Other systems, for example in WO2019103998A2, describe nanobiologics that are able to target the myeloid compartment, the nanobiologics comprising phospholipids and apo A1 and a small molecule drug. The drawback of these nanobiologics is that due to their hydrophobic core they do not allow the incorporation of polar structures such as nucleic acids, e.g. DNA and RNA.

In WO2017048789A1 dendrimer materials are described with a limited number of ionizable groups per molecule and with non-natural sulfide groups that are prone to *in vivo* oxidation. In the preparation of these materials, a multifunctional molecule is reacted with a bifunctional molecule to arrive at an intermediate that accordingly may be hard to attain in pure (non-crosslinked) form.

Therefore, there is a need for improved and alternative delivery systems for therapeutic nucleic acids to the myeloid compartment.

The above problems, among others, are solved by the invention as defined in the appended claims.

Summary

The current disclosure constitutes nanoparticle platform technology for nucleic acid therapeutic targeting and/or delivery, and more particularly for nucleic acid therapeutic targeting and/or delivery to the myeloid cell compartment. The nanoparticles as taught herein are (phospho)lipid-based nanoparticles stabilised by an apolipoprotein stabiliser (or by a derivative of an apolipoprotein, or by a mimic or a mimic derivative of an apolipoprotein). In circulation, the stabiliser and (phospho)lipids shield and/or protect the nucleic acid payload, and thereby prevent it from degradation and rapid clearance. At the same time, the nanoparticles reduce nucleic acid therapeutics' immunostimulatory-related adverse effects by limiting unwanted interactions with components in the blood, such as limiting unwanted interactions of the nucleic acid payload with components in the blood. The apolipoprotein stabiliser also acts as a targeting moiety as it is capable of directing the nanoparticle to the myeloid cell compartment. Accordingly, the disclosure enables efficient nucleic acid therapeutics delivery to the myeloid cell compartment in lymphoid organs, such as for example the bone marrow and the spleen, for effective immunotherapy.

The nanoparticles as taught herein further also comprise polyvalent molecules. The polyvalent molecule has multiple positively ionizable and/or cationic groups that can efficiently bind and capture (or complex) nucleic acids. The polyvalent molecules of the disclosure bind stronger to nucleic acids than, for example, monovalent amphiphilic molecules. Accordingly, the nucleic acids are better bound and/or retained within the

nanoparticles as taught herein. In addition, the polyvalent molecules of the disclosure may also interact with the apolipoprotein stabiliser (or by a derivative of an apolipoprotein, or by a mimic or a mimic derivative of an apolipoprotein), as this protein has an overall negative charge. As a result, the apolipoprotein (or by a derivative of an apolipoprotein, or by a mimic or a mimic derivative of an apolipoprotein) becomes better integrated in the nanoparticle of the disclosure. Hence, in circulation, e.g. in the blood of a subject, the nanoparticle of the present disclosure will release very little or none of the apolipoprotein component from the nanoparticle.

The disclosure relates in particular to nanoparticles comprising a polyvalent molecule such as but not limited to a dendrimer. Furthermore, the disclosure relates in particular to nanoparticles comprising a stabiliser material such as, but not limited to, an apolipoprotein, an apolipoprotein derivative, an apolipoprotein mimetic or an apolipoprotein mimetic derivative. The disclosure further relates to methods of treatment using the nanoparticle, for example in the treatment of a disease by stimulating or inhibiting an innate immune response. The disclosure further relates to an *in vivo*, *in vitro* or *ex vivo* method for introducing a nucleic acid in a cell using the nanoparticles.

Accordingly, in a first aspect, the disclosure relates to a nanoparticle comprising a core and an outer layer, wherein the core comprises:

- a nucleic acid;
- a polyvalent molecule;

and wherein the outer layer comprises:

