

Methods of minimising effects of radiation exposure

Field of the invention

[0001] The present invention relates to methods of minimising the effects of non-targeted and/or non-therapeutic radiation exposure in an individual.

Related applications

[0002] This application claims the benefit of priority from US provisional application no. 63/586,068 filed 28 September 2023, and Australian provisional application no. 2024900057 filed 10 January 2024, the entire disclosures of which are hereby incorporated by reference.

Background of the invention

[0003] Radiation injury is damage to tissues caused by exposure to radiation. Radiation may be ionizing radiation caused by high-energy electromagnetic waves (X-rays, gamma rays) or particles (alpha particles, beta particles, neutrons). Such radiation is emitted by radioactive substances (radioisotopes), such as uranium, radon, and plutonium. Such radiation is also produced by man-made sources, such as x-ray and radiation therapy machines. Radiation dose is measured in several different units, but all relate to the amount of energy deposited. The units include the roentgen (R), the gray (Gy), and the sievert (Sv). The sievert and gray are similar, except the sievert takes into account the biologic effects of different types of radiation. The two main types of radiation exposure are irradiation and contamination. Many radiation accidents expose a person to both.

[0004] There is an urgent need for domestic security and public health preparedness to develop more effective and broader acting radiation protectors and mitigators for the increasing likelihood of radiological/nuclear attacks or accidents. Further, there is a need for radiation protectors and mitigators, in typically chronic exposure settings such as cosmic radiation exposure in space. The three types of acute radiation syndrome (ARS) are hematopoietic (H), gastrointestinal and cardiovascular/central nervous system. Survivors of ARS later manifest the delayed effects of acute radiation exposure (DEARE), a debilitating chronic illness affecting multiple organ systems. Hematopoietic DEARE, known as residual bone marrow damage (RBMD), is a latent condition with

functionally compromised hematopoietic stem cells (HSC) and depressed hematopoiesis, most evident under stress. In addition there are detrimental effects such as risk of cancers from radiation exposure to diagnostic radiation with computerized tomography which increases with repeated scans.

[0005] The four FDA licensed medical countermeasures (MedCM) for severely irradiated personnel only mitigate the effects of H-ARS and include Neupogen® (G-CSF), Neulasta® (depo-G-CSF), and Leukine® (GM-CSF) to enhance white blood cell production. Nplate® (romiplostim) to increase platelet production and erythropoietin to stimulate red blood cell production. In other words, the only drugs approved for the lethal radiation mitigation setting are bone marrow growth factors to limit short term myelosuppression. There are no MedCM to mitigate the ARS or DEARE of any of the other body's organ systems.

[0006] There is a need for new and/or improved medical counter measures to protect and/or mitigate the effects of non-therapeutic and/or non-targeted radiation exposure. Preferably, where the non-therapeutic, non-targeted radiation exposure is lethal or can promote organ dysfunction or cancer unless treated.

[0007] Reference to any prior art in the specification is not an acknowledgment or suggestion that this prior art forms part of the common general knowledge in any jurisdiction or that this prior art could reasonably be expected to be understood, regarded as relevant, and/or combined with other pieces of prior art by a skilled person in the art.

Summary of the invention

[0008] In one aspect, the present invention provides a method of minimising the effects of non-targeted and/or non-therapeutic radiation exposure in an individual, the method comprising administering a sesquiterpene lactone to the individual, thereby minimising the effects of non-targeted and/or non-therapeutic radiation exposure in the individual.

[0009] In any embodiment, the non-targeted and/or non-therapeutic radiation exposure is at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%,

at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or 100% of the surface-area of the individual that is exposed to the radiation.

[0010] In any embodiment, the non-targeted and/or non-therapeutic radiation exposure is about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95% or 100% of the surface-area of the individual that is exposed to the radiation.

[0011] In any embodiment, the non-targeted and/or non-therapeutic radiation exposure is 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100% of the surface-area of the individual that is exposed to the radiation.

[0012] In any embodiment, the method comprises non-targeted and/or non-therapeutic radiation exposure to one or more organs selected from: brain, thyroid, breast, heart, liver, pancreas, bladder, ovaries, testes, bone, salivary glands, lung, stomach, intestines, kidneys, bladder, uterus, cervix, penis, inner ear, vagina, heart, skeletal muscles, thymus, spleen bone marrow, skin and hair. Preferably, the non-targeted and/or non-therapeutic radiation exposure is to one or more organs selected from: skin, testes, bladder and lung.

[0013] In any embodiment, the non-targeted and/or non-therapeutic radiation exposure is total (whole) body or systemic exposure.

[0014] In any embodiment, the radiation exposure is a dose that, if untreated, would cause acute radiation syndrome (ARS) in the individual. The ARS may be hematopoietic (H), gastrointestinal, pulmonary, genitourinary (including reproductive) or cardiovascular/central nervous system.

[0015] In any embodiment, the acute radiation syndrome comprises acute radiation injury to one or more of the skin, testes, bladder and lung.

[0016] In any embodiment, the radiation exposure is a dose that, if untreated, would cause delayed effects of acute radiation exposure (DEARE) in the individual to any organ.

[0017] In any embodiment, the radiation exposure is a dose of radiation expected to cause death to 50 percent of an exposed population within 30 days (LD 50/30); 50 percent of an exposed population within 60 days (LD 50/60); or 70 percent of an exposed population within 30 days (LD 70/30).

[0018] In any embodiment, the radiation exposure is equal to, or greater than, about 3 Gy; equal to, or greater than, about 4 Gy; equal to, or greater than, about 5 Gy; equal to, or greater than, about 6 Gy; equal to, or greater than, about 7 Gy; equal to, or greater than, about 8 Gy; equal to, or greater than, about 9 Gy or equal to, or greater than, about 10Gy.

[0019] In any embodiment, the radiation exposure is equal to, or greater than, 3 Gy; equal to, or greater than, 4 Gy; equal to, or greater than, 5 Gy; equal to, or greater than, 6 Gy; equal to, or greater than, 7 Gy; equal to, or greater than, 8 Gy; equal to, or greater than, 9 Gy or equal to, or greater than, 10Gy.

[0020] In any embodiment, the radiation exposure is acute exposure or chronic exposure.

[0021] Acute exposure may be radiation exposure over a relatively short time period. Acute exposure may therefore be for a period of equal to, or less than, about 12 hours; equal to, or less than, about 10 hours; equal to, or less than, about 8 hours; equal to, or less than, about 6 hours; equal to, or less than, about 4 hours; equal to, or less than, about 2 hours; or equal to, or less than, about 1 hour. Typically, the radiation exposure is continuous over this time period.

[0022] Chronic exposure may be radiation exposure over a relatively long time period. Chronic exposure may therefore be for a period of equal to, or greater than about 12 hours; equal to, or greater than about 24 hours; equal to, or greater than about 2 days; equal to, or greater than about 3 days; equal to, or greater than about 4 days; equal to, or greater than about 5 days; equal to, or greater than about 6 days; equal to, or greater than about 7 days equal to, or greater than about 2 weeks; equal to, or greater than about 1 month; equal to, or greater than about 2 months; equal to, or greater than about 3 months; equal to, or greater than about 6 months or equal to, or greater than about 1 year. Typically, the radiation exposure is continuous over this time period.

