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(54) Title: PHARMACEUTICAL COMPOSITIONS COMPRISING INTEGRATION-PROMOTING PEPTIDES

(57) Abstract: The present invention provides pharmaceutical compositions of an integration-promoting peptide and hydroxyl halide salts of said peptide. The pharmaceutical compositions of the present invention are stable and maintain the peptide soluble upon administration. Methods of treating viral infection and cancer with the peptide salts and compositions thereof are also provided.



WO 2020/039445 A1

PHARMACEUTICAL COMPOSITIONS COMPRISING INTEGRATION- PROMOTING PEPTIDES

FIELD OF THE INVENTION

5 The present invention relates to stable pharmaceutical compositions of integration-promoting peptides and to halide salts of said peptides.

BACKGROUND OF THE INVENTION

10 It was previously suggested that by cooperation between retrovirus-derived integrase enzymes, accessory DNA and integration promoting agents, the integrase enzymes are capable of translocating into the nuclei of target cells, where they induce double-stranded breaks (DSBs) in the chromosomal DNA. It was hypothesized that the accumulation of DSBs exhausts the natural DNA repair capabilities of the cell, and drive the cell to apoptosis. WO 2010/041241 describes several integration-promoting peptides derived
15 from the HIV-1 integrase protein, and their effect on HIV-infected cells.

 WO 2018/215999 further exemplified use of such integration-promoting peptides in methods for inducing DNA breaks in specifically-targeted cells, in particular cancer and HIV-infected cells, thereby promoting cell death.

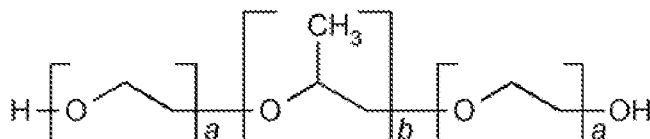
20 Peptide compositions are inherently unstable due to sensitivity towards chemical and physical degradation. Chemical degradation involves change of covalent bonds, oxidation, hydrolysis, racemization or crosslinking. Physical degradation involves conformational changes relative to the native structure of the peptide, i.e. secondary and tertiary structure, such as aggregation, precipitation or adsorption to surfaces. In addition, many peptides, particularly hydrophobic ones, have low solubility in physiological conditions. There is a
25 need for development of pharmaceutical compositions and formulations which allow high stability and bioavailability and convenient administration of such peptides.

SUMMARY OF THE INVENTION

30 The present invention is directed to stable pharmaceutical composition for parenteral administration of specific peptides, in particular peptides comprising the amino acid sequence TAVQMAVFIHNFKRK (SEQ ID NO: 2) derived from the HIV-1 protein integrase. These peptides were previously shown as being insoluble in physiological pH and osmolarity conditions. The compositions of the present invention comprise, according

to some embodiments, from 0.1 to 30 mg/ml of the peptide or a salt thereof, and are in conformity with requirements from parenteral compositions, i.e. the composition is isotonic and has a physiologically acceptable pH between 4 to 9. Specifically, intravenous and subcutaneous compositions are provided as well as solid compositions. Also provided are halide salts of the peptides used in the compositions.

According to one aspect, the present invention provides a pharmaceutical composition comprising from about 0.1 to about 30 mg/ml of a salt of a peptide of 15 to 30 amino acids that comprises the sequence TAVQMAVFIHNFKRK (SEQ ID NO: 2), or of a derivative, fragment or analog thereof, from about 0.3 wt% to about 15 wt% of a poloxamer having the structure of Formula I,



Formula I,

wherein a is an integer from 50 to 120, and b is an integer from 15 to 40 and a pharmaceutically acceptable carrier, wherein the pH of the composition is between 4 to about 7.5.

According to some embodiments, the peptide comprises the amino acid sequence WTAVQMAVFIHNFKRK (SEQ ID NO: 1), or a derivative or analog thereof.

According to some embodiments, the peptide consists of the amino acid sequence WTAVQMAVFIHNFKRK (SEQ ID NO: 1, also denoted hereinafter "INS").

According to some embodiments, the peptide contained in the pharmaceutical compositions is in the form of halide salt. According to specific embodiments, the salt of the peptide is a hydrochloride salt. According to specific embodiments, the salt of the INS is a hydrochloride salt (INS HCl).

According to some embodiments, the composition comprises from about 1 mg/ml to about 20 mg/ml of INS HCl.

According to another embodiment, a in Formula I is an integer from 60 to 90 and b is an integer from 25 to 35. In some particular embodiments, $a=80$ and $b=27$ (poloxamer 188). According to some embodiments, the pharmaceutical composition comprises from about 0.1 wt% to about 10 wt% of poloxamer 188. According to some embodiments, the composition comprises from about 1 to about 10 wt% of poloxamer 188 and from about 5 mg/ml to about 15 mg/ml of the peptide INS HCl. According to other embodiments, the

pharmaceutical composition comprises from about 0.5 wt% to about 5 wt% of poloxamer 188 and/or from about 1 mg/ml to about 10 mg/ml of the peptide INS HCl.

A pharmaceutical composition according to some embodiments further comprises a saccharide. According to some embodiments, the saccharide is selected from a disaccharide and polysaccharide. According to some embodiment, the composition comprises from about 1 wt% to about 20 wt% of the saccharide. According to other embodiments, the composition comprises from about 5 wt% to about 12 wt% of the saccharide. According to some embodiments, the saccharide is a disaccharide. According to a specific embodiment, the disaccharide is lactose.

According to some embodiments, the present invention provides a pharmaceutical composition comprising from about 0.5 to about 10 wt% of poloxamer 188, from about 5 mg/ml to about 15 mg/ml of HCl salt of a peptide consisting of the amino acid sequence WTAVQMAVFIHNFKRK (SEQ ID NO: 1), and from about 5 to about 12 wt% of lactose.

According to other embodiments, the pharmaceutical composition comprises from about 8 to 12 wt% lactose, from about 0.5 to about 2 wt% of poloxamer 188, and from about 1 mg/ml to about 10 mg/ml of INS HCl. According to further embodiments, the pharmaceutical composition comprises from about 8 to 12 wt% lactose, from about 0.8 to about 2 wt% of poloxamer 188, and from about 5 mg/ml to about 10 mg/ml of INS HCl. In yet other embodiments, the pharmaceutical composition comprises from about 8 to 12 wt% lactose, from about 0.8 to about 2 wt% of poloxamer 188, and from about 1 mg/ml to about 15 mg/ml of INS HCl.

According to some embodiments, the pharmaceutical composition further comprises lentivirus particles.

According to some embodiments, the pharmaceutical composition of the present invention further comprises a targeting moiety, e.g. a moiety that directs the active ingredients to a specific population of cells.

According to some embodiments, the composition is a liquid or semi-liquid composition. According to another embodiment, the composition is a solution. According to specific embodiments the solution is aqueous solution. According to yet another embodiment, the composition is a stable composition.

According to one embodiment, the composition is a parenteral composition, i.e. a composition suitable for parenteral administration such as intravenous, intramuscular and subcutaneous administration.

According to some embodiments, the composition further comprising an additional active agent. According to some embodiments, the active agent is an anti-viral agent. According to some specific embodiments, the anti-viral agent is a protease inhibitor. According to yet other embodiments, the additional active agent is an anti-cancer agent.

5 According to some embodiment, the pharmaceutical composition is a solid composition. According to some embodiments, the solid composition is a lyophilized composition. According to some embodiments, the solid composition is in the form of powder.

10 According to another aspect, the present invention provides a stable and soluble pharmaceutical composition comprising the peptide INS (SEQ ID NO: 1), for use in treating a disease, wherein treating comprises destroying a specific population of target cells. According to some embodiments, the composition is for use in treating subjects positive for HIV-1 and for treatment of acquired immune deficiency syndrome (AIDS). According to some embodiments, the composition is for use in treating cancer.

15 According to some embodiments, the composition for use of the present invention is administered parenterally. According to some embodiments, the composition for use is administered via a route selected from the group consisting of: intramuscular (IM), intravenous (IV) and subcutaneous (SC). According to yet other embodiments, the composition is administered directly to the diseased or injured site, for example
20 intratumorally.

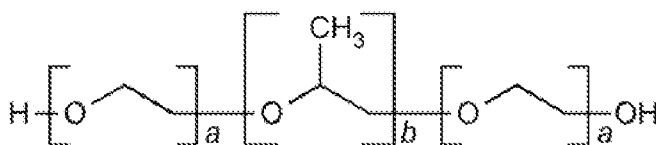
 According to some embodiments, the composition is administered in combination with: (i) a linear molecule of double-stranded DNA (dsDNA) comprising long term repeat (LTR) sequences recognized by the integrating enzyme of (ii), and (ii) an integrating enzyme, capable of entering the nuclei of cells after binding to the LTR sequences of the
25 dsDNA molecule of (i) and creating multiple double strand breaks (DSBs) in the chromosomal DNA of the cells. According to some embodiments, the linear molecule of dsDNA comprising LTR sequences recognized by the integrating enzyme is part of a lentivirus particle.

 According to some embodiments, the composition is administered in combination
30 with a targeting moiety. According to some embodiments the targeting moiety is a molecule capable of binding to a cell receptor or marker. According to some embodiments the targeting moiety is an antibody capable of binding to a tumor antigen. According to a specific embodiment, the targeting moiety is an antibody against CD24.

According to some embodiments, the composition is administered together with lentiviruses or lentivirus particles.

According to some embodiment, the composition is administered in combination with (i) a lentivirus particle comprising a linear molecule of double-stranded DNA (dsDNA) comprising long term repeat (LTR) sequences recognized by the integrating enzyme of (ii), (ii) an integrase, capable of entering the nuclei of cells after binding to the LTR sequences of the dsDNA molecule of (i) and creating multiple double strand breaks (DSBs) in the chromosomal DNA of the cells, and (iii) an antibody capable of binding CD24 expressed in cancer cells.

According to a further aspect, the present invention provides a solid pharmaceutical composition comprising a salt of a peptide consisting of the amino acid sequence of SEQ ID NO: 1, and a poloxamer having a structure of Formula I,



Formula I,

wherein a is an integer from 50 to 120, and b is an integer from 15 to 40 and wherein the weight ratio between said salt and said poloxamer is from about 10:5 to about 1:1500. According to some embodiments, the salt of said peptide is hydrochloride salt (INS HCl) and the poloxamer is poloxamer 188. According to other embodiments, the weight ratio between said salt and said poloxamer 188 is from about 10:1 to about 1:1000. According to some embodiments, the weight ratio in said solid composition between said salt and said poloxamer 188 is from about 2:1 to about 1:20 or from about 1:1 to about 1:5. According to some embodiments, the solid pharmaceutical composition further comprises a saccharide selected from a disaccharide and polysaccharide. In some embodiments, the disaccharide is lactose. According to one embodiment, the weight ratio between said salt and lactose is from about 4:1 to about 1:2000. According to some embodiments, the weight ratio between said salt and poloxamer 188 is between about 3:1 to 1:20 and the weight ratio between said salt and lactose is between about 4:1 to about 1:24. According to some embodiments, the solid pharmaceutical composition is a lyophilized composition.

According to one embodiment, the solid pharmaceutical composition of the present invention upon reconstitution provides a stable parenteral pharmaceutical composition

according to the present invention. Thus, according to some embodiments, the present invention provides reconstituted pharmaceutical compositions suitable for parenteral administration. According to one embodiment, such a reconstituted pharmaceutical composition is for use in treating a disease selected from cancer, HIV-1 infection and AIDS.

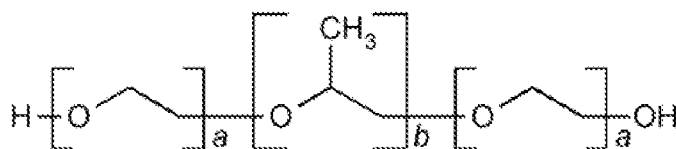
According to another aspect, the present invention provides a hydrogen halide salt of a peptide comprising the amino acid sequence of SEQ ID NO: 2, wherein said peptide consists of from 15 to 30 amino acids. According to some embodiments, the peptide comprises the amino acid sequence of SEQ ID NO: 1. According to some embodiments, the peptide consists of an amino acid sequence selected from SEQ ID NO: 1 and SEQ ID NO: 2. According to some embodiments, the hydrogen halide salt is a hydrochloride salt. In some embodiments, the present invention provides a hydrochloride salt of the peptide denoted INS consisting of the amino acid sequence set forth in SEQ ID NO: 1.

The present invention also provides methods of treating a disease comprising administering to a subject in need thereof a stable pharmaceutical composition comprising therapeutically effective amount of the peptide INS, or of a salt thereof. According to some embodiment, the subject is a human subject.

According to some embodiments, treatment results in destroying a specific population of target cells. According to some embodiments the specific population of target cells comprises cells selected from HIV-infected cells and tumor cells.

According to some embodiments, the composition is administered parenterally. According to some embodiment, the treated disease is selected from viral infection and cancer. According to some embodiments, the viral infection is HIV-1 infection. According to a specific embodiment, the treated disease is AIDS caused by HIV-1 infection. According to some embodiments, the method of treatment is part of a treatment regimen comprising administration of at least one protease inhibitor. According to some embodiments, the treatment method is part of a regimen comprising administration of a cocktail of at least two anti-HIV-1 agents.

According to some embodiments, a method of treatment an HIV-1 infection is provided comprising administering to a subject in need thereof a pharmaceutical composition comprising a salt of a peptide consisting of the amino acid sequence of SEQ ID NO: 1, and a poloxamer having a structure of Formula I,



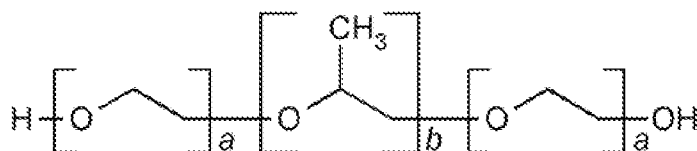
Formula I,

wherein a is an integer from 50 to 120, and b is an integer from 15 to 40 and wherein the weight ratio between said salt and said poloxamer is from about 10:5 to about 1:1500, in combination with treatment with at least one additional anti-HIV-1 agent.

5 According to some embodiments, a method of treatment an HIV-1 infection is provided comprising administering to a subject in need thereof a pharmaceutical composition comprising 0.5 to about 10 wt% of poloxamer 188, from about 5 mg/ml to about 15 mg/ml of HCl salt of a peptide consisting of the amino acid sequence WTAVQMAVFIHNFKRK (SEQ ID NO: 1), and from about 5 to about 12 wt% of lactose.

10 According to some embodiments, the method of treating HIV-1 infection further comprising administering to said subject at least one protease inhibitor. According some specific embodiments, the method comprises administering of the protease inhibitors lopinavir and ritonavir.

15 According to some embodiment, a method of treating cancer is provided comprising administering to a subject in need thereof, a pharmaceutical composition comprising a salt of a peptide consisting of the amino acid sequence of SEQ ID NO: 1, and a poloxamer having a structure of Formula I,



Formula I,

20 wherein a is an integer from 50 to 120, and b is an integer from 15 to 40 and wherein the weight ratio between said salt and said poloxamer is from about 10:5 to about 1:1500.

25 According to a specific embodiment, the method of treatment cancer comprises administering to a subject in need thereof a pharmaceutical composition comprising 0.5 to about 10 wt% of poloxamer 188, from about 5 mg/ml to about 15 mg/ml of HCl salt of a peptide consisting of the amino acid sequence WTAVQMAVFIHNFKRK (SEQ ID NO: 1), and from about 5 to about 12 wt% of lactose.

According to some embodiments, the method of treating cancer comprises co-administration of (i) a linear molecule of double-stranded DNA (dsDNA) comprising long term repeat (LTR) sequences recognized by the integrating enzyme of (ii), and (ii) an integrating enzyme, capable of entering the nuclei of cells after binding to the LTR sequences of the dsDNA molecule of (i) and creating multiple double strand breaks (DSBs) in the chromosomal DNA of the cells. According to some embodiments, the linear molecule of dsDNA comprising LTR sequences recognized by the integrating enzyme is part of a lentivirus particle.

According to some embodiments, the method of treating cancer comprises administering a composition according to the present invention in combination with a targeting moiety. According to some embodiments the targeting moiety is a molecule capable of binding to a cell receptor or marker. According to some embodiments the targeting moiety is an antibody capable of binding to a tumor antigen. According to a specific embodiment, the targeting moiety is an antibody against CD24.

According to some embodiments, the method of treating cancer comprises administering a pharmaceutical composition comprising from about 8 to 12 wt% lactose, from about 0.8 to about 2 wt% of poloxamer 188, and from about 1 mg/ml to about 15 mg/ml of the peptide INS HCl, in combination with (i) a lentivirus particle comprising a linear molecule of double-stranded DNA (dsDNA) comprising long term repeat (LTR) sequences recognized by the integrating enzyme of (ii), (ii) an integrase, capable of entering the nuclei of cells after binding to the LTR sequences of the dsDNA molecule of (i) and creating multiple double strand breaks (DSBs) in the chromosomal DNA of the cells, and (iii) an antibody capable of binding a tumor antigen expressed in cancer cells. According to some embodiments, the antibody is directed to human CD24.

According to some embodiments the cancer is a solid cancer. According to yet other embodiments, the cancer is selected from the group consisting of: sarcoma, lung cancer and pancreatic cancer. According to other embodiments, the cancer treatable with the compositions of the present invention is characterized by over expression of CD24. According to specific embodiments, the cancer characterized by over expression of CD24 is selected from the group consisting of: ovarian cancer, breast cancer, prostate cancer, bladder cancer, renal cancer, pancreatic cancer, lung cancer, sarcoma and nonsmall cell carcinoma.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1 shows HPLC chromatograms of the peptide INS (SEQ ID NO: 1) in water.

Figure 2 shows images of selected formulations comprising 5 mg/ml of the peptide INS (SEQ ID NO: 1) F4, F7 and F10 (numbers 1, 3, and 5 in the figure, respectively) and their dilution in PBS (numbers 2, 4, and 6 in the figure, respectively).

Figure 3 shows HPLC chromatograms of the Formulation F6 at t=0 and at t=24h.

Figure 4 shows HPLC chromatograms of Formulation F4 maintained at room temperature or at 4° at t=0 and at t=24h.

Figure 5 shows HPLC chromatograms of Formulation F7 maintained at room temperature or at 4° at t=0 and at t=24h.

Figure 6 shows HPLC chromatograms of Formulation F10 maintained at room temperature or at 4°C at t=0 and at t=24h.

Figure 7 shows HPLC chromatograms of Formulation F43 (5 mg/ml of INS) maintained at different conditions at t=0 (7) , t=24h (6: 5°C; 5: 25°C) and after 1 week (2: 25°C; 3: 5°C) or lyophilized (1: -20°C 7 days; 4: -20°C 1 day); 8 – INS standard 0.5 mg/ml.

Figure 8 shows HPLC chromatograms of Formulation F44 maintained at different conditions at t=0(7), t=24h (6: 5°C; 5: 25°C) and after 1 week (2: 25°C; 3: 5°C) or lyophilized (1: -20°C 7 days; 4: -20°C 1 day); 8 – INS standard 0.5 mg/ml.

Figure 9 shows dynamic light scattering (DLS) image of Formulation 43 at initial state; mean density 1.4 nm.

Figure 10 shows DLS image of Formulation 43 maintained at 4°C for 1 week; mean density 12 nm.

Figure 11 shows DLS image of Formulation 43 maintained at 25°C for 1 week; mean density 14.9 nm.

Figure 12 shows DLS image of Formulation 43 maintained at -20°C for 1 week; mean density 13.4 nm.

Figure 13 shows DLS image of Formulation 44 at initial state; mean density 2.8 nm.

Figure 14 shows DLS image of Formulation 44 maintained at 4°C for 1 week; mean density 14.5 nm.

Figure 15 shows DLS image of Formulation 44 maintained at 25°C for 1 week; mean density 10.8 nm.

Figure 16 shows DLS image of Formulation 44 maintained at -20°C for 1 week; mean density 13.4 nm.

Figures 17A and 17B show images of Lyophilized formulation F43 (**Fig. 17A**) and its reconstituted solution (**Fig. 17B**).

Figure 18 shows HPLC chromatograms of lyophilized-reconstituted formulation at t=0 (5- filtered 0.22µm; and 6 – non-unfiltered),, t=24h (3- filtered 0.22µm; and 4 – non-unfiltered), and t=7 days (1- filtered 0.22µm; and 2 – non-unfiltered); 7 – INS standard; 8 – blank: 9.5wt% lactose + 0.8 wt% poloxamer P188.

Figure 19 shows the efficacy of INS treatment, together with lentiviruses, in lung cancer model.

Figure 20 shows the efficacy of INS treatment, together with lentiviruses, in pancreatic cancer model at day 17.

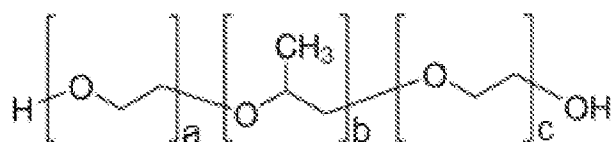
Figures 21A and 21B show representative examples from two different HIV patients, of viral (HIV-1) load in human subjects after 5 weeks of twice weekly SC administration of INS, 0.05-0.2 mg/kg for the patient presented in Figure 21A and 0.2-0.4 mg/kg for the patient presented by Figure 21B, in a formulation comprising 1% poloxamer 188 and 9% lactose anhydrous formulation at pH ~5.

Figures 22A and 22B show representative examples from two different HIV patients (001/A012 and 007/A028), of viral (HIV-1) load in two human subjects after 5 weeks of SC administration of 0.2 or 0.4 mg/kg INS, in a formulation comprising 1% poloxamer 188 and 9% lactose anhydrous formulation at pH ~5, twice a week, in combination with two antiviral drugs, lopinavir 800 mg plus ritonavir 200 mg daily.

Figure 23 shows a representative example of CD4 T-cell count in an HIV patient (009/A056) treated only with INS (0.2-0.4mg/kg) for 5 weeks and additional 5 weeks with INS (0.4mg/kg), in combination with lopinavir 800 and ritonavir as described above.

DETAILED DESCRIPTION OF THE INVENTION

According to one aspect, the present invention provides a pharmaceutical composition comprising from about 0.1 to about 30 mg/ml of a salt of a peptide comprising the amino acid sequence set forth in SEQ ID NO: 2 (TAVQMAVFIHNFKRK), from about 0.3 wt% to about 15 wt% of a poloxamer having the structure of Formula O,



Formula O,

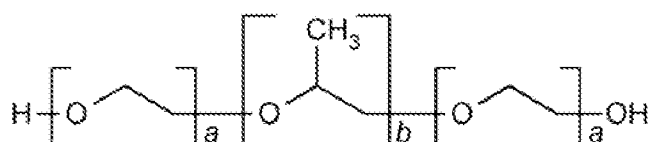
and a pharmaceutically acceptable carrier, wherein the pH of the composition is between 4 to about 7.5 and wherein a and c are each independently an integer from 50 to 120, and b is an integer from 15 to 40, and the peptide consists of 15 to 40 amino acids.

The term “poloxamer” as used herein refer to non-ionic poly (ethylene oxide) (PEO)–poly (propylene oxide) (PPO) copolymers.

According to one embodiment, the peptide comprises the amino acid sequence set forth in SEQ ID NO: 1 (WTAVQMAVFIHNFKRK). According to some embodiments, the peptide consists of 10 to 30 amino acids. According to one embodiment, the peptide consists of the amino acid sequence WTAVQMAVFIHNFKRK (SEQ ID NO: 1), denoted “INS”. Thus, the term “peptide consisting of amino acid sequence SEQ ID NO: 1”, “peptide consisting of amino acid sequence WTAVQMAVFIHNFKRK” and “INS” are used herein interchangeably. According to another embodiment, the peptide consists of the amino acid sequence set forth in SEQ ID NO: 2 (TAVQMAVFIHNFKRK).

According to any one of the above embodiments, any water-soluble salt of a peptide of the invention is contemplated. According to one embodiment, the salt is trifluoroacetate salt. According to another embodiment, the salt is acetate salt. According to one embodiment, the salt is hydroxyl halide salt. According to one embodiment, the hydroxyl halide salt is selected from HCl, HBr and HI salt.