- an apolipoprotein, an apolipoprotein derivative, an apolipoprotein mimetic and/or an apolipoprotein mimetic derivative;
- a phospholipid,
- a sterol; and optionally,
- a filler molecule,

wherein the polyvalent molecule has formula (I):

$$[core]_x – [BU]_y – [TU]_z \qquad (I)$$

wherein the core (i.e. core of the polyvalent molecule) is a nitrogen or is a C_1-C_{18} linear, branched, or cyclic group that contains 1 to 15 nitrogen heteroatoms and that optionally contains 1 to 4 oxygen heteroatoms; and

emanating further away from the core (representing the second generational layer BU), and this one may be different from the third, the fourth and the fifth BU emanating further away from the core; and

wherein the terminal units (TU) are individually and independently chosen from a hydrogen and a C_1-C_{30} alkyl, aryl, arylene-alkyl or alkylene-aryl group that optionally contains 1 to 8 heteroatoms that are individually and independently selected from the group consisting of O and N, with the proviso that not all TUs in the polyvalent molecule are hydrogens; and

wherein z represents the total number of TU groups that are attached to the polyvalent molecule, where z is 1 to 128; and

wherein the polyvalent molecule as according to formula (I) is polyvalent in positively ionizable and/or cationic groups, where the cumulative number of positively ionizable and cationic groups in the polyvalent molecule is 2 or higher.

In a second aspect, the disclosure relates to a composition comprising the nanoparticle according to the first aspect of the disclosure and a physiologically acceptable carrier, preferably wherein the composition is a pharmaceutical composition.

In a third aspect, the disclosure relates to a nanoparticle according to the first aspect of the disclosure, or the composition according to the second aspect of the disclosure for use as a medicament.

In a fourth aspect, the disclosure relates to a nanoparticle according to the first aspect of the disclosure, or the composition according to the second aspect of the disclosure for use in delivering a nucleic acid to the myeloid compartment or the spleen.

The disclosure also relates to a nanoparticle according to the first aspect of the disclosure, or the composition according to the second aspect of the disclosure for use in the treatment of a disease by stimulating or inhibiting an innate immune response, preferably wherein said disease is a cancer, a cardiovascular disease, an autoimmune disorder, or a xenograft rejection.

In a fifth aspect, the disclosure relates to use of the nanoparticle according to the first aspect of the disclosure, or the composition according to the second aspect of the disclosure in the manufacture of a medicament for the treatment of a disease by stimulating or inhibiting an innate immune response.

In a sixth aspect, the disclosure relates to a method for producing a nanoparticle, comprising the step of:

a) mixing, preferably rapid mixing, of (lipid) components in organic solvent with a nucleic acid in an aqueous buffer to produce nanoparticles, wherein the (lipid) components comprise a phospholipid, a sterol, a polyvalent molecule, and optionally a filler material (e.g. a tri-glyceride); and wherein the aqueous buffer has a pH of 5.5 or lower, preferably 5.0 or lower; and

b) mixing, preferably rapid mixing, of the lipid nanoparticles produced under a. with an apolipoprotein, an apolipoprotein derivative, an apolipoprotein mimetic and/or an apolipoprotein mimetic derivative, to produce the nanoparticle of

the disclosure at a pH between 5.5 and 9.0, preferably at a pH between 6.0 and 8.0, more preferably at a pH between 6.5 and 8.0.

In a seventh aspect, the disclosure relates to the nanoparticle according to the first aspect of the disclosure, wherein the nanoparticle is obtainable or obtained by the method of the sixth aspect.

In an eighth aspect, the disclosure relates to an *in vitro* or *ex vivo* method for introducing a nucleic acid in a cell, the method comprising contacting the nanoparticle according to the first aspect of the disclosure or the composition according to the second aspect of the disclosure with the cell.

The disclosure also relates to an *in vivo* method for introducing a nucleic acid in a cell, the method comprising contacting the nanoparticle according to the first aspect of the disclosure or the composition according to the second aspect of the disclosure with the cell.

The disclosure also relates to a method for the *in vivo* delivery of a nucleic acid, the method comprising administering the nanoparticle according to the first aspect of the disclosure or the composition according to the second aspect of the disclosure to a subject.

In a ninth aspect, the disclosure relates to use of the nanoparticle according to the first aspect of the disclosure or the composition according to the second aspect of the disclosure in the manufacture of a medicament for the *in vivo* delivery of a nucleic acid to a subject.

The disclosure also relates to a method for treating a disease or disorder in a subject in need thereof by stimulating or inhibiting an innate immune response, the method comprising administering a therapeutically effective amount of the nanoparticle according to the first aspect of the disclosure or the composition according to the second aspect of the disclosure to the subject.