[0023] In any embodiment, the radiation is ionising radiation. The ionising radiation may be electromagnetic radiation or particle radiation, preferably arising from a nuclear accident or attack. The particle radiation may be selected from alpha particles, beta particles and neutrons.

[0024] In any embodiment, the radiation exposure is irradiation.

[0025] In any embodiment, the radiation exposure is contamination. The contamination of the individual may be external or internal. Where the contamination is internal, it may have occurred or be occurring via ingestion, inhalation, or breaks in the skin.

[0026] In any embodiment, the radiation exposure is cosmic radiation exposure. In this embodiment, the radiation exposure of the individual may be continuous for greater than 12 hours, 24 hours, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 2 weeks, 1 month, 2 months, 3 months or 6 months.

[0027] In any embodiment, the individual is operating in a radiological or nuclear environment.

[0028] In any embodiment, the individual has not been diagnosed with cancer.

[0029] In any embodiment, the individual does not have a detectable tumour.

[0030] In any embodiment, wherein the individual is non-murine. In any embodiment, the individual is human.

[0031] In any embodiment, the sesquiterpene lactone is administered after the radiation exposure.

[0032] In any embodiment, the sesquiterpene lactone is administered during and after exposure to radiation.

[0033] In any embodiment, the sesquiterpene lactone is administered after exposure to radiation and not before or during exposure.

[0034] In any embodiment, the sesquiterpene lactone is administered during and after exposure to radiation, and not before exposure.

[0035] In any embodiment, the sesquiterpene lactone is administered before, during and after exposure to radiation.

[0036] In any embodiment, the method minimises the effects of non-targeted and/or non-therapeutic radiation-induced cell senescence in the individual.

[0037] In any embodiment, the method minimises the effects of non-targeted and/or non-therapeutic radiation-induced fibrosis in the individual.

[0038] In any embodiment, the method minimises the effects of non-targeted and/or non-therapeutic radiation-induced skin injury in the individual.

[0039] In any embodiment, the method minimises the effects of non-targeted and/or non-therapeutic radiation-induced hypospermia in the individual.

[0040] In any embodiment, the method minimises the effects of non-targeted and/or non-therapeutic radiation-induced inflammation in the individual.

[0041] In any embodiment, the sesquiterpene lactone is parthenolide or a pharmaceutically acceptable salt, solvate, tautomer, N-oxide, stereoisomer and/or prodrug thereof. In any embodiment, the sesquiterpene lactone is dimethylaminoparthenolide (DMAPT) or a pharmaceutically acceptable salt, solvate, tautomer, N-oxide, stereoisomer and/or prodrug thereof. Preferably, the sesquiterpene lactone is dimethylaminoparthenolide (DMAPT) or a pharmaceutically acceptable salt thereof. More preferably, the pharmaceutically acceptable salt of DMAPT is a fumarate salt.

[0042] In any embodiment, the sesquiterpene lactone is in a composition, preferably an oral or topical composition. The oral or topical composition may be any composition described herein.

[0043] In any embodiment, the sesquiterpene lactone is orally administered to the individual.

[0044] In any embodiment, the sesquiterpene lactone is topically administered to the individual.

[0045] In any embodiment, the individual is receiving or has received G-CSF or analog or derivative thereof or a composition comprising G-CSF or analog or derivative thereof.

[0046] In any embodiment, the method further comprises administering G-CSF or analog or derivative thereof or a composition comprising G-CSF or analog or derivative thereof.

[0047] In another aspect, the present invention provides a method of preventing or reducing the risk of cancer, caused by non-targeted and/or non-therapeutic radiation exposure, in an individual, the method comprising administering a sesquiterpene lactone to the individual, thereby preventing cancer in the individual caused by non-targeted and/or non-therapeutic radiation exposure.

[0048] In another aspect, the present invention provides use of a sesquiterpene lactone in the manufacture of a medicament for preventing or reducing the risk of cancer, caused by non-targeted and/or non-therapeutic radiation exposure, in an individual.

[0049] In another aspect, the present invention provides a sesquiterpene lactone for use in for preventing or reducing the risk of cancer, caused by non-targeted and/or non-therapeutic radiation exposure, in an individual.

[0050] In another aspect, the present invention provides a kit comprising (a) a sesquiterpene lactone as defined herein and (b) G-CSF or analog or derivative thereof, or a composition comprising G-CSF or analog or derivative thereof.

[0051] In another aspect, the present invention provides use of a sesquiterpene lactone in the manufacture of a medicament for minimising the effects of non-targeted and/or non-therapeutic radiation exposure in an individual. In one embodiment, the individual has received, or is receiving, G-CSF or analog or derivative thereof, or a composition comprising G-CSF or analog or derivative thereof.

[0052] In another aspect, the present invention provides a sesquiterpene lactone for use in minimising the effects of non-targeted and/or non-therapeutic radiation exposure in an individual.

[0053] In another aspect, the present invention provides use of (a) a sesquiterpene lactone in the manufacture of a first medicament and (b) G-CSF or analog or derivative thereof, or a composition comprising G-CSF or analog or derivative thereof in the manufacture of a second medicament the first and second medicaments for minimising the effects of non-targeted and/or non-therapeutic radiation exposure in an individual.

[0054] In another aspect, the present invention provides use of a (a) sesquiterpene and (b) G-CSF or analog or derivative thereof, or a composition comprising G-CSF or analog or derivative thereof, in the manufacture of a medicament for minimising the effects of non-targeted and/or non-therapeutic radiation exposure in an individual.

[0055] In another aspect, the present invention provides a sesquiterpene lactone for use in minimising the effects of non-targeted and/or non-therapeutic radiation exposure in an individual. In one embodiment, the individual has received, or is receiving, G-CSF or analog or derivative thereof, or a composition comprising G-CSF or analog or derivative thereof.

[0056] In another aspect, the present invention provides (a) sesquiterpene and (b) G-CSF or analog or derivative thereof, or a composition comprising G-CSF or analog or derivative thereof, for use in minimising the effects of non-targeted and/or non-therapeutic radiation exposure in an individual.

[0057] In another aspect, the present invention provides a method of preventing or treating radiation-induced hypospermia in an individual, the method comprising administering a sesquiterpene lactone to the individual, thereby preventing or reducing radiation-induced hypospermia in the individual. In one embodiment, the radiation exposure is non-targeted and/or non-therapeutic radiation exposure.

[0058] In another aspect, the present invention provides use of a sesquiterpene lactone in the manufacture of a medicament for preventing or treating radiation-induced hypospermia in an individual. In one embodiment, the radiation exposure is non-targeted and/or non-therapeutic radiation exposure.

[0059] In another aspect, the present invention provides a sesquiterpene lactone for use in preventing or treating radiation-induced hypospermia in an individual. In one embodiment, the radiation exposure is non-targeted and/or non-therapeutic radiation exposure.