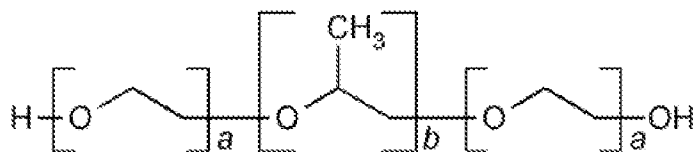
According to one embodiment, a and c in Formula O, are each independently an integer from 55 to 115, from 60 to 110, from 65 to 105, from 70 to 100 or from 75 to 90. According to another embodiment, b is an integer from 15 to 35, or from 20 to 30. According to some embodiment, $a=c$. According to such embodiment, the poloxamer has a structure of Formula I:



Formula I.

According to one embodiment, the present invention provides a pharmaceutical composition comprising from about 0.1 to about 30 mg/ml of a salt of a peptide consisting of 15 to 30 amino acid and comprising the amino acid sequence set forth in SEQ ID NO: 1 (WTAVQMAVFIHNFKRK), from about 0.3 wt% to about 15 wt% of a poloxamer having

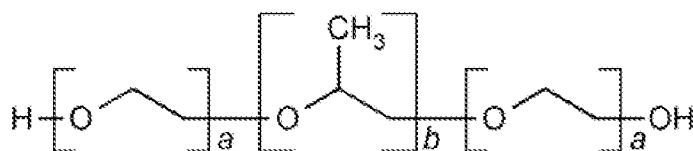
the structure of Formula I, and a pharmaceutically acceptable carrier, wherein the pH of the composition is between 4 to about 7.5 and



Formula I,

wherein a is an integer from 50 to 120, and b is an integer from 15 to 40.

- 5 According to one embodiment, the present invention provides a pharmaceutical composition comprising from about 0.1 to about 30 mg/ml of a salt of a peptide consisting of the amino acid sequence set forth in SEQ ID NO: 1, from about 0.3 wt% to about 15 wt% of a poloxamer having the structure of Formula I, and a pharmaceutically acceptable carrier, wherein the pH of the composition is between 4 to about 7.5 and

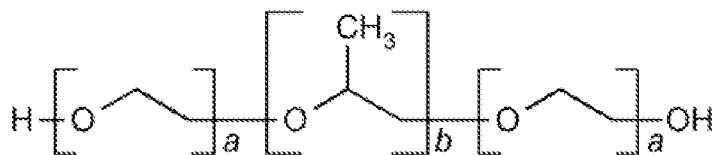


Formula I,

wherein a is an integer from 50 to 120, and b is an integer from 15 to 40.

- The term “pharmaceutical composition” as used herein refers to a composition comprising at least one active agent as disclosed herein optionally formulated together with one or more pharmaceutically acceptable carriers. Formulation of the pharmaceutical composition may be adjusted according to their intended use and administration route. In particular, the pharmaceutical composition may be formulated using a method known in the art so as to provide rapid, continuous or delayed release of the active ingredient after administration to mammals. According to one embodiment, the pharmaceutical composition is formulated for a parenteral administration.

- 20 Thus in some embodiments, the present invention provides a parenteral pharmaceutical composition comprising from about 0.1 to about 30 mg/ml of a salt of a peptide consisting of amino acid sequence SEQ ID NO: 1, from about 0.3 wt% to about 15 wt% of a poloxamer having the structure of Formula I,



Formula I,

and a pharmaceutically acceptable carrier, wherein the pH of the composition is between 4 to about 7.5 and wherein a is an integer from 50 to 120, and b is an integer from 15 to 40, and the pharmaceutical composition is configured for parenteral administration.

5 The term "pharmaceutically acceptable carrier" or "pharmaceutically acceptable excipient" as used herein refers to any and all solvents, dispersion media, preservatives, antioxidants, coatings, isotonic and absorption delaying agents, surfactants, fillers, disintegrants, binders, diluents, lubricants, glidants, pH adjusting agents, buffering agents, enhancers, wetting agents, solubilizing agents, surfactants, antioxidants the like, that are
 10 compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art.

Pharmaceutical compositions adapted for parenteral administration include, but are not limited to, aqueous and non-aqueous sterile injectable solutions or suspensions, which can contain antioxidants, buffers, bacteriostats and solutes that render the compositions
 15 substantially isotonic with the blood of an intended recipient. Such compounds do not decrease the stability or the solubility of the peptide.

According to one embodiment, a is an integer from 55 to 115, from 60 to 110, from 65 to 105, from 70 to 100 or from 75 to 90. According to another embodiment, b is an integer from 15 to 35, or from 20 to 30. According to some embodiments, a is an integer
 20 from 60 to 90 and b is an integer from 25 to 35. According to one embodiment, $a=80$ and $b=27$, and such a poloxamer is referred hereinafter as poloxamer 188. According to some embodiments, poloxamer 188 has a molecular weight between 7600 to 9600. According to one embodiment, poloxamer 188 has a molecular weight of 7800 to 9400, 8000 to 9200 or 8400 to 8800 Dalton.

25 According to one embodiment, the composition comprises from 0.1% to 5 wt% poloxamer. According to another embodiment, the composition comprises from 5wt% to 10wt% poloxamer. According to a further embodiment, the composition comprises from 10 wt% to 15 wt% poloxamer. According to certain embodiments, the composition comprises from 15 wt% to 20 wt% poloxamer. According to one embodiment, the

composition comprises from 0.5 wt% to 12 wt%, from 0.6 wt% to 10wt%, from 0.8 wt% to 8 wt%, from 1 wt% to 6 wt%, from 2 wt% to 5 wt%, from 3 wt% to 4 wt% of poloxamer. According to one embodiment, the pharmaceutical composition comprises from 0.5 wt% to 2wt%, from 0.6wt% to 1.8 wt% from 0.7 wt% to 1.6 wt% from 0.8 wt% to 1.4 wt% or
5 from 0.9 wt% to 1.2 wt% of the poloxamer. According to another embodiment, the pharmaceutical composition comprises from 0.6 wt% to 1.2 wt% of the poloxamer. According to one embodiment, poloxamer is poloxamer 188.

According to some embodiment, the composition comprises from 0.1 to 30 mg/ml of INS (SEQ ID NO: 1) salt. According to one embodiment, the composition comprises from
10 0.5 mg/ml to 25 mg/ml, from 1 mg/ml to 20 mg/ml, from 2.5 mg/ml to 15 mg/ml, from 5 mg/ml to 12 mg/ml, from 6 mg/ml to 10 mg/ml or from 7 mg/ml to 8 mg/ml of INS salt. According to another embodiment, the pharmaceutical composition comprises from 0.5 to 12 wt% from 0.7 to 10 wt% or from 1 to 10 wt% of INS as a salt. According to some embodiment, the pharmaceutical composition comprises 1, 2, 2.5, 3, 4, 5, 6, 7, 7.5, 8, 9, or
15 10 wt% of the peptide consisting of SEQ ID NO: 1. According to any one of the above embodiments, the peptide of the present invention is a salt. According to one embodiment, the peptide consists of amino acid sequence SEQ ID NO: 1. According to one embodiment, the composition comprises a trifluoroacetate salt of INS. According to another embodiment, the composition comprises an acetate salt of INS. According to one
20 embodiment, the composition comprises a hydroxyl halide salt of INS. According to one embodiment, the hydroxyl halide salt is selected from HCl, HBr and HI salt. According to one embodiment, the composition comprises an HCl salt of INS, denoted also as "INS HCl".

According to the teaching of the present invention, the concentration or the amount
25 when refers to INS salt relates to the peptide component of the salt. Thus, the statement that the composition comprises 10 mg/ml of INS HCl means that the composition comprises 10 mg/ml of the peptide consisting of SEQ ID NO:1. To calculate the content of the whole INS salt, the number should be multiplied by a correction factor of about 1.1, depending on the salt.

30 According to one embodiment, the composition comprises from 0.5 mg/ml to 25 mg/ml, from 1 mg/ml to 20 mg/ml, from 2.5 mg/ml to 15 mg/ml, from 5 mg/ml to 12 mg/ml from 6 mg/ml to 10 mg/ml or from 7 mg/ml to 8 mg/ml of INS peptide as HCl salt. According to one embodiment, the pharmaceutical composition comprises from 0.5 to 12

wt% from 0.7 to 10 wt% or from 1 to 10 wt% of the peptide set forth in SEQ ID NO: 1 as HCl salt. According to some embodiment, the pharmaceutical composition comprises 1, 2, 2.5, 3, 4, 5, 6, 7, 7.5, 8, 9, or 10 wt% of the peptide set forth in SEQ ID NO: 1 as HCl salt.

According to one embodiment, the composition comprises from 0.6 to 10 wt% of poloxamer 188 and from 1 mg/ml to 20 mg/ml, from 2.5 mg/ml to 15 mg/ml, from 1 to 10 mg/ml, from 2 to 10 mg/ml or from 5 mg/ml to 10 mg/ml of the peptide INS HCl. According to another embodiment, the composition comprises from 0.8 to 8 wt% poloxamer 188 and from 1 mg/ml to 20 mg/ml, from 2.5 mg/ml to 15 mg/ml or from 5 mg/ml to 10 mg/ml of the peptide INS HCl. According to a further embodiment, the composition comprises from 1 to 5 wt% poloxamer 188 and from 1 mg/ml to 20 mg/ml, from 2.5 mg/ml to 15 mg/ml, from 1 to 10 mg/ml, from 2 to 10 mg/ml or from 5 mg/ml to 10 mg/ml of the peptide INS HCl. According to yet another embodiment, the composition comprises from 0.8 to 2 wt% poloxamer 188 and from 1 mg/ml to 20 mg/ml, from 2.5 mg/ml to 15 mg/ml, from 1 to 10 mg/ml, from 2 to 10 mg/ml or from 5 mg/ml to 10 mg/ml of INS HCl.

According to one embodiment, the composition comprises from 0.3 wt% to 5 wt% of poloxamer 188 and from 1 mg/ml to 10 mg/ml of the peptide INS HCl. According to another embodiment, the composition comprises from 0.5 wt% to 2 wt% poloxamer 188 and from 1 to 5 mg/ml, from 1 to 5 or from 2 mg/ml to 4 mg/ml of the peptide INS HCl.

According to any one of the above embodiments, the pharmaceutical composition has a pH of about 4 to 7, about 4.5 to about 6.5 or from about 5 to about 6.

According to any one of the above embodiments, the pharmaceutical composition further comprises a saccharide. According to one embodiment, the saccharide is selected from a disaccharide or polysaccharide. According to one embodiment, the saccharide is a disaccharide. According to one embodiment, the disaccharide is selected from lactose, sucrose, lactulose, maltose, trehalose, cellobiose, and chitobiose. According to one embodiment, the disaccharide is lactose.

According to another embodiment, the saccharide is a polysaccharide. According to one embodiment, the polysaccharide is dextran.

According to some embodiments, the pharmaceutical composition comprises from about 1 wt% to about 20 wt% of the saccharide. According to one embodiment, the composition comprises from 2 wt% to 18 wt%, from 4 wt% to 16 wt%, from 6 wt% to 14

wt%, from 8 to 12 wt% of the saccharide. According to one embodiment, the saccharide is a disaccharide.

According to one embodiment, the composition comprises from 1 wt% to 20 wt%, from 2 wt% to 18 wt%, from 4 wt% to 16 wt%, from 6 wt% to 14 wt% from 8 to 12 wt% of lactose. According to one embodiment, the composition comprises from 6 to 12 wt% of lactose. According to some embodiment, the lactose is anhydrous.

According to some embodiments, the pharmaceutical composition is devoid of a compound selected from Tween and ethanol.

According to one embodiment, the pharmaceutical composition comprises from about 0.5 to about 10 wt% of poloxamer 188, from about 1 mg/ml to about 20 mg/ml of HCl salt of the peptide consisting of amino acid sequence WTAVQMAVFIHNFKRK (SEQ ID NO: 1) and from about 5 to about 12 wt% of lactose.

According to another embodiment, the pharmaceutical composition of the present invention comprises from about 0.8 to about 2 wt% of poloxamer 188, from about 1 mg/ml to about 15 mg/ml or to about 10 mg/ml of the peptide INS HCl and from about 8 to about 12 wt% of lactose.

According to any one of the above embodiments, the pH of the composition is from 4.5 to 6.

According to yet another embodiment, the pharmaceutical composition comprises from about 0.8 to about 2 wt% of poloxamer 188, from about 10 mg/ml to about 20 mg/ml of INS HCl and from about 8 to about 12 wt% of lactose. According to a further embodiment, the pharmaceutical composition comprises from about 8 to 12 wt% lactose, from about 0.5 to about 2 wt% of poloxamer 188, and from about 1 mg/ml to about 5 mg/ml of the peptide INS HCl. According to one embodiment, the pharmaceutical composition comprises from about 8 to 12 wt% lactose, from about 0.8 to about 2 wt% of poloxamer 188, from about 5 mg/ml to about 10 mg/ml of the peptide INS HCl. According to another embodiment, the pharmaceutical composition comprises from about 8 to 12 wt% lactose, from about 0.8 to about 2 wt% of poloxamer 188, and from about 1 mg/ml to about 15 mg/ml of the peptide INS HCl. According to yet another embodiment, the pharmaceutical composition comprises from about 8 to 12 wt% lactose, from about 0.5 to about 2 wt% of poloxamer 188, and from about 1 mg/ml to about 12 mg/ml of the peptide INS HCl. According to some embodiments, the pH of the composition is from 4.5 to 6.

According to one embodiment, the pharmaceutical composition of the present invention comprises from about 0.5 to about 10 wt% of poloxamer 188, from about 5 mg/ml to about 15 mg/ml of HCl salt of the peptide consisting of amino acid sequence SEQ ID NO: 1 and from about 5 to about 12 wt% of lactose. According to one
5 embodiment, the composition comprises from 2.5 to 20 mg/ml of the peptide INS HCl, 1% Poloxamer 188 and 9% Lactose anhydrous, and the composition has pH of about 5. According to another embodiment, the composition comprises from 2.5 to 20 mg/ml of the peptide INS HCl, 0.8% Poloxamer 188 and 9.5% Lactose anhydrous, and the composition has pH of about 5. According to some such embodiments, the composition comprises 2.5,
10 5, 10, 15 or 20 mg/ml of the peptide INS HCl. According to other embodiments, the pH is from about 4.5 to about 5.5

According to some embodiment, the pharmaceutical composition further comprises at least one buffer. The terms "buffer," "buffering system," and/or "buffer solution" refer to compounds which reduce the change of pH upon addition of small amounts of acid or base,
15 or upon dilution. The term "buffering agent" refers to a weak acid or weak base in a buffer solution. According to one embodiment, the buffer is an acetate buffer.

According to any one of the above embodiments, the composition is a liquid composition. According to another embodiment, the composition is a semi-liquid composition. According to some embodiments the liquid or semi-liquid composition is an
20 aqueous composition.

According to any one of the above embodiments, the composition is a solution. The term "solution" as used herein refers to a clear, homogeneous liquid dosage form that contains one or more chemical substances dissolved in a solvent or in a mixture of mutually miscible solvents.

25 According to one embodiment, the composition is a clear solution. As used herein, the term "clear solution" refers to essentially transparent solutions devoid of particles above 100 nm. Alternatively, the term "clear solution" refers to essentially transparent solutions devoid of particles above 50 nm. Alternatively, the term "clear solution" refers to essentially transparent solutions devoid of particles above 40 nm.

30 The terms "substantially devoid", "essentially devoid", "devoid", "does not include" and "does not comprise" may be used interchangeably and refer to composition that does not include, contain or comprise a particular compound or particles, e.g. said composition comprises less than 0.1 %, less than 0.01 %, or less than 0.001 % of the such compounds

or particles. In some embodiments, the term devoid contemplate composition comprising traces of the devoid compounds or particles.

According to any one of the above embodiments, the composition is a stable composition.

5 The term “stable” as used herein refer to a composition, for example a solution, in which at least 80% of the peptide remains dissolved for at least 24 hours at room temperature (about 25°C). According to one embodiment, at least 80 wt% of the peptide maintains dissolved for at least 2 days, at least 5 days, at least 7 days or at least 14 days. According to another embodiment, at least 85 wt% of the peptide remains dissolved for at
10 least 2 days, at least 5 days, at least 7 days or at least 14 days. According to a certain embodiment, at least 90 wt% or 95 wt% of the peptide remains dissolved for at least 2 days, at least 5 days, at least 7 days or at least 14 days.

According to one embodiment, the composition is stable upon dilution with a phosphate buffered saline (PBS). According to one embodiment, the composition remains
15 clear solution upon dilution with PBS. According to another embodiment, the composition remains a stable for at least 1, 2, 3, 5, 7 10 or 14 days upon dilution in PBS. According to one embodiment, the dilution is 2, 5 or 10 times dilution. According to one embodiment, no more that 10%, 5% or 3% of the INS peptide precipitates upon dilution of the pharmaceutical composition with PBS. According to one embodiment, dilution is 10 times
20 dilution.

According to any one of the above embodiments, the pharmaceutical composition of the present invention is a parenteral composition, i.e. the composition is formulated for parenteral administration. According to one embodiment, parenteral administration is selected from intravenous, intramuscular and subcutaneous administration. Thus,
25 according to one embodiment, the pharmaceutical composition of the present invention is formulated for an administration route selected from intravenous, intramuscular and subcutaneous administration. According to one embodiment, the pharmaceutical composition is for subcutaneous administration.

According to any one of the above embodiments, the pharmaceutical composition of
30 the present invention further comprising an additional active agent. According to some embodiments the additional active ingredient is an anti-viral agent. According to some embodiments, the active agent is a protease inhibitor. According to some embodiments, the protease inhibitor is selected from darunavir, lopinavir, ritonavir and a combination

thereof. according to another embodiment, the active agent is selected from atazanavir (ATV), amprenavir, fosamprenavir (APV), tipranavir (TPV), indinavir, saquinavir, lopinavir/ritonavir, prezista, nelfinavir (NFV) and azidothymidine (AZT).

According to yet other embodiments, the additional active ingredient is an anti-cancer agent.

According to some embodiments, the pharmaceutical composition of the present invention further comprises lentivirus particles.

The term "active agent" refers to an agent that has biological activity, pharmacologic effects and/or therapeutic utility.

According to any one of the above embodiments, the pharmaceutical composition of the present invention is for use in treating a disease treatable with the peptide consisting of amino acid sequence WTAVQMAVFIHNFKRK (SEQ ID NO: 1) or a salt thereof. According to some embodiments, the disease treatable with a peptide of SEQ ID NO: 1 is selected from cancer and viral infection.

According to one embodiment, the disease is cancer. According to another embodiment, the disease is acquired immune deficiency syndrome (AIDS). According to one embodiment, the AIDS is caused by human immunodeficiency virus 1 (HIV-1) infection. According to one embodiment, the disease is a viral disease caused by lentivirus.

As used herein, the term "cancer" refers to all types of cancer, neoplasm or malignant tumors found in mammals, including leukemias, lymphomas, melanomas, neuroendocrine tumors, carcinomas and sarcomas. Exemplary cancers that may be treated with a compound, pharmaceutical composition, or method provided herein include lymphoma, sarcoma, bladder cancer, bone cancer, brain tumor, cervical cancer, colon cancer, esophageal cancer, gastric cancer, head and neck cancer, kidney cancer, myeloma, thyroid cancer, leukemia, prostate cancer, breast cancer (e.g. triple negative, ER positive, ER negative, chemotherapy resistant, herceptin resistant, HER2 positive, doxorubicin resistant, tamoxifen resistant, ductal carcinoma, lobular carcinoma, primary, metastatic), ovarian cancer, pancreatic cancer, liver cancer (e.g., hepatocellular carcinoma), lung cancer (e.g. non-small cell lung carcinoma, squamous cell lung carcinoma, adenocarcinoma, large cell lung carcinoma, small cell lung carcinoma, carcinoid, sarcoma), glioblastoma multiforme, glioma, melanoma, prostate cancer, castration-resistant prostate cancer, breast cancer, triple negative breast cancer, glioblastoma, ovarian cancer, lung cancer, squamous cell carcinoma (e.g., head, neck, or esophagus), colorectal cancer, leukemia, acute myeloid

leukemia, lymphoma, B cell lymphoma, or multiple myeloma. Additional examples include, cancer of the thyroid, endocrine system, brain, breast, cervix, colon, head & neck, esophagus, liver, kidney, lung, non-small cell lung, melanoma, mesothelioma, ovary, sarcoma, stomach, uterus or medulloblastoma, Hodgkin's disease, Non-Hodgkin's lymphoma, multiple myeloma, neuroblastoma, glioma, glioblastoma multiforme, ovarian cancer, rhabdomyosarcoma, primary thrombocytosis, primary macroglobulinemia, primary brain tumors, cancer, malignant pancreatic insulanoma, malignant carcinoid, urinary bladder cancer, premalignant skin lesions, testicular cancer, lymphomas, thyroid cancer, neuroblastoma, esophageal cancer, genitourinary tract cancer, malignant hypercalcemia, endometrial cancer, adrenal cortical cancer, neoplasms of the endocrine or exocrine pancreas, medullary thyroid cancer, medullary thyroid carcinoma, melanoma, colorectal cancer, papillary thyroid cancer, hepatocellular carcinoma, Paget's Disease of the Nipple, Phyllodes tumors, lobular carcinoma, ductal carcinoma, cancer of the pancreatic stellate cells, cancer of the hepatic stellate cells, or prostate cancer. According to some embodiments, the cancer is a solid cancer. According to one embodiment, the cancer is a colon cancer. According to another embodiment, the cancer is a pancreatic cancer.

The term “treating” a condition or patient refers to taking steps to obtain beneficial or desired results, including clinical results. Beneficial or desired clinical results include, but are not limited to, ameliorating abrogating, substantially inhibiting, slowing or reversing the progression of a disease, condition or disorder, substantially ameliorating or alleviating clinical or esthetical symptoms of a condition, substantially preventing the appearance of clinical or esthetical symptoms of a disease, condition, or disorder, and protecting from harmful or annoying symptoms. Treating further refers to accomplishing one or more of the following: (a) reducing the severity of the disorder; (b) limiting development of symptoms characteristic of the disorder(s) being treated; (c) limiting worsening of symptoms characteristic of the disorder(s) being treated; (d) limiting recurrence of the disorder(s) in patients that have previously had the disorder(s); and/or (e) limiting recurrence of symptoms in patients that were previously asymptomatic for the disorder(s).

According to some embodiments, treating AIDS comprises reducing a viral load. According to one embodiment, treating of AIDS comprises reducing the HIV-1 RNA count to below 1000 copies/ml, below 800 copies/ml, below 600 copies/ml, below 300 copies/ml or below 100 copies/ml.

According to some embodiments, treating AIDS comprises reducing a viral load by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95% or at least 97% in comparison to the baseline.

5 According to other embodiments, treating AIDS comprises increasing the count of CD4+ T-cells by at least 20% in comparison to the base line. According to another embodiment, treating AIDS comprises increasing the count of CD4+ T-cells by at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95 % in comparison to the base line. According to a further embodiment, treating
10 AIDS comprises increasing the count of CD4+ T-cells by at least 97% or at least 98% in comparison to the base line. According to another embodiment, treating AIDS comprises increasing the count of CD4+ T-cells 2, 2.5, 3, 4, 5 or 10 times in comparison to the base line.

The pharmaceutical composition of the present invention may be administered by
15 any know method. The term "administering" or "administration of" a substance, a compound, an agent or a composition comprising any of them, to a subject can be carried out using one of a variety of methods known to those skilled in the art. For example, a compound or a composition comprising said compound, can be administered, intravenously, arterially, intradermally, intramuscularly, intraperitoneally, intravenously,
20 subcutaneously, ocularly, sublingually, orally (by ingestion), intranasally (by inhalation), intraspinally, intracerebrally, and transdermally (by absorption, e.g., through a skin duct). A compound or a composition can also appropriately be introduced by rechargeable or biodegradable polymeric devices or other devices, e.g., patches and pumps, or formulations, which provide for the extended, slow or controlled release of the compound
25 or agent. According to one embodiment, the composition is administered intravenously. According to another embodiment, the composition is administered intramuscularly. According to a further embodiment, the composition is administered subcutaneously.

