and clinical development as a drug (in particular against cancer), while improving patient compliance and reducing the frequency of dosing double-stranded polyribonucleotide molecules with well-defined safety margin and therapeutic effects.

SUMMARY

The present disclosure relates to a composition comprising particles wherein
(i) each of said particles comprises a complex of at least one double-stranded polyribonucleotide, or a salt or solvate thereof, and at least one polyalkyleneimine, or a salt and/or solvate thereof;
(ii) at least 95%, or at least 90%, of said particles has a diameter of less than or equal to 600 nm, preferably, less than or equal to 300 nm (for example, between 140 and 250 nm); and
(iii) said particles have a z-average diameter of less than or equal to 200 nm, preferably less than or equal to 150 nm, in particular, as measured according to ISO 22412.

In one aspect, the present disclosure refers to a polyinosinic-polycytidylic acid [poly(I:C)] molecule composition comprising double-stranded polyribonucleotides formed by single-stranded poly(I) and poly(C) molecules, respectively, or a salt and/or solvate thereof, wherein (a) between 5% and 60%, preferably between 10% and 30%, of the double-stranded polyribonucleotides have less than 400 base pairs; (b) between 20% and 45% or between 15% and 30%, preferably between 20% and 30%, of the double-stranded polyribonucleotides, have between 400 and 850 base pairs; (c) between 20% and 70%, preferably between 50% and 60%, of the double-stranded polyribonucleotides have between 850 and 5000 base pairs; and (d) between 0% and 10%, preferably 1% or less, of the double-stranded polyribonucleotides have more than 5000 base pairs.

In one aspect, the present disclosure refers to a polyinosinic-polycytidylic acid [poly(I:C)] molecule composition comprising double-stranded nucleotides formed by single-stranded poly(I) and poly(C) molecules respectively, or a salt and/or solvate thereof, wherein the composition is obtainable by: (a) dissolving the poly(I) molecules in PBS to form a first aqueous solution; (b) dissolving the poly(C) molecules in PBS to form a second aqueous solution; (c) mixing the first aqueous solution and the second aqueous solution at a 1:1 stoichiometry; (d) applying the resulting solution containing double-stranded poly(I:C) on a GPC (Gel Permeation Chromatography) column.

In a preferred example, the present disclosure relates to an aqueous composition comprising particles wherein
(i) each of said particles comprises a complex of at least one double-stranded polyribonucleotide, or a salt or solvate thereof, and at least one linear polyalkyleneimine, or a salt and/or solvate thereof, wherein said double-stranded polyribonucleotide is polyinosinic-polycytidylic acid [poly(I:C)] and the average molecular weight of said linear polyalkyleneimine is between 17 and 23 kDa;
(ii) at least 90% of said particles has a mono-modal diameter distribution below 300 nm;
(iii) said particles have a z-average diameter of less than or equal to 150 nm, as measured according to ISO 22412; and
(iv) said composition has a zeta potential equal or superior to 30 mV, according to ISO 13099.

The present disclosure also relates to an aqueous composition comprising particles as disclosed herein wherein:
(i) each of said particles is formed by making a complex of at least one double-stranded polyribonucleotide, or a salt or solvate thereof, and at least one linear polyalkyleneimine, or a salt and/or solvate thereof, wherein said double-stranded polyribonucleotide is polyinosinic-polycytidylic acid [poly(I:C)] and the average molecular weight of said linear polyalkyleneimine is between 17 and 23 kDa;
(ii) at least 90% of said particles has a mono-modal diameter below 300 nm;
(iii) said particles have a z-average diameter of less than or equal to 150 nm, as measured according to ISO 22412; and
(iv) said composition has a zeta potential equal or superior to 30 mV, according to ISO 13099;

wherein said particles are formed at the ratio of the number of moles of nitrogen of said polyalkyleneimine to the number of moles of phosphorus of said double-stranded polyribonucleotide in said composition being equal to or greater than 2.5.

The present disclosure also relates to a composition obtainable by lyophilisation of the aqueous composition as disclosed herein.

In addition, the present disclosure relates to a composition, as disclosed herein, for use as a medicament.

Moreover, the present disclosure relates to a composition, as disclosed herein, for use in treatment of a cell growth disorder characterized by abnormal growth of human or animal cells.

