

A method of treating cancer and pharmaceutical composition

Field of the Invention

The present invention relates to polyinosinic:polycytidylic acid (PIC) compositions and their use in the treatment or prevention of cancer, or in the alleviation of symptoms of a cancer, in particular cancer-induced malignant effusion. In some aspects cancer is prevented or treated by a method involving administration of a PIC containing composition to the mucosa of the subject.

Background

It has been an exciting era for cancer therapeutics. The success of immune checkpoint inhibitors in treating various cancers and adoptive transfer of genetically modified T cells in treating blood cancers seems to represent a turning point for our battle against cancer.

Polyinosinic acid : polycytidylic acid (polyIC or PIC) is a known TLR 3 agonist that mimics pathogenic dsRNA, capable of initiating a series of innate and adaptive immune responses in mammals. It has been identified as a candidate vaccine adjuvant and as a general immune stimulatory molecule in some mice and clinical studies.

However the stability of PIC has limited its usefulness in clinical applications. PIC can be rapidly broken down by nucleases in primates and presents certain risks for inflammation and local toxicity. To achieve its therapeutic effect while minimizing the side effects, PIC needs to be stabilized with its toxicity carefully tuned.

Recently, there were reports of PIC and its derivatives' direct cytotoxic effect on tumor cell lines. The inhibition of tumor growth and induction of cell apoptosis was achieved through direct activation of TLR3, MDA-5 and/or RIG-I. However, early clinical experiences were not satisfactory. There were also attempts to use PIC as an adjuvant for cancer vaccines without much success.

Mucosal Immunity

Not until recently, we have confirmed the importance of our immune system in combating cancer. The mucus-covered epithelial surfaces, such as the respiratory, digestive and urogenital tracts, are some of the major sites of host defense, where failure may lead to fetal infection of external pathogens or cancerous development. There is clear compartmentalization of the body towards the systemic immunity and mucosal immunity. Two features of this compartmentalization are 1) immune responses induced within one compartment are largely confined in expression to that particular compartment; 2) the lymphocytes are restricted to particular compartments by expression of homing receptors that are specifically expressed within the tissues of the compartment. Therefore, one would largely differentiate the mucosal immune system from the rest of the peripheral lymphoid system by the site of action and the lymphocytes repertoire.

In particular, the dominant antibody isotype of the mucosal system is IgA. IgA can be also found in the peripheral blood system but is almost exclusively produced as a dimer with the ratio of IgA1 to IgA2 approximately 4:1, whereas the IgA in the gut is polymeric with a ratio about 3:2. Higher proportion of IgA2 renders the antibody more resistant to intestinal proteolytic enzymes. The T cells of the digestive tract is also unique and can be divided into two types, the ones who bear the conventional $\alpha:\beta$ T-cell receptors and a second class bearing TCR $\gamma:\delta$ and $\alpha:\beta$ T-cells paired with CD8 $\alpha:\alpha$ homodimer in mice. The receptors of this second class of T cells do not bind to the usual MHC class IA ligands but to a number of different ligands, including MHC class IB molecules.

We believe mucosal immunity is also an indispensable part of immunosurveillance against viral infection or cancer and a potent therapeutic area worth exploring. For viral infections and cancers that arise from the mucosal surfaces, mucosal immunity may provide first line of defense by directly eliminating the pathogens at the site of infection or inhibiting cancerous growth. However, mucosal immune therapy is rarely used in combating viral infection or epithelium related cancer, probably due to immune tolerance at the mucosal site that is designed by nature so that no intense immune response follows the food intake or inhalation of foreign substances.

A further advantage of mucosal immunity is the site of application. Compared to

injection, mucosal delivery such as vaginal, rectal, intranasal administration, or inhalation enables patients' self-care without the intervention of medical staff and saves medical resources.

Radiation Therapy

Radiation therapy or radiotherapy is widely applied to treat primary cancer or recurring cancer. It may also be used as (neo)adjuvant or palliative therapy for local disease control or symptomatic relief when cancer is incurable. The intense ionizing radiation works by damaging the DNA of targeted tissue that leads to cellular deaths. The effects of ionizing radiation are at least two fold, where intended cellular damage is usually accompanied with surrounding tissue damage and other acute or late side effects, including nausea, vomiting, intestinal discomfort, swelling, skin damage, mouth, throat and stomach scores, fibrosis and epilation etc.

A recent clinical report mentioning the abscopal (systemic relief of cancer, or tumor regression distant from treatment site) effect of radiotherapy combining immunologic checkpoint inhibitor, cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), received vast interest in the field. Several pre-clinical studies elucidated the underlying mechanism for such effect. Generally, it is believed that the effect was generated by release of tumor antigen during radiation and subsequent immune activation.

Up-regulation of some important antigen presenting molecules and co-stimulatory signals are also part of the immune stimulatory effects generated by radiation. In particular, enhanced expression of MHC-1 molecule and generation of tumor specific cytotoxic T cells directly contribute to systemic relief of cancer. Release of danger associated molecular patterns (DAMPs) and ablation of regulatory cells helps to enhance immune responses in some cases. Generating effective immune responses and immunogenic cell death mediated by radiation is an area of controversy.

However, radiation alone is rarely effective in eradicating cancer. Therefore, to successfully restore the host immune system against cancer may require simultaneous activation of multi-specific immune response involving various immunities.

Summary of Invention

The invention is related to a method of treating cancer. The method comprises administering a pharmaceutical composition or medicament containing polyinosinic:polycytidylic acid (PIC), wherein the anti-cancer agent is administered to the mucosa of the subject. The invention is also related to a pharmaceutical formulation or medicament for mucosal delivery of an anti-cancer agent comprising polyinosinic:polycytidylic acid (PIC) and at least one stabilising agent. The invention is further related to the pharmaceutical formulation or medicament in treating cancer and cancer-induced malignant effusion, wherein the pharmaceutical composition or medicament is effective in controlling cancerous growth.

In one aspect of the present invention a method of treating cancer in a subject in need of treatment is provided, the method comprising administering to the subject a pharmaceutical composition or medicament comprising polyinosinic:polycytidylic acid (PIC), wherein the pharmaceutical composition or medicament is administered to the mucosa of the subject.

In another aspect of the present invention polyinosinic:polycytidylic acid (PIC) is provided for use in a method of treating cancer in a subject, the method comprising administering a pharmaceutical composition or medicament comprising PIC to the mucosa of the subject.

In another aspect of the present invention the use of PIC in the manufacture of a medicament for use in a method of treatment of cancer is provided, the method comprising administration of the medicament to the mucosa of the subject.

The pharmaceutical composition or medicament may comprise PIC and at least one stabilising agent. The stabilising agent may be selected from the group consisting of kanamycin, poly-lysine, protamine, chitin, chitosan, spermine and glucosamine. In some embodiments the pharmaceutical composition or medicament comprises Kanamycin. In some embodiments the pharmaceutical composition or medicament comprises 500 to 10000 µg PIC per unit dose and 500 to 10000 IU kanamycin per unit dose.

