in SEQ ID NO: 40, for example SEQ ID NO: 1, or a variant thereof having at least about 80% or about 85% identity thereto, but with an alternative substitution instead of a mutation (deletion, addition or substitution) at position
20 241 in reference to SEQ ID NO: 40 or 1 (which would read for example 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). Accordingly, R241E mutation could be replaced by R241X mutation, X being selected from the group consisting of A, C, D, E, F, G, H, I, K, L, M, N, O, P, Q, S, T, U, V, W and Y, preferably A, C, D, E, G, H, N, P, Q, S, T, and V, for example A, D, E or N. In a particular aspect, X is selected from the group
25 consisting of A, D, N and Q. Optionally, X is D. In other words, the Cyclic GMP-AMP synthase (cGAS) has a sequence as set forth in SEQ ID NO: 40 or 1, or a variant thereof having at least about 80% or about 85% identity thereto, but it has an amino acid X in position 241 as defined above and said X is not R, or it has several amino acids in position 241 one or several of them, except the first one of them, being a R.

In a particular aspect, the nucleic acid construct envisioned herein, preferably the mRNA envisioned herein
30 comprises or consists essentially of a nucleic acid sequence as described under SEQ ID NO: 3.

In another particular aspect, the nucleic acid construct envisioned herein, preferably the mRNA envisioned herein comprises or consists essentially of a nucleic acid sequence as described under SEQ ID NO: 6.

The nucleic acid molecule can be vectorized by any means available to the person skilled in the art such as viral vectors, lipid-based or LNP nanoparticles (for example liposomes, micelles, nano-emulsions), pseudoviral particles, or dendrimer nanoparticles.

Thus, the present disclosure relates to a vector nanoparticle comprising a nucleic acid molecule comprising
5 a Nuclear Localization Signal (NLS) sequence and a sequence encoding a Cyclic GMP-AMP synthase (cGAS) as herein defined. In a particular aspect, the Cyclic GMP-AMP synthase (cGAS) has a sequence as set forth in the present description, typically 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, said variant is constitutively active, i.e., it is capable of producing cGAMP when present
10 in a cell or subject (even in the absence of exogenous DNA).

In a particular aspect, the vector is a nanoparticle, for example a lipid-based nanoparticle, a viral nanoparticle or a dendrimer nanoparticle.

In a particular aspect, the vector is not a viral vector.

In another particular aspect, the vector is not in (i.e. is not used to modify) a genetically modified cancer
15 cell.

In a further particular aspect, the vector is not in (i.e. is not used to modify) an anti-tumor (or “anti-tumor effector”) immune cell, such as an anti-tumor lymphocyte, T cell, natural killer (NK) cell, NK-T cell or macrophage. In particular, the vector is not a CAR-T cell.

In another particular aspect, the vector is not a dendrimer nanoparticle.

20

Lipid-based nanoparticles

The present disclosure relates to a lipid-based nanoparticle comprising a nucleic acid molecule comprising a Nuclear Localization Signal (NLS) sequence and a sequence encoding a Cyclic GMP-AMP synthase (cGAS) as defined herein. In a particular aspect, the Cyclic GMP-AMP synthase (cGAS) has a sequence as set forth
25 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.

The lipid-based nanoparticle according to the invention is particularly formulated either as a liposome or a lipid-based nanoparticle (LNP). The lipid-based nanoparticle also encompasses similar nanoparticles such as but not limited to micelles and nano-emulsions. Preferably, the lipid-based nanoparticle
30 envisioned herein is a LNP.

The lipid-based nanoparticles of the disclosure can be generated in particular using components, compositions, and methods as generally known in the art, for example such as disclosed in WO

2017004143; WO2017049245; WO2017112865; WO2017218704; WO2015164674; WO2017031232; WO2017099823; WO2016118724; WO2016118724; WO2017223135; WO2014152211; WO2015038892; WO2017049074; WO2013090648; WO2017180917; WO2017075531 and WO2017117528 all of which are incorporated by reference herein in their entirety.

- 5 In a particular aspect, the lipid-based nanoparticle comprises one or more ionizable or cationic lipid(s), one or more helper lipid(s), one or more sterol(s), and/or one or more polyethylene glycol (PEG)-modified lipid(s).

In some aspects, the lipid-based nanoparticle according to the invention comprises one or more ionizable or cationic lipid(s). As used herein, the term "ionizable or cationic lipid" refers to a lipid molecule positively charged in an acidic environment. Preferably the ionizable or cationic lipid has a pKa in the range of 6.0-6.5.

Examples of ionizable cationic lipids are described in WO 2016/021683, WO 2015/011633, WO 2011/153493, WO 2013/126803, WO 2010/054401, WO 2010/042877, WO 2016/104580, WO 2015/005253, WO 2014/007398, WO 2017/117528, WO 2017/075531, WO 2017/00414, WO 15 2015/199952, US 2015/0239834, WO2019/131839, WO2023/182756, all of which are incorporated by reference herein in their entirety.

Ionizable cationic lipids can particularly be selected from the group consisting of 1,2-dioleoyl-3-trimethylammonium propane (DOTAP); N,N-dimethyl-2,3-dioleoyloxypropylamine (DODMA), 1,2-di-Octadecenyl-3-trimethylammonium propane (DOTMA), 3-(N-(N',N'-dimethylaminoethane)-carbamoyl)cholesterol (DC-Chol), dimethyldioctadecylammonium (DDAB); 1,2-dioleoyl-3-dimethylammonium-propane (DODAP); 1,2-diacyloxy-3-dimethylammonium propanes; 1,2-dialkyloxy-3-dimethylammonium propanes; dioctadecyldimethyl ammonium chloride (DODAC), 1,2-distearoyloxy-N,N-dimethyl-3-aminopropane (DSDMA), 2,3-di(tetradecoxy)propyl-(2-hydroxyethyl)-dimethylazanium (DMRIE), 1,2-dimyristoyl-sn-glycero-3-ethylphosphocholine (DMEPC), 1,2-dimyristoyl-3-trimethylammonium propane (DMTAP), 1,2-dioleoyloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide (DORIE), and 2,3-dioleoyloxy- N-[2(spermine carboxamide)ethyl]-N,N-dimethyl-1-propanamium trifluoroacetate (DOSPA), 1,2-dilinoleyloxy-N,N-dimethylaminopropane (DLinDMA), 1,2-dilinolenyloxy-N,N-dimethylaminopropane (DLenDMA), dioctadecylamidoglycyl spermine (DOGS), 3-dimethylamino-2-(cholest-5-en-3-beta-oxybutan-4-oxy)-1-(cis,cis-9,12-oc-tadecadienoxy)propane (CLinDMA), 2-[5'-(cholest-5-en-3-beta-oxy)-3'-oxapentoxy]-3-dimethyl-1-(cis,cis-9',12'-octadecadienoxy)propane (CpLinDMA), N,N-dimethyl-3,4-dioleoyloxybenzylamine (DMOBA), 1,2-N,N'-dioleylcarbamyl-3-dimethylaminopropane (DOcarbDAP), 2,3-Dilinoleyloxy-N,N-dimethylpropylamine (DLinDAP), 1,2-N,N'-Dilinoleyloxy-3-dimethylaminopropane (DLincarbDAP), 1,2-Dilinoleyloxy-3-dimethylaminopropane (DLinCDAP), 2,2-dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane (DLin-K-DMA),

2,2-dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (DLin-K-XTC2-DMA), 2,2-dilinoleyl-4-(2-dimethylaminoethyl)-[1,3]-dioxolane (DLin-KC2-DMA), heptatriaconta-6,9,28,31-tetraen-19-yl-4-(dimethylamino)butanoate (DLin-MC3-DMA), N-(2-Hydroxyethyl)-N,N-dimethyl-2,3-bis(tetradecyloxy)-1-propanaminium bromide (DMRIE), (±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(cis-9-tetradecenyl)-1-propanaminium bromide (GAP-DMORIE), (±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(dodecyloxy)-1-propanaminium bromide (GAP-DLRIE), (±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(tetradecyloxy)-1-propanaminium bromide (GAP-DMRIE), N-(2-Aminoethyl)-N,N-dimethyl-2,3-bis(tetradecyloxy)-1-propanaminium bromide (PAE-DMRIE), N-(4-carboxybenzyl)-N,N-dimethyl-2,3-bis(oleoyloxy)propan-1-aminium (DOBAQ), 2-({8-[(3P)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propan-1-amine (Octyl-ClinDMA), 1,2-dimyristoyl-3-dimethylammonium-propane (DMDAP), 1,2-dipalmitoyl-3-dimethylammonium-propane (DPDAP), N1-[2-((1S)-1-[(3-aminopropyl)amino]-4-[di(3-amino-propyl)amino]butylcarboxamido)ethyl]-3,4-di(oleoyloxy)-benzamide (MVL5), 1,2-dioleoyl-sn-glycero-3-ethylphosphocholine (DOEPC), 2,3-bis(dodecyloxy)-N-(2-hydroxyethyl)-N,N-dimethylpropan-1-aminium bromide (DLRIE), N-(2-aminoethyl)-N,N-dimethyl-2,3-bis(tetradecyloxy)propan-1-aminium bromide (DMORIE), di((Z)-non-2-en-1-yl) 8,8'-(((2(dimethylamino)ethyl)thio)carbonyl)azanediyl)dioctanoate (ATX), N,N-dimethyl-2,3-bis(dodecyloxy)propan-1-amine (DLDMA), N,N-dimethyl-2,3-bis(tetradecyloxy)propan-1-amine (DMDMA), Di((Z)-non-2-en-1-yl)-9-((4-(dimethylaminobutanoyl)oxy)heptadecanedioate (L319), N-Dodecyl-3-((2-dodecylcarbamoylethyl)-2-[(2-dodecylcarbamoylethyl)-2-((2-dodecylcarbamoylethyl)-2-[2-(2-dodecylcarbamoylethylamino)-ethyl]-amino)-ethylamino]propionamide (lipidoid 98Niz-5), 1-[2-[bis(2-hydroxydodecyl)amino]ethyl-2-[4-[2-[bis(2-hydroxydodecyl)amino]ethyl]piperazin-1-yl]ethyl]amino]dodecan-2-ol (lipidoid C12-200), [(4-hydroxybutyl)azanediyl]di(hexane-6,1-diyl) bis(2-hexyldecanoate) (ALC-0315), 9-Heptadecan-1-yl 8-((2-hydroxyethyl)[6-oxo-6-(undecyloxy)hexyl]amino)octanoate (SM-102), ((2-(piperidin-4-yl)ethyl)azanediyl)bis(hexane-6,1-diyl) bis(2-hexyldecanoate), ((2-(piperazin-1-yl)ethyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate), ((4-aminobenzyl)azanediyl)bis(hexane-6,1-diyl) bis(2-hexyldecanoate), ((2-(6-aminopyridin-3-yl)ethyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate), ((3-(2-methyl-1H-imidazol-1-yl)propyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate), ((3-(2,4-dimethyl-1H-imidazol-1-yl)propyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate), Heptadecan-9-yl 8-((3-(2,4-dimethyl-1H-imidazol-1-yl)propyl)(8-(nonyloxy)-8-oxooctyl)amino)octanoate, Bis (2-hexyldecyl) 7,7'-((2-(6-aminopyridin-3-yl)ethyl)azanediyl)diheptanoate, ((2-(6-aminopyridin-3-yl)ethyl)azanediyl)bis(hexane-6,1-diyl)bis(2-(2-(butyldisulfanyl)ethyl)octanoate), (((3-(2-methyl-1H-imidazol-1-yl)propyl)azanediyl)bis(ethane-2,1-diyl))bis(oxy))bis(propane-3, 1-diyl) bis(2-hexyldecanoate), ((3-((2,4-dimethyl-1H-imidazol-1-yl)methoxy)propyl)azanediyl) bis(hexane-6,1-diyl) bis(2-hexyldecanoate) and any mixtures thereof.

Preferably, the ionizable cationic lipid is any one disclosed in WO2017/004143, the disclosure of which being incorporated herein by reference.

In some aspects, the lipid-based nanoparticle according to the invention comprises a helper lipid. As used herein, the term “helper lipid” refers to a class of lipid molecules that increases particle stability, fluidity, tolerability and/or biodistribution of lipid-based nanoparticles.

For instance, the helper lipid can be selected from the group consisting of 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-dilinoleoyl-sn-glycero-3-phosphocholine (DLPC), 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-diundecanoyl-sn-glycero-3-phosphocholine (DUPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), 1,2-di-O-octadecenyl-sn-glycero-3-phosphocholine (18:0 Diether PC), 1-oleoyl-2-cholesterylhemisuccinoyl-sn-glycero-3-phosphocholine (OChemPC), 1-hexadecyl-sn-glycero-3-phosphocholine (C16 Lyso PC), 1,2-dilinolenoyl-sn-glycero-3-phosphocholine, 1,2-diarachidonoyl-sn-glycero-3-phosphocholine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphocholine, 1-stearoyl-2-oleoyl-sn-glycero-3-phosphocholine (SOPC), ethyl phosphatidylcholine (EPC), 1-oleoyl-2-hydroxy-sn-glycero-3-phosphocholine (18 :1 Lyso PC), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine (ME 16:0 PE), 1-hexadecyl-2-(9Z-octadecenoyl)-sn-glycero-3-phosphoethanolamine (C16-18:1), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinoleoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinolenoyl-sn-glycero-3-phosphoethanolamine, 1,2-diarachidonoyl-sn-glycero-3-phosphoethanolamine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphoethanolamine, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (POPE), 1,2-di-O-phytanoyl-sn-glycero-3-phosphoethanolamine (4ME), 1-stearoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (SOPE), 1,2-dielaidoyl-sn-glycero-3-phosphoethanolamine (DEPE), N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-phosphatidylethanolamine (NBD-PE), N-(lisamineRhodamine B sulfonyl)-phosphatidylethanolamine (Rh-PE), 1-oleoyl-2-hydroxy-sn-glycero-3-phosphoethanolamine (18 :1 Lyso PE), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-methyl (18 :1 Monomethyl PE), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N,N-dimethyl (18 :1 Dimethyl PE), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(hexanoylamine) (18 :1 Caproylamine PE), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(biotinyl) (18 :1 Biotinyl PE), sn-(3-oleoyl-2-hydroxy)-glycerol-1-phospho-sn-1'-(3'-oleoyl-2'-hydroxy)-glycerol (BMP-S,S), sn-(3-(9Z-octadecenoyl)-2-hydroxy)-glycerol-1-phospho-sn-3'-(1'-(9Z-octadecenoyl)-2'-hydroxy)-glycerol (BMP-S,R), 1,2-dioleoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt (DOPG), 1,2-Diacyl-sn-glycero-3-phospho-L-serine (DSPS), L- α -phosphatidylserine (PS), 1,2-dioleoyl-sn-glycero-3-phosphate (PA), 1,2-dioleoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (PG), 1,2-dioleoyl-sn-glycero-3-phosphomethanol (18 :1 Phosphatidymethanol), 1,2-dioleoyl-sn-glycero-3-phosphoethanol (18 :1 Phosphatidylethanol), 1,2-dioleoyl-sn-glycero-3-phosphopropanol (18 :1

Phosphatidylpropanol), 1,2-dioleoyl-sn-glycero-3-phospho-L-serine (18:1 PS, DOPS), 1,2-distearoyl-sn-glycero-3-phospho-L-serine (18:0 PS), N-oleoyl-D-erythro-sphingosine (Ceramide), Sphingomyelin (SM), Phosphatidylinositol (PI), 9A1P9, 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), 1,2-di-O-octadecenyl-3-trimethylammonium propane (DOTMA), Dimethyldioctadecylammonium (18:0 DDAB), and
5 any mixtures thereof.