[0060] In one embodiment of this aspect, the radiation exposure is a dose that, if untreated, would cause delayed effects of acute radiation exposure (DEARE) in the individual to any organ.

[0061] In one embodiment of this aspect, the radiation exposure is a dose that, if untreated, would cause acute radiation syndrome (ARS) in the individual.

[0062] In another aspect, the present invention provides a method of preventing or treating radiation-induced cell senescence in an individual, the method comprising administering a sesquiterpene lactone to the individual, thereby preventing or reducing radiation-induced cell senescence in the individual.

[0063] In one embodiment of this aspect, the radiation exposure is non-targeted and/or non-therapeutic radiation exposure.

[0064] In one embodiment of this aspect, the radiation-induced cell senescence is present in any organ, including any organ described herein. Preferably the radiation-induced cell senescence is in the colorectum or testis.

[0065] In one embodiment of this aspect, the radiation exposure is a dose that, if untreated, would cause delayed effects of acute radiation exposure (DEARE) in the individual to any organ.

[0066] In one embodiment of this aspect, the radiation exposure is a dose that, if untreated, would cause acute radiation syndrome (ARS) in the individual.

[0067] In another aspect, the present invention provides use of a sesquiterpene lactone in the manufacture of a medicament for preventing or treating radiation-induced cell senescence in an individual.

[0068] In one embodiment of this aspect, the radiation exposure is non-targeted and/or non-therapeutic radiation exposure.

[0069] In one embodiment of this aspect, the radiation exposure is a dose that, if untreated, would cause delayed effects of acute radiation exposure (DEARE) in the individual to any organ.

[0070] In one embodiment of this aspect, the radiation exposure is a dose that, if untreated, would cause acute radiation syndrome (ARS) in the individual.

[0071] In another aspect, the present invention provides a method of preventing or treating radiation-induced cell senescence in an individual, the method comprising administering a sesquiterpene lactone to the individual, thereby preventing or reducing radiation-induced cell senescence in the individual.

[0072] In one embodiment of this aspect, the radiation exposure is non-targeted and/or non-therapeutic radiation exposure.

[0073] In one embodiment of this aspect, the radiation-induced cell senescence is present in any organ. Preferably the radiation-induced cell senescence is in the colorectum or testis.

[0074] In one embodiment of this aspect, the radiation exposure is a dose that, if untreated, would cause delayed effects of acute radiation exposure (DEARE) in the individual to any organ.

[0075] In one embodiment of this aspect, the radiation exposure is a dose that, if untreated, would cause acute radiation syndrome (ARS) in the individual.

[0076] In another aspect, the present invention provides a sesquiterpene lactone for use in preventing or treating radiation-induced cell senescence in an individual.

[0077] In one embodiment of this aspect, the radiation exposure is non-targeted and/or non-therapeutic radiation exposure.

[0078] In one embodiment of this aspect, the radiation exposure is a dose that, if untreated, would cause delayed effects of acute radiation exposure (DEARE) in the individual to any organ.

[0079] In one embodiment of this aspect, the radiation exposure is a dose that, if untreated, would cause acute radiation syndrome (ARS) in the individual.

[0080] In another aspect, the present invention provides a method of preventing or treating radiation-induced cell senescence in an individual, the method comprising administering a sesquiterpene lactone to the individual, thereby preventing or reducing radiation-induced cell senescence in the individual.

[0081] In one embodiment of this aspect, the radiation exposure is non-targeted and/or non-therapeutic radiation exposure.

[0082] In one embodiment of this aspect, the radiation-induced cell senescence is present in any organ. Preferably the radiation-induced cell senescence is in the colorectum or testis.

[0083] In one embodiment of this aspect, the radiation exposure is a dose that, if untreated, would cause delayed effects of acute radiation exposure (DEARE) in the individual to any organ.

[0084] In one embodiment of this aspect, the radiation exposure is a dose that, if untreated, would cause acute radiation syndrome (ARS) in the individual.

[0085] In another aspect, the present invention provides a method of preventing or reducing radiation-induced fibrosis in an individual, the method comprising administering a sesquiterpene lactone to the individual, thereby preventing or treating radiation-induced fibrosis in the individual.

[0086] In one embodiment of this aspect, the radiation exposure is non-targeted and/or non-therapeutic radiation exposure.

[0087] In one embodiment of this aspect, the radiation-induced fibrosis is present in any organ, including any organ described herein. Preferably, the radiation-induced fibrosis is in the lung or bladder.

[0088] In one embodiment of this aspect, the radiation exposure is a dose that, if untreated, would cause delayed effects of acute radiation exposure (DEARE) in the individual to any organ.

[0089] In one embodiment of this aspect, the radiation exposure is a dose that, if untreated, would cause acute radiation syndrome (ARS) in the individual.

[0090] In another aspect, the present invention provides use of a sesquiterpene lactone in the manufacture of a medicament for preventing or reducing fibrosis in an individual.

[0091] In one embodiment of this aspect, the radiation exposure is non-targeted and/or non-therapeutic radiation exposure.

[0092] In one embodiment of this aspect, the radiation-induced fibrosis is present in any organ. Preferably, the radiation-induced fibrosis is in the lung or bladder.

[0093] In one embodiment of this aspect, the radiation exposure is a dose that, if untreated, would cause delayed effects of acute radiation exposure (DEARE) in the individual to any organ.

[0094] In one embodiment of this aspect, the radiation exposure is a dose that, if untreated, would cause acute radiation syndrome (ARS) in the individual.

[0095] In another aspect, the present invention provides a sesquiterpene lactone for use in preventing or reducing radiation-induced fibrosis in an individual.

[0096] In one embodiment of this aspect, the radiation exposure is non-targeted and/or non-therapeutic radiation exposure.

[0097] In one embodiment of this aspect, the radiation-induced fibrosis is present in any organ. Preferably, the radiation-induced fibrosis is in the lung or bladder.

[0098] In one embodiment of this aspect, the radiation exposure is a dose that, if untreated, would cause delayed effects of acute radiation exposure (DEARE) in the individual to any organ.

[0099] In one embodiment of this aspect, the radiation exposure is a dose that, if untreated, would cause acute radiation syndrome (ARS) in the individual.

[0100] In another aspect, the present invention provides a method of minimising the effects of radiation-induced injury to a second organ, wherein the radiation is applied to a first organ, the method comprising administering a sesquiterpene lactone to the second organ, thereby minimising the effects of radiation-induced injury to the second organ.

[0101] In one embodiment of this aspect, the radiation exposure is a dose that, if untreated, would cause delayed effects of acute radiation exposure (DEARE) in the individual to any organ.

[0102] In one embodiment of this aspect, the radiation exposure is a dose that, if untreated, would cause acute radiation syndrome (ARS) in the individual.

[0103] In one embodiment, the second organ is skin and the first organ is not skin.