Furthermore, the present disclosure relates to a process to manufacture the composition, as disclosed herein, which comprises:

(i) preparing an aqueous solution of at least one double-stranded polyribonucleotide, or a salt or solvate thereof, and an aqueous solution of at least one polyalkyleneimine, or a salt or solvate thereof, wherein either or both solutions optionally further comprise a pharmaceutically acceptable carrier, organic solvent, excipient and/or adjuvant;

(ii) independently filtering each solution through a filter having a pore diameter of less than or equal to 500 nm to form sterilized solutions;

(iii) mixing the resulting sterilized solutions in a mixing chamber to form an aqueous composition by addition of one of said solutions into the other solution in said mixing chamber, optionally by injection, at a rate of greater than or equal to 1 mL/min, or by simultaneous addition of each of said solutions into said mixing chamber, optionally by injection, at a rate of greater than or equal to 1 mL/min; and optionally

(iv) filtering the resulting aqueous composition through a filter having a pore diameter of less than or equal to 600 nm to form a filtrate, or centrifuging the resulting aqueous composition at greater than or equal to 22480 m/s^2 to form a supernatant; and/or

(v) lyophilising the resulting aqueous composition, filtrate or supernatant.

In one aspect, the present disclosure refers to a method for producing the composition disclosed herein, comprising: (a) dissolving the poly(I) molecules in PBS to form a first aqueous solution; (b) separately dissolving the poly(C) molecules in PBS to form a second aqueous solution; (c) filtering each respective solution obtained from step (a) and step (b) using a membrane; (d) concentrating each respective permeate obtained for each respective filtration step (c) for each respective solution over a membrane to form a respective retentate for each respective solution; (e) mixing each respective retentate obtained for each respective the first aqueous solution (a) and the second aqueous solution (b) with a PBS solution; (f) determining the optical density of each respective retentate obtained in step (e) for each respective solution (a) and (b) and adjusting the total volume to a 1:1 stoichiometry; (g) combining the respective poly(I) solution with the poly(C) solution obtained at step (e) using heat; (h) cooling the resulting combined solution to room temperature; (i) filtering the solution obtained from step (h); (j) applying the solution obtained from step (i) on a gel permeation column (GPC); and (k) purifying and desalting the resulting solution.

In one aspect, the present disclosure refers to a method for producing the composition disclosed herein, comprising: (a) dissolving the poly(I) molecules in PBS to form a first solution, wherein (i) 80-95% poly(I) molecules comprises less than 400 bases, (ii) 5-20% poly(I) molecules comprises 400-850 bases, (iii) 0-5% poly(I) molecules comprises 850-5000 bases, and (iv) 1% or less poly(I) molecules comprises at least 5000 bases; (b) separately dissolving the poly(C) molecules in PBS to form a second solution, wherein (i) 20-82% poly(C) molecules comprises less than 400 bases, (ii) 15-40% poly(C) molecules comprises 400-850 bases, (iii) 3-50% poly(C) molecules comprises 850-5000 bases, and (iv) 1% or less of poly(C) molecules comprises at least 5000 bases; (c) filtering each respective solution obtained from step (a) and step (b) using a membrane; (d) concentrating each respective permeate obtained for each respective filtration step (c) for each respective solution over a membrane to form a respective retentate for each respective solution; (e) mixing each respective retentate obtained for each respective solution (a) and (b) with a PBS solution; (f) determining the optical density of each respective retentate obtained in step (e) for each respective solution (a) and (b) and adjusting the total volume to a 1:1 stoichiometry; (g) combining the respective poly(I) solution with the poly(C) solution obtained at step (e) using heat; (h) cooling the resulting combined solution to room temperature; (i) filtering the solution obtained from step (h); (j) applying the solution obtained from step (i) on a gel permeation column (GPC); and (k) purifying and desalting the resulting solution.

In one aspect, the present disclosure refers to the composition obtained by the method disclosed herein, wherein glucose or mannitol is added to the solution before step (i).

Preferably, the present disclosure also relates to a composition comprising particles wherein:

(i) each of said particles comprises a complex of at least one double-stranded polyribonucleotide, or a salt or solvate thereof, and at least one polyalkyleneimine, or a salt and/or solvate thereof, wherein

(a) said double-stranded polyribonucleotide is polyinosinic-polycytidylic acid [poly(I:C)] or polyadenylic-polyuridylic acid [poly(A:U)], wherein at least 60% of said double-stranded polyribonucleotides have at least 850 base pairs, at least 70% of said double-stranded polyribonucleotides have between 400 and 5000 base pairs, and between 20% and 45% of said double-stranded polyribonucleotides have between 400 and 850 base pairs; and

(b) said polyalkyleneimine comprises at least 95% polyethyleneimines, wherein the weight average molecular weight of said polyalkyleneimine is between 17 and 23 kDa and the polydispersity index is < 1.5, and wherein the ratio of the number of moles of nitrogen of said polyalkyleneimine to the number of moles of phosphorus of said double-stranded polyribonucleotide in said composition is between 2.5 and 5.5;

(ii) at least 99% of said particles has a diameter of less than or equal to 600 nm; and

(iii) said particles have a z-average diameter of between 30 nm and 150 nm.