Preferably, the helper lipid is 1,2-distearoyl-sn-glycero-3-phosphocholine.

In some aspects, the lipid-based nanoparticle disclosed herein comprises one or more molecules comprising polyethylene glycol (PEG). Accordingly, the lipid-based nanoparticle may comprise PEG or PEG-modified lipids.

10 As used herein, the term “PEG lipid” refers to polyethylene glycol (PEG)-lipids. Non-limiting examples of PEG lipids include PEG-modified phosphatidylethanolamine and phosphatidic acid, PEG-ceramide conjugates (e.g., PEG-CerC14 or PEG-CerC20), PEG-modified dialkylamines and PEG-modified 1,2-diacyloxypropan-3-amines. Such lipids are also referred to as PEGylated lipids.

In some aspect, the lipid moiety of the PEG-lipids includes those having lengths of from about C14 to about
15 C22, preferably from about C14 to about C16.

Optionally, the PEG may present a molecular weight within the range from 0.5 to 50 kiloDaltons (kD), more preferably from 1 to 20 kD. In some aspect, a PEG moiety, for example a mPEG-NH₂, has a size of about 1000, 2000, 5000, 10,000, 15,000 or 20,000 Daltons. In a very particular aspect, the PEG-lipid is PEG 2000-DMG.

20 A PEG lipid may be selected in particular from the non-limiting group including PEG-modified phosphatidylethanolamines, PEG-modified phosphatidic acids, PEG-modified ceramides, PEG-modified dialkylamines, PEG-modified diacylglycerols, PEG-modified dialkylglycerols, and any mixture thereof.

In some aspects, the PEG-lipid includes, but are not limited to 1,2-dimyristoyl-sn-glycerol methoxypolyethylene glycol (PEG-DMG), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-
25 [amino(polyethylene glycol)] (PEG-DSPE), PEG-disteryl glycerol (PEG-DSG), PEG-dipalmitoyl, PEG-dioleoyl, PEG-distearyl, PEG-diacylglycamide (PEG-DAG), PEG-dipalmitoyl phosphatidylethanolamine (PEG-DPPE), or PEG-1,2-dimyristoylpropyl-3-amine (PEG-c-DMA).

Preferably, the PEG lipid is any one disclosed in WO2017/004143, the disclosure of which being incorporated herein by reference.

30 In some aspects, the lipid-based nanoparticle of the invention comprises one or more sterol. The sterol can be selected in particular from the group consisting of cholesterol, fecosterol, sitosterol, ergosterol,

campesterol, stigmasterol, brassicasterol, tomatidine, ursolic acid, alpha-tocopherol, and any mixtures thereof. Preferably, the sterol is cholesterol.

In a very specific aspect, the lipid-based nanoparticle comprises a lipid mixture. Particularly, the lipid-based nanoparticle of the invention comprises an ionizable or cationic lipid, a helper lipid, a sterol and a PEG lipid.

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

Preferably, in the lipid-based nanoparticle of the invention, the ionizable or cationic lipid is from about 10 mol % to about 70 mol % of the total lipids present in the nanoparticle, the helper lipid is from about 5 mol% to about 70 mol % of the total lipids present in the nanoparticle, the sterol is from about 10 mol% to about 70 mol% of the total lipids present in the nanoparticle, and the PEG lipid is from about 0.5 mol% to about 4 mol% of the total lipids present in the nanoparticle.

The lipid-based nanoparticle according to the invention may also comprise one or more functionalized lipids. For example, a lipid may be functionalized with an alkyne group that, when exposed to an azide under appropriate reaction conditions, may undergo a cycloaddition reaction. In particular, a lipid bilayer may be functionalized in this fashion with one or more groups useful in facilitating membrane permeation, cellular recognition and/or imaging.

In some aspects, the lipid-based nanoparticle may comprise an imaging agent in particular for MRI (magnetic resonance imaging), PET (Positron Emission Tomography), SPECT (Single Photon Emission Computed Tomography), ultrasound, radiography, X-ray tomography and optical imaging (fluorescence, bioluminescence, diffusion...). These imaging agents can make it possible to follow the position of the lipid-based nanoparticles especially after their administration to a patient. Examples of imaging agents include but are not limited to paramagnetic gadolinium chelates, paramagnetic lanthanide chelate (DOTA, DO3A, DTPA, PCTA), especially with a membrane lipophilic part, and, iron gluconates, and iron sulfates or iron oxide comprised in the cavity of the lipid-based nanoparticle, or instance, magnetite (Fe₃O₄), maghemite (γ-Fe₂O₃), wüstite (FeO), hematite (α-Fe₂O₃), or combinations thereof, for example such as described in WO2021194672 the disclosure thereof being incorporated herein by reference.

The amount of a mRNA molecule in a lipid-based nanoparticle may depend on the size, composition, desired target and/or application, or other properties of the lipid-based nanoparticle. For example, the amount of mRNA useful in the lipid-based nanoparticle may also depend on the size, sequence, and other characteristics of the mRNA. The relative amounts of a mRNA molecule and other elements (e.g., lipids) in a lipid-based nanoparticle may also vary. In some aspects, the wt/wt ratio of the lipid component to a mRNA molecule in a lipid-based nanoparticle may be from about 5:1 to about 60:1, such as 5:1, 6:1, 7:1,

8:1, 9:1, 10:1, 11:1, 12:1, 13:1, 14:1, 15:1, 16:1, 17:1, 18:1, 19:1, 20:1, 25:1, 30:1, 35:1, 40:1, 45:1, 50:1, and 60:1. The amount of a mRNA molecule in a lipid-based nanoparticle may, for example, be measured using absorption spectroscopy (e.g., ultraviolet-visible spectroscopy).

5 Synthesis and production methods

In an aspect, the invention relates to a method for producing a lipid-based nanoparticle comprising a nucleic acid molecule such as described herein.

The manufacture of LNPs are for example described in Semple et al., 2010, Nat Biotechnol., 28(2): 172-176; Akinc *et al.*, 2010, Mol Ther., 18(7): 1357-1364; Basha *et al.*, 2011, Mol Ther, 19(12): 2186-2200; 10 Leung et al., 2012, J Phys Chem C Nanomater Interfaces, 116(34): 18440-18450; Lee *et al.*, 2012, Int J Cancer., 131(5): E781-90; Belliveau *et al.*, 2012, Mol Ther nucleic Acids, 1: e37; Jayaraman *et al.*, 2012, Angew Chem Int Ed Engl., 51(34): 8529-8533; Mui *et al.*, 2013, Mol Ther Nucleic Acids. 2, e139; Maier *et al.*, 2013, Mol Ther., 21(8): 1570-1578; and Tam *et al.*, 2013, Nanomedicine, 9(5): 665-74, each of which are incorporated by reference in their entirety.

15 The invention particularly concerns an *in vitro* method for producing a lipid-based nanoparticle comprising a nucleic acid molecule comprising any Nuclear Localization Signal (NLS) sequence and any sequence encoding a Cyclic GMP-AMP synthase (cGAS) 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:

20 (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 comprising a Nuclear Localization Signal (NLS) sequence and a sequence encoding a Cyclic GMP-AMP synthase (cGAS) as defined herein above, preferably as set forth in SEQ ID NO: 40, or any nucleic acid sequence of interest 25 as herein described such as SEQ ID NO: 1, or a variant thereof having at least about 80% or about 85% identity thereto, and a mutation at position 241 in reference to SEQ ID NO: 40 or 1, for example a R241 mutation such as a deletion, an addition or a substitution, for example a R241E, R241D, R241N, or R241A substitution, preferably mRNA molecules; and

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

30 Any type of mixing device known or used for the generation of LNPs may be used to perform the method according to the invention. Examples of suitable mixing devices include, but are not limited to, T-mixer, U-

mixer, V-mixer, NanoAssembler (Precision Nanosystems), Impigement Jet Mixing (Knauer), Microfluidizer M700 or M805 (Microfluidics International Corporation).

Suitable flow rates for mixing the first composition and the second composition are known to the man skilled in the art.

- 5 Preferably, the pH of the second solution is comprised between about 3 to about 6, preferably between about 4 and about 5, even more preferably between about 4 and about 4.5. Preferably, the pH of the acidic aqueous solution (i.e., the second composition) is inferior to the pKa of the ionizable lipid in the first solution.

10 Optionally, the synthesis method comprises a prior step of mRNA synthesis and purification. mRNA synthesis and purification methods are known to the man skilled in the art and are for example described in Baierdörfer *et al.*, 2019; *Molecular Therapy. Nucleic Acids*, 15, pp. 26–35, WO2014152031 and WO2016193206 which are incorporated herein by reference.

In vitro transcription of an expression vector, in particular a plasmid DNA (pDNA) template, is typically used to produce functional synthetic mRNA. The pDNA is linearized with a selected unique restriction site
15 enzyme. After digestion, the linearized pDNA may be purified using methods such as the phenol-chloroform protocol, tangential flow filtration (TFF).

Following linearization, *in vitro* transcription and capping can be performed in a mixed solution of recombinant RNA polymerase (T7, T3 or SP6) and nucleoside triphosphates, plus a cap analog such as CleanCap® Reagent (TriLink) or ARCA (Anti-Reverse Cap Analog). The modified nucleoside such as N1-
20 Methylpseudouridine-5'-Triphosphate (N1-Methylpseudo-UTP, 1-Methylpseudo-UTP) can be used instead of GTP to suppress the innate immune system. Alternatively, capping may be achieved by performing the transcription without a cap analog, instead employing the vaccinia virus-encoded capping complex (capping enzyme, 2'-O-Methyltransferase, GTP, and S-adenosyl methionine (SAM)). Finally, the length of the poly A tail of the template pDNA can be extended by use of poly-A enzyme.

- 25 Linearized pDNA template is generally removed using deoxyribonuclease, or DNase. Cellulose-based purification can be used for the removal of dsRNA.

Verification of the purity or dosage of the synthesized mRNA can be determined by any methods known to the man skilled in the art, such as an agarose gel and/or spectrophotometry.

Synthesized mRNA can be stored at -20°C prior to their use in the synthesis methods, preferably in a
30 solution suitable for storage, such as a solution comprising phosphate buffered saline (PBS) and glycerol, or a nuclease free water.

Expression vectors that can be used in the present invention include non-exhaustively eukaryotic expression vectors, in particular mammalian expression vectors, virus-based expression vectors, baculovirus expression vectors, plant expression vectors, and plasmid expression vectors. Suitable expression vector can be derived from viruses such as baculoviruses, papova viruses such as SV40, vaccinia
5 viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, especially lentiviruses, or combinations thereof.

Accordingly, the present disclosure relates to an expression vector comprising a nucleic acid molecule such as described herein above.

Any eukaryotic cell can be used in the method for the production of suitable mRNA. For instance, cells
10 used for the production can be a mammalian cell, for example COS-1 cells, CHO (Chinese hamster ovary) (US 4,889,803; US 5,047,335), HEK (human embryonic kidney) cell lines such as 293 and 293T cell lines and HL-116 cell lines, Vero cell lines, BHK (baby hamster kidney) cell lines; a plant cell (e.g., *N. bethamiana*); an insect cell such as *Spodoptera frugiperda* (Sf)-derived cells (such as Sf-9 cells), SF21, Hi-5, Express Sf+, and S2 Schneider cells, in particular with baculovirus-insect cell expression system; or an avian cell.

15 Accordingly, the present disclosure relates to a recombinant host cell comprising a nucleic acid construct or an expression vector as described above. In a particular aspect, the recombinant host cell is not a cancer cell or an anti-tumor immune cell, in particular an anti-tumor T lymphocyte such as a CAR-T cell.

Optionally, after the mixing of the first and second solutions, the synthesis method may comprise a step of removing the ethanol solvent and adjusting the pH of the solution to a range from about 6.5 to about
20 7.5, preferably to 7. This step can be carried by any method known in the art, such as dialysis or buffer exchange. Any suitable buffer can be used such as phosphate buffered saline (PBS).

Optionally, the method of the invention may further comprise a step of purification and/or concentration of the lipid nanoparticles. For example, diafiltration or Tangential flow filtration (TFF) can be used.

Optionally, the method further comprises a step of LNP characterization.

25 Lipid-based nanoparticles or a solution comprising LNPs may be characterized by a variety of methods. For example, microscopy (e.g., transmission electron microscopy or scanning electron microscopy) may be used to examine the morphology and size distribution of a LNP or of a composition comprising LNPs. Dynamic light scattering or potentiometry (e.g., potentiometric titrations) may be used to measure zeta potentials. Dynamic light scattering may also be utilized to determine particle sizes. Instruments such as
30 the Zetasizer Nano ZS (Malvern Instruments Ltd, Malvern, Worcestershire, UK) may also be used to measure multiple characteristics of a LNP or of a composition comprising LNPs, such as particle size, polydispersity index, and zeta potential.