[0104] In one embodiment, the radiation-induced skin injury is one or more selected from: erythema, altered sensation, itching, edema, blistering, desquamation, ulcer, necrosis, hair loss and onycholysis.

[0105] In one embodiment, the radiation applied to the first organ is for the treatment of a cancer. In one embodiment, the radiation is external beam radiation.

[0106] In one embodiment, the cancer is a head and neck cancer.

[0107] In one embodiment, the sesquiterpene lactone is administered topically, preferably the sesquiterpene lactone is topically administered in a composition. The topical composition could be any one described herein.

[0108] In another aspect, the present invention use of a sesquiterpene lactone in the manufacture of a medicament for minimising the effects of radiation-induced injury to a second organ, wherein radiation has been, or being, applied to a first organ, and wherein the medicament is to be administered to the second organ.

[0109] In one embodiment of this aspect, the radiation exposure is a dose that, if untreated, would cause delayed effects of acute radiation exposure (DEARE) in the individual to any organ.

[0110] In one embodiment of this aspect, the radiation exposure is a dose that, if untreated, would cause acute radiation syndrome (ARS) in the individual.

[0111] In one embodiment, the second organ is skin and the first organ is not skin.

[0112] In one embodiment, the radiation-induced skin injury is one or more selected from: erythema, altered sensation, itching, edema, blistering, desquamation, ulcer, necrosis, hair loss and onycholysis.

[0113] In one embodiment, the radiation applied to the first organ is for the treatment of a cancer. In one embodiment, the radiation is external beam radiation.

[0114] In one embodiment, the cancer is a head and neck cancer.

[0115] In one embodiment, the sesquiterpene lactone is administered topically, preferably the sesquiterpene lactone is topically administered in a composition. The topical composition could be any one described herein.

[0116] In another aspect, the present invention provides a sesquiterpene lactone for use in minimising the effects of radiation-induced injury to a second organ, wherein radiation has been, or is being, applied to a first organ, comprising administering the sesquiterpene lactone to the second organ.

[0117] In one embodiment of this aspect, the radiation exposure is a dose that, if untreated, would cause delayed effects of acute radiation exposure (DEARE) in the individual to any organ.

[0118] In one embodiment of this aspect, the radiation exposure is a dose that, if untreated, would cause acute radiation syndrome (ARS) in the individual.

[0119] In one embodiment, the second organ is skin and the first organ is not skin.

[0120] In one embodiment, the radiation-induced skin injury is one or more selected from: erythema, altered sensation, itching, edema, blistering, desquamation, ulcer, necrosis, hair loss and onycholysis.

[0121] In one embodiment, the radiation applied to the first organ is for the treatment of a cancer. In one embodiment, the radiation is external beam radiation.

[0122] In one embodiment, the cancer is a head and neck cancer.

[0123] In one embodiment, the sesquiterpene lactone is administered topically, preferably the sesquiterpene lactone is topically administered in a composition. The topical composition could be any one described herein.

[0124] In another aspect, the present invention provides a method of preventing or treating radiation-induced skin injury in an individual, the method comprising administering a sesquiterpene lactone topically to the individual, thereby preventing or treating radiation-induced skin injury in the individual. In one embodiment, the radiation exposure is non-targeted and/or non-therapeutic radiation exposure.

[0125] In one embodiment, the sesquiterpene lactone is administered topically, preferably the sesquiterpene lactone is topically administered in a composition in a composition. The topical composition could be any one described herein.

[0126] In any embodiment, the skin injury is one or more selected from: erythema, altered sensation, itching, edema, blistering, desquamation, ulcer, necrosis, hair loss and onycholysis.

[0127] In one embodiment of this aspect, the radiation exposure is a dose that, if untreated, would cause delayed effects of acute radiation exposure (DEARE) in the individual to any organ.

[0128] In one embodiment of this aspect, the radiation exposure is a dose that, if untreated, would cause acute radiation syndrome (ARS) in the individual.

[0129] In another aspect, the present invention provides use of a sesquiterpene lactone in the manufacture of a medicament for preventing or radiation-induced skin injury in an individual. In one embodiment, the radiation exposure is non-targeted and/or non-therapeutic radiation exposure.

[0130] In one embodiment, the sesquiterpene lactone is administered topically, preferably the sesquiterpene lactone is topically administered in a composition in a composition. The topical composition could be any one described herein.

[0131] In any embodiment, the skin injury is one or more selected from: erythema, altered sensation, itching, edema, blistering, desquamation, ulcer, necrosis, hair loss and onycholysis.

[0132] In one embodiment of this aspect, the radiation exposure is a dose that, if untreated, would cause delayed effects of acute radiation exposure (DEARE) in the individual to any organ.

[0133] In one embodiment of this aspect, the radiation exposure is a dose that, if untreated, would cause acute radiation syndrome (ARS) in the individual.

[0134] In another aspect, the present invention provides a sesquiterpene lactone for use in preventing or treating radiation-induced skin injury in an individual. In one embodiment, the radiation exposure is non-targeted and/or non-therapeutic radiation exposure.

[0135] In one embodiment, the sesquiterpene lactone is administered topically, preferably the sesquiterpene lactone is topically administered in a composition in a composition. The topical composition could be any one described herein.

[0136] In any embodiment, the skin injury is one or more selected from: erythema, altered sensation, itching, edema, blistering, desquamation, ulcer, necrosis, hair loss and onycholysis.

[0137] In one embodiment of this aspect, the radiation exposure is a dose that, if untreated, would cause delayed effects of acute radiation exposure (DEARE) in the individual to any organ.

[0138] In one embodiment of this aspect, the radiation exposure is a dose that, if untreated, would cause acute radiation syndrome (ARS) in the individual.

[0139] As used herein, except where the context requires otherwise, the term "comprise" and variations of the term, such as "comprising", "comprises" and "comprised", are not intended to exclude further additives, components, integers or steps.

[0140] Further aspects of the present invention and further embodiments of the aspects described in the preceding paragraphs will become apparent from the following description, given by way of example and with reference to the accompanying drawings.

Brief description of the drawings

[0141] **Figure 1. DMAPT limits the early stages of radiation induced fibrosis in bladder.** Collagen fraction in bladder visualized using Masson's trichrome staining (n = 7-8) in mice that received LD50 whole body dose in the presence or absence of DMAPT, or sham exposure. Representative staining images have been provided with regions of positively-stained collagen highlighted in black for visualisation purposes. *p<0.05, **p<0.005, ****p<0.0001.

[0142] **Figure 2. DMAPT reduces radiation-induced hypospermia.** Types of tubule damage observed in the testis of mice after radiation exposure +/- DMAPT (n = 4, 1 SD). Top bar, thin/damaged membrane; middle bar, vacuoles present; bottom bar hypospermia.