Brief description of the figures

Fig. 1. Exemplary polyvalent molecule structures, with their respective codes.

Fig. 2. Exemplary polyvalent molecule structures, with their respective codes.

Fig. 3. Exemplary polyvalent molecule structures, with their respective codes.

Fig. 4. Reference molecule structures of DLin-MC3-DMA and ALC-0315.

Fig. 5. Schematic representation of the production process of exemplary RNA containing apolipoprotein nanoparticles (aNP). The lipidic components are solubilized in organic solvent and are mixed with RNA (for example siRNA) solubilized in buffered water at a low pH (for example pH 4). The formed particles are dialyzed in PBS pH 7.4

and then mixed with apolipoprotein (for example apolipoprotein A1). Finally, the particle solutions are filtered and centrifuged. The reference LNP nanoparticles contain the ionizable cationic lipid DLin-MC3-DMA and do not contain tri-caprylin (TG) nor apolipoprotein A1 (for LNPs the second mixing step is not required). The reference aNPs (containing DLin-MC3-DMA) and the exemplary aNPs (containing polyvalent ionizable molecules) according to the invention do not contain polyethylene glycol

CLAIMS

1. A nanoparticle comprising a core and an outer layer, wherein the core comprises: - a nucleic acid;

- a polyvalent molecule;

and wherein the outer layer comprises:

- an apolipoprotein, an apolipoprotein derivative, an apolipoprotein mimetic and/or an apolipoprotein mimetic derivative;

- a phospholipid;

- a sterol; and optionally

- a filler molecule,

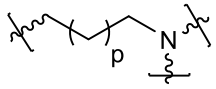
wherein the polyvalent molecule has formula (I):

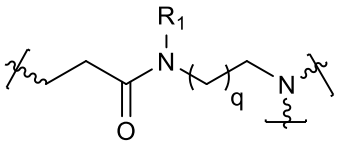
$$[core]_x – [BU]_y – [TU]_z \qquad (I)$$

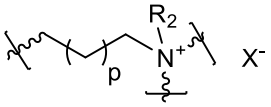
wherein the core is a nitrogen or is a C_1-C_{18} linear, branched, or cyclic group that contains 1 to 15 nitrogen heteroatoms and that optionally contains 1 to 4 oxygen heteroatoms; and

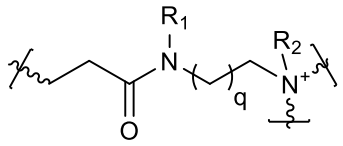
wherein x represents the number of connections from the core to the branching units BU (or to the terminal units TU, when y = 0), where x is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or 12, and where these connections are all made from nitrogen atoms of the core to carbon atoms of the branching unit BU (or of the terminal unit TU); and

wherein the branching unit BU has formula (IIa), (IIb), (IIc) or (IId):

(IIa) 

(IIb) 

(IIc) 

(IId) 

wherein (IIa) represents an n-alkylene-nitrogen spacer that is connected at the CH_2-end to the core or to a BU that is closer to the core, and that is connected at the N-end to two terminal units (TUs) or to two branching units (BUs) that are closer

to the TUs, where the connections are given in wavy bonds, and wherein p is 1, 2, 3 or 4; and

wherein (IIb) represents an amide containing n-alkylene-nitrogen spacer that is connected at the CH_2-end to the core or to a BU that is closer to the core, and that is connected at the N-end to two terminal units (TUs) or to two branching units (BUs) that are closer to the TUs, where the connections are given in wavy bonds, wherein q is 1, 2, 3, 4 or 5, and wherein R_1 is a hydrogen, a methyl, an ethyl, an n-propyl or an iso-propyl group; and

wherein (IIc) and (IId) are defined as (IIa) and (IIb), respectively, and wherein R_2 is a methyl, ethyl, n-propyl, benzyl, acetamide ($-CH_2-C(O)NH_2$) or 2-hydroxy-ethylene group, and wherein X^- is the counter anion to the quaternary amine cation moiety; and

wherein y represents the specific and discrete generation number of the polyvalent molecule, where this number indicates how many successive BU layers are incorporated in the polyvalent molecule, as counted from traveling from the inner core to the outer TU groups, and where y is 0, 1, 2, 3, 4 or 5; and