The efficiency of encapsulation of mRNA molecules describes the amount of mRNA encapsulated or otherwise associated with a LNP after preparation, relative to the initial amount provided. The encapsulation efficiency is preferably desirably high (e.g., close to 100%). The encapsulation efficiency may be measured, for example, by comparing the amount of mRNA in a solution containing the lipid-based nanoparticle before and after breaking up the lipid-based nanoparticle with one or more organic solvents or detergents. Fluorescence may be used to measure the amount of free mRNA in a solution. For the lipid-based nanoparticles described herein, the encapsulation efficiency may be of at least 50%, for example 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%. In some aspects, the encapsulation efficiency is of at least 70%. In certain aspects, the encapsulation efficiency is of at least 80%, preferably at least 90%, even more preferably at least 95%.

In particular, the mRNA encapsulation efficiency is of at least 95%, preferably as determined using a Quant-iT Ribogreen assay (R11490, Life Technologies).

Virus-based nanoparticles

The present disclosure relates to a virus or viral-based nanoparticle comprising a nucleic acid molecule comprising a Nuclear Localization Signal (NLS) sequence and a sequence encoding a Cyclic GMP-AMP synthase (cGAS) as herein defined. In a particular aspect, the Cyclic GMP-AMP synthase (cGAS) has 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.

A viral vector comprising viral nucleic acid encapsidated in a protein capsid can deliver the viral vector nucleic acid to cells or tissues. Depending on the particular vector, the viral vector may further comprise a viral envelope. Examples of viral vectors that can be used for gene delivery include adenovirus vectors, recombinant adeno-associated virus (AAV), retrovirus vectors such as lentiviral vectors. Different serotypes exist within different types of viruses. The different serotypes can provide for different activities, such as cell or tissue tropism and likelihood of generating a host immune response. The term "serotype" broadly refers to both serologically distinct viruses as well as viruses not serologically distinct that can be within a subgroup or a variant of a given serotype. Serologic distinctiveness can be determined based on the lack of cross-reactivity between antibodies to one capsid as compared to another capsid. Such cross-reactivity differences are usually due to differences in capsid protein sequences/antigenic determinants (e.g., due to VP1, VP2, and/or VP3 sequence differences of AAV serotypes).

Adenoviruses are non-enveloped double-stranded DNA viruses. Recombinant adenovirus vectors comprise recombinant adenovirus nucleic acid lacking one or more protein involved in viral replication,

and further comprise an adenoviral capsid. Recombinant adenovirus vectors can be produced containing different amounts of adenoviral DNA. The adenovirus (Ad) genome is flanked by hairpin-like inverted terminal repeats (ITRs) varying in length from 30-371 bp at its termini. The ITRs serve as self-priming structures that promote primase-independent DNA replication. A packaging signal located at the left arm of the genome is required for viral genome packaging. (Liu and Seol (2020) BMB Reports; 53(11):565-575; and Bulcha *et al.*. (2021) Sig. Transduct. Target Ther. 6:53.).

In certain aspects, the recombinant adenovirus vector is a third- generation vector, which are also referred to as “gutless” or “helper-dependent”. Gutless vectors can be produced from recombinant adenovirus nucleic acid where all, or substantially all viral sequences, except for the ITRs and the packaging signal, are not present. Gutless adenovirus vectors are high capacity vectors able to accommodate up to about 36 kb of DNA insert. Preferred recombinant adenovirus nucleic acid is about 27 kb to about 37 kb. Stuffer sequences can be added to recombinant adenovirus nucleic acid to increase nucleic acid size and capsid incorporation. Preferred stuffer sequences avoid coding sequences, repetitive sequences, recombination sequences, and immunogenic sequences. (Liu and Seol (2020) BMB Reports, 53(11):565-575; Bulcha *et al.*, (2021) Sig. Transduct. Target Ther. 6:53; and Sandig *et al.*, PNAS (2000) 97(3); 1002-1007, each of which are hereby incorporated by reference herein in their entirety.).

In certain aspects, recombinant adenovirus vectors can be produced based on rare human serotypes or chimpanzee serotypes. The use of chimpanzee and rare human serotypes may be helpful in reducing host immune response against recombinant adenovirus vectors due to preexisting immunity (Guo *et al.*, (2018) Human vaccines & immunotherapeutics, 14(7): 1679-1685 and Bulcha *et al.*, (2021) Sig. Transduct. Target Ther. 6:53.).

Adenovirus vectors can be produced by supplying viral proteins needed for vector production in trans using, for example, appropriate helper viruses or plasmids and cell lines. (Liu and Seol (2020) BMB Reports; 53(11):565-575; and Bulcha *et al.*. (2021) Sig. Trans duct. Target Ther. 6:53.).

Recombinant adeno-associated viral vector are based on the adeno-associated virus. The adeno-associated virus is a single-strand DNA virus containing a 4.7-kb genome flanked by 145-nt ITRs on both ends of the genome. ITR activity is important for self-priming and packaging, and may also provide additional activity such as promoter activity. AAV 5' and 3 ITRs can vary' in size and the 5' and 3' inverted repeats need not be exact inverted repeats.

A rAAV vector contains AAV recombinant nucleic acid and a viral capsid. The rAAV recombinant nucleic acid lacks one or more AAV proteins involved in viral replication. In certain embodiments, the rAAV vector contains an AAV 5' and/or 3' ITR along with a DNA insert. In certain embodiments. rAAV nucleic acid comprise a 5' ITR and/or 3' ITR independently selected from 5' and 3' ITRs provided in AAV1, AAV2, AAV3,

AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAVrh.10, AAVrh.74 and AAV3B ITRs. In further embodiments 5' and 3' ITRs are present, and both ITRs are from the same serotype genome.

Recombinant adeno-associated viral vectors typically accept inserts of DNA having a size range generally about 4 kb to about 5.2 kb. If needed, stuffer sequences can be used to increase rAAV nucleic acid size and packaging efficiency. In certain embodiments the rAAV nucleic acid including stuffer is less than 5.5 kb. In further embodiments the rAAV nucleic acid including stuffer is less than 5.2 kb, less than 5.1 kb, less than 5.0 kb, less than 4.9 kb, less than 4.8 kb, less than 4.7 kb, less than 4.6 kb; between 4 kb to 5.2 kb, 3.0 kb to 5.5 kb, 4.0 kb to 5.0 kb, or 4.3 kb to 4.8 kb; or about 4.2 kb, about 4.3 kb, about 4.4 kb, about 4.5 kb, about 4.6 kb, about 4.7 kb, about 4.8 kb, about 4.9 kb. or about 5.0 kb. Preferred stuffer sequences avoid coding sequences, repetitive sequences, recombination sequences, and immunogenic sequences.

In certain embodiments the rAAV is a self-complementary adeno-associated virus vector (scAAV) or short hairpin adeno-associated virus vector (shAAV). scAAV and shAAV provide for a double-stranded rAAV nucleic acid that can be incorporated into an AAV capsid. scAAV and shAAV comprise inverted dimeric repeats providing intramolecular double-stranded DNA. scAAV can be produced by mutating an ITR terminal resolution site so that rep fails to nick the terminal resolution site. shAAV can utilize a short hairpin to produce double-stranded AAV nucleic acid. scAAV and shAAV being double-stranded DNA provide an advantage in circumventing the DNA synthesis step required for single-stranded rAAV nucleic acid upon entry into a cell. A potential disadvantage of scAAV and shAAV is the size of DNA inserts that can be incorporated is reduced by about half compared to single stranded rAAV nucleic acid (U.S. Patent No. 10,457,940; Xie *et al.*, Mol Ther. (2017) 25(6): 1363-1374; and McCarty Mol. Ther. (2008) 16(10): 1648-1656.

Naturally occurring AAV capsids contain viral proteins VP1, VP2 and VP3 in a ratio of about 1: 1: 10. AAV vectors can be produced where all three viral proteins are based upon a particular serotypes or where one, two or all three viral protein are based on different serotypes.

Recombinant AAV capsid and nucleic acid can be based on the same serotype (or subgroup or variant), or can be different from each other. In certain embodiments, a rAAV nucleic acid has the same serotype genome (e.g., ITRs) as the encapsidating capsid protein.

In different embodiments, the rAAV capsid comprises a protein having a sequence identity' of at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.1%, at least 99.2%, at least 99.3%, at least 99.4%, at least 99.5%, at least 99.9% or 100% identical to a VP1, VP2 or VP3 of any known AAV.

The AAV genome contains two main genes: rep and cap. Transcription from the rep gene is initiated from two different promoters resulting in the production of nonstructural proteins designated Rep78, Rep68, Rep52, and Rep40. The rep proteins function in genome replication and/or encapsidation. The cap gene encodes for structural proteins making up the capsid (VP1, VP2 and Vp3); a non-structural assembly-activating protein (APP), which performs functions related to capsid assembly; and the membrane-associated accessory' protein, which may be associated with production phases of the replication cycle. (Maurer and Weitzman (2020) Hum. Gene Ther. 31(9- 10): 499-511, hereby-incorporated by reference herein in its entirety).

AAV requires helper virus functions to complete its replication cycle. Helper virus functions can be supplied by different viruses in permissive cell lines. Permissive cell lines are cell lines able to support viral replication. Examples of helper viruses for AAV include adenovirus, HSV-1, HPV-16, and HBoV1 which can be used in conjunction with, for example, permissive primate cells; and baculovirus which can be used in conjunction with, for example, permissive insect cells such as sf9. (Maurer and Weitzman (2020) Hum. Gene Ther. (2020) 31(9-10):499-511 and Meier *et al.* (2020) Viruses 19:12(6):662, both of which are herein incorporated by reference herein in their entirety).

Recombinant AAV can be produced by supplying viral proteins needed for vector production in trans using, for example, appropriate helper viruses or plasmids and cell lines. In certain embodiments, rAAV is produced using a rAAV vector genome plasmid. The plasmid comprises that portion of the rAAV nucleic acid ultimately packaged or encapsidated to form a viral (e.g., rAAV) vector. The "plasmid backbone," contains elements important for propagation and recombinant virus production. Except for possible 3' ITR and/or 5' ITR cloning remnants the plasmid backbone is not itself packaged or encapsidated into virus particles. The vector genome plasmid may contain regions such as an origin of replication and a selectable marker. Additional sites that may be present include cloning sites.

Retroviruses are enveloped RNA viruses that, after infection of a host cell, reverse transcribe their RNA genomes into a DNA intermediate, or provirus. All viruses containing an RNA genome and producing an RNA-dependent DNA polymerase are contained in the retroviral family. The family is divided into three subfamilies: (1) Oncovirinae, including all the oncogenic retroviruses, and several closely related non-oncogenic viruses; (2) Lentivirinae, the "slow retroviruses" such as the human immunodeficiency virus (HIV) and visna virus; and (3) Spumavirinae, the "foamy" retroviruses that induce persistent infections, generally without causing any clinical disease.

Retroviruses contain at least three types of proteins encoded by the viral genome, i.e., gag proteins (the group antigen internal structural proteins), pol proteins (the RNA-dependent DNA polymerase and the protease and integrase proteins), and env proteins (the viral envelope protein or proteins). In addition to genes encoding the gag, pol, and env proteins, the genome of the retrovirus includes two long terminal

repeat (LTR) sequences, one at the 5' and one at the 3' end of the virus. These 5' and 3' LTRs promote transcription and polyadenylation of viral mRNAs and participate in the integration of the viral genome into the cellular DNA of the host.

5 The provirus can be stably integrated into the host's cellular DNA. Gene products encoded by the provirus are then expressed by the host cell to produce retroviral virions, thereby replicating the virus. Because the retroviral genome can be manipulated to include exogenous nucleotide sequence(s) of interest for expression in a target cell, retroviral vectors are important tools for stable gene transfer into mammalian cells.

10 Many proposed gene therapy applications use retroviral vectors to take advantage of the ability of these naturally infectious agents to transfer and efficiently express recombinant nucleotide sequences in susceptible target cells. Retroviral vectors suitable for use in such applications are generally defective retroviral vector that are capable of infecting the target cell, reverse transcribing their RNA genomes, and integrating the reverse transcribed DNA into the target cell genome, but are incapable of replicating within the target cell to produce infectious retroviral particles (e.g., the retroviral genome transferred into the target cell is defective in gag, and/or in pol (see, e.g., Coffin, J., In: RNA Tumor Viruses, Weiss, R. *et al.*, (ed) 15 Cold Spring Harbor Laboratory, Vol. 2, pp. 36-73, 1985).

Retroviral vectors and packaging cells (helper cells) have been developed to introduce recombinant nucleic acid molecules into mammalian cells without the danger of the production of replicating infectious virus. This methodology uses two components, a retroviral vector and a packaging cell. The retroviral vector 20 contains long terminal repeats (LTRs), the foreign DNA to be transferred, and a packaging sequence. This retroviral vector will not reproduce by itself because the genes that encode the structural and envelope proteins are not included within the vector. The packaging cell contains genes encoding the gag, pol, and env proteins, but does not contain the packaging signal, so that the cell can only form empty virus particles by itself. With this method, the retroviral vector is introduced into the packaging cell, to create a cell able 25 to produce virus. The cell manufactures viral particles containing only the retroviral vector DNA, and therefore has been considered safe.

The above described examples of viral vectors should be considered as examples only. Other viral vectors could be envisioned such as for example herpes simplex vectors.

30 **Dendrimer nanoparticles**

The present disclosure relates to a dendrimer nanoparticle comprising a nucleic acid molecule comprising a Nuclear Localization Signal (NLS) sequence and a sequence encoding a Cyclic GMP-AMP synthase (cGAS) as herein defined. In a particular aspect, the Cyclic GMP-AMP synthase (cGAS) has 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.

Dendrimers are highly branched macromolecules that can be employed as gene delivery vectors. Their unique molecular architecture and key properties such as globular shape and a high density of surface functional groups make these nanoscale materials relevant vehicles for nucleic acid delivery. They help in complexing nucleic acid of interest along with providing stability and higher transfection efficiency *in vitro* and *in vivo*. Dendrimer condenses nucleic acids into nanoparticles by ionic interactions and prevents the (therapeutic) nucleic acid of interest from endosomal and nuclear degradation.