The pharmaceutical composition or medicament may further comprise calcium chloride, which may also act as a stabilising agent. The amount of calcium chloride may be 0.1 to 5.0 mmol per unit dose.

The method of treatment of a cancer may be treatment or prevention of cancer. Treatment or prevention may be of a malignant effusion, e.g. cancer-induced malignant effusion.

Administration of the pharmaceutical composition or medicament to the mucosa may involve delivery to gastric mucosa, enteral mucosa, lung mucosa or airway mucosa.

The pharmaceutical composition or medicament may be delivered or administered vaginally, rectally, intranasally, intragastrically, enterally, ocularly, orally, by inhalation, ophthalmically, topically, pulmonary, or transdermally.

The pharmaceutical composition or medicament may be delivered or administered by inhalation, e.g. via the assistance of inhaler, spray, applicator or nebulizer.

The pharmaceutical composition or medicament may be delivered or administered 5 times per day, 4 times per day, 3 times per day, twice per day, once per day, on alternative days, 6 times per week, 5 times per week, 4 times per week, 3 times per week, twice per week, weekly, four times per month, 3 times per month, twice per month, monthly, or bimonthly.

The pharmaceutical composition or medicament may be delivered or administered in conjunction with another administration of the pharmaceutical composition or medicament by a route selected from the group consisting of intradermal injection, intramuscular injection, subcutaneous injection, intravenous infusion, intraperitoneal perfusion, intrapleural perfusion, intrapericardial injection.

The pharmaceutical composition or medicament may be delivered or administered by systemic administration and by mucosal delivery or administration.

The pharmaceutical composition or medicament may be administered during and/or via an intestinal, gastric intubation, chest drainage, thoracentesis, temporary or permanent pleural catheter insertion, thoroscopic pleurodesis, pericardial intraluminal stenting or fenestrated drainage, or surgical shunting procedure.

The ratio of polyinosinic acid and polycytidylic acid in the pharmaceutical composition or medicament may be selected from the group consisting of 0.8:1.0, 0.9:1.0, 1.0:1.0, 1.0:1.1, 1.0:1.2.

The pharmaceutical composition or medicament may be a liquid or fluid formulation in which a unit dose is provided in a volume of the liquid or fluid between 0.1 ml and 10.0 ml.

The pharmaceutical composition or medicament may further comprise an antigen. The source of the antigen may be a human, non-human animal, plant, bacteria, fungus, virus, parasite or cancer antigen.

The pharmaceutical composition or medicament may act as an adjuvant in enhancing immunogenic responses in the subject host against virus, bacteria, fungus, parasite or cancer.

The pharmaceutical composition or medicament may be effective in controlling cancerous growth and reducing tumor size. The pharmaceutical composition or medicament may be effective in controlling cancer-induced malignant effusion.

The method of treatment may further comprise administering a therapeutically effective amount of radiation targeted against the underlying cancer. The radiation may be given before, after or during the administration of the pharmaceutical composition or medicament.

A kit comprising the pharmaceutical composition or medicament and a device for mucosal delivery or administration, and instructions for administration of the pharmaceutical composition or medicament is also provided.

Description

The present invention may be understood more readily by reference to the following detailed description of certain embodiments of the invention and the Examples included herein.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skilled in the art to which this invention belongs. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited.

It must be noted that as used herein and in the appended claims, the singular forms "a," "and," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a polyinosinic:polycytidylic acid composition" includes a plurality of such compositions.

Pharmaceutical Formulation and Medical Use

The invention concerns a pharmaceutical formulation for mucosal delivery of an anti-cancer agent, comprising polyinosinic:polycytidylic acid (PIC). The anti-cancer agent may further comprise a stabilising agent. The anti-cancer agent may be immunomodulating. The mucosal delivery of the anti-cancer agent is capable of inducing mucosal immunity at the deposition site. The mucosal delivery may further induce systemic immunity at remote site from the deposition.

The inventors have surprisingly found that the pharmaceutical formulation for mucosal delivery of an anti-cancer agent comprising a polyinosinic:polycytidylic acid (PIC) when delivered or administered to the mucosa is effective in controlling cancerous growth and reducing tumor size at the site of deposition and/or remote site of deposition. Thus in one embodiment, the present invention is related to the use of the anti-cancer agent in a method of treatment of cancer and cancer-induced malignant effusion. In further embodiment, the anti-cancer agent further alleviates cancer-induced malignant effusion.

PIC is typically a double stranded polymer comprising one strand of inosinic acid polymer (polyinosinic acid; polyI) and one strand of cytidylic acid polymer (polycytidylic acid; polyC). The polymer backbone may be a deoxyribonucleic acid backbone or ribonucleic acid backbone. In one embodiment, PIC is a double strand RNA analog. In another embodiment, PIC is an oligonucleotide analog. In preferred embodiments the polyI is polyriboinosinic acid and polyC is polyribocytidylic acid. The resulting mixture is polyriboinosinic:polyribocytidylic acid, i.e. a double stranded RNA (dsRNA)-like polymer.

In a particular embodiment, the ratio between polyinosinic acid (PI): polycytidylic acid (PC) which mixed to form polyinosinic:polycytidylic acid (PIC) is selected from the group consisting of 0.8:1.0, 0.9:1.0, 1.0:1.0, 1.0:1.1, 1.0:1.2. Preferably, the ratio is 1.0:1.0.

In one aspect, the stabilising agent is an amine, selected from the group consisting of kanamycin, poly-lysine, protamine, chitin, chitosan, spermine and glucosamine. The positively charged amine group on the stabilising agent should be capable of neutralizing the negative charges of PIC. The stabilized PIC forms 3D structures that is interconnected and resistant to nuclease.

In another aspect, the stabilising agent is an aminoglycoside antibiotic selected from the group consisting of Dihydrostreptomycin, Mannosidostreptomycin, Amikacin, Butyrosin, Dibekacin, Destomycin, Gentamicin, Kanamycin, Lividomycin, Netilmicin, Neomycin, Paromomycin, Ribostamycin, Sisomicin, Streptomycin, Streptothricin, Tobramycin and Validamycin. In some preferred embodiments the aminoglycoside is Kanamycin.

In a particular aspect, the pharmaceutical formulation of the current invention comprises PIC and kanamycin, wherein the amount of PIC is 500 to 10,000 ug per unit dose; and the amount of kanamycin is 500 to 10,000 IU per unit dose. In some embodiments, the amount of PIC is 500 ug, 600 ug, 700 ug, 800 ug, 900 ug, 1000 ug, 1100 ug, 1200 ug, 1300ug, 1500 ug, 1600 ug, 1700 ug, 1800 ug, 1900 ug, 2000 ug, 2500 ug, 3000 ug, 3500 ug, 4000 ug, 4500 ug, 5000 ug, 6000 ug, 7000 ug, 8000 ug, 9000 ug, 10,000 ug or any value selected from the values between 500 and 10,000 ug per unit dose. In some embodiments, the amount of kanamycin is 500 IU, 600 IU, 700 IU, 800 IU, 900 IU, 1000 IU, 1100 IU, 1200 IU, 1300IU, 1500 IU, 1600 IU, 1700 IU, 1800 IU, 1900 IU, 2000 IU,

2500 IU, 3000 IU, 3500 IU, 4000 IU, 4500 IU, 5000 IU, 6000 IU, 7000 IU, 8000 IU, 9000 IU, 10,000 IU or any value selected from the values between 500 and 10,000 IU per unit dose.