wherein for each separate generational layer the BU can independently be selected from formulas (IIa), (IIb), (IIc) or (IId), i.e. the first BU emanating from the core, representing the first generational layer BU, can be different from the second BU emanating further away from the core (representing the second generational layer BU), and this one may be different from the third, the fourth and the fifth BU emanating further away from the core; and

wherein the terminal units (TU) are individually and independently chosen from a hydrogen and a C_1-C_{30} alkyl, aryl, arylene-alkyl or alkylene-aryl group that optionally contains 1 to 8 heteroatoms that are individually and independently selected from the group consisting of O and N, with the proviso that not all TUs in the polyvalent molecule are hydrogens; and

wherein z represents the total number of TU groups that are attached to the polyvalent molecule, where z is 1 to 128; and

wherein the polyvalent molecule as according to formula (I) is polyvalent in positively ionizable and/or cationic groups, where the cumulative number of positively ionizable and cationic groups in the polyvalent molecule is 2 or higher.

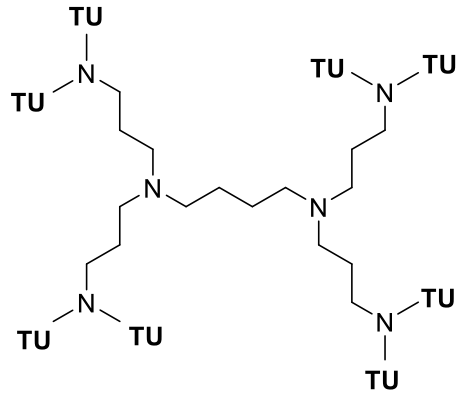
2. The nanoparticle according to claim 1, wherein the polyvalent molecule has formula (I):

$$[core]_x – [BU]_y – [TU]_z \qquad (I)$$

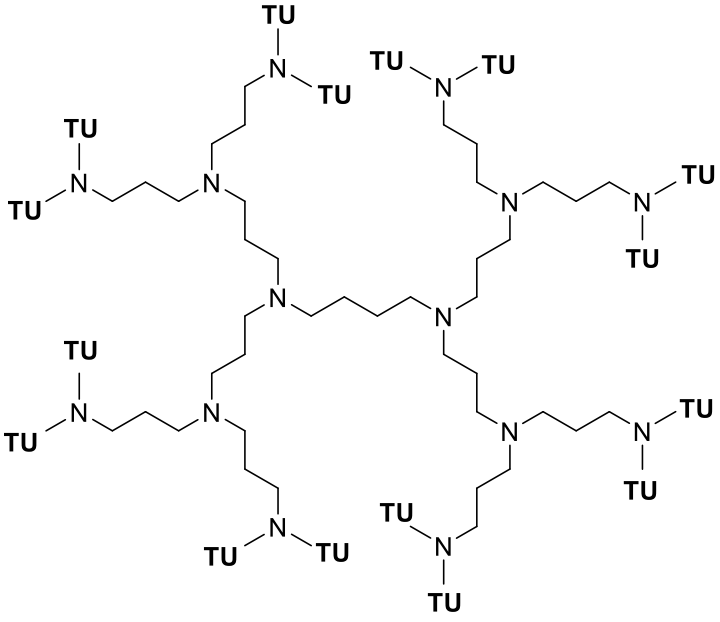
wherein y = 0, 1, 2, or 3.

3. The nanoparticle according to any one of the preceding claims, wherein the polyvalent molecule is a first, second or third generation dendrimer selected from a poly(propylene imine) (PPI) dendrimer or a polyamidoamine (PAMAM) dendrimer, most preferably a PPI dendrimer, or a modification thereof.