In a particular aspect of the invention, the vector is a one-component multifunctional ionizable amphiphilic Janus dendrimer (IAJD) delivery system known by the skilled person in the art (cf. for example Zhang *et al.*, "One-Component Multifunctional Sequence-Defined Ionizable Amphiphilic Janus Dendrimer Delivery Systems for mRNA" J Am Chem Soc. 2021 Aug 11; 143(31): 12315 – 12327).

Pharmaceutical compositions

The present invention also relates to a pharmaceutical or veterinary composition comprising a nucleic acid or lipid-based nanoparticle as described hereabove, preferably as the active ingredient or compound and optionally a pharmaceutically acceptable carrier or excipient.

As used herein, a "pharmaceutical composition" refers to a preparation of one or more of the active agents, such as comprising a nucleic acid, a vector, for example a lipid-based nanoparticle, in particular a LNP, a viral nanoparticle or a dendrimer nanoparticle, according to the invention, with optional other chemical components such as physiologically suitable carriers and excipients. The purpose of a pharmaceutical composition is to facilitate administration of the active agent to an organism. Compositions of the present invention can be in a form suitable for any conventional route of administration or use. An "acceptable vehicle" or "acceptable carrier" as referred to herein, is any compound or combination of compounds that are known to those skilled in the art to be useful in formulating pharmaceutical compositions.

The pharmaceutical composition may be prepared by mixing a nucleic acid, a vector nanoparticle, for example lipid-based nanoparticle, viral nanoparticle or dendrimer nanoparticle, as disclosed herein and having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients, antioxidant, and/or stabilizers in the form of lyophilized formulations or aqueous solutions.

Preferably, the pharmaceutical composition is mixed with auxiliary agents such as pharmaceutically acceptable carriers, excipients, salts, antioxidant and/or stabilizers which do not deleteriously interact with the nucleic acid, vector nanoparticle, for example lipid-based nanoparticle, viral nanoparticle or dendrimer

nanoparticle, of the invention and does not impart any undesired toxicological effects. Suitable carriers, excipients, antioxidants, and/or stabilizers are well known in the art and have been for example described in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980). Preferably such components meet the standards of the United States Pharmacopoeia (USP), the European Pharmacopoeia (EP), the
5 British Pharmacopoeia, and/or the International Pharmacopoeia.

The means of making a suitable pharmaceutical composition have been described in the art (see, for instance, Remington: The Science and Practice of Pharmacy, Lippincott Williams & Wilkins, 21st edition (2005)).

Particularly, the pharmaceutical composition according to the invention can be formulated for any
10 conventional route of administration including a topical, enteral, oral, parenteral, intranasal, intravenous, intramuscular, or subcutaneous.

Preferably, the nucleic acid, vector nanoparticle, for example lipid-based nanoparticle, viral nanoparticle or dendrimer nanoparticle, according to the invention or the pharmaceutical composition according to the invention comprising said nucleic acid, vector nanoparticle, for example lipid-based nanoparticle, viral
15 nanoparticle or dendrimer nanoparticle, is formulated for intravenous administration or is formulated so as to be suitable for subcutaneous, intramuscular, intratumoral or intravenous injection, preferably intravenous injection, more preferably intravenous administration.

Preferably, the pharmaceutical composition comprising the vector nanoparticle, for example the lipid-based nanoparticle, viral nanoparticle or dendrimer nanoparticle, is relatively homogenous. A
20 polydispersity index may be used to indicate the homogeneity of the composition, e.g., the particle size distribution of the vector nanoparticle, for example of the lipid-based nanoparticle, viral-based nanoparticle or dendrimer nanoparticle, comprised in the composition. A small (e.g., less than 0.3) polydispersity index generally indicates a narrow particle size distribution. Preferably, the pharmaceutical composition has a polydispersity index from about 0.010 to about 0.1.

25 Preferably, the pharmaceutical composition comprising the lipid-based nanoparticle has a polydispersity index (PI) of 0.02 to 0.06, in particular as measured by dynamic light scattering using a Zetasizer Nano ZS (Malvern Instruments Ltd, Malvern, UK).

Preferably, the mean hydrodynamic diameter of the lipid-based nanoparticle comprised in the pharmaceutical composition is comprised between 50 nm and 100 nm, preferably between 70 nm and 90
30 nm, more preferably between 75 nm and 85 nm, most preferably of about 80 nm. The mean hydrodynamic diameter can particularly be measured by any methods known to the man skilled in the art, such as dynamic light scattering using a Zetasizer Nano ZS (Malvern Instruments Ltd, Malvern, UK)

Pharmaceutical compositions according to the invention may be formulated to release the active ingredients (e.g., the nucleic acid, vector nanoparticle, for example lipid-based nanoparticle, viral nanoparticle or dendrimer nanoparticle, of the invention) substantially immediately upon administration or at any predetermined time or time period after administration. The pharmaceutical composition in
5 some aspects can employ time-released, delayed release, and sustained release delivery systems such that the delivery of the composition occurs prior to, and with sufficient time to cause, sensitization of the site to be treated. Means known in the art can be used to prevent or minimize release and absorption of the composition until it reaches the target tissue or organ, or to ensure timed-release of the composition.

It will be understood by one skilled in the art that the formulations of the invention may be isotonic with
10 human blood, that is the formulations of the invention have essentially the same osmotic pressure as human blood. Such isotonic formulations generally have an osmotic pressure from about 250 mOSm to about 350 mOSm. Isotonicity can be measured by, for example, a vapor pressure or ice-freezing type osmometer.

Pharmaceutical composition typically must be sterile and stable under the conditions of manufacture and
15 storage. Prevention of presence of microorganisms may be ensured both by sterilization procedures (for example by microfiltration), and/or by the inclusion of various antibacterial and antifungal agents.

In some embodiments, the pharmaceutical composition includes one or more pharmaceutically acceptable excipients or accessory ingredients such as, but not limited to, one or more solvents, dispersion media, diluents, dispersion aids, suspension aids, surface active agents, buffering agents and/or
20 preservatives.

Surface active agents and/or emulsifiers may include, but are not limited to, natural emulsifiers (e.g., acacia, alginic acid, sodium alginate, cholesterol, and lecithin), sorbitan fatty acid esters (e.g., polyoxyethylene sorbitan monolaurate [TWEEN®20], polyoxyethylene sorbitan [TWEEN® 60], polyoxyethylene sorbitan monooleate [TWEEN®80], sorbitan monopalmitate [SPAN®40], sorbitan
25 monostearate [SPAN®60], sorbitan tristearate [SPAN®65], glyceryl monooleate, sorbitan monooleate [SPAN®80]), polyoxyethylene esters (e.g., polyoxyethylene monostearate [MYRJ® 45], polyoxyethylene hydrogenated castor oil, polyethoxylated castor oil, polyoxymethylene stearate, and SOLUTOL®), sucrose fatty acid esters, polyethylene glycol fatty acid esters (e.g., CREMOPHOR®), polyoxyethylene ethers, (e.g., polyoxyethylene lauryl ether [BRIJ® 30]), poly(vinyl-pyrrolidone), diethylene glycol monolaurate,
30 triethanolamine oleate, sodium oleate, potassium oleate, ethyl oleate, oleic acid, ethyl laurate, sodium lauryl sulfate, PLURONIC®F 68, POLOXAMER® 188, cetrimonium bromide, cetylpyridinium chloride, benzalkonium chloride, docusate sodium and/or combinations thereof.

Examples of preservatives may include, but are not limited to, antioxidants, chelating agents, free radical scavengers, antimicrobial preservatives, antifungal preservatives, alcohol preservatives and/or acidic preservatives.

- 5 Examples of buffering agents include, but are not limited to, citrate buffer solutions, acetate buffer solutions, phosphate buffer solutions, ammonium chloride, calcium carbonate, calcium chloride, calcium citrate, calcium glutubionate, calcium gluceptate, calcium gluconate, d-gluconic acid, calcium glycerophosphate, calcium lactate, calcium lactobionate, propanoic acid, calcium levulinate, pentanoic acid, dibasic calcium phosphate, phosphoric acid, tribasic calcium phosphate, calcium hydroxide phosphate, potassium acetate, potassium chloride, potassium gluconate, potassium mixtures, dibasic
10 potassium phosphate, monobasic potassium phosphate, potassium phosphate mixtures, sodium acetate, sodium bicarbonate, sodium chloride, sodium citrate, sodium lactate, dibasic sodium phosphate, monobasic sodium phosphate, sodium phosphate mixtures, tromethamine, amino-sulfonate buffers (e.g., HEPES), magnesium hydroxide, aluminum hydroxide, alginic acid, pyrogen-free water, isotonic saline, Ringer's solution and/or ethyl alcohol.
- 15 Formulations of the pharmaceutical compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. Such preparatory methods include the step of bringing active ingredient into association with one or more excipients and/or one or more other accessory ingredients, and then, if necessary and/or desirable, shaping and/or packaging the product into a desired single- or multi-dose unit.
- 20 A pharmaceutical composition in accordance with the invention may be prepared, packaged, and/or sold in bulk, as a single unit dose, and/or as a plurality of single unit doses.

- Relative amounts of the nucleic acids, vector nanoparticles, for example lipid-based nanoparticles, viral nanoparticles or dendrimer nanoparticles, pharmaceutically acceptable excipients and/or any additional ingredients in a pharmaceutical composition in accordance with the present disclosure may vary,
25 depending upon the identity, size, and/or condition of the subject treated and further depending upon the route by which the composition is to be administered. The amount of nucleic acid, vector nanoparticles, for example lipid-based nanoparticles, viral nanoparticles or dendrimer nanoparticles, as disclosed herein which can be combined with a carrier material to produce a single dosage form will generally be that amount of the nucleic acid molecules, vector nanoparticles, for example lipid-based
30 nanoparticles, viral nanoparticles or dendrimer nanoparticles, which produces a therapeutic effect. Preferably, pharmaceutical compositions described herein will generally be administered in such amounts and for such a time as is necessary or sufficient to induce an immune response.

Therapeutic uses

The nucleic acid, vector nanoparticle, for example lipid-based nanoparticle, viral nanoparticle or dendrimer nanoparticle, as defined herein and the pharmaceutical composition comprising such as defined above have numerous *in vitro* and *in vivo* utilities and applications. Particularly, the nucleic acid
5 molecules, vector nanoparticles, for example lipid-based nanoparticles, viral nanoparticles or dendrimer nanoparticles, as defined and the pharmaceutical compositions provided herein may be used in therapeutic methods and/or for therapeutic purposes.

The present invention relates to a nucleic acid, vector nanoparticle, for example lipid-based nanoparticle, viral nanoparticle or dendrimer nanoparticle, as defined herein or a pharmaceutical composition
10 comprising it for use as a medicament or vaccine.

The present invention relates to a nucleic acid, vector nanoparticle, for example lipid-based nanoparticle, viral nanoparticle or dendrimer nanoparticle, as defined herein or a pharmaceutical composition comprising it for use in the treatment of a disorder or disease, in particular a STING-mediated disease or disorder such as a cancer or an infection.

As used herein, the term "treatment", "treat" or "treating" refers to any act intended to ameliorate the health status of patients such as therapy, prevention, prophylaxis and retardation of the disease. In certain aspects, such term refers to the amelioration or eradication of a disease or symptoms associated with a disease, such as according to the present disclosure, the disruption or the delay in the resolution of the inflammation leading to inflammation associated disease. In other aspects, this term refers to minimizing
20 the spread or worsening of the disease resulting from the administration of one or more therapeutic agents to a subject with such a disease.

The invention also relates to the use of a nucleic acid, a vector nanoparticle, for example a lipid-based nanoparticle, viral nanoparticle or dendrimer nanoparticle, as defined herein, a combination of a) said nucleic acid, vector nanoparticle, lipid-based nanoparticle, viral nanoparticle or dendrimer nanoparticle,
25 and b) a distinct therapeutic agent (such as an anti-cancer or antiviral agent), or a pharmaceutical composition comprising such for treating a disease or disorder, such as a cancer or an infection, in a subject. It also concerns the use a nucleic acid, of a vector nanoparticle, for example a lipid-based nanoparticle, viral nanoparticle or dendrimer nanoparticle, of a combination or pharmaceutical composition as disclosed herein, in the manufacture of a medicament for treating a disease or disorder,
30 such as a cancer or an infection, in a subject.

Finally, it relates to a method for treating a disease or a disorder, such as a cancer or an infection, in a subject comprising administering a therapeutically effective amount of a pharmaceutical composition, a

combination, a nucleic acid, a vector nanoparticle, for example a lipid-based nanoparticle, a viral nanoparticle or a dendrimer nanoparticle, as disclosed herein.

By "method for treating" is meant a process that is intended to produce a beneficial change in the condition of an individual, e.g., mammal, especially human. Human and veterinary treatments are both contemplated. A beneficial change can include one or more of: restoration of function, reduction of symptoms, limitation or retardation of a disease, disorder, or condition, or prevention, limitation or retardation of deterioration of a patient's condition, disease or disorder.

Particularly, the invention concerns a treatment method that comprises: (a) identifying a patient in need of treatment; and (b) administering to the patient a therapeutically effective amount of the nucleic acid, of the vector nanoparticle, for example a lipid-based nanoparticle, a viral nanoparticle or a dendrimer nanoparticle, of a combination or of a pharmaceutical composition as described herein.

"An effective amount" or a "therapeutic effective amount" as used herein refers to the amount of active agent (i.e., the nucleic acid or the vector nanoparticle, for example the lipid-based nanoparticle, viral nanoparticle or dendrimer nanoparticle, as disclosed herein) required to confer therapeutic effect on the subject, either alone or in combination with one or more other active agents, e.g., the amount of active agent that is needed to treat the targeted disease or disorder, or to produce the desired effect. The "effective amount" will vary depending on the agent(s), the disease and its severity, the characteristics of the subject to be treated including age, physical condition, size, gender and weight, the duration of the treatment, the nature of concurrent therapy (if any), the specific route of administration and like factors within the knowledge and expertise of the health practitioner. These factors are well known to those of ordinary skill in the art.