[0143] Figure 3. DMAPT reduces radiation-induced senescence in colorectal tissue. (A) Schematic overview of the pre-clinical study. In brief, C57BL/6J mice were treated thrice weekly +/-DMAPT, every week from one week pre-irradiation to the study endpoint. Mice received 10x fractions of 2 Gy radiation over the course of two weeks. (B) Quantification of Senescence-Associated β -Galactosidase (SA- β -Gal) staining in mouse colorectal tissues (n = 7-9 mice per treatment group, ** p < 0.005, *** p < 0.001, **** p < 0.0001). (C) Representative images of SA- β -Gal staining in murine colorectal tissue (regions of positively-stained SA- β -Gal have been highlighted in black for visualisation purposes).

[0144] Figure 4. DMAPT reduces radiation-induced senescence in testis. (A) Schematic overview of the pre-clinical study. In brief, C57BL/6J mice were treated thrice weekly +/-DMAPT, every week from one week pre-irradiation to the study endpoint. Mice received 10x fractions of 2 Gy radiation over the course of two weeks. (B) Quantification of Senescence-Associated β -Galactosidase (SA- β -Gal) staining in mouse testis (n = 9 mice per treatment group, **** p < 0.0001). (C) Representative images of SA- β -Gal staining in murine testis tissue (regions of positively-stained SA- β -Gal have been highlighted in black for visualisation purposes).

[0145] Figure 5. DMAPT reduces markers of fibrosis in lung. (A) Schematic overview of the pre-clinical study. In brief, C57BL/6J mice received high-dose whole-body irradiation. Mice were treated +/-DMAPT on the day of irradiation and once a day for the subsequent three days. (B) Fold-change in RNA expression (by RT-qPCR) of ARS biomarkers (Wnt3 and Ddb2) in murine lung tissues (n = 7-9 mice per treatment group, ± 1 SD).

[0146] Figure 6. DMAPT reduces markers of fibrosis in bladder. (A) Schematic overview of the pre-clinical study. In brief, C57BL/6J mice received high-dose whole-body irradiation. Mice were treated +/- DMAPT on the day of irradiation and once a day for the subsequent three days. (B) Fold-change in RNA expression (by RT-qPCR) of ARS biomarkers (Wnt3 and Ddb2) in murine bladder tissues (n = 7-9 mice per treatment group, ± 1 SD).

[0147] Figure 7. DAMPT topical composition prevents and treats radiation-induced skin injury. Pictures representing skin condition before and after 70Gy

radiation treatment administered over 35 sessions. A topical DMAPT composition was applied daily as detailed in Example 6 below.

Detailed description of the embodiments

[0148] It will be understood that the invention disclosed and defined in this specification extends to all alternative combinations of two or more of the individual features mentioned or evident from the text or drawings. All of these different combinations constitute various alternative aspects of the invention.

[0149] Reference will now be made in detail to certain embodiments of the invention. While the invention will be described in conjunction with the embodiments, it will be understood that the intention is not to limit the invention to those embodiments. On the contrary, the invention is intended to cover all alternatives, modifications, and equivalents, which may be included within the scope of the present invention as defined by the claims.

[0150] One skilled in the art will recognize many methods and materials similar or equivalent to those described herein, which could be used in the practice of the present invention. The present invention is in no way limited to the methods and materials described. It will be understood that the invention disclosed and defined in this specification extends to all alternative combinations of two or more of the individual features mentioned or evident from the text or drawings. All of these different combinations constitute various alternative aspects of the invention.

[0151] All of the patents and publications referred to herein are incorporated by reference in their entirety, unless otherwise specified.

[0152] For purposes of interpreting this specification, terms used in the singular will also include the plural and vice versa.

[0153] The present invention is based on the surprising finding that administration of a sesquiterpene lactone can minimise the effects of non-targeted, for example whole body, and/or non-therapeutic radiation exposure in an individual. In particular, administration of a sesquiterpene lactone to an individual can have a radioprotective or radiomitigative effect where that individual has been exposed to an amount of, or

duration of, radiation that would otherwise be lethal, cause organ dysfunction or increase risk of cancer without intervention.

Forms and sources of radiation

[0154] The radiation as described herein, may be in the form of non-ionising or ionising radiation. When the radiation is in the form of non-ionising radiation, the radiation is typically ultraviolet radiation.

[0155] Radiation exposure can damage DNA, RNA, and proteins directly, but more often, the damage to these molecules is indirect, caused by highly reactive free radicals generated by radiation's interaction with intracellular water molecules. Large doses of radiation can cause cell death, and lower doses may interfere with endogenous molecular repair systems, homeostasis, and cellular proliferation. Damage to these and other cellular components may result in progressive tissue hypoplasia, atrophy, and eventually fibrosis as well as cancer.

[0156] Exposure to ionizing radiation can be classified into 3 exposure situations. The first, planned exposure situations, result from the deliberate introduction and operation of radiation sources with specific purposes, as is the case with the medical use of radiation for diagnosis or treatment of patients, or the use of radiation in industry or research. The second type of situation, existing exposures, is where exposure to radiation already exists, and a decision on control must be taken – for example, exposure to radiation in homes or workplaces or exposure to natural background radiation from the environment. The last type, emergency exposure situations, result from unexpected events requiring prompt response such as nuclear accidents or malicious acts.

[0157] As used herein, "ionising radiation" refers to alpha particles, beta particles, gamma rays, x-rays, neutrons, high-speed electrons, high-speed protons, and other particles capable of producing ions. When ionizing radiation passes through material such as air, water, or living tissue, it produces ions by breaking molecular bonds and displace (or remove) electrons from atoms or molecules. The effects of external radiation on the body may appear within minutes (acute radiation syndrome) or develop many months or years after exposure (delayed effects of acute radiation exposure). In general, exposure to higher doses of radiation produce symptoms more rapidly.

[0158] The term “irradiation” refers to a situation or the condition of exposure to radiation of the recipient individual. In some embodiments, the radiation is ionizing radiation. In other embodiments, the radiation is non-ionizing radiation. Radiation includes electromagnetic radiation, which includes X-rays and/or gamma rays. The term radiation also encompasses radioactive particle radiation. The term also encompasses radiation resulting from the decay of radioactive elements.

[0159] Preferably, the radiation is ionising radiation. The ionising radiation may be electromagnetic radiation or particle radiation, preferably arising from a nuclear accident or attack. The particle radiation may be selected from alpha particles, beta particles and neutrons.

[0160] Ionising radiation may be produced by natural background sources, including cosmic radiation and terrestrial radiation.

[0161] “Cosmic radiation” is a source of natural background radiation, which originates in outer space and is composed of penetrating ionizing radiation. Cosmic radiation consists of high-energy charged particles, x-rays and gamma rays produced in space. Charged particles react with the earth’s atmosphere to produce secondary radiation which reaches the earth. Differences in elevation, atmospheric conditions, and the Earth’s magnetic field can change the amount (or dose) of cosmic radiation that an individual receives. In preferred embodiments, the radiation exposure is cosmic radiation exposure.

[0162] “Terrestrial radiation” refers to the portion of the natural radiation that is emitted by naturally occurring radioactive materials, such as uranium, thorium, and radon originating from the earth.