The precise dose to be employed also depends on the route of administration, and the progression of the disease or disorder, and should be decided according to the judgment of
30 the practitioner and each patient's circumstances. Typical dosage of the INS peptide in a composition according to the present invention, is in the range 0.01 to 5 mg/kg/dose. According to some embodiments, the INS peptide is administered in the dosage of 0.01 to 2, 0.05 to 1.5, 0.1 to 1.2, 0.2 to 1, 0.4 to 0.8 0.3 to 0.7 or 0.4 to 0.6 mg/kg/dose. According

to some embodiments, the composition comprises from 1 to 30 mg of the peptide INS HCl, from 2.5 to 20 mg, from 5 to 15 mg or 10 mg of the peptide INS HCl. According to some embodiments, the pharmaceutical composition is administered in the amount of 10 to 50 mg/dose, 15 to 40 mg/dose, 20 to 30 mg/dose. The composition may be administered as a single daily dose or in multiple doses. The administration schedule can be taken once-daily, twice-daily, thrice-daily, once-weekly, twice-weekly, thrice-weekly, once-monthly, twice-monthly, thrice-monthly, or any other administration schedule known to those of skill in the art. According to some embodiments, the pharmaceutical composition is administered twice a week. According to some embodiment, the composition is administered in a dose of 10 to 40 or 15 to 30 mg twice a week. The administration may be continuous, i.e., every day, or intermittently. The terms "intermittent" or "intermittently" as used herein means stopping and starting at either regular or irregular intervals. For example, intermittent administration can be administration in one to six days per week or it may mean administration in cycles (e.g. daily administration for two to eight consecutive weeks, then a rest period with no administration for up to one week) or it may mean administration on alternate days.

According to one embodiment, treatment of AIDS is affected as described in WO 2010/041241. According to some embodiment, treatment of AIDS comprises administering the composition of the present invention in combination with at least one additional active agent, for example an anti-HIV agent. According to some embodiments, the anti-HIV agent is a protease inhibitor. According to some embodiments, the protease inhibitor is selected from lopinavir, darunavir, ritonavir and a combination thereof.

According to one embodiment, treatment of AIDS comprises administering the composition of the present invention in combination with at least one protease inhibitor. According to some embodiments, the composition is administered in combination with lopinavir and ritonavir. According to some embodiments, treating comprises administering the composition of the present invention from 1 to 4 times a week in combination with 400 to 800 mg of lopinavir once a day and/or 100 to 300 mg of ritonavir once a day, wherein the dose of the INS peptide is from 0.1 to 1 mg/kg/dose. According to one embodiment, treatment of AIDS comprises administering to the subject in need of such treatment, 2 times a week, a composition comprising 0.2 to 0.4 mg/kg of INS peptide in combination with daily administration of 800 mg lopinavir and 200 mg ritonavir. According to any one

of the above embodiments, the composition of the present invention is administered subcutaneously.

According to one embodiment, treating of AIDS results in reducing the HIV-1 RNA count to below 600 copies/ml. According to another embodiment, treating AIDS results in
5 increasing the count of CD4+ T-cells by at least 80%, at least 85, at least 90%, at least 95%, or at least 98 % in comparison to the base line.

According to one embodiment, co-administration of the composition of the present invention and optionally of an at least one additional active agents, is performed in a regimen selected from a single combined composition, separate individual compositions
10 administered substantially at the same time, and separate individual compositions administered under separate schedules and include treatment regimens in which the agents are not necessarily administered by the same route of administration or at the same time. The composition of the present invention and of an additional active agent are administered in a sequential manner in either order. According to other embodiments, the
15 composition of the present invention and of an additional active agent are administered in a substantially simultaneous manner, i.e. administered with only a short time interval between them. In some embodiments, the time interval is in the range of from 0.5 to 60 minutes. In other embodiment the co-administration is a simultaneous administration.

According to another embodiment, treatment of cancer is performed as described in
20 WO2018215999. According to some embodiment, treatment comprises administering the composition of the present invention in combination with any other known treatment.

According to one embodiment, the composition of the present invention is administered in combination with:

(i) a linear molecule of double-stranded DNA (dsDNA) comprising long term repeat
25 (LTR) sequences recognized by the integrating enzyme of (ii), and

an integrating enzyme, capable of entering the nuclei of cells after binding to the LTR sequences of the dsDNA molecule of (i) and creating multiple double strand breaks (DSBs) in the chromosomal DNA of the cells.

According to some embodiment, the composition of the present invention is
30 administered in combination with (i) a lentivirus particle comprising a linear molecule of double- stranded DNA (dsDNA) comprising long term repeat (LTR) sequences recognized by the integrating enzyme of (ii), (ii) an integrase, capable of entering the nuclei of cells after binding to the LTR sequences of the dsDNA molecule of (i) and creating multiple

double strand breaks (DSBs) in the chromosomal DNA of the cells, and (iii) an antibody capable of binding CD24 or CD20 presented by the cancer cells.

According to some embodiments, the pharmaceutical composition is co-administered with viral particles. According to one embodiment, the viral particles are lentiviral particles. According to some embodiments, the viral particles such as lentiviral particles comprise an antibody capable of binding CD24 or CD20. According to one embodiment, the viral particles such as lentiviral particles comprise a single chain antibody directed against CD24 or CD20.

The term "integrating enzyme" as used herein refers to any enzyme capable of creating double stranded breaks (DSBs) in the chromosomal DNA of a human cell. The "integrating enzyme" may optionally be further capable of incorporating a double stranded DNA molecule into the gap formed by the DSBs. Non-limiting examples of integrating enzymes are the integrase enzymes of retroviruses, such as the integrase enzyme of HIV-1.

In certain embodiments, the term "HIV-1 integrase" as used herein relates e.g. to the protein having the UniProtKB Entry Q76353 (Q76353_9HIV1). In certain embodiments, the term "HIV-2 integrase" as used herein relates e.g. to the protein having the UniProtKB Entry D5LQ24 (D5LQ24_9HIV2). Due to the high sequence diversity in HIV genes, many other variants of HIV-1 integrase and HIV-2 integrase are further considered as "integrating enzymes" according to the present invention.

Retroviral integrase is an enzyme produced by a retrovirus (such as HIV) that enables its genetic material to be integrated into the DNA of the infected cell. Since retroviruses are rapidly and constantly changing, the exact sequence of retroviral integrase is practically impossible to follow. In certain embodiments, the integrating enzyme is an integrase enzyme selected from the group consisting of HIV-1 integrase, HIV-2 integrase, active fragments thereof, and active analogs thereof. Each possibility represents a separate embodiment of the invention.

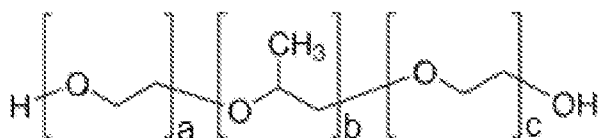
According to some embodiments, the pharmaceutical composition is administered twice a week to provide from 10 to 40 mg of INS HCl peptide salt per dose in combination with lentiviral particles comprising a single chain antibody directed against CD24, e.g. 10^8 to 10^{10} IU of the particles.

One particular such a method is described in WO2018215999.

According to some embodiments, the pharmaceutical composition according to any one of the above embodiments is a lyophilized composition.

According to another aspect, the present invention provides a solid pharmaceutical composition comprising a salt of a peptide comprising amino acid sequence

5 TAVQMAVFIHNFKRK and a poloxamer having the structure of Formula O

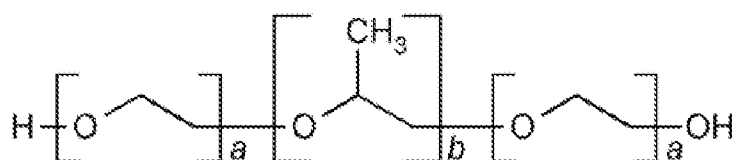


Formula O,

wherein a and c are each independently an integer from 50 to 120, and b is an integer from 15 to 40, and the peptide consists of 15 to 40 amino acids. According to one embodiment, the weight ratio between said salt and said poloxamer is from about 10:1 to about 1:1500.

10 According to one embodiment, the peptide comprises the amino acid sequence WTAVQMAVFIHNFKRK (SEQ ID NO: 1). According to one embodiment, the peptide consists of amino acid sequence WTAVQMAVFIHNFKRK (SEQ ID NO: 1, INS). According to another embodiment, the peptide consists of amino acid sequence TAVQMAVFIHNFKRK (SEQ ID NO: 2).

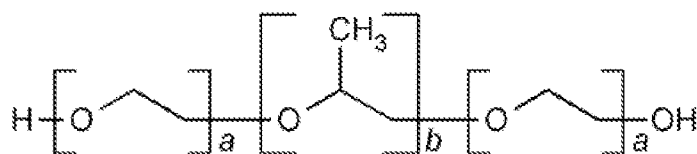
15 According to one embodiment, a and c are each independently an integer from 55 to 115, from 60 to 110, from 65 to 105, from 70 to 100 or from 75 to 90. According to another embodiment, b is an integer from 15 to 35, or from 20 to 30. According to some embodiment, $a=b$. According to such embodiment, the poloxamer has the structure of Formula I:



Formula I.

20 According to one embodiment, the salt is hydroxyl halide salt. According to one embodiment, the hydroxyl halide salt is selected from HCl, HBr and HI salt. According to one embodiment, the composition comprises the peptide salt INS HCl.

25 According to one embodiment, the present invention provides a solid pharmaceutical composition comprising a salt of peptide consisting of amino acid sequence SEQ ID NO: 1 and a poloxamer having a structure of Formula I,



Formula I,

wherein a is an integer from 50 to 120, and b is an integer from 15 to 40. According to one embodiment, the weight ratio between said INS salt and said poloxamer is from about 10:1 to about 1:1500. According to another embodiment, the weight ratio between said salt and said poloxamer is from 9:1 to 1:1250, from 8:1 to 1:1100, from 7:1 to 1:800, from 6:1 to 1:600, from 5:1 to 1:400, from 4:1 to 1:200, from 3:1 to 1:100 from 2:1 to 1:50 or from 1:1 to 1:25. According to another embodiment, the weight ratio between said salt and said poloxamer is from 5:1 to 1:50, from 4:1 to 1:40, from 3:1 to 1:30, from 2:1 to 1:20 or from 1:1 to 1:10.

According to one embodiment, the poloxamer is as defined hereinabove. According to one embodiment, the poloxamer is poloxamer 188.

According to one embodiment, the peptide salt is HCl salt of INS. According to one embodiment, the present invention provides a solid pharmaceutical composition comprising an HCl salt of INS and a poloxamer 188, wherein the weight ratio between said salt and said poloxamer is from about 10:1 to about 1:1000. According to another embodiment, the weight ratio between INS HCl and poloxamer 188 is from 5:1 to 1:50, from 4:1 to 1:40, from 3:1 to 1:30, from 2:1 to 1:20 or from 1:1 to 1:10. According to one embodiment, the weight ratio between the INS HCl and the poloxamer 188 is from about 2:1 to about 1:20.

According to any one of the above embodiments, the solid pharmaceutical composition of the present invention further comprises a saccharide selected from a disaccharide and a polysaccharide. According to one embodiment, the saccharide is as defined hereinabove. According to one embodiment, the saccharide is a disaccharide. According to one embodiment, the disaccharide is lactose.

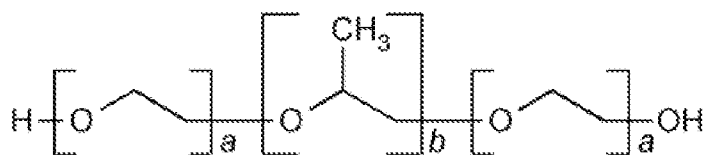
According to one embodiment, the weight ratio between the INS HCl and the lactose is from about 1:2000 to about 5:1.

According to one embodiment, the weight ratio between the INS HCl and the lactose is from about 1:1000 to about 4:1, from 1:500 to 3:1, from 1:300 to 2:1, from 1:100 to 1:1, or from 1:50 to 1:10. According to another embodiment, the weight ratio between the INS

HCl and the lactose is from 5:1 to 1:30, from 4:1 to 1:24, from 3:1 to 1:20, from 2:1 to 1:15 or from 1:1 to 1:10.

According to one embodiment, the present invention provides a solid pharmaceutical composition comprising the peptide salt INS HCl, poloxamer 188 and lactose, wherein the weight ratio between INS HCl and poloxamer 188 is between about 3:1 to 1:20 and the weight ratio between INS HCl and lactose is between about 4:1 to about 1:24.

According to one embodiment, the present invention provides a solid pharmaceutical composition comprising from 0.9 wt% to about 70 wt% of an INS salt of and a poloxamer having a structure of Formula I,



Formula I,

wherein a is an integer from 50 to 120, and b is an integer from 15 to 40. According to some embodiments, the weight ratio between said INS peptide salt and said poloxamer is from about 10:1 to about 1:1500. According to another embodiment, the weight ratio between said peptide salt and said poloxamer is from 9:1 to 1:1250, from 8:1 to 1:1100, from 7:1 to 1:800, from 6:1 to 1:600, from 5:1 to 1:400, from 4:1 to 1:200, from 3:1 to 1:100 from 2:1 to 1:50 or from 1:1 to 1:25. According to another embodiment, the weight ratio between said peptide salt and said poloxamer is from 5:1 to 1:50, from 4:1 to 1:40, from 3:1 to 1:30, from 2:1 to 1:20 or from 1:1 to 1:10.

According to one embodiment, the poloxamer is as defined hereinabove. According to one embodiment, the poloxamer is poloxamer 188. According to one embodiment, the salt is HCl salt of INS. According to one embodiment, the present invention provides a solid pharmaceutical composition comprising from about 4.5 wt% to about 60 wt% of the peptide salt INS HCl and poloxamer 188. According to another embodiment, the composition comprises from about 1.5 wt% to about 50 wt% of the peptide salt INS HCl.

According to any one of the above embodiments, the solid pharmaceutical composition of the present invention further comprises a saccharide selected from a disaccharide and polysaccharide. According to one embodiment, the saccharide is as defined hereinabove. According to one embodiment, the saccharide is a disaccharide. According to one embodiment, the disaccharide is lactose.

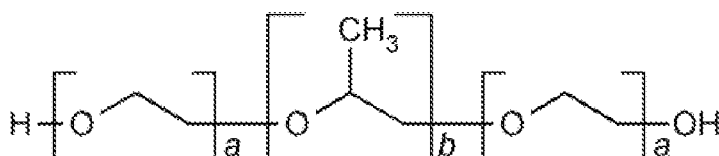
According to one embodiment, the weight ratio between the peptide INS HCl and lactose is from about 1:2000 to about 5:1.

According to one embodiment, the present invention provides a solid pharmaceutical composition comprising from about 2 wt% to about 22 wt% INS HCl peptide, from about 3.5 wt% to about 63 wt% poloxamer 188 and from about 30 wt% to about 92 wt% of lactose. According to another embodiment, the solid pharmaceutical composition comprising from about 1.75 wt% to about 18.5 wt% INS HCl peptide, from about 5 wt% to about 19.5 wt% poloxamer 188 and from about 95 wt% to about 89 wt% of lactose. According to yet another embodiment, the solid pharmaceutical composition comprising from about 2 wt% to about 20 wt% INS HCl peptide, from about 8 wt% to about 9.5 wt% poloxamer 188 and from about 75 wt% to about 88 wt% of lactose.

According to one embodiment, the solid pharmaceutical composition is a lyophilized composition.

According to any one of the above embodiments, the solid pharmaceutical composition of the present invention upon a reconstitution forms a liquid pharmaceutical composition of the present invention, e.g. a parenteral pharmaceutical composition.

According to any one of the above embodiments, the solid pharmaceutical composition of the present invention upon a reconstitution comprises from about 0.1 to about 30 mg/ml of a salt of a peptide consisting of amino acid sequence WTAVQMAVFIHNFKRK (SEQ ID NO: 1), and from about 0.3 wt% to about 15 wt% of a poloxamer, wherein the pH of the composition is between 4 to about 7.5 and the poloxamer has a structure of Formula I,



Formula I,

wherein a is an integer from 50 to 120, and b is an integer from 15 to 40.

According to another embodiment, the present invention provides a reconstituted solid pharmaceutical composition of the present invention. According to some embodiments, the reconstituted pharmaceutical composition is for use in treating a disease selected from cancer and AIDS.

According to another aspect, the present invention provides a method of treating a disease, the method comprises parenterally administering to a subject in need thereof an effective amount of the pharmaceutical composition comprising a peptide consisting of amino acid sequence WTAVQMAVFIHNFKRK (SEQ ID NO: 1), of the present invention. According to some embodiments, the present invention provides a method of treating a disease in a subject in need thereof, the method comprises parenterally administering a therapeutically effective amount of a reconstituted solid pharmaceutical composition of the present invention.

According to some embodiments, the disease is selected from a viral infection and cancer. According to some embodiments, the viral infection is of HIV. According to one embodiment, administration is selected from the group consisting of intravenous, intramuscular and subcutaneous administration. According to another embodiment, the disease is AIDS caused by HIV-1. According to one embodiment, the method of treating is as described in WO 2010/041241. According to one embodiment, the disease is cancer. According to another embodiment, treatment of cancer is affected as described in WO2018215999. According to some embodiments, the composition of the present invention is administered in combination with at least one additional therapeutically active agent. According to some embodiments, the active agent is a protease inhibitor. According to some embodiments, the protease inhibitor is selected from lopinavir, darunavir, ritonavir and a combination thereof.

According to some embodiments, the composition of the present invention is administered in combination with lentivirus particles, as described hereinabove or in WO 2018/215999. According to some embodiment, the lentivirus particles comprise a moiety that specifically bind a tumor antigen. According to some embodiments, the lentivirus particles comprises single chain antibody directed against CD24 or CD20.

According to some embodiment, the INS HCl peptide is administered once a week, twice a week or 3 times a week. According to some embodiment, the INS HCl peptide is administered in an amount of 0.01 to 2, 0.05 to 1.5, 0.1 to 1.2, 0.2 to 1, 0.4 to 0.8 0.3 to 0.7 or 0.4 to 0.6 mg/kg/dose. According to other embodiments, the method comprises administering from 10 to 50 mg/dose, 15 to 40 mg/dose, 20 to 30 mg/dose of INS HCl, e.g. twice a week. According to one embodiment, the INS HCl peptide is co-administered with lentivirus particles comprising single chain antibody directed against CD24.

According to another aspect, the present invention provides a pharmaceutical composition comprising from about 5 wt% to about 15 wt % of Cremophor RH40, from about 1 to about 20 mg/ml of an INS salt, and a pharmaceutically acceptable carrier. According to one embodiment, the salt is as defined herein above. According to one
5 embodiment, the peptide salt is INS HCl . According to one embodiment, the composition comprises from 5.5 wt% to 14.5 wt%, from 6 wt% to 14 wt%, from 7 wt% to 13 wt% or from 8 wt% to 12 wt% of Cremophor RH40. According to one embodiment, the composition comprises 10 wt% of Cremophor RH40. According to another embodiment, the composition comprises from about 2 mg/ml to about 18 mg/ml, from about 4 mg/ml to
10 about 16 mg/ml, from about 6 mg/ml to about 14 mg/ml or from about 8 mg/ml to about 12 mg/ml of the INS HCl peptide. According to one embodiment, the pH of the composition is from 4.5 to about 5.5

According to one embodiment, the present invention provides a pharmaceutical composition comprising from about 8 wt% to about 12 % of Cremophor RH40, from about
15 5 to about 10 mg/ml of the peptide INS HCl, and a pharmaceutically acceptable carrier, wherein the pH of the composition is from about 4.5 to about 5.5.

The term “Cremophor RH 40” refers to PEG-40 castor oil or Polyoxyl 40 hydrogenated castor oil.

According to another aspect, the present invention provides a pharmaceutical
20 composition comprising from about 10 wt% to about 30 wt% propylene glycol (PG), from about 0.3 to about 1.5 wt% Tween 80, from about 1 mg/ml to about 8 mg/ml of the peptide salt INS HCl, and a pharmaceutically acceptable carrier. According to one embodiment, the pH of the composition is from about 4.5 to about 6. According to one embodiment, the composition comprises from 10 wt% to 30 wt%, from about 12 wt% to 28 wt%, from 14
25 wt% to 26 wt%, from 16 wt% to 24 wt% from 18 wt% to 22 wt% of (PG). According to one embodiment, the composition comprises from 0.4 wt% to 1.4 wt%, from 0.6 to 1.2 wt% of Tween 80. According to one embodiment, the present invention provides a pharmaceutical composition comprising from 15 to 25 wt% of PG, from 0.6 to 1 wt% of Tween 80 and from 4 to 8 mg/ml of the peptide salt INS HCl.

30 According to another aspect, the present invention provides a hydrogen halide salt of a peptide comprising amino acid sequence selected from WTAVQMAVFIHNFKRK (SEQ ID NO: 1) and TAVQMAVFIHNFKRK (SEQ ID NO: 2), wherein said peptide consists of from 15 to 30 amino acids.

According to one embodiment, the peptide consists of the amino acid sequence WTAVQMAVFIHNFKRK (SEQ ID NO: 1, INS). According to another embodiment, the peptide consists of amino acid sequence TAVQMAVFIHNFKRK (SEQ ID NO: 2). According to one embodiment, the hydrogen halide is selected from HCl, HBr, and HI.

5 According to one embodiment, the present invention provides an hydrochloride salt of the peptide INS. According to another embodiment, the present invention provides an HBr salt of the peptide INS. According to yet another embodiment, the present invention provides an HCl or HBr salt of a peptide consisting of the amino acid sequence TAVQMAVFIHNFKRK (SEQ ID NO: 2).

10 According to another embodiment, the present invention provides a pharmaceutical composition comprising a peptide salt according to any one of the above embodiments.

The terms "comprising", "comprise(s)", "include(s)", "having", "has" and "contain(s)," are used herein interchangeably and have the meaning of "consisting at least in part of". When interpreting each statement in this specification that includes the term

15 "comprising", features other than that or those prefaced by the term may also be present. Related terms such as "comprise" and "comprises" are to be interpreted in the same manner. The terms "have", "has", "having" and "comprising" may also encompass the meaning of "consisting of" and "consisting essentially of", and may be substituted by these terms. The term "consisting of" excludes any component, step or procedure not specifically

20 delineated or listed. The term "consisting essentially of" means that the composition or component may include additional ingredients, but only if the additional ingredients do not materially alter the basic and novel characteristics of the claimed compositions or methods.

Throughout this application, various embodiments of this invention may be presented in a range format. It should be understood that the description in range format is merely for

25 convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from

30 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

As used herein, the term “about”, when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of +/-10%, or +/-5%, +/-1%, or even +/-0.1% from the specified value.

Having now generally described the invention, the same will be more readily understood through reference to the following examples, which are provided by way of illustration and are not intended to be limiting of the present invention.

EXAMPLES

The present invention provides stock formulations of INS peptide at the concentration of up to 10 mg/ml. These formulations are stable upon administration to physiological liquids, e.g. they may be diluted in PBS up to 10 times and maintain clear solution upon dilution without precipitation of the peptide.

Materials and Methods

The peptide denoted INS, having the sequence WTAVQMAVFIHNFKRK (SEQ ID NO: 1) was prepared as described in WO 2010/041241, stored at -20°C, and used at concentration of 2-10 mg/ml.

Chemicals that were used for preparation of the formulations of the present invention are summarized in **Table 1**.

Table 1. Chemicals used in development of the formulations

Name	Manufacture	Batch No.
Trifluoroacetic acid	Alfa Aesar	U10D816
Acetonitrile (ACN)	Merck	I0896730 725
PEG 400	Sinopharm Chemical Reagent Co. Ltd	20170717
Propylene glycol (PG)	SIGMA-ALDRICH	MKBZ1609V
Ammonium bicarbonate	N/A	T067101
HP-β-CD	Kunshan RSK	170801
SBE-β-CD	ROQUETTE	N/A
Phosphate-buffered saline (PBS)	Thermo scientific	NZD1121
Cremophor EL	BASF	4126664760
1N NaOH	STA-DFR	N/A
POLOXAMER P188 (Kolliphor [®] P 188)	BASF	N/A
Solutol HS 15	Sigma-Aldrich	BCBN9145V

Cremophor RH 40	SIGMA	BCBG5390V
Tween 80	SPECTRUM CHENICAL	3DK0061
L-Arginine	N/A	13040003
28% Ammonioium hydroxide	Alfa Aesar	10183704
D-glucose	ENOX	20110801
Anhydrous Lactose	Deceling	N/A
D-Mannitol	Roquette	E519K
D-Sorbitol	SCR	F20110509
1% Tween 80	STA-DFR	FR00110-1-180327-01
1% Poloxamer P188	STA-DFR	FR00110-1-180327-03
20% HP- β -CD (Hydroxy propyl beta Cyclodextrin)	STA-DFR	N/A
Sodium acetate Trihydrate	Sinopharm Chemical Reagent Co. Ltd	20150109
9.5% Lactose+0.8% Poloxamer P188 (w/v)	STA-DFR	FR00110-5-180509-01

Preparation of buffers and solutions

0.1M citrate buffer pH 6: 11.5ml 0.1M citric acid (0.1M) were mixed with 88.5ml sodium citrate (0.1M).