- Cancer

In a particular aspect, the invention provides a nucleic acid, vector nanoparticle, for example lipid-based nanoparticle, viral nanoparticle or dendrimer nanoparticle, combination, or pharmaceutical composition as defined herein for use in the treatment of a subject having a cancer.

It relates in particular to a nucleic acid, a vector nanoparticle, for example lipid-based nanoparticle, viral nanoparticle or dendrimer nanoparticle, a combination, or a pharmaceutical composition as defined herein for use in prevention or treatment of a cancer, in a subject.

The term "cancer" as used herein is defined as disease characterized by the rapid and uncontrolled growth of aberrant cells. Cancer cells can spread locally or through the bloodstream and lymphatic system to other parts of the body. The term "cancer" encompasses disease or disorder such as cancer, pre-cancerous

syndromes and tumor metastasis. The cancer may be a solid or a liquid cancer. Preferably the cancer is a solid cancer. Preferably, the cancer is a STING mediated cancer.

Examples of cancer diseases and conditions in which a composition of the invention may have beneficial antitumor effects include, but are not limited to, cancers 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; carcinoma, in particular adenocarcinoma (for example colon adenocarcinoma), melanoma, or hepatocellular carcinoma, 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 mesotheliomas; brain stem glioma; pituitary adenoma; bronchial adenoma; chondromatous hamartoma; mesothelioma; Hodgkin's Disease or a combination of one or more of the foregoing cancers.

In a particular aspect, the cancer is a cancer induced by a virus or associated with immunodeficiency. Such a cancer can be selected from the group consisting of Kaposi sarcoma (e.g., associated with Kaposi sarcoma herpes virus); cervical, anal, penile and vulvar squamous cell cancer and oropharyngeal cancers (e.g., associated with human papilloma virus); B cell non-Hodgkin lymphomas (NHL) including diffuse large B cell lymphoma, Burkitt lymphoma, plasmablastic lymphoma, primary central nervous system lymphoma, HHV-8 primary effusion lymphoma, classic Hodgkin lymphoma, and lymphoproliferative disorders (e.g., associated with Epstein-Barr virus (EBV) and/or Kaposi sarcoma herpes virus); hepatocellular carcinoma (e.g., associated with hepatitis B and/or C viruses); Merkel cell carcinoma (e.g., associated with Merkel cell polyoma virus (MPV)); and cancer associated with human immunodeficiency virus infection (HIV) infection.

In another embodiment, the invention provides the use of a nucleic acid, a vector nanoparticle, for example a lipid-based nanoparticle, viral nanoparticle or dendrimer nanoparticle, a combination, or a pharmaceutical composition as defined herein, in the manufacture of a medicament for treating a cancer.

The invention also provides a method of treating a cancer in a subject, comprising administering to the subject a therapeutically effective amount of a nucleic acid, a vector nanoparticle, for example a lipid-based nanoparticle, viral nanoparticle or dendrimer nanoparticle, a combination, or a pharmaceutical composition as defined herein, preferably such that the subject is treated from cancer.

The present invention relates to a nucleic acid, a vector nanoparticle, for example a lipid-based nanoparticle, viral nanoparticle or dendrimer nanoparticle, a combination, or a pharmaceutical composition as defined herein for use as a vaccine adjuvant, in particular a cancer vaccine adjuvant.

As used herein, the term “adjuvant” refers to substances which are added and/or co-formulated in an immunization to the active antigen in order to enhance or elicit or modulate the humoral and/or cell-mediated immune response against the active antigen. Preferably, the adjuvant is also able to enhance or elicit the innate immune response.

- Infectious disease

In some aspect, the nucleic acid, the vector nanoparticle, for example the lipid-based nanoparticle, viral nanoparticle or dendrimer nanoparticle, the combination, or the pharmaceutical composition as defined herein are for use in the treatment of an infectious disease, such as a viral infection, especially a chronic viral infection.

The invention also provides the use of a nucleic acid, a vector nanoparticle, for example a lipid-based nanoparticle, viral nanoparticle or dendrimer nanoparticle, a combination, or a pharmaceutical composition as defined herein in the manufacture of a medicament for treating an infectious disease.

Accordingly, an aspect of the invention provides a method of treating an infectious disease in a subject comprising administering to the subject a therapeutically effective amount of a nucleic acid, a vector nanoparticle, for example a lipid-based nanoparticle, viral nanoparticle or dendrimer nanoparticle, a combination, or a pharmaceutical composition as defined herein, preferably such that the subject is treated for the infectious disease.

Any suitable infection may be treated with a nucleic acid, a vector nanoparticle, for example a lipid-based nanoparticle, viral nanoparticle or dendrimer nanoparticle, a combination, or a pharmaceutical composition as defined herein.

Some examples of pathogenic viruses causing infections treatable by the nucleic acid, the vector nanoparticle, for example the lipid-based nanoparticle, viral nanoparticle or dendrimer nanoparticle, the combination or the pharmaceutical composition of the invention include HIV, hepatitis (A, B, or C), herpes virus (e.g., VZV, HSV-1, HAV-6, HSV-II, and CMV, Epstein Barr virus), adenovirus, influenza virus, flaviviruses, echovirus, rhinovirus, coxsackie virus, coronavirus, respiratory syncytial virus, mumps virus, rotavirus, measles virus, rubella virus, parvovirus, vaccinia virus, HTLV virus, dengue virus, papillomavirus, molluscum virus, poliovirus, rabies virus, JC virus and arboviral encephalitis virus.

Particularly, the nucleic acid, the vector nanoparticle, for example the lipid-based nanoparticle, viral nanoparticle or dendrimer nanoparticle, the combination, or the pharmaceutical composition as defined herein are used to treat patients that have chronic viral infection, such infection being caused by viruses selected from the group consisting of Retroviruses, Anellovirus, Circovirus, Herpesvirus, Varicella zoster virus (VZV), Cytomegalovirus (CMV), Epstein-Barr virus (EBV), Polyomavirus BK, Polyomavirus, Adeno-associated virus (AAV), Herpes simplex type 1 (HSV-1), Adenovirus, Herpes simplex type 2 (HSV-2), Kaposi's sarcoma herpesvirus (KSHV), Hepatitis B virus (HBV), GB virus C, Papilloma virus, Hepatitis C virus (HCV), Human immunodeficiency virus (HIV), Hepatitis D virus (HDV), Human T cell leukemia virus type 1 (HTLV1), Xenotropic murine leukemia virus-related virus (XMLV), Rubella virus, German measles, Parvovirus B19, Measles virus, and Coxsackie virus.

Some examples of pathogenic parasites causing infectious diseases treatable by the nucleic acid, the vector nanoparticle, for example the lipid-based nanoparticle, viral nanoparticle or dendrimer nanoparticle, or the combination or pharmaceutical composition comprising such, of the invention include *Entamoeba histolytica*, *Balantidium coli*, *Naegleria fowleri*, *Acanthamoeba sp.*, *Giardia lamblia*, *Cryptosporidium sp.*, *Pneumocystis carinii*, *Plasmodium vivax*, *Babesia microti*, *Trypanosoma brucei*, *Trypanosoma cruzi*, *Leishmania donovani*, *Toxoplasma Gondii*, and *Nippostrongylus brasiliensis*.

Some examples of pathogenic bacteria causing infectious disease treatable by the nucleic acid, the vector nanoparticle, for example the lipid-based nanoparticle, viral nanoparticle or dendrimer nanoparticle, or the combination or pharmaceutical composition of the invention include chlamydia, rickettsia bacteria, mycobacteria, staphylococci, streptococci, pneumococci, meningococci, gonococci, klebsiella, proteus, serratia, pseudomonas, legionella, diphtheria, salmonella, bacilli, cholera, tetanus, botulism, anthrax, plague and leptospirosis.

- Neurological diseases

In some aspect, the nucleic acid, the vector nanoparticle, for example the lipid-based nanoparticle, viral nanoparticle or dendrimer nanoparticle, the combination, or pharmaceutical composition as defined herein are for use in the treatment of a neurological disease, in particular a neurodegenerative disease or disorder or a disease associated with neuroinflammation.

The invention also provides the use of a nucleic acid, a vector nanoparticle, for example a lipid-based nanoparticle, viral nanoparticle or dendrimer nanoparticle, a combination, or a pharmaceutical composition as defined herein in the manufacture of a medicament for treating a neurological disease.

Accordingly, an aspect of the invention provides a method of treating a neurological disease in a subject comprising administering to the subject a therapeutically effective amount of a nucleic acid, a vector

nanoparticle, for example a lipid-based nanoparticle, viral nanoparticle or dendrimer nanoparticle, a combination, or a pharmaceutical composition as defined herein, preferably such that the subject is treated for the neurological disease.

5 Particularly, neurological disorders include disorders that involve the central nervous system (brain, brainstem and cerebellum), the peripheral nervous system (including cranial nerves), and the autonomic nervous system (parts of which are located in both central and peripheral nervous system).

Preferably, the nucleic acid, the vector nanoparticle, for example the lipid-based nanoparticle, viral nanoparticle or dendrimer nanoparticle, the combination, or the pharmaceutical composition as defined herein are for use in the treatment of a neurological disease selected from the group consisting of
10 Parkinson's disease, Alzheimer's disease, Huntington's disease, multiple sclerosis, Amyotrophic lateral sclerosis (ALS) and Frontotemporal Lobar Degeneration (FTD).

Particularly, the nucleic acid, vector nanoparticle, for example lipid-based nanoparticle, viral nanoparticle or dendrimer nanoparticle, combination, or pharmaceutical composition as defined herein are for use in the treatment of multiple sclerosis. "Multiple sclerosis" refers to an autoimmune neurodegenerative
15 disease, which is marked by inflammation within the central nervous system with lymphocyte attack against myelin produced by oligodendrocytes, plaque formation and demyelization with destruction of the myelin sheath of axons in the brain and spinal cord, leading to significant neurological disability over time.

Combination or Combined therapies

20 In an aspect, the invention refers to a combination of a) a nucleic acid, vector nanoparticle, for example lipid-based nanoparticle, viral nanoparticle or dendrimer nanoparticle of the invention, and b) a distinct therapeutic agent, preferably an anti-cancer or antiviral agent. Such combination may particularly be comprised in a pharmaceutical composition such as described herein.

In some embodiments, the nucleic acid, the vector nanoparticle, for example the lipid-based nanoparticle,
25 viral nanoparticle or dendrimer nanoparticle, or the pharmaceutical composition as defined herein may be used in combination with another therapeutic agent or therapy, in particular for the treatment of cancer or of an infectious disease.

The present invention also relates to a method for treating a disease, such as a cancer or an infectious disease, in a subject, comprising administering to said subject a therapeutically effective amount of the
30 nucleic acid, the vector nanoparticle, for example the lipid-based nanoparticle, viral nanoparticle or dendrimer nanoparticle, or the pharmaceutical composition as defined herein and a therapeutically effective amount of an additional or second (distinct) therapeutic agent or therapy.

Accordingly, also provided herein are combined therapies with any of the nucleic acid, the vector nanoparticle, for example the lipid-based nanoparticle, viral nanoparticle or dendrimer nanoparticle, or the pharmaceutical composition as defined herein, and a suitable second therapeutic agent, for the treatment of a disease such as cancer or infectious disease.

- 5 In an aspect, the nucleic acid, the vector nanoparticle, for example the lipid-based nanoparticle, viral nanoparticle or dendrimer nanoparticle, or the pharmaceutical composition as defined herein, and the second agent can be present in a unique pharmaceutical composition.

Alternatively, the terms "combination therapy" or "combined therapy", as used herein, embrace administration of these two agents (e.g., a nucleic acid, vector nanoparticle, for example lipid-based
10 nanoparticle, viral nanoparticle or dendrimer nanoparticle, as described herein and an additional or second (distinct) suitable therapeutic agent) in a sequential manner, that is, wherein each therapeutic agent is administered at a different time, as well as administration of these therapeutic agents in a substantially simultaneous manner. Sequential or substantially simultaneous administration of each agent may be by any appropriate route. The agents can be administered by the same route or by different routes.

- 15 Particularly, the nucleic acid, vector nanoparticle, for example lipid-based nanoparticle, viral nanoparticle or dendrimer nanoparticle, as disclosed herein is to be injected subcutaneously, intramuscularly, intratumorally or intravenously, preferably intravenously while the other agent(s) of the combination may be administered by any appropriate route such as by topical, enteral, oral, parenteral, intranasal, intravenous, intramuscular, or subcutaneous route.

- 20 In an aspect, the additional therapeutic agent can be selected in the non-exhaustive list comprising alkylating agents, angiogenesis inhibitors, antibodies, antimetabolites, antimitotic, antiproliferative, antivirals, aurora kinase inhibitors, activators of death receptor pathway, Bcr-Abl kinase inhibitors, antibody drug conjugates, Bruton's tyrosine kinase (BTK) inhibitors, cyclin-dependent kinase inhibitors, cell cycle inhibitors, cyclooxygenase-2 inhibitors, leukemia viral oncogene homolog (ErbB2) receptor
25 inhibitors, growth factor inhibitors, heat shock protein (HSP)-90 inhibitors, histone deacetylase (HDAC) inhibitors, hormonal therapies, antibiotics, Jak2 inhibitors, mammalian target of rapamycin inhibitors, microRNAs, mitogen-activated extracellular signal-regulated kinase inhibitors, non-steroidal anti-inflammatory drugs (NSAIDs), poly ADP (adenosine diphosphate)-ribose polymerase (PARP) inhibitors, platinum chemotherapeutics, polo-like kinase (Plk) inhibitors, phosphoinositide-3 kinase (PI3K) inhibitors,
30 proteasome inhibitors, purine analogs, pyrimidine analogs, receptor tyrosine kinase inhibitors, small inhibitory ribonucleic acids (siRNAs), topoisomerase inhibitors, ubiquitin ligase inhibitors, hypomethylating agents, checkpoints inhibitors, peptide vaccine, epitopes or neoepitopes from tumor antigens, as well as combinations of one or more of these agents.

In a particular embodiment, the additional agent is an anti-cancer agent or an anti-viral agent.