[0163] Alternatively, ionising radiation may be produced by “human-made sources”. Such sources include, but are not limited to: medical procedures, such as x-ray diagnostics, nuclear medicine, and radiation therapy; generation of electricity and heat at nuclear power plants and transport nuclear engines, and also by radionuclide sources in spacecrafts; nuclear fuel cycle; Sterilizing of good and pasteurization of food using industrial irradiators (industrial accelerators and gamma irradiators); Non-destructive testing (NDT) and quality control in manufacturing, building and construction and transport; Control of technical processes, level of liquids in vessels, defining parameters

of products and environmental samples such as thickness, moisture content (radiation gauges); Exploration of fossils, well logging and control of drill to stop; Manufacturing of IR measuring equipment; Research of substances' atomic-molecular structure (X-ray fluorescence analysis, X-ray diffraction analysis, electron-microscope investigation, activation analysis, neutronography, etc.); Control of technological processes in various areas using radioactive tracers; Applications in medicine using X-ray machines, accelerators, radionuclide sources; Manufacturing of radionuclides for different purposes, including radiopharmaceuticals for nuclear medicine; Management of materials contained natural radionuclides in concentrations increasing the natural ones (Naturally Occurring Radioactive Materials, NORM); Transportation of nuclear and radioactive materials; Activities at radioactive contaminated territories; Elimination of radioactive material from nuclear accidents; Radioactive waste management; Using X-ray examination systems for luggage and cargoes control, X-ray inspection at public places and sites, nuclear forensics.

[0164] Accordingly, radiation exposure may be more frequent, and the risk of radiation injury and or radiation symptoms may be higher, for individuals that are occupationally exposed to radiation or in individuals who receive repeated medical diagnostic procedures that utilise ionising radiation, for example, CT scans. In particular, occupationally exposed individuals typically work in the following areas: fuel cycle facilities, industrial radiography, radiology departments (medical), nuclear medicine departments, radiation oncology departments, nuclear power plants and government and university research laboratories. In any embodiment, the individual is operating in a radiological or nuclear environment.

[0165] The present invention is directed to radioprotection or radiomitigation in situations where the purpose, or primary outcome, is not therapeutic. For example, the methods of the invention are not directed to therapeutic exposure for the treatment of cancer. However, if the individual was accidentally or inadvertently exposed to a lethal dose of radiation whilst receiving therapeutic radiotherapy for the treatment of cancer, then one embodiment of the invention would be directed to the radiomitigation in that scenario. Again, in that case the radiation exposure could be lethal, cause organ dysfunction or increase the risk of cancer unless intervention by a method described herein.

Non-targeted and/or non-therapeutic radiation exposure

[0166] As used herein, “non-targeted radiation exposure” may refer to radiation exposure that is non-image guided. Typically, the non-targeted radiation exposure is non-therapeutic. For example, the non-targeted radiation exposure may be at a radiation dose that is lower, or higher, than a dose that would be used in a therapeutic setting. Alternatively, the non-targeted radiation exposure event was not intended to treat, prevent or diagnose a medical condition, for example the individual did not receive radiation as part of a standard clinical protocol for treating or preventing, or diagnosing, a medical condition. In another example, the non-targeted radiation exposure may have been received at a substantially higher dose than intended for the standard clinical protocol due to error.

[0167] In any embodiment, the non-targeted radiation exposure was not provided for therapeutic purposes, for example the radiation exposure will not be radiotherapy as may be utilized for treatment of cancer or other therapy.

[0168] In any embodiment, the non-therapeutic radiation exposure may be provided for diagnosing a medical condition, for example where the individual receives repeated diagnostic CT scans.

[0169] In any embodiment, the non-targeted and/or non-therapeutic radiation exposure is at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or 100% of the surface-area of the individual that is exposed to the radiation.

[0170] In any embodiment, the non-targeted and/or non-therapeutic radiation exposure is about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95% or 100% of the surface-area of the individual that is exposed to the radiation.

[0171] In any embodiment, the non-targeted and/or non-therapeutic radiation exposure is 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100% of the surface-area of the individual that is exposed to the radiation.

[0172] In any embodiment, at least a substantial portion (e.g. an entire limb and/or torso and/or entire head region) of an individual may be exposed to the radiation.

[0173] Accordingly, the radiation exposure may result in damage to one or more systems or organs in an individual. In any embodiment, the radiation exposure may result in damage to two or more systems or organs in an individual.

[0174] Organs that may be exposed to radiation include, but are not limited to, the brain, the thyroid, breast, the heart, the liver, the pancreas, the bladder, the ovaries, the testes, bone, the salivary glands, the oesophagus, upper airways, the lung, the stomach, intestines, the kidneys, bladder, uterus, cervix, penis, inner ear, vagina, heart, skeletal muscles, thymus, spleen bone marrow, skin and hair. In preferred embodiments, the organs are one or more selected from the group consisting of: breast, ovaries, testes, lung, stomach, colon and bone marrow.

[0175] In general the major systems that may be exposed to radiation include, but are not limited to, the central nervous system, gastrointestinal system, hematopoietic system, respiratory system, cardiovascular system, immune system, muscular system and reproductive system. In preferred embodiments, the system is one or more selected from the group consisting: hematopoietic system, respiratory system, reproductive system and gastrointestinal system.

[0176] In any embodiment, the non-targeted and/or non-therapeutic radiation exposure is total body (i.e. whole body) or systemic exposure.

[0177] In any embodiment, the non-targeted and/or non-therapeutic radiation exposure may be internal or external. Internal exposure to radiation may occur when a radionuclide is inhaled, ingested or otherwise enters into the bloodstream (for example, through wounds). External exposure may occur when airborne radioactive material (such as dust, liquid, or aerosols) is deposited on skin or clothes. Exposure to radiation may also result from irradiation from an external source, such as medical radiation exposure from X-rays. In any embodiment, the non-targeted and/or non-therapeutic radiation exposure may be both internal and external.

[0178] In any embodiment, the radiation exposure is irradiation or contamination. Irradiation is exposure to radiation but not radioactive material (ie, no contamination is involved). Contamination is the unintended contact with and retention of radioactive

material, usually as dust or liquid. Contamination of the individual may be external or internal. Where the contamination is internal, it may have occurred or be occurring via ingestion, inhalation, or breaks in the skin. Once in the body, radioactive material may be transported to various sites (eg, bone marrow), where it continues to emit radiation until it is removed or decays.

Radiation dose

[0179] The term "radiation dose" refers to the total amount of radiation absorbed by material or tissues, generally expressed in centigrays (cGy), radiation unit (rad), grays (Gy), roentgen (rem) or sieverts (Sv). The term "radiation dose rate" refers to the radiation dose (dosage) absorbed per unit of time.

[0180] Gray (Gy) is the SI unit of absorbed dose. One gray is equal to an absorbed dose of 1 Joule/kilogram (100 rads). Rad is the special unit of absorbed dose. One rad is equal to an absorbed dose of 100 ergs/gram or 0.01 joule/kilogram (0.01 gray). Rem is the special unit of any of the quantities expressed as dose equivalent. The dose equivalent in rems is equal to the absorbed dose in rads multiplied by the quality factor (1 rem=0.01 sievert). Sievert is the SI unit of any of the quantities expressed as dose equivalent. The dose equivalent in sieverts is equal to the absorbed dose in grays multiplied by the quality factor (1 Sv=100 rems).