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For anionic component of the formulation, or has a functional group which may be anionic (e.g., COOH may be COO⁻), then a salt may be formed with a suitable cation. Examples of suitable inorganic cations include, but are not limited to, alkali metal ions such as Na⁺ and K⁺, alkaline earth cations such as Ca²⁺ and Mg²⁺, and other cations such as Al³⁺. Examples of suitable organic cations include, but are not limited to, ammonium ion (i.e., NH₄⁺) and substituted ammonium ions (e.g., NH₃R⁺, NH₂R₂⁺, NHR₃⁺, NR₄⁺).

In a preferred embodiment, the pharmaceutical formulation further comprises Ca²⁺. In one embodiment, the source of Ca²⁺ is calcium chloride, calcium carbonate, calcium fluoride, calcium hydroxide, calcium phosphates, or calcium sulfate, preferably the source is calcium chloride (CaCl₂). In one embodiment, the amount of CaCl₂ is 0.1 to 3.0 mmol (or mM) per unit dose. In some embodiments, the amount of CaCl₂ is 0.1 mmol, 0.2 mmol, 0.3 mmol, 0.4 mmol, 0.5 mmol, 0.6 mmol, 0.7 mmol, 0.8 mmol, 0.9 mmol, 1.0 mmol, 1.5 mmol, 2.0 mmol, 2.5 mmol, 3.0 mmol, or any value selected from the values between 0.1 and 3.0 mmol per unit dose.

For cationic component of the formulation, or has a functional group which may be cationic (e.g., NH₂ may be NH₃⁺), then a salt may be formed with a suitable anion. Examples of suitable inorganic anions include, but are not limited to, those derived from the following inorganic acids: hydrochloric, hydrobromic, hydroiodic, sulfuric, sulfurous, nitric, nitrous, phosphoric, and phosphorous.

Examples of suitable organic anions include, but are not limited to, those derived from the following organic acids: 2-acetoxybenzoic, acetic, ascorbic, aspartic, benzoic, camphorsulfonic, cinnamic, citric, edetic, ethanedisulfonic, ethanesulfonic, fumaric, glucoheptonic, gluconic, glutamic, glycolic, hydroxymaleic, hydroxynaphthalene carboxylic, isethionic, lactic, lactobionic, lauric, maleic, malic, methanesulfonic, mucic, oleic, oxalic, palmitic, pantoic, pantothenic, phenylacetic, phenylsulfonic, propionic,

pyruvic, salicylic, stearic, succinic, sulfanilic, tartaric, toluenesulfonic, and valeric. Examples of suitable polymeric organic anions include, but are not limited to, those derived from the following polymeric acids: tannic acid, carboxymethyl cellulose.

Unless otherwise specified, a reference to a particular compound also include salt forms thereof.

The formulation may further comprise pharmaceutically acceptable ingredients well known to those skilled in the art, including, but not limited to, pharmaceutically acceptable carriers, adjuvants, excipients, diluents, fillers, buffers, preservatives, antioxidants, lubricants, stabilisers, solubilisers, surfactants (e.g., wetting agents), masking agents, colouring agents, flavouring agents, and sweetening agents.

The term "pharmaceutically acceptable" as used herein pertains to compounds, ingredients, materials, compositions, dosage forms, etc., which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of the subject in question (e.g., human) without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio. Each carrier, adjuvant, excipient, etc. must also be "acceptable" in the sense of being compatible with the other ingredients of the formulation. Suitable carriers, adjuvants, excipients, etc. can be found in standard pharmaceutical texts, for example, A. Gennaro (2000) "Remington: The Science and Practice of Pharmacy," 20th edition, Lippincott, Williams, & Wilkins;- Pharmaceutical Dosage Forms and Drug Delivery Systems (1999) H.C. Ansel et al., eds., 7th ed., Lippincott, Williams, & Wilkins; and Handbook of Pharmaceutical Excipients (2000) A.H. Kibbe et al., eds., 3rd ed. Amer. Pharmaceutical Assoc.

The formulation may be in solid or liquid form. In particular, the liquid form may be liquid solution, liquid drops, liquid suspension, emulsion, spray, inhalant, aerosol, syrup, slurry, or enema. Liquid forms for oral administration may contain a liquid carrier in addition to the above agents. Suitable liquid carriers include water, oils, such as olive oil, peanut oil, sesame oil, sunflower oil, safflower oil, arachis oil, coconut oil, liquid paraffin, ethylene glycol, propylene glycol, polyethylene glycol, ethanol, propanol, isopropanol, glycerol, fatty alcohols, triglycerides, or mixtures thereof. The solid forms may be solid capsule,

soft capsule, microcapsule, tablet, dragee, granule or powder. The solid forms may be reconstituted into liquid form with saline or other commonly used physiological acceptable dissolvent.

In one embodiment, the pharmaceutical formulation described in the current invention can be prepared into clear aqueous solution. In one embodiment, the volume of the final preparation is between 0.1 to 10.0 ml per unit dose. In some embodiments, the volume of the final preparation is 0.1 ml, 0.2 ml, 0.3 ml, 0.4 ml, 0.5 ml, 0.6 ml, 0.7 ml, 0.8 ml, 0.9 ml, 1.0 ml, 2.0 ml, 3.0 ml, 4.0 ml, 5.0 ml, 6.0 ml, 7.0 ml, 8.0 ml, 9.0 ml, 10.0 ml, or any value selected from the values between 0.1 and 10.0 ml per unit dose.

For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous and intraperitoneal administration. In this connection, sterile aqueous media, which can be employed, will be known to those of skill in the art in light of the present disclosure. Exemplary injection media, which can be used in the present invention, include a buffer with or without dispersing agents and/or preservatives, and edible oil, mineral oil, cod liver oil, squalene, mono-, di- or triglyceride, and a mixture thereof.

The exact amount of such compositions required will vary from subject to subject, depending on the species, age, weight, and general conditions of the subject, the severity of the disease, infection, or condition that is being treated or prevented, the particular compound used, its mode administration, and the like. An appropriate amount may be determined by one of ordinary skill in the art using only routine experimentation given the teachings herein. Following an initial administration, subjects may receive one or several booster immunizations adequately spaced.