For instance, the nucleic acid, the vector nanoparticle, for example the lipid-based nanoparticle, viral nanoparticle or dendrimer nanoparticle, the combination of a) said nucleic acid, vector nanoparticle, for example lipid-based nanoparticle, viral nanoparticle or dendrimer nanoparticle as disclosed herein, and
5 b) a distinct therapeutic agent, or pharmaceutical composition of the invention can be combined with an anti-cancer treatment selected in the group consisting of surgery, chemotherapy, radiotherapy, immunotherapy (e.g., checkpoint inhibitors such as anti-CTLA-4 immunotherapy or anti-PD-1/PD-L1 immunotherapy), cytokine treatment (e.g., interferons, GM-CSF, G-CSF, IL-2), cell therapy (e.g., CAR-T cells), cancer vaccines, antibiotics and probiotics and any combination thereof.

- 10 In a particular aspect, the nucleic acid, in particular the free nucleic acid or nucleic acid comprised in a vector nanoparticle, in particular a viral nanoparticle, is not used in combination with an anti-tumor immune cell, in particular lymphocyte, such as a T cell in particular a CAR-T cell.

- In some aspects, wherein the disease to be treated is cancer, the nucleic acid, the vector nanoparticle, for example lipid-based nanoparticle, viral nanoparticle or dendrimer nanoparticle, or the pharmaceutical
15 composition according to the invention may be used in combination with a chemotherapeutic agent. Chemotherapeutic agents can include, but are not limited to, aminoglutethimide, amsacrine, anastrozole, asparaginase, bcr, bicalutamide, bleomycin, buserelin, busulfan, campothecin, capecitabine, carboplatin, carmustine, chlorambucil, cisplatin, cladribine, clodronate, colchicine, cyclophosphamide, cyproterone, cytarabine, dacarbazine, dactinomycin, daunorubicin, dienestrol, diethylstilbestrol, docetaxel,
20 doxorubicin, epirubicin, estradiol, estramustine, etoposide, exemestane, filgrastim, fludarabine, fludrocortisone, fluorouracil, fluoxymesterone, flutamide, gemcitabine, genistein, goserelin, hydroxyurea, idarubicin, ifosfamide, imatinib, interferon, irinotecan, irinotecan, letrozole, leucovorin, leuprolide, levamisole, lomustine, mechlorethamine, medroxyprogesterone, megestrol, melphalan, mercaptopurine, mesna, methotrexate, mitomycin, mitotane, mitoxantrone, nilutamide, nocodazole, octreotide,
25 oxaliplatin, paclitaxel, pamidronate, pentostatin, plicamycin, porfimer, procarbazine, raltitrexed, rituximab, streptozocin, suramin, tamoxifen, taxol, temozolomide, teniposide, testosterone, thioguanine, thiotepa, titanocene dichloride, topotecan, trastuzumab, tretinoin, trimetrexate, vinblastine, vincristine, vindesine, vinorelbine, 6-mercaptopurine, 6-thioguanine, cytarabine (CA), 5-azacytidine, hydroxyurea, deoxycoformycin, 4-hydroxyperoxycyclophosphoramide, 5-fluorouracil (5- FU), 5-fluorodeoxyuridine (5-
30 FUDR) and methotrexate (MTX).

In some aspects, wherein the disease to be treated is multiple sclerosis, the nucleic acid, the vector nanoparticle, for example the lipid-based nanoparticle, viral nanoparticle or dendrimer nanoparticle, or the pharmaceutical composition according to the invention may be used in combination with interferon therapy (an interferon protein, IFN).

Subject, regimen and administration

A subject in need of a treatment may be a human having, at risk for, or suspected of having a disease such as a cancer or an infectious disease. Such a patient can be identified by routine medical examination.

- 5 As used herein, the term “subject”, “host”, “individual,” or “patient” refers to human, including adult and child. The subject to treat may be a human, particularly a human at the prenatal stage, a new-born, a child, an infant, an adolescent or an adult, in particular an adult of at least 30 years old, 40 years old, preferably an adult of at least 50 years old, still more preferably an adult of at least 60 years old. However, the term “subject” can also refer to non-human animals, in particular mammals, more particularly domestic animals
10 such as pets or farm animals, for example dogs, cats, horses, cows, bovines, pigs, sheep and non-human primates, among others.

In one embodiment, a subject who needs a treatment is a patient having, suspected of having, or at risk for a cancer. In some aspects, the subject is a cancerous subject identified as resistant to an immune-checkpoint inhibitor, in particular to an anti-CTLA4 antibody.

- 15 In one embodiment, a subject who needs a treatment is a patient having, suspected of having, or at risk for an infectious disease or a neurological disease.

Diagnostic of the subject in need of treatment can be performed by any suitable methods, including routine examination.

- The form of the pharmaceutical compositions, the route of administration and the dose of administration
20 can be adjusted by the man skilled in the art according to the type and severity of the disease, and to the patient, in particular its age, weight, size, sex, and/or general physical condition.

- Conventional methods, known to those of ordinary skill in the art of medicine, can be used to administer the nucleic acid, the vector nanoparticle, for example the lipid-based nanoparticle, viral nanoparticle or dendrimer nanoparticle, the pharmaceutical composition or combined therapy as disclosed herein to a
25 subject, depending upon the type of diseases to be treated or the site of the disease e.g., administered orally, parenterally, enterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir. Preferably, the nucleic acid, the vector nanoparticle, for example the lipid-based nanoparticle, viral nanoparticle or dendrimer nanoparticle, the pharmaceutical composition or the combined therapy as disclosed herein is administered via subcutaneous, intra-cutaneous, intravenous,
30 intramuscular, intra-articular, intra-arterial, intra-synovial, intra-tumoral, intra-sternal, intra-thecal, intra-lesion, or intracranial injection or infusion techniques.

Preferably, the nucleic acid, lipid-based nanoparticles, viral nanoparticle or dendrimer nanoparticle, combination or pharmaceutical composition are administrated or are to be administrated by systemic route (versus by local route). Administration can take place by enteral or parenteral administration. Examples of systemic routes usable in the context of the present invention include intravenous, inhalation, ocular, intraocular, cutaneous, subcutaneous, transdermal, intramuscular, intratumoral, intraarticular and intrathecal routes.

Preferably, the nucleic acid, the vector nanoparticle, for example the lipid-based nanoparticles, viral nanoparticles or dendrimer nanoparticles, the combination or the pharmaceutical composition are administrated or are to be administrated by subcutaneous, intramuscular, intratumoral or intravenous route, preferably intravenous route.

In a particular aspect, the systemic route is not the intratumoral (IT) route, in particular if the product administered is a viral vector, or a dendrimer, comprising a nucleic acid of the invention as herein described.

Dosing regimens may consist of a single dose or a plurality of doses over a period of time.

Preferably, the treatment with the nucleic acid, the vector nanoparticle, for example the lipid-based nanoparticle, viral nanoparticle or dendrimer nanoparticle, the combination or the pharmaceutical composition according to the invention is administered regularly, preferably between every day, every week or every month, more preferably between every day and every one, two, three or four weeks. In a particular embodiment, the treatment is administered several times a day, preferably 2 or 3 times a day.

The duration of treatment with the nucleic acid, the vector nanoparticle, for example the lipid-based nanoparticle, viral nanoparticle or dendrimer nanoparticle, the combination or the pharmaceutical composition according to the invention is preferably comprised between 1 day and 20 weeks, more preferably between 1 day and 10 weeks, still more preferably between 1 day and 4 weeks, even more preferably between 1 day and 2 weeks. Alternatively, the treatment may last as long as the disease persists.

The nucleic acid, the vector nanoparticle, for example the lipid-based nanoparticle, viral nanoparticle or dendrimer nanoparticle, the combination or the pharmaceutical composition according to the invention may be provided every one, two, three or four weeks, preferably by intravenous administration.

The nucleic acid, the vector nanoparticle, for example the lipid-based nanoparticle, viral nanoparticle, or dendrimer nanoparticle, the combination or the pharmaceutical composition according to the invention may be provided at an effective dose range from about 1 pg/kg body weight to about 1 ng, 1 µg, 1 mg or 1 g/kg body weight, 1 ng/kg body weight to about 1 µg, 1 mg or 10 mg/kg body weight, 0.1 or 1 µg/kg body weight to about 1 mg or 10 mg/kg body weight, 10 µg/kg body weight to about 1 or 10 mg/kg body weight or from 100 µg/kg body weight to 1 or 10 mg/kg body weight, from about 1 ng/kg body weight to

about 1 mg/kg body weight, 1 µg/kg body weight to about 1 mg/kg body weight, 10 µg/kg body weight to about 1 mg/kg body weight or from 100 µg/kg body weight to 1 mg/kg body weight. For example, the effective dose range is between 0.1 µg/kg body weight, for example 0.125 µg/kg body weight, and 2, 5 or 10 µg/kg body weight.

- 5 In some cases, the nucleic acid, the vector nanoparticle, for example the lipid-based nanoparticle, viral nanoparticle or dendrimer nanoparticle, the pharmaceutical composition or the combined therapy according to the invention can be administered at a subtherapeutic dose.

In some embodiments, the subject has already received at least one line of treatment (e.g., an anticancer or antiviral treatment), preferably several lines of treatment, prior to the administration of the nucleic acid, the vector nanoparticle, for example the lipid-based nanoparticle, viral nanoparticle or dendrimer nanoparticle, the pharmaceutical composition or the combined therapy of the invention.

10

The methods and uses as disclosed herein may be used for veterinary applications, e.g., canine and feline applications. If desired, the methods as disclosed herein may also be used with domestic animals such as cats and dogs and also farm animals, such as ovine, avian, bovine, porcine and equine breeds.

15

Kits

Any of the nucleic acid, vector nanoparticle, for example lipid-based nanoparticle, viral nanoparticle or dendrimer nanoparticles, combinations or compositions as described herein may be included in a kit provided by the present invention. The present disclosure particularly provides kits for use in preventing or treating diseases or disorders (e.g., cancer or viral infection).

20

Preferably, the term "kit" means one more component(s) or composition(s) packaged in a container, recipient or otherwise. A kit can hence be described as a set of products and/or utensils that are sufficient to achieve a certain goal, which can be marketed as a single unit. The kits of this invention are particularly in suitable packaging.

- 25 In some aspects, the kit comprises :

- i) a lipid mixture of an ionizable cationic lipid, a helper lipid, a sterol and a polyethylene glycol-lipid, in particular as described herein, preferably in an ethanolic solution; and
 - (ii) nucleic acid molecules or means to synthesize nucleic acid molecules, preferably mRNAs, comprising a Nuclear Localization Signal (NLS) sequence and a sequence encoding a Cyclic GMP-AMP synthase (cGAS), preferably as set forth in SEQ ID NO: 40, or any nucleic acid sequence of interest as herein described such as SEQ ID NO: 1, or a variant thereof having at least about 80% or about 85% identity thereto and a
- 30

mutation at position 241 in reference to SEQ ID NO: 40 or 1, for example a R241 mutation, such as a deletion, an addition or a substitution, for example a R241E, R241D, R241N, or R241A substitution.

Alternatively, the kit comprises a first solution and a second solution wherein:

5 (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, in particular as described herein; and

(ii) the second solution is an acidic aqueous solution and comprises nucleic acid molecules, preferably mRNA molecules, comprising a Nuclear Localization Signal (NLS) sequence and a sequence encoding a Cyclic GMP-AMP synthase (cGAS) as described herein, preferably as set forth in SEQ ID NO: 40, or any nucleic acid sequence of interest as herein described such as SEQ ID NO: 1, or a variant thereof having at
10 least about 80% or about 85% identity thereto and a mutation at position 241 in reference to SEQ ID NO: 40 or 1, for example a R241 mutation, such as a deletion, an addition or a substitution, for example a R241E, R241D, R241N, or R241A substitution.

Such a kit is particularly for use in the synthesis methods provided herein, so as to obtain any of the lipid-based nanoparticles as disclosed herein. Once obtained, such lipid-based nanoparticles could then be
15 intended for administration to a patient as described herein.

In another aspect, the kit comprises :

i) a nucleic acid molecule, for example a plasmid, said nucleic acid expressing a mRNA coding for lentiviral vector proteins, and
ii) a nucleic acid molecule, for example a plasmid, expressing a mRNA coding for a fusion protein, said
20 nucleic acid comprising a Nuclear Localization Signal (NLS) sequence and a sequence encoding a Cyclic GMP-AMP synthase (cGAS), preferably as set forth in SEQ ID NO: 40, or any nucleic acid sequence of interest as herein described such as SEQ ID NO: 1, or a variant thereof having at least about 80% or about 85% identity thereto and a mutation at position 241 in reference to SEQ ID NO: 40 or 1, for example a R241 mutation, such as a deletion, an addition or a substitution, for example a R241E, R241D, R241N, or R241A
25 substitution.

The nucleic acid molecule of step i) is typically used to transfect mammalian cells such as for example HEK-293T cells, in order to produce lentiviral particles using standard methods.

In another aspect, the kit comprises :

i) dendrimers in solution, and
30 ii) nucleic acid molecules or means to synthesize nucleic acid molecules, preferably mRNAs, comprising a Nuclear Localization Signal (NLS) sequence and a sequence encoding a Cyclic GMP-AMP synthase (cGAS), preferably as set forth in SEQ ID NO: 40, or any nucleic acid sequence of interest as herein described such

as SEQ ID NO: 1, or a variant thereof having at least about 80% or about 85% identity thereto and a mutation at position 241 in reference to SEQ ID NO: 40 or 1, for example a R241 mutation, such as a deletion, an addition or a substitution, for example a R241E, R241D, R241N, or R241A substitution.

In some aspects, the kit includes, preferably in suitable container means, the pharmaceutical composition, combination, nucleic acid or vector nanoparticle, for example lipid-based nanoparticles, viral nanoparticles or dendrimer nanoparticles, of the present invention.

In some embodiments, in particular when the kit is for use in the treatment of a disease, the kit may further include an additional agent for treating cancer or an infectious disease.

The additional agent i) may be provided in a single formulation comprising the pharmaceutical composition and/or nucleic acid and/or vector nanoparticle, for example lipid-based nanoparticle, viral nanoparticle or dendrimer nanoparticle, of the present invention, or ii) may be provided in the kit in a separate (i.e., second) composition. Particularly, the kit described herein may include one or more additional therapeutic agents such as those described in the "Combined Therapy" described hereabove. The kit(s) may be tailored to a particular cancer for an individual and comprise(s) respective second cancer therapies for the individual as described hereabove.