[0181] The term "LD_x/y" refers to the average dose of radiation which results in death of x % of subjects by y days. For example, the terms LD₅₀/30 and LD₅₀/60 refer to the average dose of radiation which results in death of 50% of the subjects by 30 or 60 days, respectively.

[0182] Radiation damage to tissue and/or organs depends on the dose of radiation received, or the absorbed dose which is expressed in a unit called the gray (Gy). The potential damage from an absorbed dose depends on the type of radiation and the sensitivity of different tissues and organs.

[0183] The effective dose is used to measure ionizing radiation in terms of the potential for causing harm. The sievert (Sv) is the unit of effective dose that takes into account the type of radiation and sensitivity of tissues and organs. It is a way to measure ionizing radiation in terms of the potential for causing harm. The Sv takes into account the type of radiation and sensitivity of tissues and organs.

[0184] Beyond certain thresholds, radiation can impair the functioning of tissues and/or organs and can produce acute effects such as skin redness, hair loss, radiation burns, acute radiation syndrome or cancer. These effects are more severe at higher doses and higher dose rates, e.g. repeated doses. For instance, the dose threshold for acute radiation syndrome is about 1 Sv (1000 mSv).

[0185] In some embodiments, the exposure is such that, if left untreated, it would generally be lethal to the subject within about 1-2 days (e.g., exposures of greater than 30 Gy ionizing radiation (IR)), 2 days to 2 weeks (e.g., exposures of about 8-30 Gy IR), or about 2-4 weeks (e.g., exposures of about 2-8 Gy IR).

[0186] In any embodiment, the radiation exposure is a dose of radiation expected to cause death to 50 percent of an exposed population within 30 days (LD 50/30); 50 percent of an exposed population within 60 days (LD 50/60); or 70 percent of an exposed population within 30 days (LD 70/30).

[0187] The radiation dose may be about 0.1 Gy (or 10 rads) or greater, about 0.2 Gy (or 20 rads) or greater, about 0.3 Gy (or 30 rads) or greater, about 0.4 Gy (or 40 rads) or greater, about 0.5 Gy (or 50 rads) or greater, about 0.6 Gy (or 60 rads) or greater, about 0.7 Gy (or 70 rads) or greater, about 0.8 Gy (or 80 rads) or greater, about 0.9 Gy (or 90 rads) or greater, about 1 Gy (or 100 rads) or greater, about 2.0 Gy (or 200 rads), about 3.0 Gy (or 300 rads) or about 4.0 Gy (or 400 rads) or greater.

[0188] Alternatively, the radiation dose may be 0.1 Gy (or 10 rads) or greater, 0.2 Gy (or 20 rads) or greater, 0.3 Gy (or 30 rads) or greater, 0.4 Gy (or 40 rads) or greater, 0.5 Gy (or 50 rads) or greater, 0.6 Gy (or 60 rads) or greater, 0.7 Gy (or 70 rads) or greater, 0.8 Gy (or 80 rads) or greater, 0.9 Gy (or 90 rads) or greater, 1 Gy (or 100 rads) or greater, 2.0 Gy (or 200 rads), 3.0 Gy (or 300 rads) or 4.0 Gy (or 400 rads) or greater.

[0189] The radiation dose may be penetrating or non-penetrating. In any embodiment, the radiation dose may be a deep-dose or shallow-dose.

[0190] As used herein, "deep-dose" refers to dose equivalent at a tissue depth of 1 cm (1000 mg/cm²) with external whole-body exposure.

[0191] As used herein, "shallow-dose" refers to the dose equivalent at a tissue depth of 0.007 cm (7 mg/cm²).

[0192] In any embodiment, the radiation exposure has occurred in a high radiation area or a very high radiation area.

[0193] “High radiation area” means an area, accessible to individuals, in which radiation levels from radiation sources external to the body could result in an individual receiving a dose equivalent in excess of 0.1 rem (1 mSv) in 1 hour at 30 centimeters from the radiation source or 30 centimeters from any surface that the radiation penetrates.

[0194] “Very high radiation area” means an area, accessible to individuals, in which radiation levels from radiation sources external to the body could result in an individual receiving an absorbed dose in excess of 500 rads (5 grays) in 1 hour at 1 meter from a radiation source or 1 meter from any surface that the radiation penetrates.

Duration of radiation exposure

[0195] The radiation exposure may be a low radiation dose over a long period of time through continuous or intermittent exposure, as referred to herein as chronic exposure. For example, astronauts are exposed to continuous cosmic radiation while in space. Alternatively, the radiation exposure may be a high or lethal radiation dose, typically higher than therapeutic radiation levels, for a relatively short period of time, also referred to herein as acute exposure. The acute exposure may occur from single or multiple exposure events.

[0196] In any embodiment, the chronic exposure may be for a period of equal to, or greater than about 12 hours; equal to, or greater than about 24 hours; equal to, or greater than about 2 days; equal to, or greater than about 3 days; equal to, or greater than about 4 days; equal to, or greater than about 5 days; equal to, or greater than about 6 days; equal to, or greater than about 7 days equal to, or greater than about 2 weeks; equal to, or greater than about 1 month; equal to, or greater than about 2 months; equal to, or greater than about 3 months; equal to, or greater than about 6 months or equal to, or greater than about 1 year.

[0197] In any embodiment, the chronic exposure may be for a period of equal to, or greater than 12 hours; equal to, or greater than 24 hours; equal to, or greater than 2 days; equal to, or greater than 3 days; equal to, or greater than 4 days; equal to, or greater than 5 days; equal to, or greater than 6 days; equal to, or greater than 7 days

equal to, or greater than 2 weeks; equal to, or greater than 1 month; equal to, or greater than 2 months; equal to, or greater than 3 months; equal to, or greater than 6 months or equal to, or greater than 1 year.

[0198] In any embodiment, the radiation exposure of the individual may be continuous for greater than 12 hours, 24 hours, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 2 weeks, 1 month, 2 months, 3 months or 6 months.

[0199] In any embodiment, the chronic exposure may be intermittent. The intermittent exposure may be received as repeated short term doses, such as may be received from repeated medical diagnostic procedures utilising ionisation radiation (eg CT scans). For example, individuals whom are in hospital for acute stay or undergoing surveillance for cancer recurrence may be subjected to intermittent and repeated radiation exposure for diagnostic purposes. The frequency of the intermittent exposure may be such that the cumulative exposure over a period of time may be lethal, cause organ dysfunction or increase the risk of cancer. The radiation dose for each repeat exposure may be a radiation dose as described herein. Preferably, the radiation dose is about 0.1 Gy, about 0.2 Gy, about 0.3 Gy, about 0.4 Gy, about 0.5 Gy, about 0.6 Gy, about 0.7 Gy, about 0.8 Gy, about 0.9 Gy, about 1 Gy or from about 0.1 to about 1 Gy. Alternatively, the radiation dose is 0.1 Gy, 0.2 Gy, 0.3 Gy, 0.4 Gy, 0.5 Gy, 0.6 Gy, 0.7 Gy, 0.8 Gy, 0.9 Gy, 1 Gy or from 0.1 to 1 Gy. In an exemplary embodiment, the intermittent exposure is from repeated diagnostic chest CT scans. In this embodiment, each repeat chest CT scan may expose the individual to about, or up to, 0.8 Gy radiation.