In some embodiments the pharmaceutical composition or medicament is administered to the mucosa of the subject, which is termed "mucosal delivery". Mucosa refers to a mucous membrane lining a body cavity or surrounding an internal organ. Mucosa usually composes layers of epithelial cells overlying connective tissue. Examples of mucosa can be found in the eye, ears, inside the nose, inside the mouth, lining the gastric wall,

the intestinal ducts, the airways of the lung(s) and trachea, the urethral opening, anus, tongue and vagina.

As such, suitable routes of mucosal delivery may include intranasal, intragastrical, or enteral delivery, by inhalation, suppository, oral administration, or intrapleural perfusion.

The pharmaceutical composition or medicament may be formulated appropriately, e.g. in liquid or fluid form capable of rapid absorption by the mucosa. Suitable formulations may include spray formulations (e.g. a spray formulation), inhalation formulations (e.g. powder or aerosol which may be suitable for delivery by an inhaler or nebuliser), or suppository.

In some embodiments mucosal delivery is to the airway, e.g. to the mucosa or lining of the lung or airways (e.g. trachea, bronchioles, bronchioli, alveoli). Delivery may be by spray application or inhalation. In some embodiments mucosal delivery is directly to the gastric wall, intestinal duct or esophagus. Delivery may be by intubation or any specific oral formulation that allow the pharmaceutical formulation to reach the targeted sites.

Antigens

The pharmaceutical formulation of current invention may further comprise an antigen. The source of antigen may be a human, non-human animal, plant, bacteria, fungus, virus, parasite or cancer antigen.

In some embodiments, the cancer antigen may be tumor associated antigens (TAA), mutated neo antigens, tumor suppressor genes, over expressed cellular proteins, tumor antigens produced by oncogenic viruses, altered cell surface glycolipids or glycoproteins and cell type specific differentiation antigens.

Examples of such antigens include carbonic anhydrase IX, alpha-fetoprotein, α -actinin-4, antigen specific to A33 antibody, ART-4, B7, Ba 733, BAGE, BrE3-antigen, CA125, CAMEL, CAP-1, CASP-8/m, CCCL19, CCCL21, CD1, CD1a, CD2, CD3, CD4, CD5, CD8, CD11A, CD14, CD15, CD16, CD18; B cell specific antigens (i.e. CD19, CD20, CD21, CD22, CD27, CD37, CD72, CD78, DC79a, CD79b, CD138, CD150, PD1/CD279,

GCET-1, hFCRL1/CD307a, FCRL2/CD307b, CXCR5/CD185, B7-DC/CD273, MRC/CD200, CD130, CXCR4/CD184, and Siglec-5/14), HMGB-1, hypoxia inducible factor (HIF-1), HSP70-2M, HST-2, insulin growth factor-1 (IGF-1), KC4-antigen, KS1-4, Le-Y, LDR/FUT, macrophage migration inhibitory factor (MIF). Other examples include CD147, CD154, CDC27, CDK-4/m, CDKN2A, colon-specific antigen, gp100, GROB, HLA-DR, HM1.24, human chorionic gonadotropin (HCG) and its subunits, mesothelin, prostate stem cell antigen (PSCA), WT-1, EphA2, CEA, HER-2/Neu/ErbB2, MAGE-A1, MAGE-A2, MAGE-A3, MAGE-A4, NY-ESO-1, NY-CO-8, NY-CO-9, NY-CO-13, NY-CO-16, NY-CO-20, NY-CO-38, NY-CO-45, K-Ras, H-Ras, N-Ras, Trp-2, MART-1, MUC1, MUC2, MUC3, MUC4, MUM-1/2, MUM-3, NCA66, NCA95, NCA90, placental growth factor, p53, prostatic acid phosphatase, PSA, PRAME, PSMA, PIGF, ILGF, ILGF-1R, IL-6, IL-25, RS5, RANTES, T101, SAGE, S100, survivin, survivin-2B, TAC, TAG-72, tenascin, TRAIL receptors, TNF- α , Tn and GAGE/PAGE family. Still other examples include hepatitis A antigens, hepatitis B surface antigen, hepatitis B core antigens, hepatitis B polymerase antigens, hepatitis C antigens, Epstein-Barr virus LMP antigens, Epstein-Barr virus EBNA antigens, HPV antigens, CMV antigens, HIV antigens.

Method and Uses

The present invention concerns a method for treating cancer and/or cancer-induced malignant effusion, comprising administering to the subject in need of treatment an anti-cancer agent comprising polyinosinic:polycytidylic acid (PIC) and at least one stabilising agent, wherein the anti-cancer agent is administered to the mucosa of the subject.

In some embodiments, the pharmaceutical formulation of an anti-cancer agent, comprising polyinosinic:polycytidylic acid (PIC) and at least one stabilising agent may be delivered or administered vaginally, rectally, intranasally, intragastrically, enterally, ocularly, orally, by inhalation, ophthalmically, topically, pulmonary, or transdermally.

In a preferred embodiment, the current invention concerns a pharmaceutical formulation for mucosal delivery of the anti-cancer agent. In one embodiment, the anti-cancer agent is delivered by inhalation via the assistance of inhaler, spray, applicator or nebulizer.

In some embodiments, the pharmaceutical formulation of the anti-cancer agent is delivered or administered 5 times per day, 4 times per day, 3 times per day, twice per day, once per day, on alternative days, 6 times per week, 5 times per week, 4 times per week, 3 times per week, twice per week, weekly, four times per month, 3 times per month, twice per month, monthly, or bimonthly.

The anti-cancer agent may also be delivered or administered in conjunction with other administration route selected from the group consisting of intradermal injection, intramuscular injection, subcutaneous injection, intravenous infusion, intraperitoneal perfusion, intrapleural perfusion, intrapericardial injection.

The anti-cancer agent, antigen or other therapies may each be separately formulated as a pharmaceutical composition or medicament and separately administered, wherein each agent is administered simultaneously or sequentially.

Simultaneous or sequential administration may be intended such that all agents reach the site of action and are present in the site of action in active form at the same time. This may allow the agents and/or other therapies to exert a synergistic preventative or treatment effect.

Simultaneous administration refers to administration of the anti-cancer agent, and/or at least one antigen, and/or other therapies together, for example as a pharmaceutical composition containing all agents, or immediately after each other and optionally via the same route of administration.

In some particular embodiments, the anti-cancer agent is administered during and/or via a chest drainage, thoracentesis, temporary or permanent pleural catheter insertion, thoroscopic pleurodesis, pericardial intraluminal stenting or fenestrated drainage, or surgical shunting procedure.

Sequential administration refers to administration of the anti-cancer agent, and/or at least one antigen, and/or other therapies followed after a given time interval by separate administration of the other agent.

The time interval may be any time interval. However, in some embodiments the time interval is selected such that the agents are expected to be present in the site of action in active forms at the same time, thereby allowing for a combined, additive or synergistic effect in treating cancer.