The components comprised in the kit according to the invention may particularly be formulated into a syringe compatible composition, in particular for intravenous administration purposes.

The kit may comprise one or more dose unit.

The instructions related to the use of the nucleic acid, the vector nanoparticle, for example the lipid-based nanoparticle, viral nanoparticle or dendrimer nanoparticle, the combination or the pharmaceutical composition as described herein generally include information as to dosage, dosing schedule, route of administration for the intended treatment, means for reconstituting the vector nanoparticle if required, for example the lipid-based nanoparticle and/or means for diluting the vector nanoparticle, for example the lipid-based nanoparticles, viral nanoparticles or dendrimer nanoparticles, of the invention. Instructions supplied in the kits of the invention are typically written instructions on a label or package insert (e.g., a paper sheet included in the kit in the form of a leaflet or instruction manual).

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Design of cGAS constructs for mRNA-LNP. (A) Overview of the cGAS-STING pathway. **(B)** Organization of mRNA-LNP. **(C)** Schematical structure of the constructs tested.

Figure 2. Expression of the mRNA-LNP using a luciferase construct. (A) Overview of the experiment. **(B)** Whole-organism luciferase signal: Top, representative images and Bottom, quantification. p/s, photons per second (n = 3 mice per condition). **(C)** Single-organ luciferase signal: Top, representative images and Bottom, quantification. "ingLN", inguinal lymph node (n = 3 mice per condition).

Figure 3. Measurement of cGAMP after IM injection. (A) Overview of the experiment. **(B)** cGAMP concentration in the different organs ("ingLN", inguinal lymph node; "Ctrl Luc", luciferase mRNA-LNP). N = 4 mice per group, Kurskal-Walli's test with Dunn's multiple correction test. * p < 0.05, ** p < 0.01.

Figure 4. Anti-tumor effect of "NLS mu-cGAS" (R241E) mRNA-LNP. (A) Overview of the experiment, 2 µg of injected mRNA-LNP. **(B)** Tumor size measured at the indicated time points. N = 6 mice per group, mixed-effect analysis with Sidak multiple comparison test. * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001.

Figure 5. Exemplification of distinct substitutions at position R241, and exemplification of a distinct NLS (NLS-MYC). Mice were injected with 0.5 µg of the indicated mRNA-LNP (lipid SM-102) (i.v. route). 16 hours later, plasma was harvested and concentrations of IFN-alpha and IFN-beta were measured using a LegendPlex assay. NLS, NLS from SV40 large T antigen. NLS-MYC, NLS from the gene MYC. Both types of NLS lead to comparable levels of IFN-alpha and IFN-beta, showing that the activity of the construct is independent from the type of NLS chosen and can be generalized to any NLS. ** p < 0.01, *** p < 0.001, **** p < 0.0001, one-way ANOVA with Dunnett test.

Figure 6. Exemplification of different lipids. Mice were injected with 0.5 µg of the indicated mRNA-LNP (ionizable lipid Acuitas or ionizable lipid SM-102) (i.v. route). 16 hours later, plasma was harvested and concentration of IFN-alpha and IFN-beta were measured using a LegendPlex assay. The ionizable lipid of mRNA-LNP allows efficient delivery of the RNA into cells via fusion with target cells. Both types of ionizable lipids lead to a functional NLS-mouse cGAS R241E product that can induce high levels of IFN-alpha and IFN-beta production, in contrast to the Luciferase construct. ** p < 0.01, *** p < 0.001, **** p < 0.0001, Kruskal-Wallis with Dunn test.

Figure 7. "NLS mouse cGAS R241E" induces anti-tumor effects and anti-tumor T cell responses. Mice were grafted with B16-OVA tumor cells. 7 days later, when tumors reached 100 mm³ in average size, mice were injected with 0.5 µg of the indicated mRNA-LNP (i.v. route). (Left) 10 days later, the anti-OVA T cell response was measured in blood cells using an interferon-gamma ELISPOT (IFNg, SFU = spot forming units, PBMC = peripheral blood mononuclear cells). The specific response compared to a "no peptide" control is

shown. (Right) Size of individual tumors at day 18 after injection of tumor cells. ns = not significant, * $p < 0.05$, Kruskal-Wallis with Dunn test.

Figure 8. "NLS mouse cGAS R241E" induces anti-tumor effect against different tumor types. Mice were grafted with MC38 or B16-OVA tumor cells. 7 days later, when tumors reached 50 mm³ in average size, mice were injected once with 0.5 µg of the indicated mRNA-LNP (i.v. route).

Figure 9. The activity of NLS mouse cGAS R241 mRNA-LNP is dose-dependent. Mice were injected with the indicated dose of mRNA-LNP (i.v. route). 16 hours later, plasma was harvested and concentrations of IFN-α and IFN-β were measured using a LegendPlex assay, and concentration of cGAMP was measured with an ELISA.

Figure 10. The mRNA-LNP "NLS mouse cGAS R241E" induces antiviral activity. Bone marrow-derived dendritic cells (BMDCs) were differentiated from bone marrow of C57BL/6 mice by incubation during 7 days with recombinant FLT3L protein at 200 ng/ml in standard cell culture conditions. At day 7, cells were aliquoted and treated with the indicated amounts of mRNA-LNP or synthetic cGAMP (final concentration is indicated). Luc, Luciferase mRNA-LNP; cGAS*, NLS mouse cGAS R241E mRNA-LNP; NT, not treated. (A) After overnight incubation, media was harvested and the concentration of IFN-α and IFN-β was measured. cGAS*mRNA LNP. (B) After overnight incubation, cells were infected with the indicated multiplicity of infection (MOI) of a Vesicular Stomatitis Virus (VSV) coding for a reporter green fluorescent protein (GFP). 8 hours later, %GFP+ cells were analyzed by flow cytometry (gated as live cells, CD11c+MHCII+).

Figure 11. The mRNA "NLS mouse cGAS R241E" is also active in the form of a lentivector. Human R255E, NLS human cGAS R255E, mouse cGAS R241E and NLS mouse cGAS R241E were cloned in the pTRIP-SFFV HIV-1 lentiviral vector with a eGFP-FLAG tag. Lentiviral vector particles were producing by transient transfection of 293FT cells using standard methods. Lentiviral particles were used to transduced human monocyte-derived dendritic cells in the presence of the Vpx protein using standard methods. 48 hours after transduction, cells were harvested to measure (left) the level of transduction and (right) the intracellular concentration of cGAMP.

Figure 12. Examples of additional substitutions at position R241, example using NLS-MYC, examples with cGAS of different origins (from different species of mammal, in particular rodents). Tumor bearing mice were injected with 0.5 µg of the indicated mRNA-LNP (i.v. route). 16 hours later, plasma was harvested and concentrations of IFN-γ were measured using a LegendPlex assay. (A) B16-OVA tumor bearing mice, Acuitas lipid. (B) B16-OVA tumor bearing mice, SM-102 lipid. (C) MC38 tumor bearing mice, SM-102 lipid. NLS, NLS from SV40 large T antigen. NLS-MYC, NLS from the gene MYC. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, one-way ANOVA with Dunnett test.

EXAMPLES

Further aspects and advantages of the present invention will be described in the following examples, which should be regarded as illustrative and not limiting.

5 Example 1. Characterization of the unexpected activity of "NLS mouse cGAS R241E"

The purpose of the invention is to use cGAS to treat tumors by activating STING. The STING pathway is a validated target for treating tumors by intra-tumoral injection, but there are very few technologies to induce an anti-tumor activity of this pathway by the systemic route.

10 cGAS is not normally activated when expressed in cells. Therefore, the inventors engineered several modifications to activate cGAS:

- A NLS was added to force cGAS entry into the nucleus; and
- Mutations R255E (human) or R241E (mice) have been introduced to detach cGAS from histone and allow its activation by chromatin (as described in SEQ ID NO: 1 (mice) and SEQ ID NO: 7 (human)).

15 Mouse and Human cGAS have slightly different biochemical properties and were therefore compared. The inventors particularly designed 4 constructs, represented in Figure 1.

20 Then, the inventors performed a pilot experiment to determine the expression of the mRNA-LNP using a Luciferase construct (Figure 2A). The inventors compared intravenous (IV), sub-cutaneous (SC) and intramuscular (IM) route. Seven hours after injection, the signal was observed. At the whole-organism level, the signal was the highest in the IV route (Figure 2B). In isolated organs, the highest signal was found in the liver and in the spleen (Figure 2C). In the SC and IM route, signal was also detected in the draining lymph node.

25 Next, the inventors set out to compare the activity of the mRNA-LNP by measuring the production of cGAMP, the product of cGAS. The mRNA-LNP were injected by the IM route and the cGAMP concentration was measured in tissues 16 hours later (Figure 3A). Unexpectedly, the "NLS mu-cGAS" (R241E) constructs led to significant levels of cGAMP across liver, spleen and right inguinal lymph node (Figure 3B), while the other constructs did not induce detectable levels of cGAMP in organs. This was unexpected because the other constructs also code for an active cGAS enzyme.

30 Based on these results, the inventors selected the "NLS mu-cGAS" construct to assess its impact on tumor growth (Figure 4). The inventors implanted mice with MCA-OVA tumors and treated them with one dose of mRNA-LNP injected by the IV, IM and SC route (Figure 4B). Strikingly, all 6 mice treated with IV route eliminated their tumors. In the IT route, 3 out of 6 mice eradicated their tumors. In the IM route, a significant reduction in tumor size was observed.

In conclusion, the “NLS mu-cGAS” (R241E) mRNA-LNP leads to unexpected high levels of cGAMP production in liver, spleen and draining lymph node, compared to the other forms of cGAS tested. Furthermore, the “NLS mu-cGAS” (R241E) mRNA-LNP leads to complete tumor eradication notably after a single IV injection.

5 Surprisingly, “NLS mu-cGAS” stands out has a much higher activity than the other constructs.

Unexpectedly, out of 4 constructs tested, NLS mu-cGAS gives very strong activation as shown by production of cGAMP (the product of cGAS) in tissues and inflammatory cytokines in the serum. Therefore, this construct provides a solution to activate cGAS with mRNA-LNP.

10 This leads to a very strong anti-tumoral effect: a single dose of product by intravenous route (2 µg) leads to complete tumor eradication.

MATERIAL AND METHODS

the inventors engineered several modifications to activate cGAS.

– A NLS in particular as described in SEQ ID NO: 9 was added to force cGAS entry into the nucleus, as described in Gentili *et al.*, Cell Rep. 2019 Feb 26;26(9):2377-2393.e13, and

15 –Mutations R255E (human, in particular as described in SEQ ID NO: 7) or R241E (mice, in particular as described in SEQ ID NO : 1) have been introduced to detach cGAS from histone and allow its activation by chromatin (as described in Volkman *et al.*, Elife . 2019 Dec 6;8:e47491, in SEQ ID NO: 1 (mice) and SEQ ID NO: 7 (human)).

mRNA production

20 Codon-optimized murine cGAS R241E, NLS murine cGAS R241E, human cGAS R255E, NLS human cGAS R255E gene containing plasmids were synthesized (Genscript, Piscataway, NJ). Plasmids were then linearized, and a T7-driven *in vitro* transcription reaction (AMB13345, Life Technologies, Carlsbad, CA) was performed to generate mRNA with 101 nucleotide long poly(A) tails. Capping of mRNA was performed in concert with transcription through addition of a trinucleotide cap1 analog CleanCap and m1Ψ-5'-
25 triphosphate (N-7113 and N-10181, both from TriLink, San Diego, CA) was incorporated into the reaction instead of UTP. Cellulose-based purification of mRNA was performed as described (Baierdörfer *et al.*, 2019; *Molecular Therapy. Nucleic Acids*, 15, pp. 26–35). mRNAs were then checked on an agarose gel before storing at -20°C.

Lipid nanoparticle formulation of mRNA

30 The inventors produced the engineered cGAS inside mRNA-LNPs, which is the platform that is used for Covid-19 vaccines. The mRNA-LNPs were produced in collaboration with Nobert Pardi from UPenn, Philadelphia. There is an R&D agreement in place for this work between Curie and UPenn.

Purified mRNAs were encapsulated in lipid nanoparticles (LNP) using a self-assembling ethanolic lipid mixture of an ionizable cationic lipid, 1,2-distearoyl-sn-glycero-3-phosphocholine, cholesterol, and a polyethylene glycol-lipid. This mixture was rapidly combined with an aqueous solution containing mRNA at acidic pH. The ionizable cationic lipid (pKa in the range of 6.0-6.5, proprietary to Acuitas Therapeutics (Vancouver, Canada) and LNP composition are described in the patent application WO 2017/004143. The average hydrodynamic diameter was ~80 nm with a polydispersity index of 0.02-0.06 as measured by dynamic light scattering using a Zetasizer Nano ZS (Malvern Instruments Ltd, Malvern, UK) and an encapsulation efficiency of ~95% as determined using a Quant-iT Ribogreen assay (R11490, Life Technologies).

10 Cell culture

MCA-OVA cells were cultured in RPMI GlutaMAX medium with 10% FBS (Gibco), penicillin-streptomycin (Gibco), 1 mM 2-mercaptoethanol, and hygromycin (60 µg/ml). B16-OVA cells were cultured in RPMI GlutaMAX medium with 10% FBS (Gibco), penicillin-streptomycin (Gibco), 1 mM 2-mercaptoethanol, geneticin (2 mg/ml), and hygromycin (60 µg/ml). MC38 cells were cultured in DMEM GlutaMAX medium with 10% FBS and penicillin-streptomycin.

Mice

All animals were used according to the protocols approved by Animal Committee of Curie Institute CEEA-IC #118 and maintained in pathogen-free conditions in a barrier facility. Experimental procedures were approved by the French "Ministère de l'enseignement supérieur, de la recherche et de l'innovation" (APAFIS#11561-2017092811134940-v2) in compliance with the international guidelines. C57BL/6J female mice (6 to 8 weeks) were purchased from Charles River Laboratories.