More preferably, the present disclosure also relates to an aqueous composition which comprises particles wherein:

(i) each of said particles comprises a complex of at least one double-stranded polyribonucleotide, or a salt or solvate thereof, and at least one polyalkyleneimine, or a salt and/or solvate thereof, wherein

(a) said double-stranded polyribonucleotide is polyinosinic-polycytidylic acid [poly(I:C)], wherein at least 60% of said poly(I:C) has at least 850 base pairs, at least 70% of said poly(I:C)

has between 400 and 5000 base pairs, and between 20% and 45% of said poly(I:C) has between 400 and 850 base pairs; and

(b) said polyalkyleneimine is polyethyleneimine (PEI), wherein the weight average molecular weight of said PEI is between 17.5 and 22.6 kDa and the polydispersity index is < 1.5, and wherein the ratio of the number of moles of nitrogen of said polyalkyleneimine to the number of moles of phosphorus of said double-stranded polyribonucleotide in said composition is between 2.5 and 4.5;

(ii) at least 99% of said particles has a diameter of less than or equal to 500 nm;

(iii) said particles have a z-average diameter of between 60 nm and 130 nm; and

(iv) said particles have a median diameter (D50%) of between 75 nm and 150 nm.

Further examples related to the preparation of such compositions in form of BO-11X formulations, their features, their analysis and their uses are provided in the Detailed Description and in the Examples below.

DESCRIPTION OF THE FIGURES

Figure 1: Functional activity of distinct BO-111 preparations following filtration or centrifugation by analyzing cell death in melanoma cells SK-Mel-103 after a 48-hour exposure. (**A**) Poly(I:C) molecules are associated with PEI to form BO-111 complexes according to the description of the 2-vial manufacturing processing in Example 1, and the resulting solution is either filtered using membrane with different pore sizes (and then using the flow-through solution for cell-based assays) or centrifuged at different speeds (and then using the supernatant for cell-based assays), generating distinct BO-111 preparations, each of them containing poly(I:C)-based complexes with a more homogeneous, lower size that is determined as Z-average (hydrodynamic diameter, expressed as d.nm). Qualitatively similar data were generated by testing cell death at 24 hours. Data on cell death are compared with sample not treated with BO-111. (**B**) Dose-response activity of a BO-111 preparation that is obtained by filtering through 0.8µm filter (hatched bars) when compared to the corresponding unfiltered BO-111 preparation (white bars). Since centrifugation or filtration causes some loss of poly(I:C) due to the sedimentation or retention of bigger complexes (respectively), the amount of poly(I:C) in each distinct BO-111 preparation was quantified by spectrophotometric analysis at wavelength of 260 nm, in order to compare correctly the effects of such preparation on cells by diluting accordingly the initial preparation at the appropriate concentration. Qualitatively similar data were generated by testing cell death at 12, 24, and 36 hours. Cell death is determined by Trypan Blue assay as described in the literature. Standard deviation is calculated using triplicate data for each condition of the same experiment.

Figure 2: Effects of PEI features on BO-111 functional activity, as determined using the same cell-based assay and BO-111 concentration of Figure 1. (**A**) Distinct BO-111 preparations were obtained by using commercial JetPEI preparations containing linear polymers having different molecular weight (expressed in kiloDalton; NT: control, not treated cells; such JetPEI preparations present a mono-modal distribution with polydispersity index that is inferior to 1.5, in particular comprised between 1.1 and 1.4). (**B**) Distinct BO-111 preparations were obtained by modifying the ratio N/P (from 1.2 to 5.3) that is calculated as: µL PEI x 150 nM/µg poly(I:C) x 3 nmol, wherein 150 nM refers to the concentration of nitrogen residues in PEI and 3 nmol refers to the number of nanomoles of anionic phosphate per 1 µg poly(I:C). The pharmacological effect of each combination is determined by comparing cell death percentages in both melanoma cells and