The time interval between different treatment may be any one of 5 minutes or less, 10 minutes or less, 15 minutes or less, 20 minutes or less, 25 minutes or less, 30 minutes or less, 45 minutes or less, 60 minutes or less, 90 minutes or less, 120 minutes or less, 180 minutes or less, 240 minutes or less, 300 minutes or less, 360 minutes or less, 420 minutes or less, 480 minutes or less, 540 minutes or less 10 hours or less, 11 hours or less, 12 hours or less, 13 hours or less, 14 hours or less, 15 hours or less, 16 hours or less, 17 hours or less, 18 hours or less, 19 hours or less, 20 hours or less, 21 hours or less, 22 hours or less, 23 hours or less, 24 hours or less, 36 hours or less, 48 hours or less, 60 hours or less, 72 hours or less, 4 days or less, 5 days or less, 6 days or less, or 7 days or less.

Methods according to the present invention may be performed, or products may be present, in vitro, ex vivo, or in vivo. The term “in vitro” is intended to encompass experiments with materials, biological substances, cells and/or tissues in laboratory conditions or in culture whereas the term “in vivo” is intended to encompass experiments and procedures with intact multi-cellular organisms. “Ex vivo” refers to something present or taking place outside an organism, e.g. outside the human or animal body, which may be on tissue (e.g. whole organs) or cells taken from the organism.

Kit

In some embodiments, a kit comprises the anti-cancer agent formulated for mucosal delivery which is suitable for administration rectally, vaginally, nasally, orally (including inhalation), ophthalmically, topically, pulmonary, ocularly or transdermally and an appropriate delivery device for example, inhaler, suppository, applicator, nebulizer or the like.

In certain embodiments, the invention is related to a kit of parts comprising a predetermined quantity of the anti-cancer agent or pharmaceutical formulation, a device

for application and, optionally, instructions for use. In some embodiments, the kit comprises a predetermined quantity of a) the anti-cancer agent comprising polyinosinic acid:polycytidylic acid and at least one stabilising agent in predetermined formulation, and b) at least one antigen, each contained in different containers, or combined in the same container. A single container may be provided or multiple containers each containing discrete unit dosages of one or both a) and b). The kit and/or container(s) and/or device are preferably sterile.

In some embodiments, the invention is packaged in a kit comprising a sterile liquid (e.g., aqueous) formulation, where the formulation is sterile, and is provided in a sterile container, a sterile vial, or a sterile syringe and optionally a needle. In some embodiments, the invention is packaged in a kit comprising the anti-cancer agent in a sterile liquid formulation in a unit dosage amount (e.g., a single dose).

There are several different types of inhalation methodologies, which can be employed in connection with the above-described purpose. First, a subject composition can be formulated with low boiling point propellants. Alternatively, the anti-cancer agent can be formulated in aqueous or ethanolic solutions and delivered by conventional nebulizers. In some embodiments, such solution formulations are aerosolized. Furthermore, the anti-cancer agent can be formulated into dry powder formulations. Such formulations can be administered by simply inhaling the dry powder formulation after creating an aerosol mist of the powder. Formulations suitable for intranasal administration include nasal sprays, nasal drops, aerosol formulations; and the like.

In certain embodiments, the kit further comprises a delivery system suitable for colocalysis, aerosol spray, or administration via gastric, intestine or tracheal intubation or instillation. As used herein, the term "aerosol" is used in its conventional sense as referring to very fine liquid or solid particles carried by a propellant gas under pressure to a site of therapeutic application. Aerosols employed in the present invention are intended for administration as fine, solid particles or as liquid mists via the respiratory tract of a subject. Various types of propellants known to one of skill in the art can be utilized. Examples of suitable propellants include, but are not limited to, hydrocarbons or other suitable gas. In the case of the pressurized aerosol, the dosage unit may be determined by providing a value to deliver a metered amount.

The kit in some embodiments will further include instructions for use, including e.g., dosage amounts and dosage frequencies. Instructions are in some embodiments printed directly on the kit. In other embodiments, instructions are printed material provided as a package insert. Instructions can also be provided in other media, e.g., electronically in digital or analog form, e.g., on an audio cassette, an audio tape, a compact disc, a digital versatile disk, and the like.

Combined therapy

Due to the sensitivity of different cancerous cell types and the location of tumor, radiotherapy is rarely curative when used alone. Recurrence is common after curative treatment. For cancers that are widely spread over the body and metastatic cancers, radiation therapy is usually used as adjuvant therapy, or used together with other treatment options such as chemotherapy or targeted therapy.

In some embodiments, the method further comprises administering the subject a therapeutically effective amount of radiation targeted against the underlying cancer.

The form of radiation techniques may be applied together with the anti-cancer agent include conventional external beam radiation (2DRT), stereotactic radiation (SRS), stereotactic body radiation therapy (SBRT), 3-dimensional conformal radiation therapy (3DCRT) and intensity-modulated radiation therapy (IMRT).

The amount of radiation used in a particular treatment is measured in Gray (Gy, an international system of units). Fractionation is another term used to describe the dosage of a planned radiation treatment regimen, where a total amount of radiation is divided into fractionated sections and given over a period of time. The amount of radiation and fractionation are usually individualized depending on the patient's tolerability and response to prior treatment. In some embodiments, the amount of radiation used in combination with the pharmaceutical formulation ranges from 1Gy to 100Gy.

Cancer

In this specification a cancer may be any unwanted cell proliferation (or any disease manifesting itself by unwanted cell proliferation), neoplasm or tumor or increased risk of or predisposition to the unwanted cell proliferation, neoplasm or tumor. The cancer may be benign or malignant and may be primary or secondary (metastatic). A neoplasm or tumor may be any abnormal growth or proliferation of cells.

In some embodiments the cancer may be lung cancer, pleural cancer, lymphoma, breast cancer or stomach cancer.

In some embodiments a cancer is one that is associated with effusion fluid. Such association may involve production of effusion fluid by the cancerous tissue, e.g. by cancer cells, or by normal cells near to or contained in the cancerous tissue, or it may involve overproduction of effusion fluid by other tissues (e.g. the lymphatic system) as a direct or indirect response to the presence of the cancer in the subject.

A cancer may be characterised by the collection of effusion fluid in one or more locations in the subject's body. Such locations may include one or more body cavities or tissue spaces. Body cavities (or serous cavities) may be formed by a serous membrane surrounding an organ or tissue and forming a sac in which fluid may collect.

For example, effusion fluid may collect in one or each (right or left) pleural cavity (the space between the visceral and parietal pleura). In another example, effusion fluid may accumulate in the peritoneal cavity (the space between the parietal peritoneum and visceral peritoneum). In another example, fluid may accumulate in the pericardial cavity surrounding the heart (formed by the parietal and visceral pericardium). In another example, fluid may accumulate in the perimetrium surrounding the uterus.

Thus, in some embodiments a cancer is one in which pleural effusion, peritoneal effusion (ascites), pericardial effusion or perimetrial effusion occurs.