- 5 50 mM phosphate buffer, pH 8.0: 25mL of the 0.2M potassium phosphate solution were mixed with 24ml of 0.2M NaOH.

50 mM borate buffer, pH 9.0: 12ml 0.2M NaOH were added to 25ml 0.2M boric acid and potassium chloride solution.

- 10 0.1M NH_4HCO_3 : about 99.87mg NH_4HCO_3 was weighed and dissolved by 12.6ml purified water.

1% Tween 80 (w/w): to 200 mg of Tween 80 were added 19.80 mL of purified water and mixed well.

5% poloxamer P188 (w/w): 1000 mg of poloxamer P188 were placed in a 20 ml volumetric flask and 19.00 mL of purified water were added and mixed well.

- 15 10%HP- β -CD (w/w): 2000 mg of HP- β -CD were placed in a 20 ml volumetric flask and add 18.00 mL of purified water were added and mixed well.

9.5% Lactose+0.8% Poloxamer P188 (w/v): 2373 mg of lactose and 200 mg of poloxamer P188 were weighed in a 25mL volumetric flask, 15 ml of purified water were

added to dissolve completely; then the solution was diluted to volume (25ml) at room temperature. The 9.5% lactose + 0.8% poloxamer P188 was used for making peptide solution formulation.

5 The stability of the solubilized INS peptide was tested using high performance liquid chromatography (HPLC) protocols presented in Tables 2 and 3.

Table 2. HPLC method for detection of INS peptide (60 min)

Column details	Germini C18 (150 * 4.6 mm, 5 μ m), 110A		
Column temperature	40°C		
Mobile phase A	0.1% TFA in water (v/v)		
Mobile phase B	0.075% TFA in ACN		
Flow rate	1 mL/min		
Gradient profile	Time (mins)	% Mobile Phase A	% Mobile Phase B
	0	95	5
	8	95	5
	43	60	40
	45	95	5
	60	95	5
Detector wavelength	200 nm		
Injection volume	10 μ L		
Needle wash solvent	H ₂ O		
Dilution	ACN/H ₂ O 1:1		

Table 3. Modified HPLC method for detection of INS peptide (15min)

Column details	Germini C18 (150 * 4.6 mm, 5 μ m), 110A		
Column temperature	40°C		
Mobile phase A	0.1% TFA in water (v/v)		
Mobile phase B	0.075% TFA in ACN		
Flow rate	1 mL/min		
Gradient profile	Time (mins)	% Mobile Phase A	% Mobile Phase B
	0	90	10
	10	60	40
	10.1	60	40
	15	90	10
Detector wavelength	200 nm		
Injection volume	10 μ L		
Needle wash solvent	H ₂ O		
Dilution	ACN/H ₂ O 1:1		

Example 1. HPLC characterization of the INS peptide

INS peptide was dissolved in pure water to form a solution of about 0.5 mg/ml and analyzed by HPLC (modified method – **Table 3**). The purity results, indicating a purity of about 98.71% are presented in Table 4 and **Figure 1**.

5

Table 4. HPLC elution of INS peptide

Retention Time	Area %	S/N
12.156	0.062	0.27
12.648	0.405	2.03
12.958	98.708	460.41
13.333	0.825	2.48

Example 2. Preliminary solubility in different pH values

10 Buffers pH solubility and stability plays an important role in drug discovery and development. Knowledge of buffer stability profiles helps in modifying pre-formulation and formulation as well as the molecular structure to improve physicochemical properties and in selecting more suitable molecules for subsequent development.

Material and methods

15 Analytical HPLC-MS was performed using an Agilent 1260 series Liquid Chromatograph/Mass Selective Detector (MSD, Single Quadrupole) equipped with an electrospray interface and a UV diode array detector. Analyses were performed on an ACE C8, 50x3.0mm, 3µm column, with an isocratic 0.1% formic acid/ACN (19%) eluent, at a rate of 1ml/min over a period of 5 minutes. UV absorption was measured at 220nm and 215-395nm. MS analysis was performed with ESI (electron spray ionization) with a
20 positive ionization method.

PBS isotonic buffer was prepared from 1 tablet of Sigma Phosphate buffer saline P4417, in 200ml of deionized water (according to product reconstitution instructions).

Acetate isotonic buffers were prepared from sodium acetate/acetic acid in water in 150mM concentration. NaOH 50% solution was used to adjust the pH.

25 NaOAc solution was prepared by dissolving 1.23gr in 100ml water. Acetic acid solution was prepared by adding 945µl of acetic acid to 100ml water.

INS HCl salt was used directly in the buffers at concentration of 5mg/ml.

The samples were further diluted to 1 mg/ml before analyzing by HPLC. The samples were prepared by dilution of 80 µl of reaction solution with 320 µl of MeOH.

Each sample was filtered through PTFE 0.45um filter vial.

The samples were run in LC-MS and data used for calculation concentration in buffer.

All injections for stability assay were performed using 20 µl due to low peak intensity with 2 µl. Final concentration was calculated accordingly.

5

Results

The results are presented in Tables 5-7. It can be seen that in acetate buffers having pH 4 and pH 6 the INS peptide showed better stability at 5°C compared to the samples at 25°C. It can be seen that the assay for both cases was already very low (about 70%) at time zero.

10 In PBS buffer having pH 7.4 the INS peptide had a no solubility at all and a white precipitate was observed.

Table 5. INS stability in acetate buffer pH=4 at 5°C and at 25°C

Time (hours)	5°C		25 °C	
	Concentration calculated from LC-MS data (mg/ml)	Assay %	Concentration calculated from LC-MS data (mg/ml)	Assay %
0	0.646	66.84	0.646	66.87
24	0.602	62.32	0.640	66.25
168	0.599	62.00	0.355	36.75

15

Table 6. INS stability in acetate buffer pH=6 at 5°C and at 25°C

Time (hours)	5°C		25 °C	
	Concentration calculated from LC-MS data (mg/ml)	Assay %	Concentration calculated from LC-MS data (mg/ml)	Assay %
0	0.729	73.49	0.729	73.49
24	0.530	53.43	0.520	52.42
168	0.438	44.15	0.218	21.98

Table 7. INS stability in PBS buffer pH=7.4 at 5°C and at 25°C

Time (hours)	Area (mAU*S)	Concentration calculated from LC-MS data (mg/ml)	Assay %
0	201.90	0.030	2.91
24	Not observed	-	-
168	Not observed	-	-

Example 3. Preliminary solubility test.

Solubility of different INS peptide salts was measured in several co-solvents and in water.

INS trifluoroacetate (TFA)

INS TFA salt was dissolved in water and DMSO up to a concentration of 10 mg/ml, however upon dilution in PBS, the INS peptide precipitated.

INS acetate

INS acetate salt was soluble in water and completely insoluble in DMSO and PBS. It could be dissolved in low pH, but precipitated when the pH was raised above 4.

INS HCl

The INS peptide could be dissolved in water and formed a solution with opalescence; the pH was 2.6, which could be ascribed to the fact that the peptide is an HCl salt. Although INS TFA showed moderate solubility, it is less recommended to be used for parenteral product, thus, the HCl salt was selected for further studies. The compound showed moderate solubility in PG (a clear solution was obtained), while it shows poor solubility in PEG400 and ethanol. Table 8 shows the solubility result of the INS HCL peptide in co-solvents and water. It should be noted that the INS HCL peptide is practically insoluble in PBS namely in physiological conditions.

Table 8. Solubility results of the INS HCL peptide in co-solvents/water

Co-solvents/Buffers	Approximate Solubility (mg/mL)
PBS	Not soluble
PEG400	<5
Ethanol	<5
Propylene glycol (PG)	5~10 (clear solution in 5mg/ml)
DMSO	≤10
H ₂ O	>10 (slightly opalescence) pH 2.60 (10mg/ml)

Example 4. Preliminary screening of formulations

The target concentration of the formulation was first set at 10mg/mL, which may be diluted up to 10 times with PBS. Dilution in PBS predicts the solubility of the peptide in physiological conditions and in particular in blood and interstitial fluid. Table 9 lists the results obtained with initial formulations. The results indicated that clear solutions of peptide formulation could be obtained if small amounts of co-solvents or surfactants were added. However, the results of a dilution test in PBS showed that opalescence was

observed in most of the formulations. Appearance of opalescence may indicate that peptide aggregates or precipitates in PBS.

Table 9. Content and dilution results of preliminary formulations

	Formulation components	Appearance	final pH	Dilution in PBS
A1	water	opalescence	2.60	opalescence
A2	10% PG, 10% Cremophor EL, and 10%, pH8 buffer ¹	clear	3.23	slight opalescence particle of about 1µm
A3	15%PG, 15% pH6 buffer ¹	gel state	N/A	N/A
A4	4% SB-β-CD, 10% pH8 buffer ¹	white precipitation	N/A	N/A
A5	25%PG,, 10% pH8 buffer ¹	gel state	N/A	N/A
A6	20% PG, 10% pH8 buffer ¹	clear	3.24	slight opalescence
A7	10% Cremophor EL, 10% pH8 buffer ¹	Clear	3.23	slight opalescence
A8	10% PEG400, 10% Cremophor EL, 10% pH8 buffer ¹	Clear	3.65	slight opalescence
A9	10% PEG400, 10%PG, 10% pH8 buffer ¹	Clear	3.44	slight opalescence
A10	10% PEG 400, 10% Cremophor EL, 10% pH9 buffer ¹	Clear	3.64	slight opalescence
A11	10%PEG 400, 10% Cremophor EL, 10% 0.1M NH ₄ HCO ₃	Clear	4.90	slight opalescence
A12	80% water, 20% 0.1M NH ₄ HCO ₃	Clear	6.25	slight opalescence

¹pH6 buffer- 0.1M citrate buffer, pH8 buffer- 50 mM phosphate buffer, pH9 buffer- 50 mM borate buffer

The results with 10mg/mL concentration were not satisfactory. Opalescence was observed in redispersibility test and the pH was very low because of the existence of HCl.

10 Example 5. Formulation screening

Based on the results obtained in Example 4, the concentration of the INS HCl peptide was reduced to 5mg/ml or 2.5 mg/ml in order to prepare a clear formulation, which would maintain clear when diluted in PBS. The pH of all formulations was adjusted to be between 5 to 6 with about 4µl 1N NaOH to meet the parenteral formulation requirement. Unless stated explicitly, the carrier in all formulation was water. The content of the tested formulations and results of their dilution in PBS are summarized in **Table 10**.

Table 10. Content and dilution results for the tested formulations

	Formulation Components	Conc. (mg/ml)	Appearance	Final pH	Redispersion in PBS
F1	1% Tween 80	5	clear	5.80	slight opalescence
F2	20% PG	5	clear	6.13	slight opalescence
F3	10% SBE-β-CD	5	white	N/A	N/A

			precipitation		
F4	5% Poloxamer P188	5	clear	5.61	clear
F5	10% HP- β -CD	5	clear	6.11	slight opalescence
F6	10% Cremophor EL	5	clear	5.49	slight opalescence
F7	10% Cremophor RH40	5	clear	5.48	clear
F8	10% Solutol HS15, 4.5% Poloxamer P188	5	clear	5.58	slight opalescence
F9	10% Cremophor EL, 4.5% Poloxamer P188	5	clear	5.82	slight opalescence
F10	20% PG, 0.8% Tween 80	5	clear	5.59	clear
F11	0.5% Poloxamer P188	5	clear	7.63	slight opalescence
F12	0.5% Poloxamer P188	8	slight opalescence	7.05	slight opalescence
F13	0.5% Poloxamer P188	10	slight opalescence	6.99	slight opalescence
F14	0.5% Poloxamer P188	2.5	clear	7.41	clear
F15	5% Poloxamer P188	10	clear	5.90	clear
F16	5% Poloxamer P188	8	clear	6.38	clear
F17	5% Poloxamer P188	6	clear	6.99	clear
F18	10% Cremophor RH40	10	clear	5.57	slight opalescence
F19	10% Cremophor RH40	8	clear	5.80	slight opalescence
F20	10% Cremophor RH40	6	clear	5.52	slight opalescence
F21	10% Cremophor EL	2.5	clear	N/A	slight opalescence
F22	1% poloxamer	5	clear	6.08	slight opalescence (0.33)*
F23	1% poloxamer P188, 0.1% Tween 80	5	clear	5.8	slight opalescence (0.02)*
F24	10% PG, 0.9% poloxamer P188, 0.09% Tween 80	5	clear	5.72	slight opalescence (0.16)*
F25	10% Glycerol, 0.9% poloxamer P188	5	clear	5.64	slight opalescence (0.09)*
F26	20% PG, 10% cremophor EL	10	clear	5.66	opalescence
F27	20% PG, 10% cremophor EL	5	clear	5.88	slight opalescence
F28	20% PG, 10% solutol HS15	10	clear	5.50	opalescence
F29	20% PG, 10% solutol HS15	5	clear	5.62	slight opalescence
F30	20% PG, 0.8% Tween 80	10	clear	6.02	opalescence
F31	20% PG, 4% poloxamer P188	5	clear	5.95	slight opalescence
F32	20% PG, 10% HP- β -CD	5	clear	5.98	slight opalescence

* value in (mg/ml) means the concentration of INS HCl peptide after filtration of the diluted solution through a 0.45 μ m filter; the concentration was measured by HPLC; the estimated concentration should be equal to 0.5 mg/ml.

- 5 As can be seen in Table 10 and **Figure 2**, formulation F4, F7, F10 and F14-17 showed the best performance. These formulations were clear and remained clear after dilution with PBS. Formulations F4, F7 and F10 were further tested for stability using HPLC.

Example 6. Stability of INS HCl peptide in formulation A6

Stability of INS in Formulation A6 at concentration of 10mg/ml for 1 day was investigated. Formulation A6 was prepared according to Table 9. The samples were diluted with water prior to HPLC analysis. The recovery of INS from formulation 6 is almost 100% after 24 hours. No impurities were detected. The purity results are listed in Table 11 and **Figure 3**.

Table 11. Peaks of INS in formulation F6 as determined by HPLC

	Area%					
Retention time (min)	32.32	32.40	32.89	33.22	33.51	33.68
Initial	0.387	0	98.125	0.779	0.177	0.532
24h	0.437	0.141	97.937	0.77	0.237	0.478

Example 7. Stability of INS HCl peptide in formulations F4, F7 and F10

To investigate the stability of formulation F4, F7 and F10 they were stored at ambient temperature and at 4°C. Samples were taken out at time zero (initial) and after 24 hours and diluted 10 times with water prior to HPLC analysis. The results were compared to the standard peptide 0.5 mg/ml in water (STD). The results are presented in Tables 12-14 and **Figures 4-6**. Based on these results, a new impurity (RRT=0.88) was observed. INS HCL demonstrated high stability in all formulations, while it seems that formulation F4 provides a little bit higher stability than the other two.

Table 12. Stability results (%) of Formulation F4

#	Relative Retention Time (RRT)						
	0.88	0.95	0.98	1.00	1.01	1.02	1.03
STD	ND	ND	0.32	98.79	0.64	ND	0.25
Initial	0.08	ND	0.36	98.38	0.64	0.07	0.47
4 °C /24h	0.07	ND	0.42	98.52	0.69	ND	0.30
RT/24h	0.16	0.06	0.52	97.51	1.01	0.22	0.53

Table 13. Stability results (%) of Formulation F7

#	RRT						
	0.88	0.98	0.99	1.00	1.01	1.02	1.03
STD	ND	0.32	ND	98.79	0.64	ND	0.25

Initial	0.20	0.38	0.05	98.19	0.65	0.06	0.47	ND
4 °C /24h	0.25	0.40	0.06	98.24	0.67	ND	0.26	0.12
RT/24h	0.30	0.43	ND	98.34	0.67	ND	0.27	ND

Table 14. Stability results (%) of Formulation F10

#	RRT									
	0.88	0.97	0.98	0.99	1.00	1.01	1.02	1.03	1.05	1.09
STD	ND	ND	0.32	ND	98.79	0.64	ND	0.25	ND	ND
Initial	1.18	ND	0.37	ND	97.29	0.64	0.08	0.44	ND	ND
4 °C /24h	1.26	ND	0.45	0.05	97.02	0.63	0.05	0.27	0.10	0.17
RT/24h	1.52	0.07	0.51	ND	96.94	0.69	ND	0.27	ND	ND

ND-Not Detected

5 Example 8. Optimization of formulations

Based on previous solubility and stability results, it seems that the opalescence in PBS may indicate aggregation of the peptide, which may be related with several factors such as pH, ionic strength, oxidation of the peptide, etc. To expand the repertoire of the formulations tested, additional excipients were used. The target concentration of the INS HCl peptide was set at 5 mg/mL. The additional excipients were added in stepwise manner, and then the mixtures were stirred at 25°C for 5 mins to facilitate dissolution. The formulations were then diluted 10 folds in PBS and tested by HPLC. Unless stated explicitly, the carrier in all formulation was water. Table 15 summarized the content of formulations and their dilution stability.

Table 15. Content and dilution results for the tested formulations

	Formulation Components	Conc. (mg/ml)	Appearance	Final pH	Redispersion in PBS
F33	1% poloxamer P188, 5% Lactose	5	clear	5.66 Add 2ul of 6% NH ₄ OH	slight opalescence
F34	1% poloxamer P188, 10% Lactose	5	clear	5.67 Add 2ul of 6% NH ₄ OH	Clear (0.46)*
F35	20% PG, 0.8% poloxamer P188, 0.08% L-Arginine	5	clear	6.08 Add 1.0 mg of L-Arginine	slight opalescence
F36	1% poloxamer P188,	5	clear	6.32	slight

	0.1% L-Arginine			Add 1.0 mg of L-Arginine	opalescence
F37	0.1% L-Arginine	5	clear	6.24 Add 1.0 mg of L-Arginine	opalescence
F38	20% HP- β -CD	5	clear	6.68 Add 1.0 mg of L-Arginine	slight opalescence
F39	20% PG, 0.8 1% Tween 80	5	clear	6.83 Add 1.0 mg of L-Arginine	slight opalescence
F40	1% poloxamer P188, 5% Glucose	5	clear	5.80 Add 2ul of 6% NH ₄ OH	slight opalescence
F41	1% poloxamer P188, 5% Mannitol	5	clear	5.78 Add 2ul of 6% NH ₄ OH	slight opalescence
F42	1% poloxamer P188, 5% Sorbitol	5	clear	5.75 Add 2ul of 6% NH ₄ OH	slight opalescence

* value in (mg/ml) means the concentration of INS after filtration of the diluted solution through a 0.45 μ m filter; the concentration was measured by HPLC; the calculated concentration equals to 0.5 mg/ml.

- 5 It was found that formulation F34 showed the best performance. The formulation remained clear after dilution in PBS with no particles detected.

Example 9. Scale up

- Formulation F34 was scaled up; two additional formulations, having a pH of 5.5 and 4.0
10 were prepared as described in Table 16 below.

Table 16. Scale-up of Formulation 34.

Formulation	Weigh of INS peptide (mg)	Drug-loading (mg/mL)	pH	Acetate (μ M)	Poloxamer P188 (%)	Lactose (mM/%)	Volume (mL)
F43	50	5	5.5	100	1	2.8/9.5	10
F44	50	5	4.0	100	1	2.8/9.5	10

- Formulation F43 and F44 were prepared and tested for peptide stability after storage at 25°C and 5°C. Stability in freeze-thaw conditions were also assessed placing samples in -
15 20°C, thawing for about 1-2h in room temperature and then returning to -20 °C (this process was counted as one time/cycle). Appearance, pH, and osmolality were recorded and the concentration was analyzed by HPLC to evaluate possible degradation. The samples were taken at time points: 0h, 24h and 1week. The formulations were filtered

through 0.45µm filters and the % recovery after filtration were measured at 1 week. At last, the aggregation and purity of the INS HCl peptide were examined by dynamic light scattering (DLS). The results are presented in Tables 17-19 and **Figures 7-16**.

5 **Table 17.** physical properties of formulations F43 and F44.

	Formulation condition	Appearance	pH	Osmolality	Purity %	Recovery %
F43	initial	Clear solution	5.58	372	98.23	95.15
	5°C-1d	Clear solution	5.66	378	97.91	N/A
	25°C-1d	Clear solution	5.65	377	97.89	N/A
	-20°C-1d, freeze and thaw 3 times	Clear solution	5.65	371	97.78	N/A
	5°C-1w	Clear solution	5.72	363	98.07	95.93
	25 °C-1w	Clear solution	5.76	363	95.85	96.70
	-20°C-1w, freeze and thaw 3 times	Clear solution	5.71	366	97.81	95.29
F44	initial	Clear solution	3.95	367	98.17	97.90
	5°C-1d	Clear solution	3.97	362	98.22	N/A
	25°C-1d	Clear solution	3.95	371	98.18	N/A
	-20°C-1d, freeze and thaw 3 times	Clear solution	3.98	366	98.26	N/A
	5°C-1w	Clear solution	4.05	355	97.76	97.68
	25°C-1w	Clear solution	4.05	358	97.01	97.24
	-20°C-1w, freeze and thaw 3 times	Clear solution	4.10	361	97.36	97.81

Table 18. HPLC analysis of formulation F43

Formulation 43	RRT						
	0.82	0.88	0.98	0.99	1.00*	1.01	1.02
initial	ND	ND	0.42	ND	98.23	0.76	0.60
5°C /24h	ND	0.13	0.70	ND	97.91	0.76	0.33
25°C /24h	ND	0.16	0.89	ND	97.87	0.85	0.42
-20°C/Lyophilized /24h	ND	0.12	0.79	ND	97.78	0.88	0.44
5°C /1W	0.11	ND	0.53	ND	98.07	0.83	0.46
25°C /1W	0.13	ND	2.34	0.13	95.85	1.18	0.37
-20°C/Lyophilized /1W	0.22	0.20	0.50	ND	97.81	0.82	0.46

* INS peptide

ND -Not Detected

Table 19. HPLC analysis of formulation F44

Formulation 44	RRT							
	0.69	0.81	0.82	0.88	0.98	1.00*	1.01	1.02
initial	ND	ND	0.11	ND	0.36	98.17	0.78	0.59
5°C /24h	ND	ND	ND	ND	0.40	98.22	0.68	0.46
25°C /24h	ND	ND	ND	ND	0.41	98.15	0.66	0.47
-20°C/Lyophilized /24h	ND	ND	ND	ND	0.35	98.26	0.68	0.47
5°C /1W	ND	ND	0.19	0.23	0.39	97.76	0.94	0.50
25°C /1W	0.13	0.12	0.24	0.31	0.63	97.01	1.07	0.48
-20°C/Lyophilized /1W	0.21	0.21	0.40	0.34	0.36	97.36	0.63	0.49

* INS peptide

ND -Not Detected

- 5 It can be clearly seen from these results that no change in the appearance, pH, osmolality was observed for formulations F43 and F44 after 1 week.

In the DLS experiments, several populations of particle size were detected. Particles with the size of around 10nm may indicate micelle formulation of the poloxamer 188 and some observed particles with the size of >40nm may be correlative to aggregation of peptide molecules. Nevertheless, no more than 2% of such aggregation particles (>20 nm) were observed. The % recovery of the INS HCL peptide as measured by HPLC, was above 95% after filtration through 0.45µm filters. The peptide aggregation or absorption to the filters may explain the slightly lower recovery value in comparison to the theoretical one. It was shown that INS HCL is relatively stable at 5°C in F43 formulation, but showed about 3-3.5% reduction when maintained at 25°C condition for 1 week. Similar trend was observed for F44; some degradation (about 1-2%) was observed at 25°C and -20°C freeze-thaw condition for 1 week.