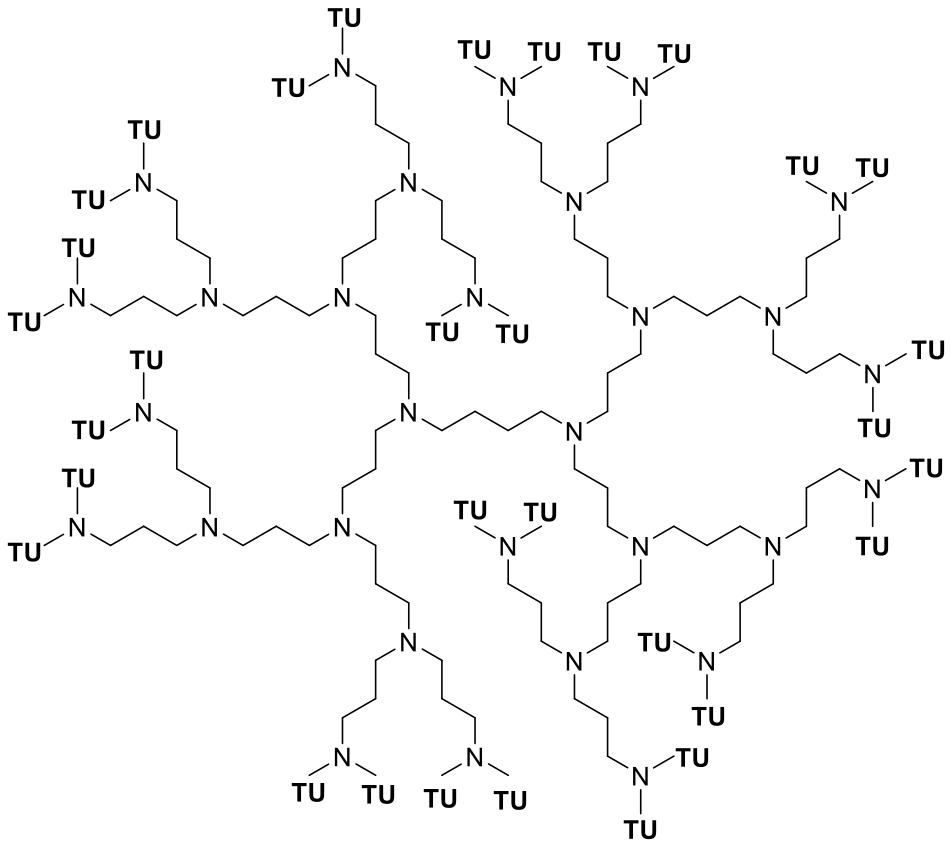
4. The nanoparticle according to any of the preceding claims, wherein the polyvalent molecule has a structure selected from:



or



or



wherein the terminal units (TU) are individually and independently chosen from a hydrogen and a C_1-C_{30} alkyl, aryl, arylene-alkyl or alkylene-aryl group that optionally contains 1 to 8 heteroatoms that are individually and independently selected from the group consisting of O and N, with the proviso that not all TUs in the polyvalent molecule are hydrogens.

5. The nanoparticle according to any one of the preceding claims, wherein the apolipoprotein, apolipoprotein derivative, apolipoprotein mimetic or apolipoprotein mimetic derivative is selected form apo A1, apo A1-Milano, apo A2, apo A4, apo A5, apo B48, apo B100, apo C-I, apo C-II, apo C-III, apo C-IV, apo D, apo E, apo F, apo H, apo L and apo M or a mimetic or derivatives thereof,

preferably selected from apo A1, apo A2, apo A4, apo A5, apo B100, apo C-I, apo C-II, apo C-III, apo C-IV and apo E or a mimetic or derivatives thereof,

more preferably selected from apo A1, apo A4, apo A5, apo B100, apo C-III and apo E or a mimetic or derivatives thereof,

most preferably selected from apo A1, apo B100 and apo E or a mimetic or derivatives thereof.

6. The nanoparticle according to any one of the preceding claims, wherein the nucleic acid is RNA, DNA, or a nucleic acid analogue,

preferably wherein the RNA is microRNA (miRNA), small interfering RNA (siRNA), piwi-interacting RNA (piRNA), small nuclear RNA (snoRNA), transfer RNA (tRNA), tRNA-derived small RNA (tsRNA), small regulatory RNA (srRNA), messenger RNA (mRNA), modified mRNA, ribosomal RNA (rRNA), self-amplifying RNA (saRNA), circular RNA (circRNA), long non-coding RNA (lncRNA), or guide RNA (gRNA) or combinations thereof and/or modifications thereof; or

preferably wherein the DNA is single stranded or double stranded DNA; or

preferably wherein the nucleic acid is an antisense oligonucleotide which is single stranded DNA or RNA consisting or comprising of nucleotide or nucleoside analogues containing modifications of the phosphodiester backbone or the 2' ribose, more preferably wherein the nucleotide or nucleoside analogues are selected from locked nucleic acid (LNA), bridged nucleic acid (BNA), morpholino or peptide nucleic acid (PNA).