All types of cancer may be associated with production of effusion fluid, partly because all types of cancer can metastasize to any of the body's serous cavities and result in malignant effusion (Olopade CA-A Cancer Journal For Clinicians Vol.41, No.3 May/June 1991). Cancers in which production of effusion fluid is known to occur include cancers

of the following type or tissues: lung cancers, pleural cancers, mesothelioma, malignant pleural mesothelioma, non-small cell lung cancer (NSCLC), small cell lung cancer (SCLC), ovarian cancers, ovarian carcinoma, uterine cancer, endometrial cancer, heart cancer, breast cancer, colon cancer, stomach cancer, gastric cancer, pancreatic cancer, kidney cancer, liver cancer, lymphatic cancer (e.g. lymphoma, non-Hodgkin lymphoma), soft tissue sarcoma, osteosarcoma, adenocarcinoma, parotid cancer (e.g. parotid adenocarcinoma), thymic carcinoma, cancers of the reproductive tract (including cervical, fallopian tube, endometrium), gastrointestinal tract, or genitourinary tract, leukemia, larynx, prostate, bile duct, hypernephroma, sinus piriformis carcinoma, thyroid cancer, melanoma and cancers of unknown primary (CUP) origin.

The development of a malignant pleural effusion is a common complication of advanced malignancies of many types of cancer, especially breast, lung (including NSCLC and SCLC) and ovarian carcinoma (Warren et al. *European Journal of Cardio-thoracic Surgery* 33 (2008) 89-94). Pleural effusions are at least known to be associated with cancers of the following type or tissue: lung, breast, lymphoma, uterus, ovarian, female reproductive tract (e.g. cervical, fallopian tube, endometrium), leukemia, pancreas, kidney, colon, stomach (gastric), mesothelioma, sarcoma, larynx, prostate, bile duct, hypernephroma, sinus piriformis carcinoma, thyroid cancer, non-Hodgkin lymphoma, malignant melanoma, reproductive tract, gastrointestinal tract, genitourinary tract, (Warren et al. *Ann Thorac Surg* 2008;85:1049-55; Warren et al. *European Journal of Cardio-thoracic Surgery* 33 (2008) 89-94; Schulze et al. *Ann Thorac Surg* 2001;71:1809-12; Olopade *CA-A Cancer Journal For Clinicians* Vol.41, No.3 May/June 1991).

Peritoneal effusions (ascites) are at least known to be associated with cancers of the following type or tissue: ovarian, epithelial related ovarian, uterus, breast, colon, gastric, pancreatic, hepatic, colon, lymphoma, mesothelioma, and cancers of unknown primary (CUP) origin (Olopade *CA-A Cancer Journal For Clinicians* Vol.41, No.3 May/June 1991)

Pericardial effusions are at least known to be associated with cancers of the following type or tissue: lung, breast, leukemia, lymphoma, sarcoma, melanoma (Olopade *CA-A Cancer Journal For Clinicians* Vol.41, No.3 May/June 1991).

Effusion fluid refers to an excess of fluid produced by a subject in direct or indirect response to the presence of a cancer in the subject. In preferred embodiments the effusion fluid collects in a body cavity such that accumulation of effusion fluid may occur where the rate of production of the effusion fluid exceeds the rate of reabsorption. Pleural effusions (sometimes called malignant pleural effusions) lead to accumulation of fluid in the pleural cavity and occur in some lung cancers, e.g. mesothelioma. Effusion fluid collecting in the peritoneal cavity is commonly referred to as ascites, and can be a symptom of a number of types of cancer including cancer of the breast, lung, colon, stomach, pancreas, ovary, endometrium as well as lymphoma. Pericardial effusion is the abnormal accumulation of fluid in the pericardial cavity. The effusion fluid is preferably exudative.

Effusion fluid can be drained from a respective body cavity by well-known aseptic procedures (e.g. see Warren et al. *Ann Thorac Surg* 2008;85:1049-55; Warren et al. *European Journal of Cardio-thoracic Surgery* 33 (2008) 89-94). In some instances, a tube or catheter is inserted in the body cavity in order to drain effusion fluid. Drainage of effusion fluid is a common part of the diagnosis, treatment and management of many forms of cancer. Drainage of effusion fluid provides a means of obtaining a sample of a subject's effusion fluid for diagnostic analysis.

In this specification treatment of malignant effusion may refer to the prevention of build-up of effusion fluid, reduction of amount of effusion fluid and/or alleviation of symptoms associated with the build-up of effusion fluid. The malignant effusion is preferably associated with a cancer, e.g. as described above. The treatment may also optionally treat, reduce or prevent progression of, reduce or prevent metastasis of, or shrink, the associated cancer.

In some embodiments in accordance with various aspects of the present invention, the malignant effusion is located in the pleural, peritoneal, and/or pericardial cavity; and the malignant effusion arises from a primary tumor originating in the lung, liver, stomach, colon, breast, ovary, pancreas, biliary duct, esophagus, brain, nose or prostate gland.

In some embodiments in accordance with various aspects of the present invention, the malignant effusion is located in the pleural, peritoneal, and/or pericardial cavity; and the

malignant effusion arises from a metastatic tumor located in the lung, liver, stomach, colon, breast, ovary, pancreas, biliary duct, esophagus, brain, nose or prostate gland.

Subject

The subject to be treated may be any animal or human. The subject is preferably mammalian, more preferably human. The subject may be a non-human mammal, but is more preferably human. The subject may be male or female. The subject may be a patient. A subject may have been diagnosed with a cancer, or be suspected of having a cancer prior to diagnosis. The subject may exhibit a malignant effusion or be at risk of developing a malignant effusion, e.g. one of pleural effusion, peritoneal effusion (ascites) or pericardial effusion.

In accordance with the present invention PIC molecules may be used to treat subjects having malignant effusion. The PIC molecules may be of any length, and may have any molecular weight.

The subject to be treated may be at risk of developing malignant effusion. The composition may modulate the immune response of the subject to prevent, reduce or alleviate malignant effusion.

Brief Description of the Figures

Embodiments and experiments illustrating the principles of the invention will now be discussed with reference to the accompanying figures in which:

Figure 1. Chart showing effect of PIC (early dose and normal dose), sorafenib and saline on size of mouse tumor xenografts.

Figure 2. Chart showing effect of PIC (early dose and normal dose), sorafenib and saline on weight of mouse tumor xenografts.

Figure 3. Chart showing malignant effusion fluid volume of different treatment groups

(ml)

Figure 4. Chart showing average IL-12 production in malignant effusion fluid post treatment (pg/ml)

Figure 5. Chart showing average IL-12 level in serum of mice post treatment (pg/ml)

Examples

The above disclosure generally describes the present invention. The following examples will be of assistance to the understanding of the present invention. These examples are described solely for purposes of illustration and are not intended to limit the scope of the invention.

Example 1. Pharmaceutical preparation of PIC

Polyinosinic acid and polycytidylic acid was mixed in a ratio between 0.8 to 1.2 to optimize PIC stability, hyperchromic effect and anti-cancer efficacy.