Example 10. Optimization and preparation of a lyophilized formulation

- 20 To obtain an isotonic solution having desired osmolality of about 300-330 mOsm/kg, the poloxamer percentage was reduced to 0.8% and the lactose percentage was reduced to 9.5%. The solution formulation F45 was prepared in a 40ml clear glass vial according to Table 20. The pH was adjusted by NH₄OH. After preparation, the solution was filtered

through a 0.22µm filter (PTFE) and pre-freeze at -50°C dry ice bath for 2 hours, and then the samples were transferred into the freeze-dryer.

Table 20. Preparation of isotonic formulation.

	INS peptide (mg) ¹	Drug-loading free INS peptide (mg/mL)	pH	Poloxamer P188 (%)	Lactose (%)	Total Volume (mL) ²
F45	57.06	5	5.05	0.8	9.5	10

¹ The correction factor for calculating the INS peptide percentage was 1.14 (1/88.11%)

² The INS HCl peptide was weighed and then a 10 ml solution containing 9.5% Lactose and 0.8% Poloxamer P188 was added, then about 20µl NH₄OH solution was added to adjust pH.

- 10 The lyophilization parameters of final product batch no. FR00110-6-180514-01 are listed in Table 21. After preparation, the lyophilized powder was stored and sealed in 40 mL clear glass vial with plastic stopper and stored at -20 °C refrigerator. Yield was calculated to be about 90.62% (solid mass value was used to roughly calculate the yield).

- 15 **Table 21.** The parameters of processing lyophilized formulation

Step	Temperature (°C)	Vacuum Degree (pa)	Time (h)	Weight of final product (mg)	Yield%
1	-50	1	5	985.11	90.62
2	-30	10	3		
3	-20	20	2		
4	-10	20	2		
5	-5	10	2		
6	0	10	2		
7	5	15	2		
8	10	15	2		
9	20	15	2		
10	30	10	1		

Example 11. Stability study of lyophilized formulation

- 20 The physical and chemical stability of lyophilized powder obtained in Example 10 was investigated. The lyophilized powder was stored at -20 °C. At each time point, about 218 mg of the lyophilized material was accurately weighed into a 4 mL glass vial and reconstituted completely with 2 mL of water after 2 mins at approximate target

concentration of 5 mg/mL (INS peptide free base) at 0h, 1d and 7ds. Formulations before filtration were used as a control (initial state) at each time point. After 2 minutes, the reconstituted formulations were filtered through a 0.22µm filter (PTFE). At the time point: 0h, 24 hours and 7 days appearance, pH and osmolality were tested and the solutions were analyzed by HPLC for the presence of degradation. The recovery percent after filtration was also calculated.

To investigate short-term in-use stability, additional 218 mg of lyophilized material was reconstituted, and the solution was stored at room temperature for 4 hours. The samples were then analyzed by HPLC to examine the purity and the recovery after filtration through a 0.22µm filter (PTFE).

Results

The results summarized in Tables 22-24 and **Figures 17-18** indicate that the lyophilized formulation showed good physical and chemical stability; no obvious changes in appearance, osmolality, or purity were observed for the reconstituted lyophilized powder.

The pH was slightly lower than in the initial control solution, i.e. before lyophilization. The recovery of the INS HCl peptide from the reconstituted lyophilized formulation was more than 95% after filtrating through 0.22µm filters (PTFE). The reconstituted INS HCl peptide was stable at room temperature for at least 4 hours showing no significant difference in comparison to control. PTFE filters were used in further experiments as it was found out that about 30% of the peptide was adsorbed to PES filters.

Table 22. Stability of lyophilized formulation

Condition	Appearance	pH	Osmolality	Concentration of INS peptide free base (mg/mL) ¹	Purity%	Recovery% (peak after filtration/peak before filtration)
Control ²	Clear solution	5.05	325	5.00	96.98	100.00
Control filtered 0.22µm	Clear solution			4.81	97.06	96.36
Reconstituted initial	Clear solution	4.56	311	4.83	97.66	100.00
Reconstituted initial filtered 0.22µm	Clear solution			4.57	97.76	94.55
Reconstituted	Clear solution	4.59	314	4.79	98.06	100.00

1 day						
Reconstituted 1 day filtered -0.22 μ m	Clear solution			4.79	97.98	99.97
Reconstituted 7 days	Clear solution			4.88	98.08	100.00
Reconstituted 7 days filtered -0.22 μ m	Clear solution	4.60	313	4.62	97.99	94.59

¹ The correction factor for calculating the INS percentage was 1.14 (1/88.11%), for 1ml reconstituted solution, it contains about 5.7 mg INS peptide, 8 mg poloxamer 188, and 95 mg lactose.

² The peptide solution before lyophilization (pH adjusted to 5.0).

5

Table 23. HPLC analysis of lyophilized-reconstituted formulation

Condition	RRT				
	0.88	0.98	1.00*	1.01	1.02
Reconstituted initial	ND	0.54	97.66	0.95	0.85
Reconstituted initial filtered 0.22 μ m	ND	0.51	97.76	0.98	0.75
Reconstituted 1 day	ND	0.52	98.06	0.77	0.65
Reconstituted 1 day filtered -0.22 μ m	ND	0.49	97.98	0.88	0.65
Reconstituted 7 days	0.11	0.44	98.08	0.73	0.64
Reconstituted 7 days filtered -0.22 μ m	0.12	0.48	97.99	0.73	0.71

* INS peptide

ND - Not Detected

10 **Table 24.** The stability results of reconstituted solution at room temperature for 4h

Condition	Appearance	pH	Osmolality	Conc. of INS peptide free base (mg/mL)*	Purity%	Recovery% (peak after filtration/peak before filtration)
Reconstituted solution 4h at room Temp	Clear solution			4.73	97.97	100.00
Reconstituted Solution, 4h at room Temp, 0.22 μ m filtered	Clear solution	4.55	312	4.56	97.91	96.36

* The correction factor for calculating the INS percentage was 1.14 (1/88.11%), for 1ml reconstituted solution, it contains about 5.7 mg INS HCl peptide, 8 mg poloxamer 188, and 95 mg lactose.

Example 12. In vivo efficacy studyAim

Examination of cancer treatment (together with lentiviral particles targeting to tumor antigens) in tumor mice model.

Study variables and end points

1. Mortality – every day
2. Tumor measurements – twice a week
3. Tumor weight post mortem
4. Tissue preservation – kept in formalin or were snap freezed and kept at -80°C

Materials and methods*Test items:***I. INS**

INS HCl peptide having the amino acid sequence WTAVQMAVFIHNFKRK

Physical Description: White to off-white powder

Assay (NPC): 88.11%

Correction Factor: 1.135 (1/88.11%)

Storage Conditions: -10°C to -30°C

Supplier: WuXi AppTec (Wuhan) Co., Ltd.

On “Day 1” stocks of 10 mg/ml (11 mg/ml -with correction factor of 1.1) INS in formulation or in PBS was prepared by dissolving 110 mg of INS in 10 ml of a vehicle solution (1% poloxamer 9% lactose), adjusting pH to about 5 with NaOH and completing the volume with the formulation up to 10 ml. The solution was vortexed and filtered using Nylon 0.22µm filter. The content of the stock formulation is as described in Table 25.

Table 25. content of stock formulation.

Raw Material	Mg/10 ml	Function	Lot No.	Manufacturer
INS peptide	110*	Active ingredient	31-18-0034	Polypeptide
Poloxamer 188	100	Solubilizing and stabilizing agent	K49589112	Merck
Lactose anhydrous	900	Diluent	101FX50	DFE pharma

Hydrochloric acid (HCl)	QS	pH adjuster	NA	NA
Sodium hydroxide (NaOH)	QS	pH adjuster	BCBQ3682 V	Fluka analytical
Water for injection	10ml	Solvent	16K14BA1 A	Baxter

*calculated per correction factor of 1.1 (Assay 88.11%)

Serial dilutions were made to obtain formulation comprising 5 mg/ml, 2.5 mg/ml, 0.97 mg/ml and 0.49 mg/ml by diluting the stock formulation with formulation comprising 1% poloxamer and 9% lactose.

- 5 As a control, INS HCL dissolved in PBS (at pH of about 5) was used.

The concentration of INS HCl in the stock solution and in the diluted solutions were tested by Nanodrop. The stability of the stock formulation was assessed as well. The results are provided in Table 26.

10 **Table 26.** Concentration of formulations

ID #	Prepared/diluted concentrations	A280 (Abs)	Achieved Concentration
1	5mg/ml t=0	13.062	4.7 mg/ml
		13.095	4.7 mg/ml
	5mg/ml 2 weeks stability	12.883	4.6 mg/ml
		13.122	4.7 mg/ml
2	2.5mg/ml	6.645	2.4 mg/ml
		6.647	2.4 mg/ml
3	1mg/ml	2.443	0.9 mg/ml
		2.478	0.9 mg/ml
4	0.5mg/ml	1.33	0.5 mg/ml
		1.338	0.5 mg/ml
5*	0.5 mg/ml (sample #1 diluted 1:10 in PBS)	1.301	0.5 mg/ml
		1.342	0.5 mg/ml

*The final formulation after dilution is 0.5 mg/ml INS peptide in 0.1% Poloxamer and 0.9% Lactose.

II. Lentiviral particles

- 15 The Lentiviral particles were manufactured at Sirion Biotech

GFP expressing lentiviral particles with packaging containing a single chain antibody directed against CD24 as N-terminal fusion to VSVG

Lot# KA17134_L4634-LT24_V_2 (empty) – 5×10^{10} IU/ml

Lentiviral particles were diluted to $1 \times 10^8/200\mu\text{l}$ in PBS.

Tumor mice model:

Two models of cancer were generated: lung cancer and pancreatic cancer.

5 About 5×10^6 of 1975 human lung cancer cells per 0.1 ml PBS per mouse were injected to male 7 weeks old athymic nude mice (n=30). The cells were injected, subcutaneously at one site on the back of the mice.

About 5×10^6 PANC-1 human pancreatic cancer cells per 0.1 ml PBS per mouse were injected to male 7 weeks old athymic nude mice (n=22). The cells were injected, subcutaneously at one site on the back of the mice.

10 When tumors were palpable ($\sim 0.3\text{-}0.5 \text{ cm}^3$), the mice were randomly divided into three groups (**Table 27**) and the viruses and/or peptides were injected, twice a week for two weeks. The dose injected to different groups are presented in **Table 28**.

Table 27. Group allocation

	Group	# of mice
Lung cancer		
1	PBS + Formulation	7
2	LV 10^8 + INS (1.25 mg)	7
3	LV 10^8 + INS (2.5 mg)	7
Pancreatic cancer		
4	PBS + Formulation	7
5	LV 10^8 + INS (1.25 mg)	7
6	LV 10^8 + INS (2.5 mg)	7

15

Table 28. Dose calculation to different test groups.

Test Item	Group #	Dose level	Dose volume (1000/35*0.1)	Dose solution free base	Dose solution (based on purity 1.1)	Per 35g		No. of mice
		mg/kg	ml/kg	mg/ml	mg/ml	ml	mg	
INS	2	1.25	2.9	0.43	0.49	0.1	0.043	7
	5	1.25	2.9	0.43	0.49	0.1	0.043	7
INS	3	2.5	2.9	0.86	0.97	0.1	0.097	7
	6	2.5	2.9	0.86	0.97	0.1	0.097	7

Experimental procedure

The first day of the study was defined as 'Day 1'. The animals were administrated IP with either Vehicle (PBS or Formulation), lentiviral particles and SC with test items according to Table 27 on days 1, 4, 8 and 11.

Body weight and tumor volume were checked on days 3, 7, 11, 14 and 17 (the latter was measured with a caliper). At the end of the experiment, on day 17, the mice were anesthetized and then sacrificed, and the tumors were removed, as well as lung, spleen, kidney and liver.

Results

Efficacy

As can be seen from **Figure 19**, growth of lung cancer tumors in groups administrated with full treatment, lentiviral particles and INS peptide (1.25mg/kg or 2.5mg/kg), was inhibited by 50% at day 11, relative to control. At the end of the experiment, on day 17, treatment with INS 1.25mg/kg showed better results than with INS 2.5mg/kg.

As for pancreatic cancer, weight of tumors decreases in a dose dependent manner, 25% or 50% reduction was observed in groups administered with lentiviral particles and INS (1.25mg/kg or 2.5mg/kg, respectively), relative to control as shown in **Figure 20**.

Mortality: No animal was found dead or in morbid state during this study.

Body weight: Mice gained weight as expected, with no differences between groups.

Tumor measurements: As the tumor volume of the vehicle group increased above the ethical limits, the study was arrested.

Conclusions:

Based on the above findings, INS HCL and lentiviral particles targeted specifically to tumors, showed a decrease of up to 50% in growth of tumors in lung cancer and pancreatic cancer models.

This study examined for the first time SC injection of the INS peptide with the new formulation. As can be seen in **Figures 19** and **20** SC injection of INS is both safe and effective in this model.

Example 13. Phase I/IIa clinical trialStudy type and goal

A phase I/IIa pilot -Proof of Concept, dose escalation study to evaluate safety, dose, tolerability and preliminary efficacy of INS peptide to reduce viral load in HIV patients.

- 5 This is a phase I/IIa pilot -Proof of Concept, dose escalation study.

Primary Objective

To determine the dose of INS peptide that is most closely associated with a low rate of any grade Severity Harm suspected adverse reaction within 24 hours after the INS peptide administration.

10 Secondary Objectives:

- To evaluate the safety of the therapy as measured by blood tests and physical examination
- To obtain a preliminary estimate of efficacy as measured by monitoring the rates of disease response at day 35 and 42 (± 3 days).
- 15 ▪ To evaluate duration of response at six months and 1-year post administration.

Multiple Ascending Dose (MAD):

The overall goal was to identify the dose of INS peptide for treating HIV patients.

- Treatment escalating consecutive multiple subcutaneous (SC) administration doses 0.05, 0.1, 0.2, 0.3 and 0.4 mg/kg body weight INS HCL peptide in the formulations of the present invention (2.5, 5, 10, 15, and 20 mg of INS peptide administration respectively, considering 50kg person);
- 20

Study Design

The study consists of three periods: screening, treatment and follow up as described below.

Screening

- 25 Inclusion/exclusion criteria, blood chemistry, hematology, coagulation, urinalysis and vital signs are used to assure medical fitness to participate in the study.

Inclusion Criteria:

1. Male and female 18 up to 65 years,
2. HIV patients confirmed by serology (Ab for HIV-1/HIV-2) and HIV-1/HIV-2 RNA testing.
- 30 3. Viral load <400,000 copies/mL.
4. >200 CD4 cells/mm³.

5. Willingness to undergo at least 10 visits including bi-weekly SC INS administrations; for a total duration of 4-6 weeks, as well as unscheduled visits, if required.

Exclusion Criteria:

1. Any female patient who either is pregnant, intends to become pregnant, or is currently breastfeeding (all women of child-bearing age will be questioned and informed by the study physician regarding these criteria and requested to use adequate contraception).
2. Total neutrophil count of $< 1,000\text{u/L}$, Hemoglobin $< 9.0\text{gm/dl}$ or platelet count of $< 75,000\text{u/L}$; serum creatinine $> 1.5\text{mg/dl}$, direct serum bilirubin $> 85\text{ }\mu\text{mol/l}$, AST or ALT > 2.5 times ULN.
3. Positive result for HBV or HCV.
4. Administration of HAART - Highly Active Antiretroviral Therapy or any 12 weeks prior HIV treatment.
5. Any clinically significant Grade 3 or 4 laboratory abnormality according to the Division of AIDS (DAIDS) grading scale.
6. Severe morbidity (clinically, psychiatric and behaviour) such as severe Ischemic or Congestive Heart Failure, non-controlled diabetic patients, renal insufficiency, liver cirrhosis or any disease judged by the investigator to influence the study results.
7. Any significant acute illness within 1 week before the first administration of investigational medication on this study.
8. Any immunomodulating therapy (including interferon), systemic steroids, or systemic chemotherapy within 4 weeks before Day-0.
9. Patients with malignant cancer.
10. Current enrolment in an investigational drug or device study or participation in such a study within 30 days of entry into this study.
11. Any potential significant allergy or hypersensitivity to any excipient in the Gammora formulation.

Exit Criteria:

1. HAART treatment administration during the study conduct (until termination visit)
2. Viral Load $> 1\log$ from baseline
3. Any clinically significant Grade 3 or 4 laboratory abnormality according to the Division of AIDS (DAIDS) grading scale.
4. Physical examinations.

5. Blood test

Treatment

Three cohorts of 3-6 patients (per cohort) received twice a week the Multiple Ascending Doses (MAD*) for up to 8 weeks to evaluate safety, clinical dose and preliminary efficacy for a duration of up to 6 weeks.

Interim analysis was performed to decide on the safety of the low dose and continue to a higher dose SC administration.

Study Duration: 1 year including screening, treatment and post treatment follow up

Follow-up

2 weeks from baseline follow-up visit to evaluate safety, tolerability (drop-out rate due to side effects) and preliminary efficacy.

Additional follow-up visits will take place after 42 days (± 5 days), six months (± 7 days) and one year (± 10 days) from baseline.

Tested Item and dose

15 INS-HCl (GammoraTM) having the sequence WTAVQMAVFIHNFKRK (SEQ ID NO: 1) was manufactured by "Polypeptide" with purity of 98.3%.

The INS HCl is formulated as a sterile, non-pyrogenic isotonic aqueous solution for SC administration. The formulation comprises 1% poloxamer 188 and 9% lactose anhydrous at pH about 5.

20 Dose and route of administration: 0.05, 0.1, 0.2, 0.3 and 0.4 mg/kg body weight. The formulation was administered SC, twice weekly up to 8 weeks.

In cases of safety issues (excluding neutropenic fever, infections, and haemorrhages caused by HIV infection, occurred during any treatment course with INS or within 24 (± 2) of INS administration) the INS dose is reduced by half, in the following administration.

25 Dose Level Assignment*Consecutive cohorts of Multiple Ascending Dose (MAD):*

Cohort 1: Three to six (3-6) patients, each one received twice a week the decided MAD* for up to 8 weeks (up to 16 doses per patient). Subcutaneous dose: LOW; 0.05, mg/kg, with escalation to 0.2 mg/kg body weight at week 2-4.

30 Cohort 2: Three to six (3-6) patients, each one received twice a week the decided MAD* for up to 8 weeks (up to 16 doses per patient). Subcutaneous dose: MEDIUM; 0.1 mg/kg, with escalation to 0.3 mg/kg body weight at week 2-4.

Cohort 3: Three to six (3-6) patients, each one received twice a week the decided MAD* for up to 8 weeks (up to 16 doses per patient). Subcutaneous dose: HIGH; 0.2 mg/kg, with escalation to 0.4 mg/kg body weight at week 2-4.

Interim analysis was performed to decide on the safety of all cohorts' doses and continue to a higher dose, up to 0.2 mg/kg for Cohort 1, up to 0.3 mg/kg for Cohort 2 and up to 0.4 mg/kg for Cohort 3, SC administration.

Optional Part II of the study.

According to the mechanism of action of known drugs, first an increase in viral load (VL) is expected followed by a decrease. If INS works accordingly, protease inhibitor may be combined with the peptide treatment to allow significant reduction of the viral load to level of "under-detected".

The contemplated Protease Inhibitor is a drug combination Darunavir + Ritonavir (DRV/r) in dosage of 800/100mg once daily or lopinavir 800 mg and ritonavir 200 mg once a day.

Monitoring adverse events

Patients are monitored for adverse events (AE) of the INS peptide such as rash, acute allergic reaction, bronchospasm, respiratory distress, and acute vascular leak syndrome. If a severe administration related reaction occurs, the drug administration is stopped.

Blood chemistry, hematology, coagulation, and urinalysis are repeated on Days 1, 4, 8, 11, 15, 18, 22, 25 at the clinic visits. Follow-up visits are performed at day 35, 42 (± 3 days) six (6) months (± 1 week) and one (1) year (± 1 week).

Any suspected adverse event after the 1st INS peptide administration within 24 hours.

Grade Severity Harm within 42 days after the 1st INS peptide administration.

Visits schedule

Visit 1: (up to 3 days before Baseline) Screening and Enrolment. (Medical history, Clinical examination, lab, serology and RNA tests).

Patients are prospectively enrolled to the study during a routine clinic visit, after meeting inclusion and exclusion criteria.

Visit 2 (day 1): Once all inclusions/exclusions criteria are verified, physical examination and blood tests re performed to obtain baseline values. Beginning of INS peptide administration, then within 2 hours from drug administration all safety tests to be repeated.

Visit 3- 16: INS peptide administration, physical examination and blood tests are performed

Visit 17-20: Post INS peptide administration safety follow-up: can be done by telephone contact.

Visit 21: Post INS peptide administration safety follow-up. Blood chemistry, hematology, coagulation, and urinalysis will be repeated on.

5 Endpoints

Safety Endpoint

The primary safety endpoint is the frequency, severity, and duration of side effects. Number of participants with treatment-related adverse events as assessed by CTCAE v4.03, are evaluated through study completion.

10 Main adverse events evaluation is based on DAIDS Toxicity criteria (Division of AIDS DAIDS Table for Grading the Severity of Adult and Paediatric Adverse Events, Corrected Version 2.1):

- Viral load
- Lymphopenia/ Neutropenia
- 15 • Immuno-reaction over reaction /cytokine storm
- Infection surrounding the administration site
- Liver function (lab tests)
- Kidney function (lab tests)

20 *Tolerability Endpoint*

Incidence of withdrawal (drop-out rate) due to side effects is evaluated throughout the study.

Exploratory Endpoint (Preliminary efficacy)

Effectiveness of Gammora assessed by reduced Viral load (by 0.5 LOG; Viral
25 Suppression).

1. Mean Change in CD4+ Cell Count as a Measure of Efficacy and Safety.
2. Reduced viral protein p24 antigen.
3. Functional Assessment of HIV Infection (FAHI) questionnaire.

Overall study duration

30 Total study duration is approximately 370 days from signing of informed consent to last on day 365 post-INS peptide administration. Approximately 3 weeks to recruit and complete 9-18 per-protocol- patients. 10 weeks to complete last enrolled patient last drug

administration, 1 year for last patient last visit (LPLV) and additional 2 weeks for study closure.

Results

Preliminary results showed that the peptide INS HCL in a formulation according to some
5 embodiments of the present invention, is safe and well tolerable. Patients demonstrated
through 5 weeks of the experiment that the treatment with the subcutaneous administration
of INS using the composition of the present invention is safe, non-toxic and well tolerable,
exhibit no side effects and no harm to immune system (monitored by CD4 cells) meeting
its primary endpoint. In addition, the preliminary results indicated treatment efficacy as
10 measured by decrease in viral load in HIV patients. Most patients achieved significant
reduction in viral load for up to 90% from baseline during the first 5 weeks of SC
administration, meeting its exploratory endpoint. Representative examples of viral load in
two different patients are presented in **Figures 21A and 21B**.

An additional short-term part II study was conducted in which a combination of INS with
15 protease inhibitor was administered. In this study, patients were treated for 5 weeks with a
combined treatment of the subcutaneous composition as previous (0.2-0.4 mg/kg twice a
week) and commercially available lopinavir 800 mg plus ritonavir 200 mg daily. The
control group received only lopinavir 800 mg plus ritonavir 200 mg for additional 5 weeks.
According to the results, combined-treated patients demonstrated a significant viral
20 suppression and reached reduction in HIV-1 RNA count to below 300 copies/mL, namely
up to 99% reduction in viral load from baseline as shown in **Figures 22A and 22B**
representing two different patients. In addition, patients treated with INS presented a
significantly increase of CD4 T-cell count, up to 97 % relative to baseline and improved
immune recovery in comparison to the control group that showed no change as shown in
25 **Figure 23**.

Example 14. Clinical trial in human subjects to test the safety and efficacy of INS HCL peptide in cancer treatment

Treatment Objectives:

30 To evaluate the safety, tolerability and preliminary anti-tumor activity of INS peptide
(SEQ ID NO: 1), in combination with humanized anti-CD24-lentiviral (LV) particles
administered to a cancer patient diagnosed with soft tissue sarcoma, after failing first and
second-line therapy. Cancer cells from a biopsy taken from the cancer patient prior to the

treatment were positive for CD24 (by staining) therefore, the patient is eligible for treatment targeted toward CD24.