7. The nanoparticle according to any one of the preceding claims, wherein the sterol is preferably selected from sterol, cholesterol, ergosterol, hopanoids, hydroxysteroid, phytosterol, steroids, zoosterol, stigmasterol, or β-sitosterol, or combinations thereof.

8. The nanoparticle according to any one of the preceding claims, wherein:
the phospholipid is selected from a phosphatidylcholine, a phosphatidylethanolamine, a phosphatidylserine and a phosphatidylglycerol or combinations thereof, preferably wherein at least one, more preferably both, of the acyl groups in the phospholipid are derived from long chain fatty acids, even more preferably wherein said long chain fatty acids are selected from lauric acid, lauroleic acid, myristic acid, myristoleic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid and linoleic acid, or combinations thereof.

9. The nanoparticle according to any one of the preceding claims, further comprising a filler molecule, preferably wherein the filler molecule is a glyceride molecule, more preferably wherein the filler molecule is selected from a triglyceride, a modified triglyceride, and a cholesteryl ester, or combinations thereof, preferably wherein the triglyceride is derived from C6-C18 fatty acids, preferably tricaprylin and/or wherein the cholesteryl ester is cholesteryl acetate, cholesteryl caprylate and/or cholesteryl oleate.

10. The nanoparticle according to any one of the preceding claims, wherein:
the amount of apolipoprotein, apolipoprotein derivative, apolipoprotein mimetic or apolipoprotein mimetic derivative ranges from 0.1 to 90 weight%; and/or
the amount of nucleic acid ranges from 0.01 to 90 weight%;

the amount of phospholipid ranges from 0.1 to 95 weight%; and/or
the amount of sterol ranges from 0.1 to 95 weight%; and/or
the amount of polyvalent molecule ranges from 0.1 to 95 weight%,
the amount of optionally present filler comprises ranges from 0 to 95 weight%,
wherein the weight percentages are based on the combined amounts of these five components plus the optional sixth filler component, i.e. these five or six components add up to 100% of the weight of the nanoparticle.

11. A composition comprising the nanoparticle according to any one of the preceding claims and a physiologically acceptable carrier, preferably wherein the composition is a pharmaceutical composition.

12. The nanoparticle according to any one of claims 1 to 10, or the composition according to claim 11 for use as a medicament.

13. The nanoparticle according to any one of claims 1 to 10, or the composition according to claim 11 for use in delivering a nucleic acid to the myeloid compartment or the spleen.

14. Use of the nanoparticle according to any one of claims 1 to 10, or the composition according to claim 11 in the manufacture of a medicament for the treatment of a disease by stimulating or inhibiting an innate immune response.

15. The use according to claim 14, wherein said disease is a cancer, a cardiovascular disease, an autoimmune disorder or xenograft rejection.

16. A method for producing a nanoparticle, comprising the step of:

a) mixing, preferably rapid mixing, of (lipid) components in organic solvent with a nucleic acid in an aqueous buffer to produce nanoparticles, wherein the (lipid) components comprise a phospholipid, a sterol, a polyvalent molecule, and optionally a filler molecule; and wherein the aqueous buffer has a pH of 5.5 or lower, preferably 5.0 or lower; and

b) mixing, preferably rapid mixing, of lipid nanoparticles with an apolipoprotein, an apolipoprotein derivative, an apolipoprotein mimetic and/or an apolipoprotein mimetic derivative, to produce the nanoparticle at a pH between 5.5 and 9.0, preferably at a pH between 6.0 and 8.0, more preferably at a pH between 6.5 and 8.0.

17. The nanoparticle according to any one of claim 1 to 10, wherein the nanoparticle is obtainable or obtained by the method of claim 16.

18. An *in vitro* or *ex vivo* method for introducing a nucleic acid in a cell, the method comprising contacting the nanoparticle according to any one of claims 1 to 10 or the composition according to claim 11 with a cell.

19. Use of the nanoparticle according to any one of claims 1 to 10 or the composition according to claim 11 in the manufacture of a medicament for the *in vivo* delivery of a nucleic acid to a subject.