Tumor implantation

Female mice were inoculated subcutaneously on the lower right flank with 5×10^5 MCA-OVA, B16-OVA or MC38 cells in 100 µl of PBS. Mice were monitored for morbidity and mortality daily. Tumors were monitored two or three times per week. Mice were euthanized if ulceration occurred or when tumor volume reached 2000 mm³. Tumor sizes were measured using a digital caliper, and tumor volumes were calculated with the formula $(\text{length} \times \text{width}^2)/2$. After tumor implantation, mice were randomized into treatment groups using the Randmice software (Jneid *et al.*, 2023 Science Immunology, 8(79), p. eabn6612).

30 LNP injections

In the case of tumor-bearing mice, intratumoral, intramuscular or intravenous injections were initiated when tumors were palpable or reached about 100 mm³ (50 to 150 mm³). A U-100 insulin syringe or equivalent [0.33 mm (29 gauge) × 12.7 mm (0.5 ml)] was filled with 50 µl of LNP (diluted in PBS), and all

air bubbles were removed. LNP were injected by 3 different routes: intratumoral (IT), intramuscular (IM) or intravenous (IV). For IT injection, with the bevel facing the skin, the needle was introduced shallowly into the area directly adjacent to the tumor, and the needle was moved underneath the skin until it reached the inside back of the tumor, and the samples were injected slowly into the center of the tumor.

- 5 For IM injection, with the bevel facing the skin, the needle was introduced shallowly into the right leg muscle and samples were injected slowly. For IV injection, with the bevel facing down, the needle was introduced shallowly into the right retro-orbital vein and samples were injected slowly. The needle was then removed delicately to avoid reflux.

Bioluminescence imaging

- 10 Whole body luciferase signal was monitored by intra-peritoneal injection of D-luciferin (IVISbrite D-Luciferin Potassium Salt Bioluminescent Substrate, PerkinElmer #P/N 122799, or D-Luciferin potassium, MedChemExpress #HY-12591B, 3 mg in 200 μ L of PBS) and mice were sedated 5 min later with isoflurane. Images were taken 15 min after D-luciferin injection using the IVIS Lumina II imaging system (PerkinElmer) for different time of exposition. Photon fluxes were transformed into pseudocolor images using the Living
15 Image software.

- For some groups, bioluminescence of organs was measured. For this groups, after whole body measurement, mice were bled (by retro-orbital vein, with dry capillary and dry tubes) and euthanized by cervical dislocation. Ethanol was put on the abdomen (to glue the bristles). The abdomen was open and the organs of interest (inguinal and popliteal lymph nodes, liver, lung, spleen, kidneys, heart, thoracic
20 aorta, pancreas, ovaries, uterus, fat and brain) were removed, and then put in a dish and bioluminescence was measured using the IVIS Lumina II imaging system (PerkinElmer) for different time of exposition. Organs images were done within 10min after euthanasia.

cGAMP assay

- After bioluminescence measurements, the organs were sliced into small pieces (except lymph nodes and
25 ovaries), transferred to 1.5 mL tubes, placed on dry ice, and preserved at -80°C until extraction. For extraction, pieces of frozen tissue were weighed and 400 μ L of T-PER Tissue Protein extraction Reagent (ThermoScientific #78510) was added. The organs were extracted at a maximum of 60 mg of tissue/mL of buffer. The samples were kept on ice during the extraction. The tissues were then crushed manually with a pestle. Once a homogeneous mixture was obtained, the samples were incubated for 10 min in ice, and
30 centrifuged for 20 min 19090 rcf at 4°C. Supernatants were collected and stored at -80° C until the ELISA assay was performed. The 2'3'-cGAMP ELISA Kit (Cayman Chemical) was used for the quantification of cGAMP in organs according to the manufacturer's instructions. Plates were analyzed at a wavelength of 450 nm. Data were fitted to a four-parameter sigmoidal curve.

Cytokines assay

After mice were bled, the blood was kept at 4°C the whole day or overnight. The blood was centrifuged at 6000rpm, 10min, RT and the serum aliquoted, then preserved at -80°C.

LEGENDplex™ Mouse Anti-Virus Response Panel (13-plex) kit (BioLegend) was used for the quantification of IFN- α , IFN- β , MCP-1, IL-6, TNF α and IL-1 β in serum according to the manufacturer's instructions.

Example 2. Exemplification of distinct substitutions at position R241, and exemplification of a second NLS (NLS-MYC).

Inventors compared R241E, the original mutation, to other mutations: R241D, polar negative amino acid, similar to E; R241N, polar uncharged; and R241A, non-polar. All mutations lead to comparable levels of IFN- α and IFN- β , showing that the activity of the construct is independent of the substitution chosen at position R241.

Example 3. Exemplification of different lipids.

In order to confirm that the effect of NLS mouse cGAS R241 mRNA was generalizable to any LNP, inventors compared a second formulation with the SM-102 lipid. Similar to the formulation with Acuitas lipids, NLS mouse cGAS R241 mRNA formulated with the SM-102 lipid induced high levels of interferon- α and interferon- β , that were not induced by a Luciferase mRNA. They conclude that the NLS mouse cGAS R241 mRNA activity in the form of LNP is not bound to a specific type of lipids.

Example 4. "NLS mouse cGAS R241" induces anti-tumor effects and anti-tumor T cell responses.

7 days after treatment of B16-OVA tumors, inventors performed an ELISPOT on mononuclear cells isolated from peripheral blood, using a peptide that is specific against OVA. Control mice (untreated) show no detectable T cells. Mice treated with Luciferase mRNA-LNP did not show significant levels either. In contrast, treatment of mice with NLS mouse cGAS R241E induced significant levels of OVA-specific circulating T cells. This shows that NLS mouse cGAS R214 induces the priming of tumor antigen-specific CD8+T cells.

Example 5. "NLS mouse cGAS R241" induces anti-tumor effects against different tumor types.

In order to show that NLS-mouse cGAS R241E mRNA-LNP allows an anti-tumoral effect against other tumor types than MCA-OVA, a fibrosarcoma, Inventors performed similar experiments with two other tumor

types: MC38, a colon adenocarcinoma, and B16-OVA, a melanoma. Similar to MCA-OVA, a single dose of NLS mouse cGAS R241E mRNA-LNP induces a potent anti-tumor effect against MC38 and B16-OVA tumors (cf. Figure 8).

5 **Example 6. The activity of NLS mouse cGAS R241 mRNA-LNP is dose-dependent.**

To evaluate the dose-response of cGAMP production by NLS mouse cGAMP R241E, inventors performed a dose titration *in vivo* from 0.125 µg to 2 µg with a 2-fold increment. The concentration of cGAMP in serum gradually increased with the dose of mRNA-LNP, indicating the direct relationship with the dose of product and the production of cGAMP *in vivo*.

10

Example 7. The mRNA-LNP induces antiviral activity.

Inventors used an assay based on infection of dendritic cells with a Vesicular Stomatitis Virus (VSV). They used synthetic cGAMP as positive control. They measured the concentration of antiviral interferons in the supernatant. NLS mouse cGAS R241E mRNA-LNP, but not Luciferase mRNA-LNP, induced the production
15 of interferon-α and interferon-β. 5 ng and 50 ng of mRNA induced 10- and 100-fold higher concentrations, respectively, than treatment with 25 µg/ml of synthetic cGAMP (**Figure 10A**). In parallel, treated cells were infected with the virus. After 8 hours, they measure the level of infection based on the percentage of GFP-positive cells. The Luciferase mRNA-LNP showed no antiviral activity. In contrast, all doses of NLS mouse cGAS R241E mRNA-LNP tested showed antiviral activity, at all MOI, similar to synthetic
20 cGAMP (**Figure 10B**). Inventors conclude that the NLS mouse cGAS R241 construct in the form of a mRNA-LNP shows potent antiviral activities, at low doses of mRNA (ng and pg range).

Example 8. Viral nanoparticle formulation of mRNA.

To check if the mRNA NLS mouse cGAS R241 therapeutic effect was related to the essential use of a LNP
25 vector, Inventors tested a lentiviral vector. Lentiviral vectors are derived from HIV-1 and contain a mRNA coding for the gene of interest. Inventors generated lentivectors with human R255E, NLS human cGAS R255E, mouse cGAS R241E and NLS mouse cGAS R241E in fusion with GFP and FLAG tag. GFP level can be used as a measure of the expression of the proteins. With the vectors, inventors transduced human monocyte-derived dendritic cells. After transduction, the level of GFP was similar between the four
30 constructs (**Figure 11A**). In contrast, NLS mouse cGAS R241 induced significantly higher levels of cGAMP than any one of the other combinations (**Figure 11B**). Inventors conclude that a mRNA coding NLS mouse cGAS R241 is also active in the form a lentivector, and not restricted to a LNP formulation.

Example 9. Examples of different substitutions at position R241, example using NLS-MYC, examples with cGAS of different origins (from different species of mammal, in particular rodents).

Inventors defined a circulating marker correlating with the activity of the nucleic acid “NLS mouse cGAS R241E” of the invention to strongly increase cGAMP concentrations in organs. They screened several cytokines and found that the concentration of IFN- γ in the plasma was induced at significantly higher levels by NLS mouse cGAS R241E (**Figure A**), as compared to mouse cGAS R241E, human cGAS R255E and NLS human cGAS R255E. Therefore, they used IFN- γ concentration as a circulating marker to evaluate other cGAS variants on mRNA-LNP. They compared R241E, the originally identified mutation in the context of “NLS mouse cGAS”, to other mutations: R241D, polar negative amino acid, similar to E; R241N, polar uncharged; R241A, non-polar (**Figure B**). All mutations induced IFN- γ at least to the same extent as R241E if substituted to R241E in the “NLS mouse cGAS” sequence. There was no significant difference between R241E, R241D and R241A. R241N induced even higher levels of IFN- γ . These results are consistent with previously shown results (**Figure 5A, 5B**).

Inventors also compared the originally tested NLS sequence (from SV40 large T antigen) to another NLS, the NLS from the MYC gene (**Figure B**). Both types of NLS lead to comparable levels of IFN- γ , showing that the activity of the construct is independent from the type of NLS chosen and can be generalized to any NLS. These results are consistent with previously shown results (**Figure 5A, 5B**).

After having tested single substitutions, inventors confirmed that deleting position R241, or introducing a linker, is also functional. They compared R241E (the originally identified mutation) to deletion of position R241, and to R241AAA (three alanines at position R241) (**Figure 12C**). As expected, R241E induced significant levels of IFN- γ . There was no significant difference for R241AAA and R241X, as compared to R241E.

They also showed that cGAS sequences from other species, carrying a mutation analogous to R241E (and including a NLS sequence), activate STING. They tested cGAS homologs from rodents, that are closely related to mouse cGAS. They tested rat cGAS (about 88% protein sequence identity to mouse cGAS SEQ ID NO:1 or 40) and rat cGAS (about 85% identity to mouse cGAS cGAS SEQ ID NO:1 or 40) sequences with an NLS and a mutation equivalent to the mouse “NLS cGAS R241E” sequence. Both “NLS rat cGAS R244E” and “NLS rat cGAS R221E” induced IFN- γ and the differences were not significantly different from “mouse NLS cGAS R241E”. Inventors conclude that a “NLS cGAS variant” with at least about 80% or about 85% sequence identity to “NLS mouse cGAS R241E” also shows the activity. Finally, they wished to test if the results observed with the rodent cGAS variants were particularly unexpected. Human cGAS encodes K at position 187 and L at position 195, while mouse cGAS encodes for N and R at these positions. These two

residues have been identified by Zhou *et al.* ("Structure of the Human cGAS-DNA complex reveals enhanced control of immune surveillance" Cell. Vol. 174, pp.300-311, July 12, 2018) as having a critical role in the enhanced enzymatic activity of mouse cGAS as compared to human cGAS. It has been proposed by Zhou *et al.* that these two residues explain the enhanced activity of mouse cGAS over human cGAS.

- 5 Therefore, it would be expected that a non-human cGAS variant with amino acids N and R at the corresponding positions, but with a low protein sequence identity to mouse cGAS, would still behave like NLS mouse cGAS R241E. To test this hypothesis, inventors considered rhesus macaque cGAS, described in the afore mentioned paper. Macaque cGAS codes for N and R, but has only about 54% identity with mouse cGAS. Strikingly, "NLS macaque cGAS R255E" produced some IFN- γ , but it was at significantly lower levels
- 10 as compared to "NLS mouse cGAS R241E". Therefore, it was totally unexpected that the cGAS variants having about 80% sequence identity, such as rodent cGAS variants, would show this unique high potency.

- Inventors conclude that mutating, deleting, or introducing a linker at position R241 in NLS mouse cGAS, or the corresponding site of homologous sequences, in particular rodent sequences (for example position R221 of rat cGAS sequence or position R244 of rat sequence), can be substituted in the nucleic
- 15 acid sequence of the invention. NLS of different origins can also be used. Other cGAS with at least about 80% or about 85% sequence identity, for example cGAS of rodent origin, can be used. However, NLS cGAS with N and R residues (at equivalent positions to K187 and L195 in humans) but lower sequence identity, for example below 60%, cannot be used, despite the presence of these key N and R residues. This confirms the unexpected nature of inventor's findings.

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.
- 5 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.
- 10 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 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 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.
- 15 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.
- 20 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.
- 25 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.
- 30 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. 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, for use as a medicament.
21. The nucleic acid, lipid-based nanoparticle, viral nanoparticle or dendrimer nanoparticle, or pharmaceutical composition, for use according to claim 20 in combination with an anticancer or antiviral therapeutic agent.
22. The nucleic acid, lipid-based nanoparticle, viral nanoparticle, dendrimer nanoparticle, or pharmaceutical composition for use according to claim 20, or combination for use according to

claim 21, for the treatment of a disease selected from the group consisting of cancer, an infectious disease and a neurological disease.

23. The nucleic acid, lipid-based nanoparticle, viral nanoparticle, dendrimer nanoparticle, pharmaceutical composition or combination for use according to claim 22, 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.

24. The nucleic acid, lipid-based nanoparticle, viral nanoparticle, dendrimer nanoparticle, pharmaceutical composition or combination for use according to claim 22, 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 Cocksackie virus.

25. The nucleic acid, lipid-based nanoparticle, viral nanoparticle, dendrimer nanoparticle, pharmaceutical composition or combination for use according to claim 22, 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.

26. 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

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