Treatment endpoints:

5 *Primary endpoints:*

Number and frequency of treatment-related Adverse Events (SE) and Serious Adverse Events (SAE) and laboratory data

Secondary endpoints:

- Objective tumor response
- 10 • A reduction in primary tumor size to evaluate the therapeutic effectiveness of a specific short-term treatment using Computed Tomography (CT) or Magnetic Resonance Imaging (MRI). Size of the primary tumor is compared to the size of the tumor after last treatment dose.

Additional data to be evaluated:

- 15 • Progression free survival: The length of time during and after the treatment, that the patient lives with the disease but it does not get worse.

Treatment Design:

Patient with advanced or metastatic cancer positive to membranal CD24, after failing first and second-line therapy are selected by the physician to receive the compassionate
20 treatment. The patient receives a full explanation concerning the treatment and provide a signed informed consent form. The physician thoroughly assesses the patient's medical history and eligibility for receiving the treatment. An eligible patient receives a 4-week treatment of anti-CD24-LV together with INS peptide in combination with second-line gemcitabine chemotherapy plus NAB paclitaxel and other standard of care therapies as per
25 hospitals standards.

Anti-CD24-LV treatment (I.M, 2×10^9 IU/1ml, twice or three times per week) is administered along with INS peptide (S.C, 20 mg per administration) during a treatment period of 6 weeks. After last treatment dose (6 weeks), the patient followed; if improvement is observed the treatment is continued, otherwise, the treatment is
30 stopped.

Safety related parameters and preliminary efficacy parameters are evaluated throughout the treatment and follow-up period.

Composition of the Product

1. INS peptide 10 mg/ml (20mg per administration) for nonconsecutive days SC administration. INS peptide is produced by Polypeptide USA.

2. Lenti-virus particles 2×10^9 [IU/1ml] for IM twice/three times a week administration

5 Lentivirus particles are produced by Sirion Germany.

Treatment Period

Days 1-4 (baseline evaluations and dose-titration)

Day 1.

The following safety assessments and baseline evaluations are performed: Demographics (age, weight, height); concomitant medication; vital signs (breathing rate, heart rate, temperature, blood pressure); full physical examination including the following systems: skin, lymph nodes, head, eye, ear, nose and throat, respiratory, cardiovascular, gastrointestinal, neurologic and musculoskeletal; and blood tests (biochemistry: complete panel including renal function, liver function and CRP; hematology - White Blood Cells (WBC), hemoglobin, neutrophils, and platelets; coagulation – Prothrombin Time (PT), activated Partial Thromboplastin Time (PTT) and International Normalized Ratio (INR); inflammatory markers assessment; tumor specific biomarkers (such as CA19-9); and determination of membranal CD24 marker from tumor biopsy).

The physician assesses eligibility and determine whether the patient can continue to receive first titration dose.

Dose Titration

A. SC administration of INS HCl peptide in the formulation of the present invention (20mg) half an hour pre-LV administration.

B. Anti-CD24-LV full-dose administration (I.M, 2×10^9 TU/ml)

25 C. SC administration of INS peptide (20mg) 3 hours post-LV administration.

D. SC administration of INS peptide (20mg) 3 hours post last INS administration.

E. Next Day - SC administration of INS peptide (20mg) 20-24 hours post-LV administration.

In cases there are issues with AEs/SAEs that according to the physician decision justifies reduction of the daily dose, the latest tolerable dose is administered until the end of the treatment period.

Days 5-30

The following assessments are performed:

- AE/SAE assessments
- Vital signs (breathing rate, heart rate, temperature, blood pressure)
- Full physical examination including skin, lymph nodes, head, eye, ear, nose and throat, respiratory, cardiovascular, gastrointestinal, neurologic and musculoskeletal

5 • Concomitant medication

• Blood tests (blood tests are taken at the baseline, after 15 days and after 30 days of treatment as part of end of treatment assessments). Biochemistry: complete panel including renal function, liver function and CRP; Hematology - White Blood Cells (WBC), hemoglobin, neutrophils, and platelets; Coagulation – Prothrombin Time (PT), activated Partial Thromboplastin Time (PTT) and International Normalized Ratio (INR); Inflammatory markers assessment; and Tumor specific biomarkers (such as CA19-9)

Day 30 (end of treatment assessments)

Final treatment assessments are performed after last treatment dose with Anti-CD24-LV and INS peptide. The following assessments are performed: AE/SAE assessments; Vital signs (breathing rate, heart rate, temperature, blood pressure); Full physical examination including skin, lymph nodes, head, eye, ear, nose and throat, respiratory, cardiovascular, gastrointestinal, neurologic and musculoskeletal; Concomitant medication, Blood tests as described for days 5-30; Objective tumor response via using CT or MRI (as soon as possible after end of treatment assessments) and Evaluation of progression free survival.

20 *Follow-up*

If improvement is seen, the treatment is continued, otherwise, stopped. If needed, the patient continues to receive the regular second-line chemotherapy gemcitabine plus NAB paclitaxel and other standard of care therapies as per hospitals standards. Objective tumor response and progression free survival is followed.

25 Treatment criteria:

Inclusion:

1. Male or female patients aged >18 years.
2. Cancer patient proven either by histology (surgical biopsy) or cytology (CT- or endoscopic-guided), with positive to membranous CD24.
- 30 3. Patients with measurable or evaluable disease according to the response evaluation criteria in solid tumors
4. Patients eligible for second-line therapy after failing first-line therapy.

5. Recovered from reversible toxicities of prior therapy
6. Patients with a malignancy that is currently not amenable to surgical intervention, due to either medical contraindications or non-resectability of the tumor.
7. ECOG performance status 0-1 with anticipated life expectancy of ≥ 3 months.
- 5 8. Adequate baseline organ function (hematologic, liver, renal and nutritional)
9. Patients, both male and female, who are either not of childbearing potential or who agree to use a medically effective method of contraception during the study and for 3 months after the last dose of treatment drug.
10. Patients with the ability to understand and give written informed consent for receiving this treatment, including all evaluations and procedures as specified by this protocol.

Exclusion:

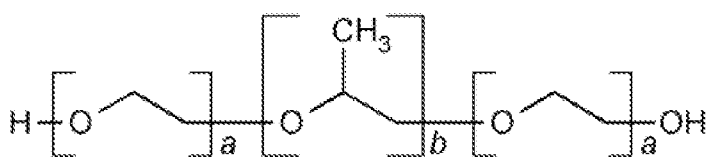
1. Active infection or other serious illness or autoimmune disease
2. Treatment with live attenuated vaccines in the last three weeks
- 15 3. Viral syndrome diagnosed during the two weeks before inclusion
4. Women who are pregnant or lactating. Women of child-bearing potential (WOCBP) and fertile men with a WOCBP partner, not using adequate birth control.
5. Patients with any hematologic abnormalities at baseline.
6. Patients with any serum chemistry abnormalities at baseline:
- 20 7. Patients with a significant cardiovascular disease or condition.
8. Patients with a history of human immunodeficiency virus (HIV) or active infection with hepatitis B virus (HBV) or hepatitis C virus (HCV).
9. Patients with inadequate recovery from any prior surgical procedure, or patients having undergone any major surgical procedure within 1 month prior to first study drug administration.
- 25 10. Patients with any other life-threatening illness, significant organ system dysfunction, or clinically significant laboratory abnormality, which, in the opinion of the Investigator, would either compromise the patient's safety or interfere with evaluation of the safety of the study drug
- 30 11. Patients with a psychiatric disorder or altered mental status that would preclude understanding of the informed consent process and/or completion of the necessary studies
12. Patients with the inability, in the opinion of the Investigator, to comply with the protocol requirements

13. Females who are pregnant or breast-feeding
14. Participation in an investigational drug or device study within 14 days of the first day of dosing on this study

5 Although the present invention has been described herein above by way of preferred embodiments thereof, it can be modified, without departing from the spirit and nature of the subject invention as defined in the appended claims.

CLAIMS

1. A pharmaceutical composition comprising from about 0.1 to about 30 mg/ml of a salt of a peptide consisting of amino acid sequence SEQ ID NO: 1 (INS), from about 0.1 wt% to about 15 wt% of a poloxamer having the structure of Formula I,



Formula I,

and a pharmaceutically acceptable carrier, wherein the pH of the composition is between 4 to about 7.5 and wherein a is an integer from 50 to 120, and b is an integer from 15 to 40.

2. The pharmaceutical composition of claim 1, wherein the salt of INS is a hydrochloride salt (INS HCl).
3. The pharmaceutical composition according to claim 1 or 2, wherein a is an integer from 60 to 90 and b is an integer from 25 to 35.
4. The pharmaceutical composition according to claim 3, wherein $a=80$ and $b=27$ (poloxamer 188).
5. The pharmaceutical composition according to claim 4, wherein the composition comprises from about 1 wt% to about 10 wt% or from about 0.5 wt% to about 5 wt% of poloxamer 188.
6. The pharmaceutical composition according to claim 5, wherein the composition comprises from about 1 mg/ml to about 20 mg/ml of the peptide INS as HCl salt.
7. The pharmaceutical composition according to claim 1, comprising from about 1 to about 10 wt% of poloxamer 188 and from about 5 mg/ml to about 15 mg/ml of the peptide INS as HCl salt.

8. The pharmaceutical composition according to claim 5, wherein the composition comprises: from about 0.5 wt% to about 3 wt% of poloxamer 188 and from 2 to 12 mg/ml of the peptide salt INS HCl.
9. The pharmaceutical composition according to any one of claims 1 to 8, further comprising a saccharide selected from a disaccharide and polysaccharide.
10. The pharmaceutical composition according to claim 9, comprising from about 1wt% to about 20 wt% of the saccharide.
11. The pharmaceutical composition according to claim 10, comprising from about 5 wt% to about 12 wt% of a disaccharide.
12. The pharmaceutical composition according to claim 11, wherein the disaccharide is lactose.
13. The pharmaceutical composition according to claim 1, comprising from about 0.5 to about 10 wt% of poloxamer 188, from about 5 mg/ml to about 15 mg/ml of HCl salt of the peptide consisting of amino acid sequence SEQ ID NO: 1 and from about 5 to about 12 wt% of lactose.
14. The pharmaceutical composition according to claim 1, comprising from about 8 to 12 wt% lactose and:
 - (i) from about 0.5 to about 2 wt% of poloxamer 188, and from about 1 mg/ml to about 5 mg/ml of INS HCl; or
 - (ii) from about 0.8 to about 2 wt% of poloxamer 188, and from about 5 mg/ml to about 10 mg/ml of INS HCl; or
 - (iii) from about 0.8 to about 2 wt% of poloxamer 188, and from about 1 mg/ml to about 15 mg/ml of INS HCl; or
 - (iv) from about 0.5 to about 2 wt% of poloxamer 188, and from about 1 mg/ml to about 12 mg/ml of INS HCl.
15. The pharmaceutical composition according to any one of claims 1 to 14, wherein the pH is from about 4 to about 6.
16. The pharmaceutical composition according to claim 1, comprising:

(i) from 2.5 to 20 mg/ml of INS HCl, 1% poloxamer 188 and 9% lactose anhydrous; or

(ii) from 2.5 to 20 mg/ml of INS HCl, 0.8% poloxamer 188 and 9.5% lactose anhydrous,

5 wherein the composition has pH of about 5.

17. The pharmaceutical composition according to any one of claims 1 to 16, wherein the composition is a liquid or semi-liquid composition.

18. The pharmaceutical composition according to claim 17, wherein the composition is an aqueous solution.

10 19. The pharmaceutical composition according to any one of claims 1 to 18, wherein the composition is a stable composition.

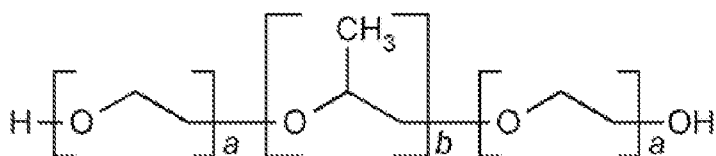
20. The pharmaceutical composition according to any one of claims 1 to 19, wherein the composition is a parenteral composition.

15 21. The pharmaceutical composition according to claim 20, wherein the composition is formulated for an administration selected from intravenous administration, intramuscular administration and subcutaneous administration.

22. The pharmaceutical composition according to any one of claims 1 to 21, further comprising an additional active agent.

20 23. A pharmaceutical composition of any one of claims 1 to 22, wherein the composition is a lyophilized composition.

24. A solid pharmaceutical composition comprising a salt of peptide consisting of amino acid sequence SEQ ID NO: 1 and a poloxamer having a structure of Formula I,



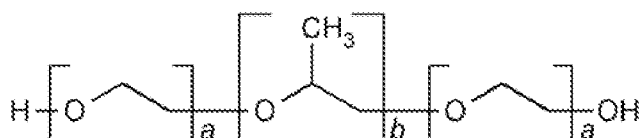
Formula I,

wherein a is an integer from 50 to 120, and b is an integer from 15 to 40 and wherein the weight ratio between said peptide salt and said poloxamer is from about 10:5 to about 1:1500.

25. The solid pharmaceutical composition of claim 24, wherein the salt of said peptide is a hydrochloride salt (INS HCl) and the poloxamer is poloxamer 188.
26. The solid pharmaceutical composition of claim 24 or 25, wherein the weight ratio between said INS peptide salt and said poloxamer 188 is from about 10:1 to about 1:1000; or wherein the weight ratio between said INS peptide salt and said poloxamer 188 is from about 2:1 to about 1:20 or from about 1:1 to about 1:5.
27. The solid pharmaceutical composition according to any one of claims 24 to 26, further comprising a saccharide selected from a disaccharide and polysaccharide.
28. The solid pharmaceutical composition of claim 27, wherein the weight ratio between said INS peptide salt and said disaccharide is from about 4:1 to about 1:2000.
29. The solid pharmaceutical composition of claim 27 or 28, wherein the disaccharide is lactose.
30. The solid pharmaceutical composition of any one of claims 24 to 29, wherein the weight ratio between said peptide salt and poloxamer 188 is between about 3:1 to 1:20 and the weight ratio between said peptide salt and lactose is between about 4:1 to about 1:24.
31. The solid pharmaceutical composition according to any one of claims 24 to 30, wherein the composition is a lyophilized composition.
32. The solid pharmaceutical composition according to claim 24, comprising from about 1.75 wt% to about 22 wt% of INS HCl, from about 3.5 wt% to about 63 wt% of poloxamer 188 and from about 30 wt% to about 92 wt% of lactose; or from about 2 wt% to about 20 wt% of INS HCl, from about 8 wt% to about 9.5 wt% of poloxamer 188 and from about 75 wt% to about 88 wt% of lactose.

33. A reconstituted pharmaceutical composition prepared from a solid composition according to any one of claims 24 to 32.

34. The reconstituted pharmaceutical composition according to claim 33, comprising from about 0.1 to about 30 mg/ml of a salt of a peptide consisting of amino acid sequence SEQ ID NO: 1, and from about 0.1 wt% to about 15 wt% of a poloxamer, wherein the pH of the composition is between 4 to about 7.5 and wherein the poloxamer has a structure of Formula I,



Formula I,

wherein a is an integer from 50 to 120, and b is an integer from 15 to 40.

35. A hydrogen halide salt of a peptide comprising amino acid sequence selected from SEQ ID NO: 1 and SEQ ID NO: 2, wherein said peptide consists of 15 to 30 amino acids.

36. The hydrogen halide salt of claim 35, wherein the peptide consists of amino acid sequence selected from SEQ ID NO: 1 and SEQ ID NO: 2.

37. The hydrogen halide salt of claim 35 or 36, wherein the hydrogen halide salt is a hydrochloride salt.

38. A pharmaceutical composition comprising the hydrogen halide salt according to any one of claims 35 to 37.

39. The pharmaceutical composition according to any one of claims 1 to 22, 33, 34 and 37 for use in treating a disease wherein treating results in destroying a specific population of target cells.

40. The pharmaceutical composition according to claim 39, wherein the disease is selected from HIV-1 infection, acquired immune deficiency syndrome (AIDS) and cancer.

41. The pharmaceutical composition for use according to claim 39 or 40, wherein the composition is administered in combination with:
- (i) a linear molecule of double-stranded DNA (dsDNA) comprising long term repeat (LTR) sequences recognized by the integrating enzyme of (ii);
- 5 (ii) an integrating enzyme, capable of entering the nuclei of cells after binding to the LTR sequences of the dsDNA molecule of (i) and creating multiple double strand breaks (DSBs) in the chromosomal DNA of the cells; and optionally (iii) a targeting moiety.
42. A method of treating a disease selected from viral infection and cancer, in a subject
10 in need thereof, the method comprising administering a pharmaceutical composition according to any one of claims 1 to 22, 33, 34 and 37.
43. The method according to claim 42 wherein the disease is HIV-1 infection or AIDS.
44. The method of claims 43, comprising co-administration of the peptide INS HCl with at least one additional anti-viral agent.
- 15 45. The method of claim 44, wherein the at least one additional anti-viral agent is a protease inhibitor.
46. The method of claim 45, comprising daily administration of lopinavir 400 to 1000 mg and ritonavir 100 to 300 mg.
47. The method according to claim 42, wherein the disease is cancer and the method
20 comprises co-administration of lentiviral particles containing an antibody directed against CD24.
48. The method of any one of claims 42 to 47, wherein the composition is administered parenterally.
49. The method of claim 48, comprising subcutaneously administering 0.05 to 1 mg/kg
25 of the peptide INS HCl.

50. The method of claim 49 wherein the pharmaceutical composition comprises from about 8 to 12 wt% lactose, from about 0.8 to about 2 wt% of poloxamer 188, and from about 1 mg/ml to about 15 mg/ml of INS HCl.