Stability was measured using a spectrophotometer before and after adding RNase to the resulting PIC samples. All PIC samples were prepared into 0.04mg/ml clear solution before testing. RNase was diluted into PBS at 0.5ug/ul and added into the PIC samples for 30 min. Optical density of different samples was recorded before and after the addition of RNase.

The PIC double helix denatures with salt. Hyperchromic effect measures the difference of absorbance at wavelength of 248nm after denaturation. Briefly, absorbance A1 and A2 were recorded before and after the addition of NaCl-phosphate salt. Hyperchromicity was calculated by formula $(A2-A1)/A1*100\%$ and the difference should be $>55\%$.

The following combination were tested and results show that any ratio of PI : PC between 0.8 to 1.2 gives stable PIC against RNase hydrolysis with satisfactory hyperchromicity.

PI : PC Ratio	Amount added	Stability against RNase	Hyperchromicity
0.8 : 1.0	0.04 mg	0.32	61.5%
1.0 : 1.0	0.04 mg	0.36	65.0%
1.2 : 1.0	0.04 mg	0.33	58.0%

Example 2. Formulation for dosing

Add stabilising agents and CaCl₂ into the above preparation of PIC containing anti-cancer agent. Top up the volume using PBS and stir the mixture under 60 degrees Celsius for 30 to 60 minutes. Filter the mixture and fill into 1ml bottle. Some particular preparations are tabulated below:

Preparations / Materials	PIC (mg)	Kanamycin (IU)	CaCl ₂ (mM)	Volume / dose	Stability
Preparation 1	0.5	600	0.1	1ml	0.31
Preparation 2	1.0	1000	0.5	1ml	0.34
Preparation 3	2.0	2000	1.0	1ml	0.33
Preparation 4	3.0	3000	1.5	1ml	0.32
Preparation 5	4.0	4000	2.0	1ml	0.34
Preparation 6	5.0	5000	2.5	1ml	0.35
Preparation 7	2.0	1200	0.2	0.5ml	0.34
Preparation 8	10.0	10,000	5.0	10ml	0.34

Example 3. Ability for IFN- γ induction in mice spleen cells

Sacrifice healthy SPF Balb/c mice, blow spleen with culture media to obtain splenocytes. Count and seed cells into 96-well plate. Culture cells 24 to 72 hours. Add different preparations of PIC containing samples and use ELISA to test for IFN- γ (each test were performed in duplicate). All test samples were able to generate comparable level of IFN- γ production.

Example 4. *In vivo* efficacy against liver and lung cancer

Revive and culture liver cancer cell line H22. Inject 2 balb/c mice to generate cancerous fluid. Obtain fluid, wash cell with DPBS, count and adjust cells to 2.5×10^7 cells/ml. Administer 5×10^6 cells (0.2 ml) into 20 mice subcutaneously (s.c) to mimic tumorous growth under the skin. Divide mice randomly into four groups (5 mice each) according to tumor size after three days as below:

Groups	Treatment	Daily Dose	Daily Dose (vol.)	Appl. Route
A	Early dose	200ug	100ul	S.C
B	Late dose	200ug	100ul	S.C
C	Sorafenib	1200ug	200ul	Oral
D	Normal Saline	100ug	100ul	S.C

Mice in group A were given anti-cancer agent containing PIC with composition as described in preparation 3 in example 1 daily 3 days post inoculation (final formulation according to preparation 2 in example 1). Treatment in other groups was started 6 days post inoculation according to the schedule depicted in the table above. Tumor growth was measured by size on days 3, 5, 6, 8, 10, 12, 14, 16 and 19, as well as by weight after sacrificing the mice one day after the final dose. Results show that both the early and high dose group produce significant tumor control effect compare to negative control and standard of care sorafenib (see figure 1 and figure 2).

Example 5. Optimized anti-cancer agent against malignant effusion

Cancer cell line H22 is used to generate malignant effusion model in female BALB/c mice 5-6 weeks old (weighted 18-20g). Draw accumulated fluid 10 days post inoculation, adjust the cell density at $1 \times 10^8/\text{ml}$ and inject total of 5×10^7 cells into healthy mice (0.5ml) to induce cancerous growth and malignant effusion.

Select mice 24h post-inoculation; thereafter divide the rest of the mice equally on day 2 and treat mice for 5 days. Mice received escalating doses of PIC containing preparation via intramuscular subcutaneous or inhalation routes and PBS control. Mice body weight, behavior, cancer development, malignant fluid reduction and survival status was recorded for analysis. Results show that inhalation route provides better malignant fluid control effect compare to intramuscular or subcutaneous routes. Figure 3 presents the results of fluid control of different treatment groups.

Example 6. Case report of anti-cancer agent treating lymphoma

Female patient 72 year old diagnosed with late stage lymphoma and systemic metastasis was consented for treatment. Patient was bed-bound and weak at the beginning of treatment. Intramuscular injection was not well tolerated due to local pain and preparation 9 was then given via intranasal spray of a total volume of 2ml in multiple applications per day. Disease progression stabilises for 8 months till the date of filing. Improved physical status was observed, such as weight gain, improved appetite, better sleep and general activity.

Example 7. Treatment with combined administration routes

A male patient 82 years old was diagnosed with late stage stomach cancer in Nov 2015. CT scan showed lung metastasis in Nov 2016 and patient started to use preparation 3 by intramuscular injection (1ml daily) thereafter with stable disease until late Jan 2017. On Jan 2017 patient was hospitalized with progressive disease accompanied by dyspnea, severe coughing, and ascites in unresponsive status.

By then, preparation 3 was given via injection, inhalation and nasointestinal tube simultaneously. Patient returned to conscious status with dyspnea, ascites and coughing

relieved after combined administration. The combined treatment was continued and the patient was able to conduct off-bed activities with stable disease till the date of filing.

Thereafter, combined treatment was able to replace diuretics with significant ascites reduction. An increase in absolute lymphocytes count was observed with an improved lymphocyte ratio and decrease in granulocyte ratio.

Example 8. Combined treatment of anti-cancer agent and radiation therapy

A female subject was recruited for treatment due to recurrent breast cancer after modified radical mastectomy. CT scan showed metastasis in both lobes of the lung. Further PET-CT revealed multiple metastases in bilateral cerebellar hemisphere and cerebellar vermis. Patient received preparation 5 (1ml daily) after consent via intramuscular injection while receiving stereotactic body radiation therapy over 6 fractions. One month after the combined treatment, CT scan showed clearance of brain metastasis with stable disease till the filing day.