FIGURE 1

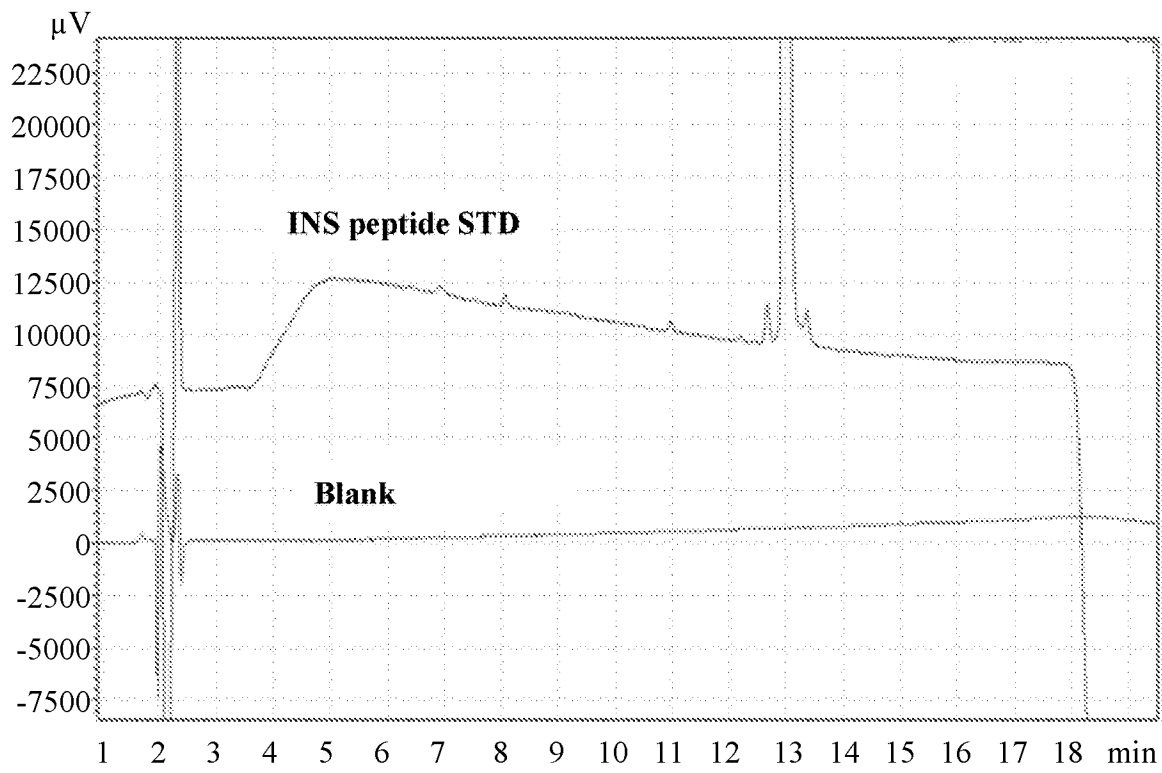


FIGURE 2

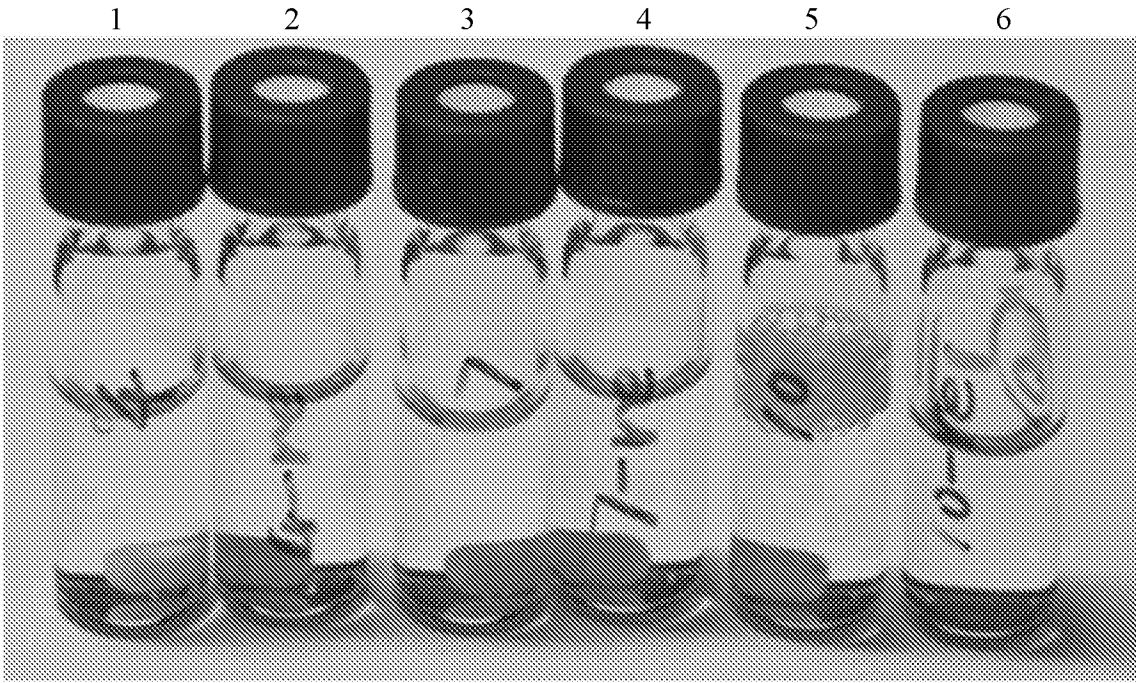


FIGURE 3

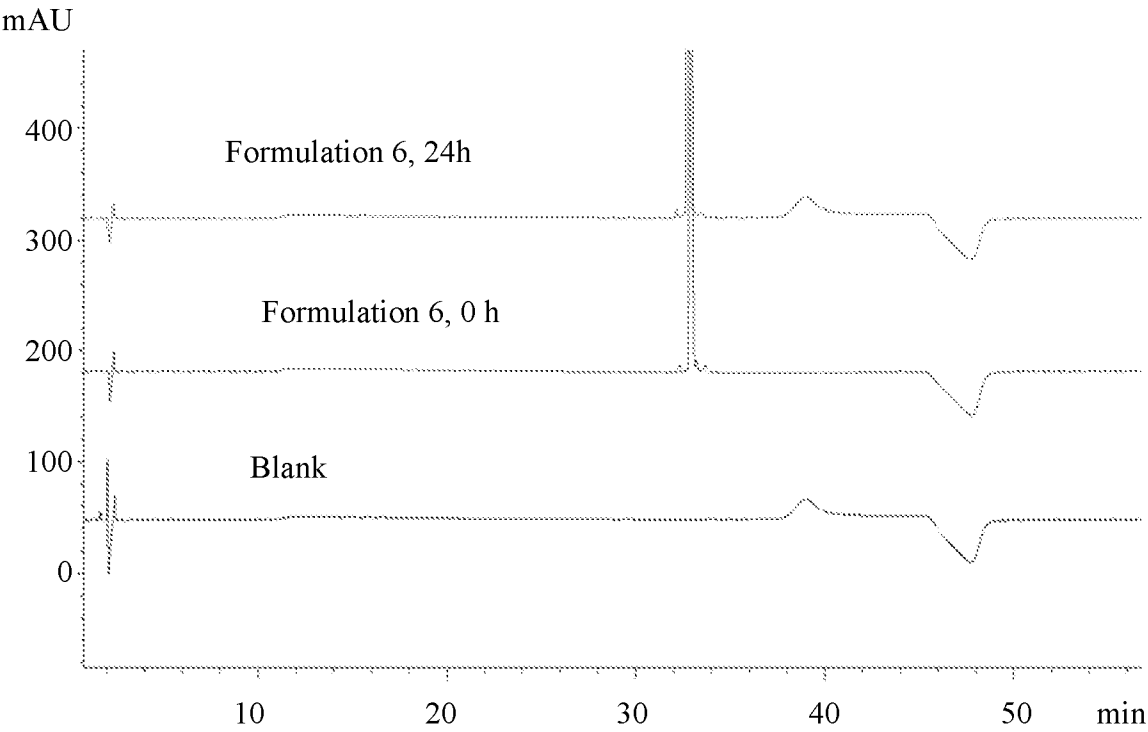


FIGURE 4

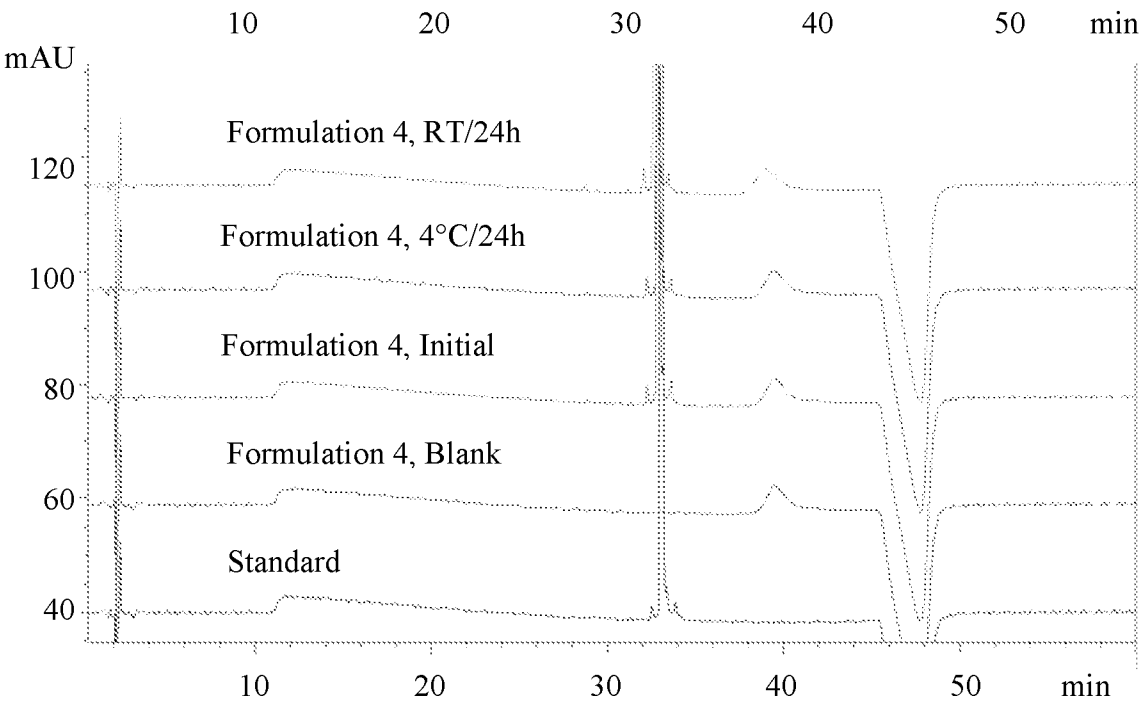


FIGURE 5

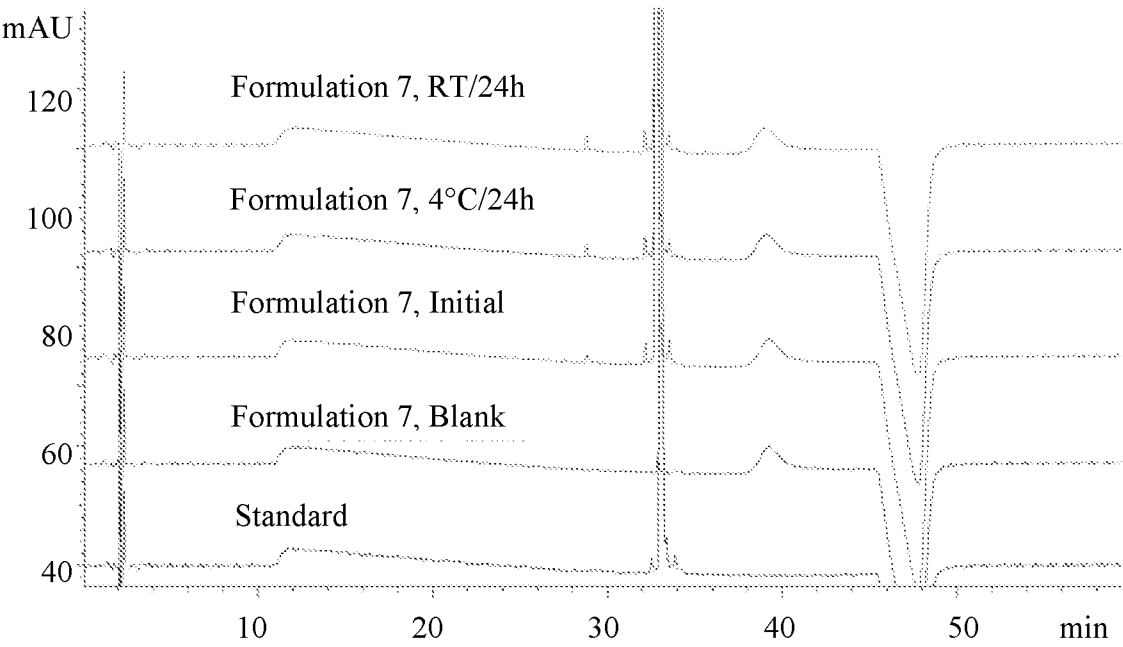


FIGURE 6

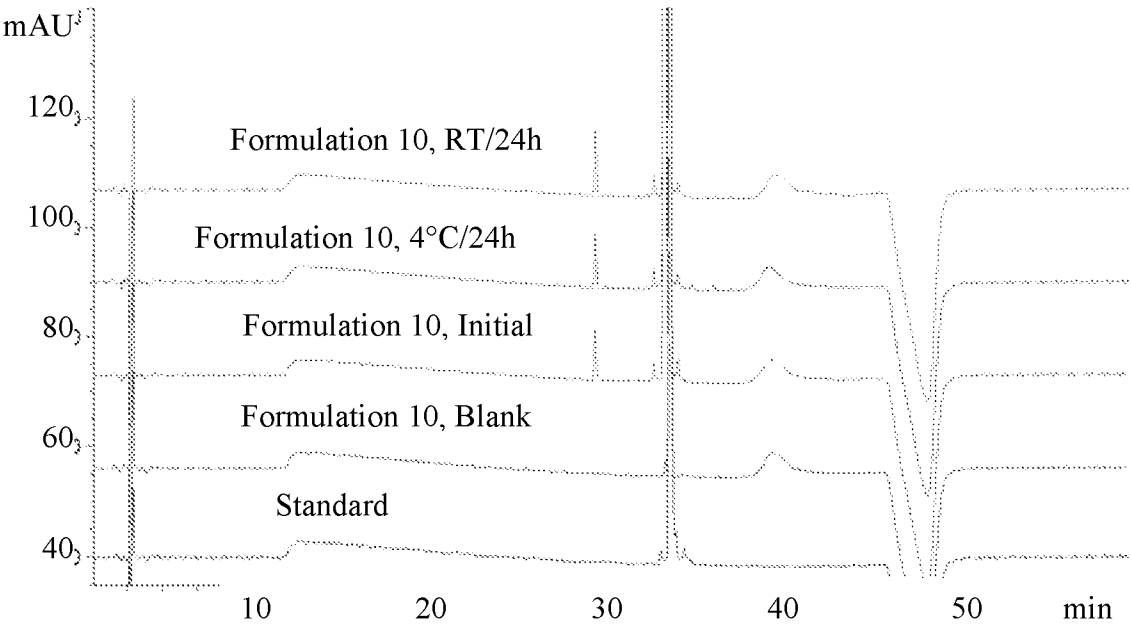


FIGURE 7

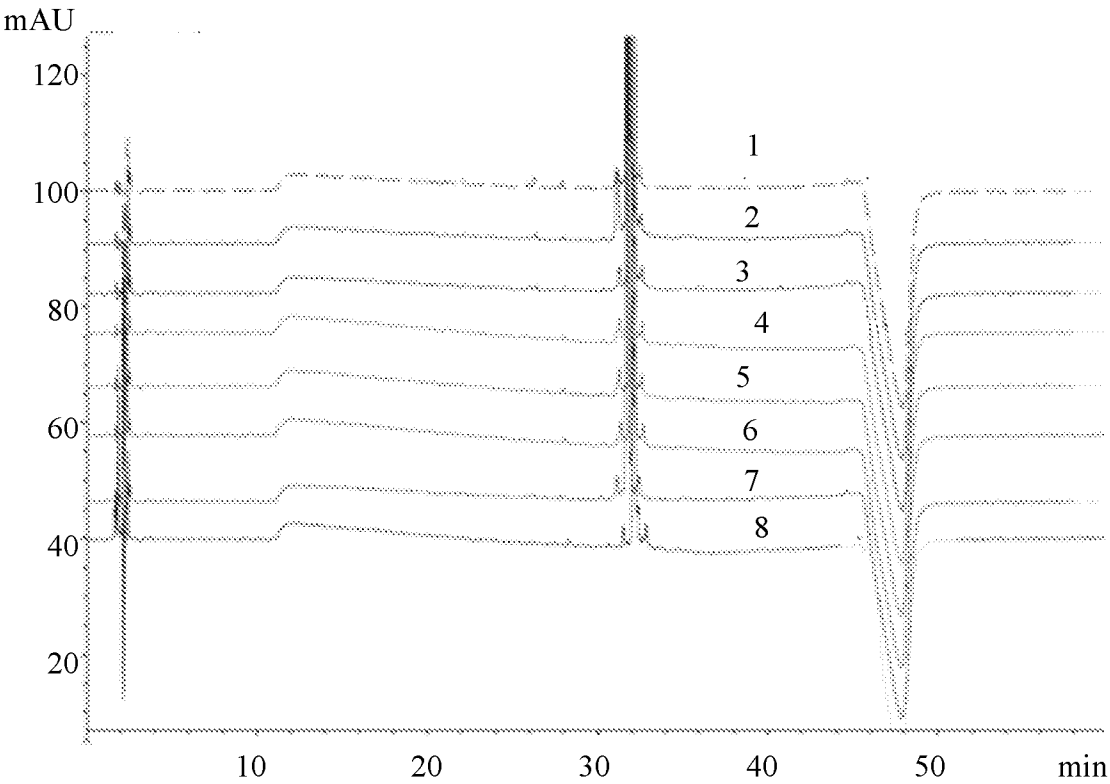


FIGURE 8

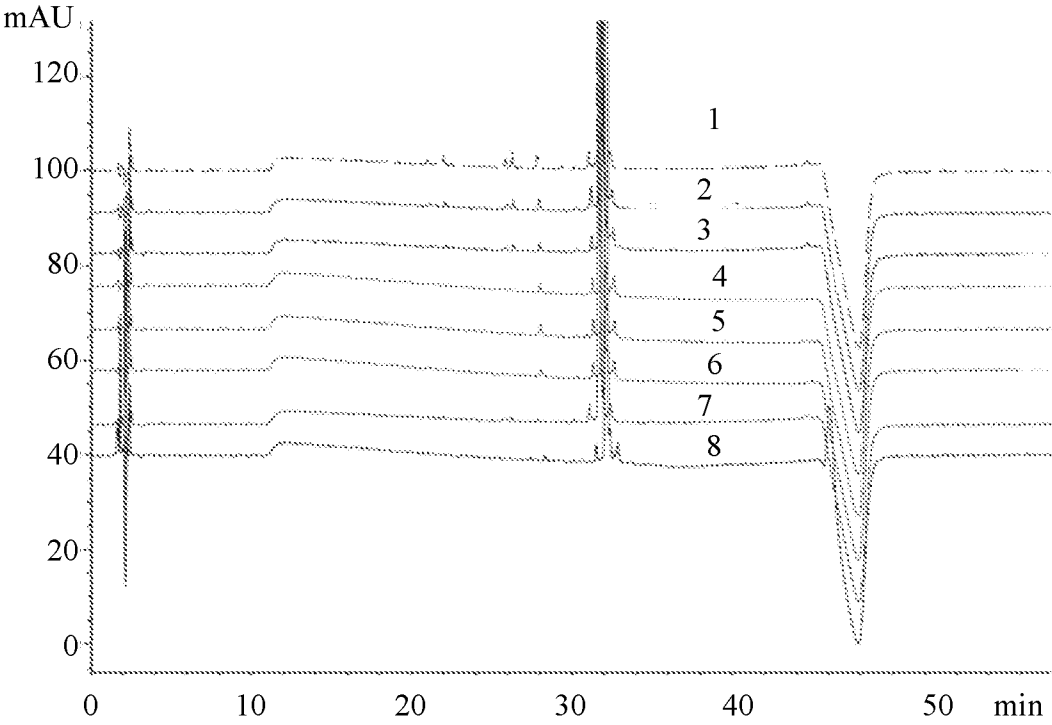


FIGURE 9

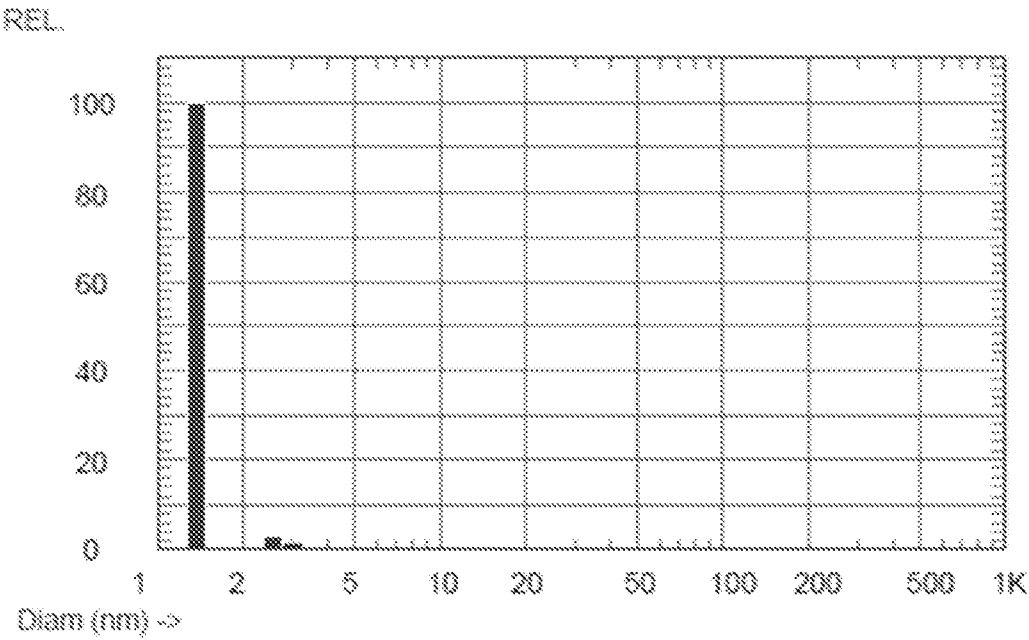


FIGURE 10

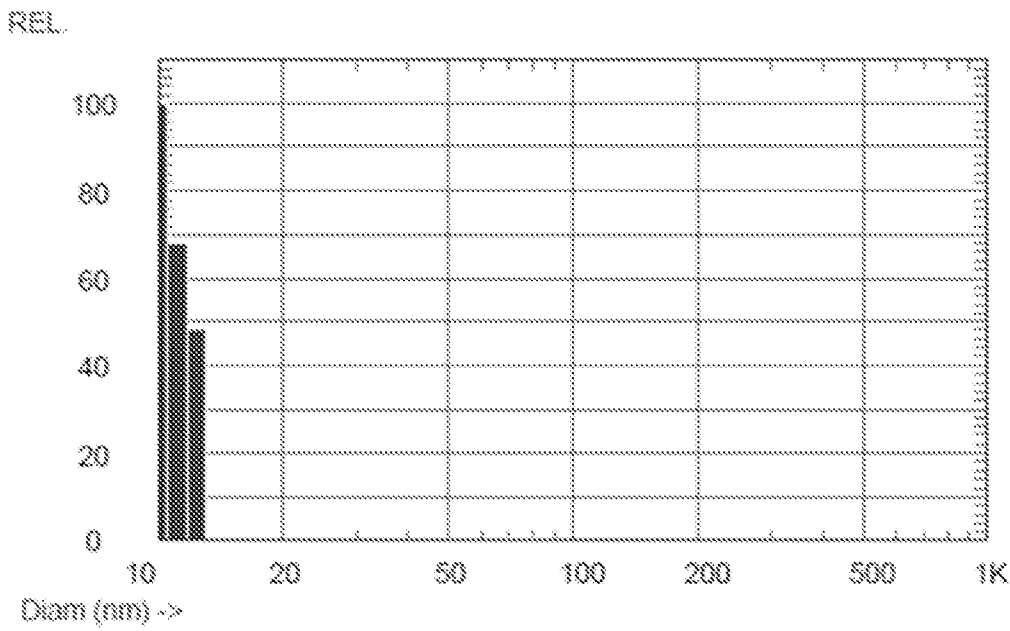


FIGURE 11

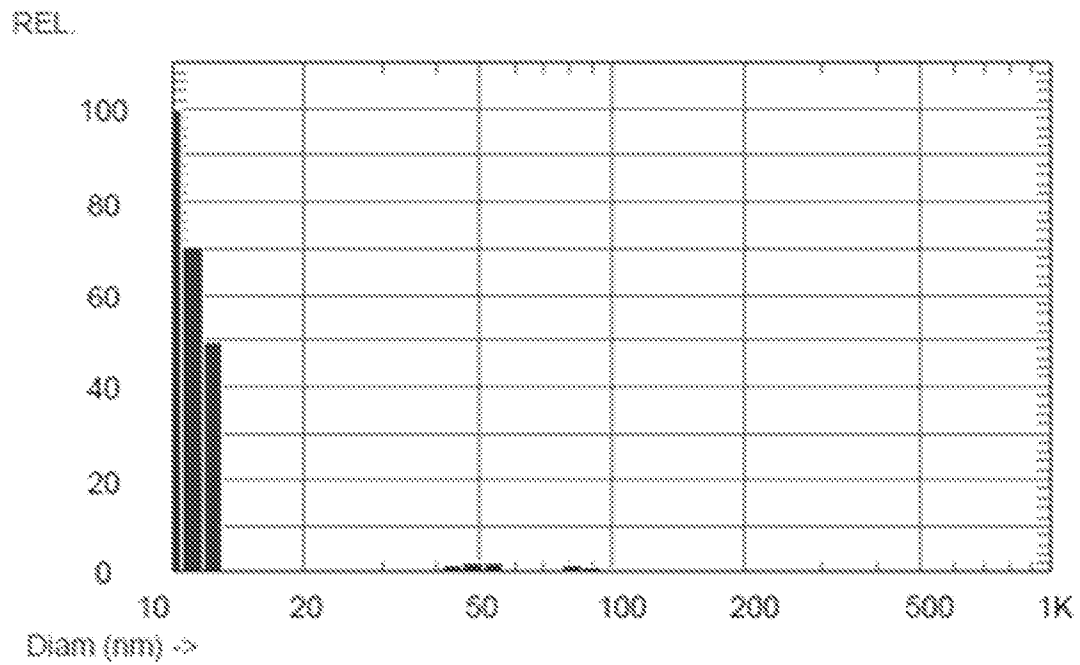


FIGURE 12

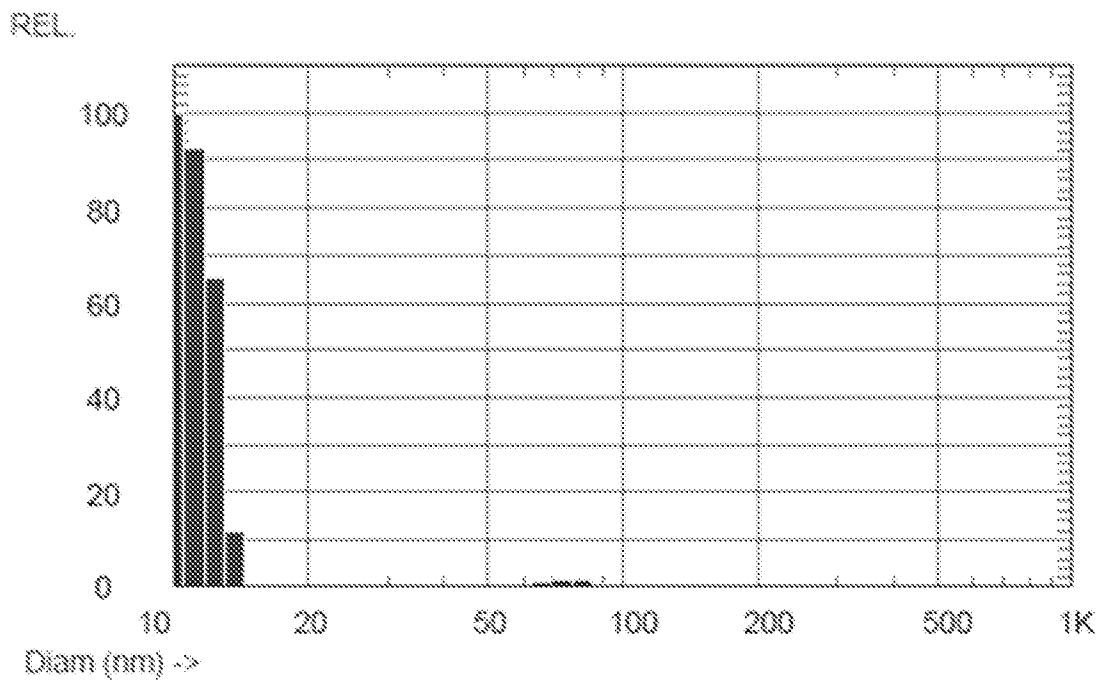


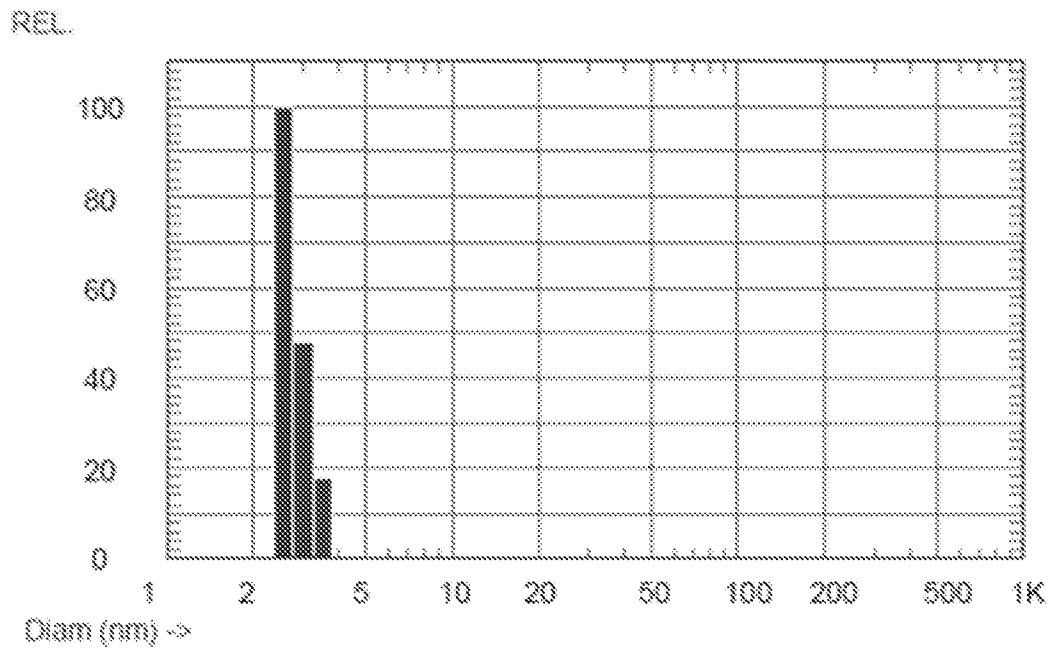
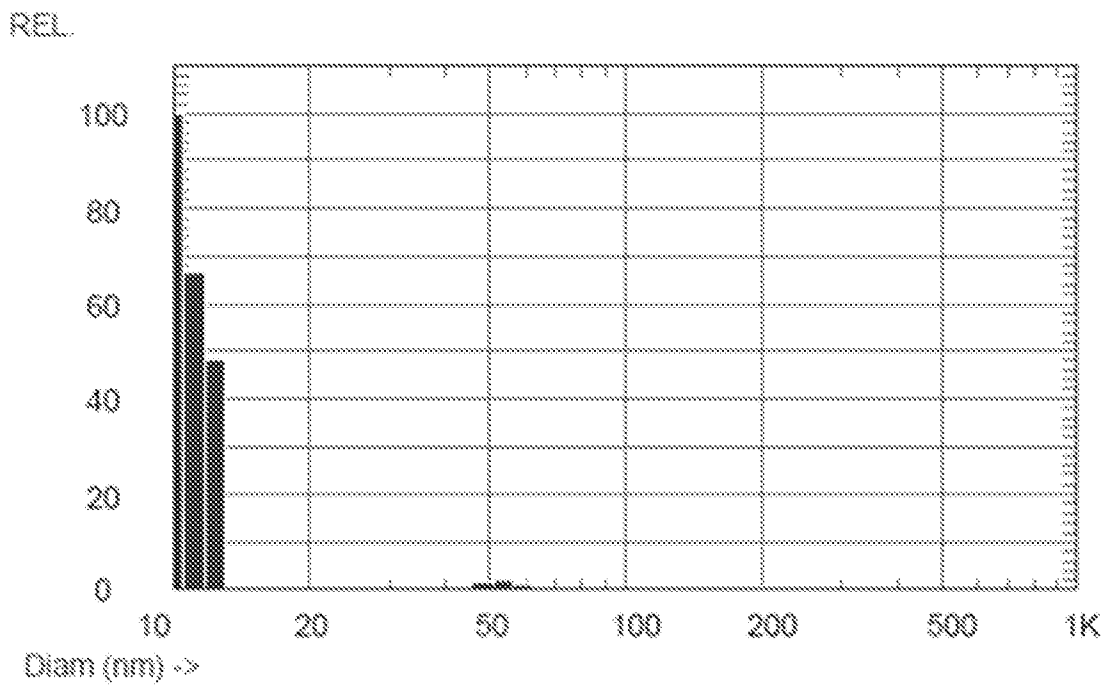
FIGURE 13**FIGURE 14**

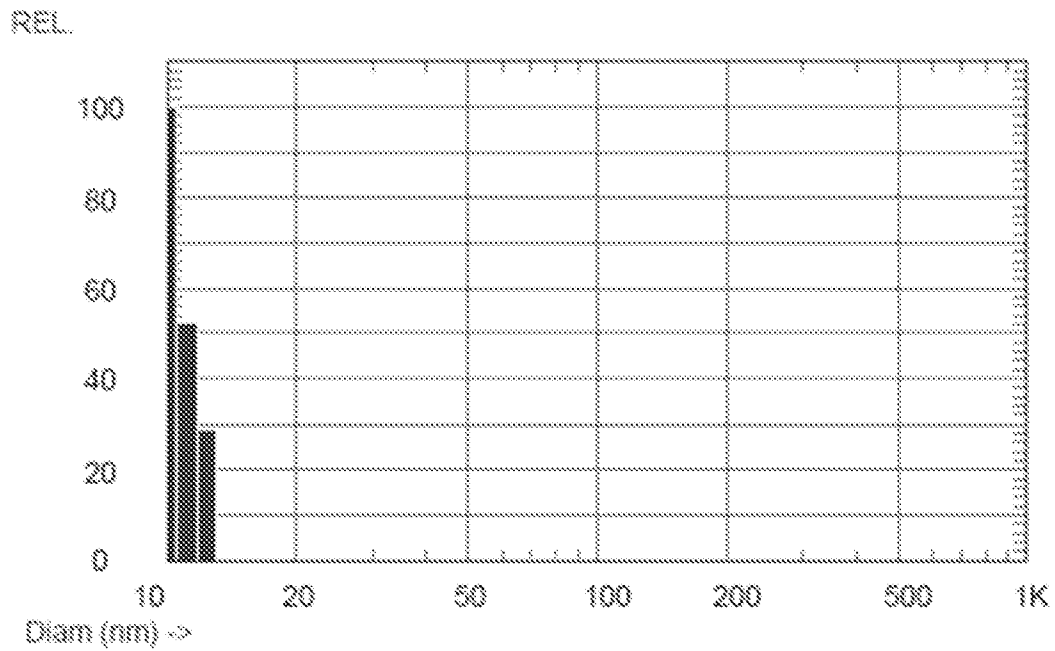
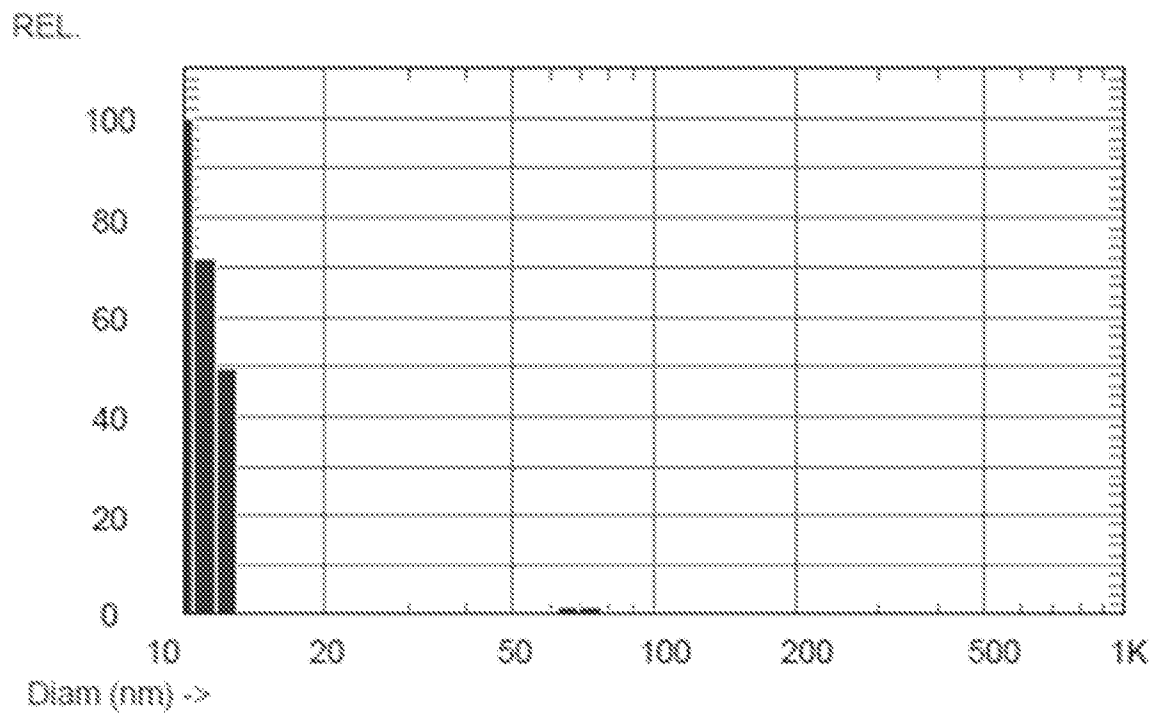
FIGURE 15**FIGURE 16**

FIGURE 17A



FIGURE 17B

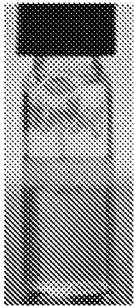


FIGURE 18

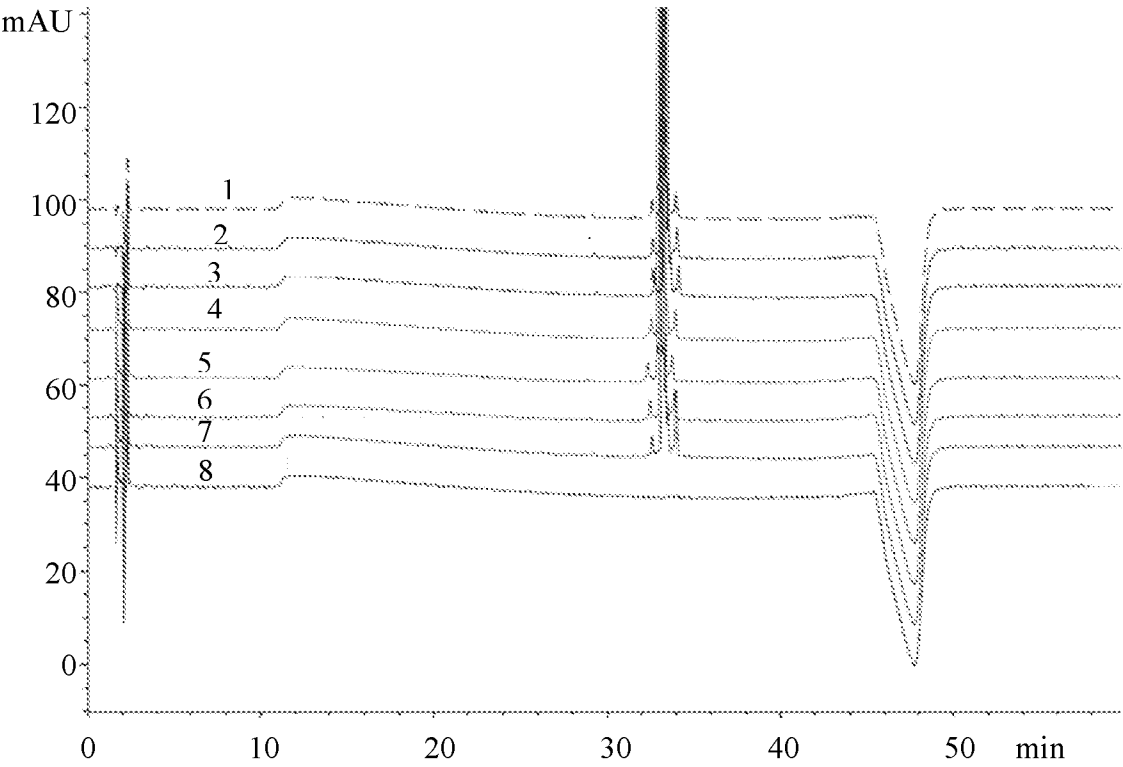


FIGURE 19

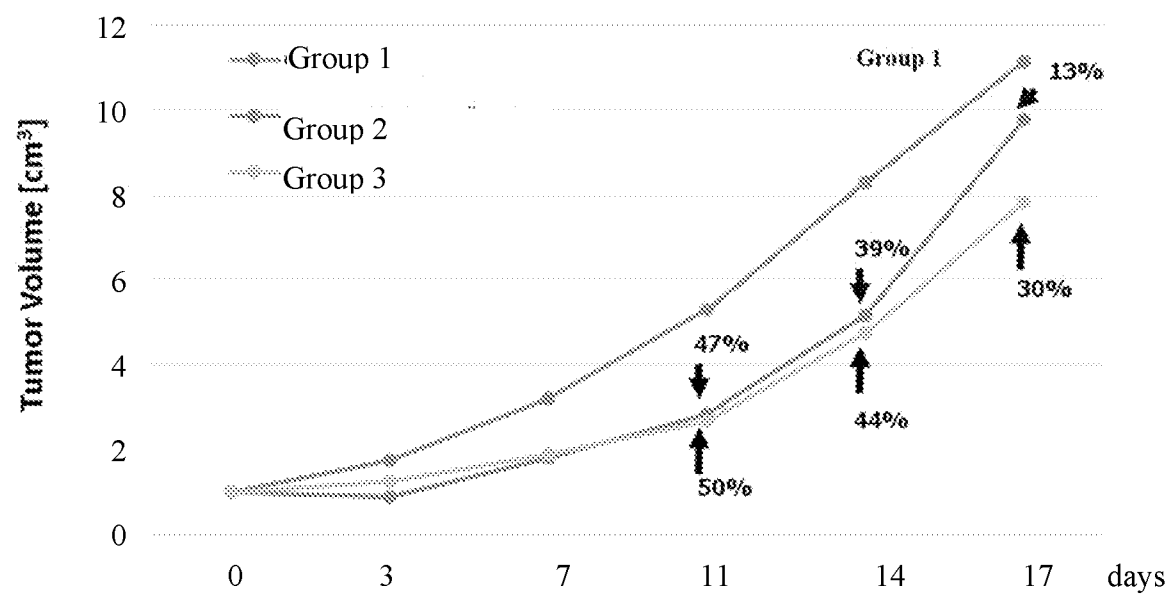


FIGURE 20

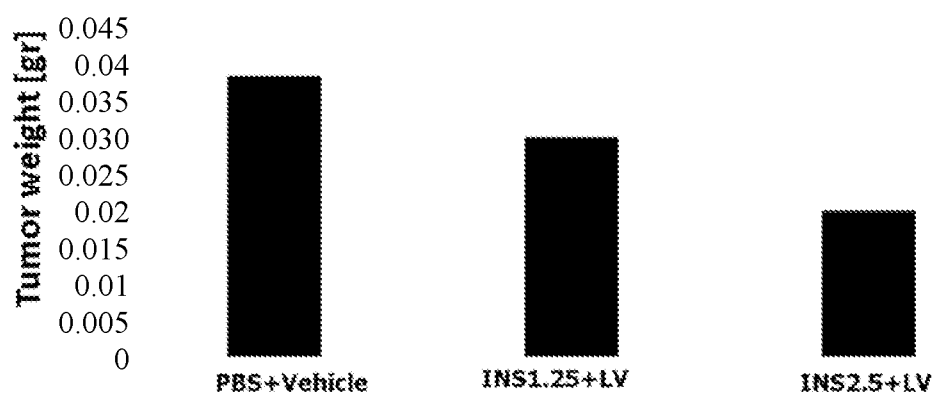


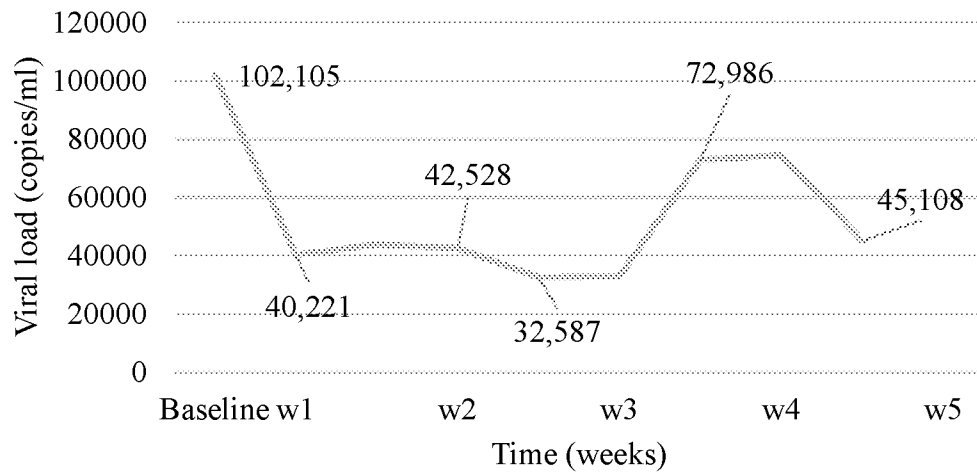
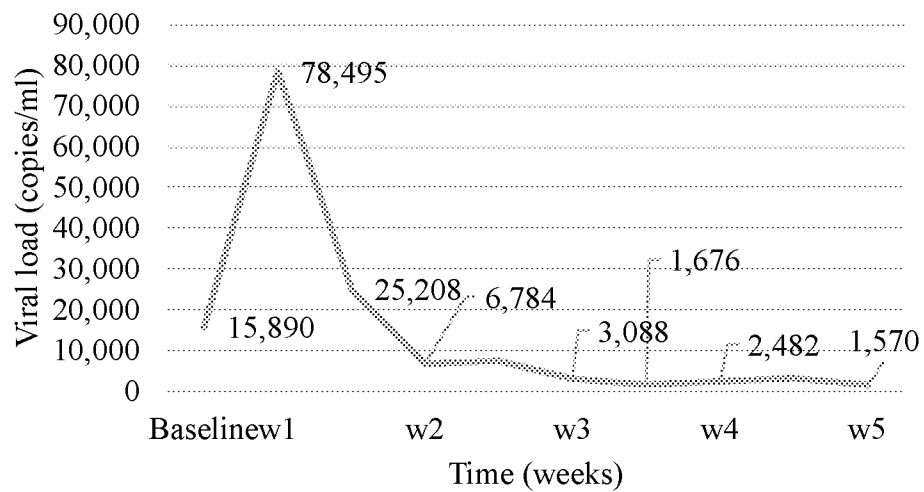
FIGURE 21A**FIGURE 21B**

FIGURE 22A

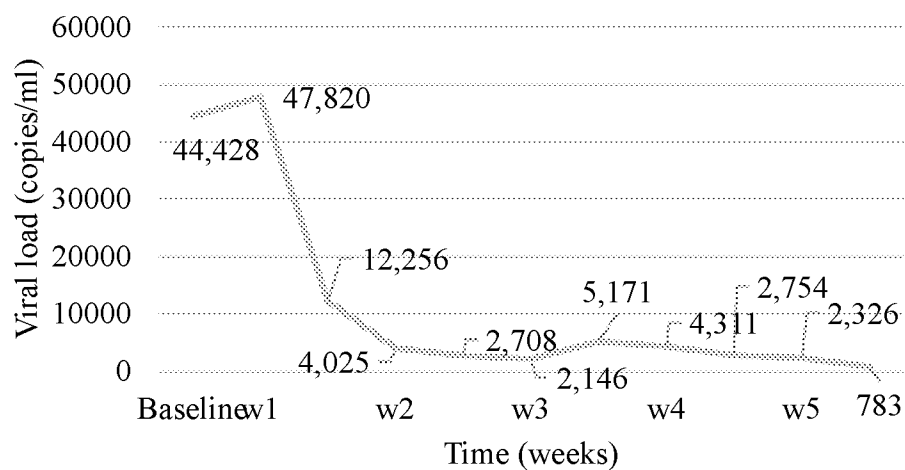


FIGURE 22B

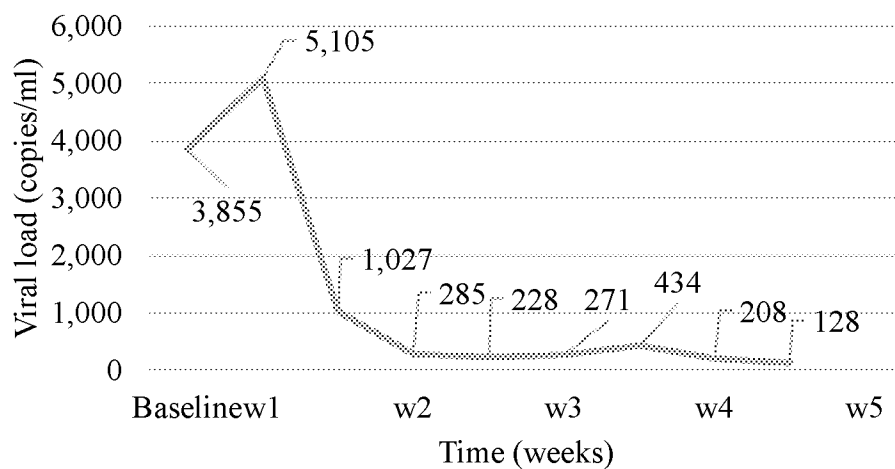
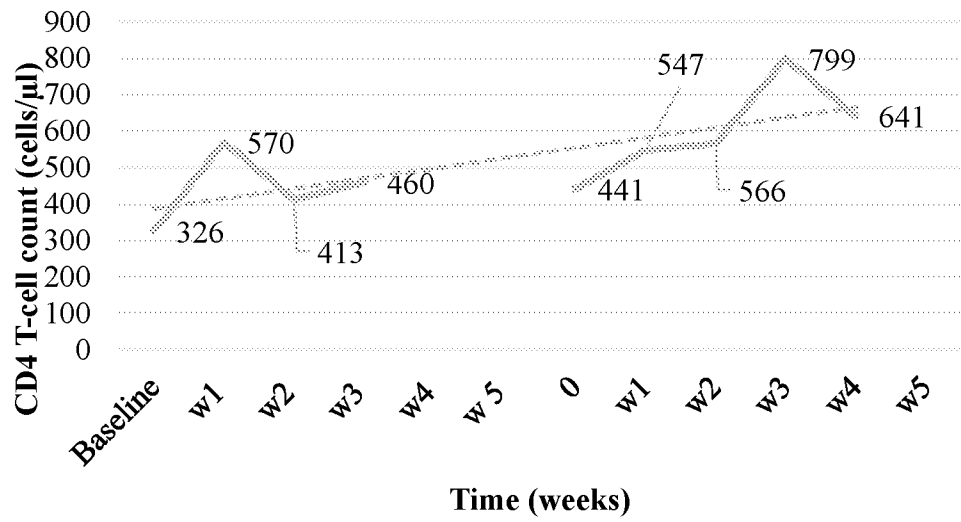


FIGURE 23



INTERNATIONAL SEARCH REPORT

International application No.

PCT/IL2019/050944

A. CLASSIFICATION OF SUBJECT MATTER IPC (20190101) C07K 7/08, A61P 31/18 CPC (20130101) C07K 7/08, A61P 31/18 According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC (20190101) C07K 7/08, A61P 31/18 CPC (20130101) C07K 7/08, A61P 31/18 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) Databases consulted: Google Patents, CAPLUS, BIOSIS, EMBASE, MEDLINE, REGISTRY, Google Scholar Search terms used: Poloxomer 188, HIV-1 integrase		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2010041241 A2 YISSUM RES DEV CO et al. 15 Apr 2010 (2010/04/15) claims 1-12; description, page 3, lines 4-6; page 15, lines 23-32	35-40
A	whole document	1-34,41-50
A	EP 1309904 A1 VALENTIS INC 14 May 2003 (2003/05/14) claims 1-10, 32-34	1-34,41-50
Y	US 4110322 A AKZONA INC 29 Aug 1978 (1978/08/29) claims 1,10-13; description column 5 (lines 34-46), column 7 (lines 29-32)	35-40
☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "D" document cited by the applicant in the international application "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 05 Nov 2019		Date of mailing of the international search report 17 Nov 2019
Name and mailing address of the ISA: Israel Patent Office Technology Park, Bldg.5, Malcha, Jerusalem, 9695101, Israel Email address: pctoffice@justice.gov.il		Authorized officer MAZEL Alexander Telephone No. 972-73-3927174

Box No. 1 Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

a. ☒ forming part of the international application as filed:

☒ in the form of an Annex C/ST.25 text file.

☐ on paper or in the form of an image file.

b. ☐ furnished together with the international application under PCT Rule 13ter. 1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

☐ in the form of an Annex C/ST.25 text file (Rule 13ter. 1(a)).

☐ on paper or in the form of an image file (Rule 13ter. 1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/IL2019/050944

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