Claims

1. A method of treating cancer and cancer-induced malignant effusion in a subject in need of treatment, the method comprising administering to the subject a pharmaceutical composition or medicament comprising polyinosinic:polycytidylic acid (PIC), wherein the pharmaceutical composition or medicament is administered to the mucosa of the subject.
2. Polyinosinic:polycytidylic acid (PIC) for use in a method of treating cancer and cancer-induced malignant effusion in a subject, the method comprising administering a pharmaceutical composition or medicament comprising PIC to the mucosa of the subject.
3. Use of PIC in the manufacture of a medicament for use in a method of treatment of cancer and cancer-induced malignant effusion, the method comprising administration of the medicament to the mucosa of the subject.
4. The method of claim 1, PIC for use in a method of treating cancer and cancer-induced malignant effusion of claim 2, or the use of claim 3, wherein the pharmaceutical composition or medicament comprises PIC and at least one stabilising agent.
5. The method of claim 4, PIC for use in a method of treating cancer of claim 4, or the use of claim 4, wherein the stabilising agent is selected from the group consisting of kanamycin, poly-lysine, protamine, chitin, chitosan, spermine and glucosamine.
6. The method of claim 4, PIC for use in a method of treating cancer of claim 4, or the use of claim 4, wherein the stabilising agent is Kanamycin.
7. The method of claim 6, PIC for use in a method of treating cancer of claim 6, or the use of claim 6, wherein the pharmaceutical composition or medicament comprises 500 to 10000 µg PIC per unit dose and 500 to 10000 IU kanamycin per unit dose.

11. The method of claims 1 or 4 to 10, PIC for use in a method of treating cancer of claims 2 or 4 to 10, or the use of claims 3 or 4 to 10, wherein the pharmaceutical composition or medicament further comprises calcium chloride.

12. The method of claim 11, PIC for use in a method of treating cancer of claim 12, or the use of claim 11, wherein the amount of calcium chloride is 0.1 to 5.0 mmol per unit dose.

13. The method of claims 1 or 4 to 12, PIC for use in a method of treating cancer of claims 2 or 4 to 12, or the use of claims 3 or 4 to 12, wherein the method is for treatment of a cancer-induced malignant effusion.

14. The method of claims 1 or 4 to 13, PIC for use in a method of treating cancer of claims 2 or 4 to 13, or the use of claims 3 or 4 to 13, wherein the mucosa is lung mucosa, intestinal mucosa or airway mucosa.

15. The method of claims 1 or 4 to 14, PIC for use in a method of treating cancer of claims 2 or 4 to 14, or the use of claims 3 or 4 to 14, wherein the ratio of polyinosinic acid and polycytidylic acid is selected from the group consisting of 0.8:1.0, 0.9:1.0, 1.0:1.0, 1.0:1.1, 1.0:1.2.

16. The method of claims 1 or 4 to 15, PIC for use in a method of treating cancer of claims 2 or 4 to 15, or the use of claims 3 or 4 to 15, wherein the pharmaceutical composition or medicament is a liquid or fluid formulation in which a unit dose is provided in a volume of the liquid or fluid between 0.1 ml and 10.0 ml.

17. The method of claims 1 or 4 to 16, PIC for use in a method of treating cancer of claims 2 or 4 to 16, or the use of claims 3 or 4 to 16, wherein the pharmaceutical composition or medicament further comprises an antigen.

18. The method of claims 1 or 4 to 17, PIC for use in a method of treating cancer of claims 2 or 4 to 17, or the use of claims 3 or 4 to 17, wherein the source of antigen is a human, non-human animal, plant, bacteria, fungus, virus, parasite or cancer antigen.

19. The method of claims 1 or 4 to 18, PIC for use in a method of treating cancer of claims 2 or 4 to 18, or the use of claims 3 or 4 to 18, wherein the pharmaceutical composition or medicament acts as an adjuvant in enhancing immunogenic responses in the subject host against virus, bacteria, fungus, parasite or cancer.

20. The method of claims 1 or 4 to 19, PIC for use in a method of treating cancer of claims 2 or 4 to 19, or the use of claims 3 or 4 to 19, wherein the pharmaceutical composition or medicament is delivered or administered vaginally, rectally, intranasally, intragastrically, enterally, ocularly, orally, by inhalation, ophthalmically, topically, pulmonary, or transdermally.

21. The method of claims 1 or 4 to 19, PIC for use in a method of treating cancer of claims 2 or 4 to 19, or the use of claims 3 or 4 to 19, wherein the pharmaceutical composition or medicament is delivered or administered by inhalation, e.g. via the assistance of inhaler, spray, applicator or nebulizer.

22. The method of claims 1 or 4 to 21, PIC for use in a method of treating cancer of claims 2 or 4 to 21, or the use of claims 3 or 4 to 21, wherein the pharmaceutical composition or medicament is delivered or administered 5 times per day, 4 times per day, 3 times per day, twice per day, once per day, on alternative days, 6 times per week, 5 times per week, 4 times per week, 3 times per week, twice per week, weekly, four times per month, 3 times per month, twice per month, monthly, or bimonthly.

23. The method of claims 1 or 4 to 22, PIC for use in a method of treating cancer of claims 2 or 4 to 22, or the use of claims 3 or 4 to 22, wherein the pharmaceutical composition or medicament is delivered or administered in conjunction with another administration of the pharmaceutical composition or medicament by a route selected from the group consisting of intradermal injection, intramuscular injection, subcutaneous injection, intravenous infusion, intraperitoneal perfusion, intrapleural perfusion, intrapericardial injection.

24. The method of claims 1 or 4 to 23, PIC for use in a method of treating cancer of claims 2 or 4 to 23, or the use of claims 3 or 4 to 23, wherein the pharmaceutical

composition or medicament is delivered or administered by systemic administration and by mucosal delivery or administration.

25. The method of claims 1 or 4 to 24, PIC for use in a method of treating cancer of claims 2 or 4 to 24, or the use of claims 3 or 4 to 24, wherein the pharmaceutical composition or medicament is administered during and/or via an intestinal intubation, gastric intubation, chest drainage, thoracentesis, temporary or permanent pleural catheter insertion, thoroscopic pleurodesis, pericardial intraluminal stenting or fenestrated drainage, or surgical shunting procedure.

26. The method of claims 1 or 4 to 25, PIC for use in a method of treating cancer of claims 2 or 4 to 25, or the use of claims 3 or 4 to 25, wherein the pharmaceutical composition or medicament is effective in controlling cancerous growth and reducing tumor size at the site of deposition and/or remote site of deposition.

27. The method of claims 1 or 4 to 26, PIC for use in a method of treating cancer of claims 2 or 4 to 26, or the use of claims 3 or 4 to 26, wherein the pharmaceutical composition or medicament is effective in controlling cancer-induced malignant effusion.

28. The method of claims 1 or 4 to 27, PIC for use in a method of treating cancer of claims 2 or 4 to 27, or the use of claims 3 or 4 to 27, wherein the method further comprises administering a therapeutically effective amount of radiation targeted against the underlying cancer.

29. The method of claim 28, PIC for use in a method of treating cancer of claim 28, or the use of claim 28, wherein the radiation is given before, after or during the administration of the pharmaceutical composition or medicament.