[0200] Chronic exposure may also indicated by an individual that has received an annual radiation dose of at least about 5 mSv, at least about 10 mSv, at least about 15 mSv, at least about 20 mSv, at least about 25 mSv, at least about 30 mSv, at least about 35 mSv, at least about 40 mSv, at least about 45 mSv or greater than about 50 mSv. The annual radiation dose may be received through continuous or intermittent radiation exposure.

[0201] In any embodiment, the acute exposure may be for a period of equal to, or less than, about 12 hours; equal to, or less than, about 10 hours; equal to, or less than, about 8 hours; equal to, or less than, about 6 hours; equal to, or less than, about 4 hours; equal to, or less than, about 2 hours; or equal to, or less than, about 1 hour. Typically, the radiation exposure is continuous over this time period.

[0202] In any embodiment, the acute exposure may be for a period of equal to, or less than, 12 hours; equal to, or less than, 10 hours; equal to, or less than, 8 hours; equal to, or less than, 6 hours; equal to, or less than, 4 hours; equal to, or less than, 2 hours; or equal to, or less than, 1 hour. Typically, the radiation exposure is continuous over this time period.

Acute radiation syndrome (ARS)

[0203] The present disclosure relates generally to methods of minimising the effects of non-targeted and/or non-therapeutic radiation exposure, such as from accidental nuclear exposure, for example from an explosion or a disaster scenario, or non-therapeutic radiation exposure.

[0204] For example, the use of ionizing radiation or nuclear devices as weapons of terrorism is now recognized as a major public health threat. In the event of a nuclear detonation, terrorist radiological (e.g., “dirty”) bomb, or attack on a nuclear power plant in a populated area, mass casualties will occur that will be in the need of immediate medical attention. At exposures approximating 4 Gy, it is estimated that 50% of individuals will die within 60 days unless there is medical intervention. The majority of deaths that occur from exposures of at least 2-10 Gy or more will result from the combined effects of immune, hematopoietic, and gastrointestinal (GI) failure, as these are the most radiosensitive tissues.

[0205] Acute radiation exposure may result from a nuclear event (such as, a nuclear explosion); a laboratory or manufacturing accident; exposure during handling of highly radioactive sources over minutes or hours; or accidental or intentional high medicinal doses.

[0206] As used herein, the term “acute radiation syndrome” or “ARS” refers to a disease or disorder associated with radiation toxicity or radiation sickness (e.g., acute radiation syndrome (ARS) including hematopoietic (bone marrow) acute radiation syndrome, gastrointestinal acute radiation syndrome, cutaneous acute radiation syndrome, cardiovascular acute radiation syndrome, and/or central nervous system (CNS) acute radiation syndrome). The term “acute radiation syndrome” or “ARS” also may include radiation-induced coagulopathy.

[0207] Acute inflammation may lead to acute radiation syndrome and the acute inflammation may persist as chronic inflammation and lead to more acute radiation syndrome and DEARE. The present invention also extends to preventing or reducing this acute and/or chronic inflammation.

[0208] Clinical characteristics of acute radiation syndrome include hematopoietic syndrome, gastrointestinal syndrome, cardiovascular syndrome and neurovascular syndrome.

[0209] Acute radiation syndrome may present with skin damage (also referred to as skin injury), which may include erythema, altered sensation, itching, edema, blistering, desquamation, ulcer, necrosis, hair loss, onycholysis, and the like. In any embodiment, acute radiation syndrome may include one or more symptoms selected from the group consisting of: neutropenia, infection caused by reduced white blood cells, haemorrhage, diarrhoea, dehydration or electrolyte imbalance and convulsion and/or coma.

[0210] As described above, acute radiation syndrome can damage radiation-sensitive systems, such as immune, hematopoietic, and gastrointestinal systems, and lead to death. In the present invention, the acute radiation syndrome may be any of the symptoms of hematopoietic syndromes, gastrointestinal syndromes, cardiovascular syndromes and neurovascular syndromes, but at the same time the acute radiation syndrome is not limited to the above symptoms.

[0211] The composition from this invention is effective in preventing, treating, or mediating the above symptoms and ultimately improves the survival rate of the subjects exposed to radiation.

[0212] In any embodiment, the acute radiation syndrome includes syndromes affected, induced or caused, by radiation dose of about 0.5 to 100 Gy, about 0.5 to 80 Gy, about 0.5 to 70 Gy, about 0.5 to 60 Gy, about 0.5 to 50 Gy, about 0.7 to 100 Gy, about 0.7 to 80 Gy, about 0.7 to 70 Gy, about 0.7 to 60 Gy, or about 0.7 to 50 Gy.

[0213] In any embodiment, the acute radiation syndrome includes syndromes affected, induced or caused, by radiation dose of 0.5 to 100 Gy, 0.5 to 80 Gy, about 0.5 to 70 Gy, 0.5 to 60 Gy, 0.5 to 50 Gy, 0.7 to 100 Gy, 0.7 to 80 Gy, 0.7 to 70 Gy, 0.7 to 60 Gy, or 0.7 to 50 Gy.

[0214] In any embodiment, the radiation exposure that, if untreated, would cause acute radiation syndrome (ARS) in the individual is about 0.5 Gy, about 0.6 Gy, about 0.7 Gy, about 0.8 Gy, about 0.9 Gy, about 1 Gy, about 2 Gy, about 3 Gy, about 4 Gy, about 5 Gy, about 10 Gy, about 15 Gy, about 20 Gy, about 25 Gy, about 30 Gy, about 35 Gy, about 40 Gy, about 45 Gy or great than about 50 Gy.

[0215] In any embodiment, the radiation exposure that, if untreated, would cause acute radiation syndrome (ARS) in the individual is 0.5 to 100 Gy, 0.5 to 80 Gy, about 0.5 to 70 Gy, 0.5 to 60 Gy, 0.5 to 50 Gy, 0.7 to 100 Gy, 0.7 to 80 Gy, 0.7 to 70 Gy, 0.7 to 60 Gy, or 0.7 to 50 Gy.

[0216] In any embodiment, the radiation exposure that, if untreated, would cause acute radiation syndrome (ARS) in the individual is at least about 0.5 Gy, at least about 0.6 Gy, at least about 0.7 Gy, at least about 0.8 Gy, at least about 0.9 Gy, at least about 1 Gy, at least about 2 Gy, at least about 3 Gy, at least about 4 Gy, at least about 5 Gy, at least about 10 Gy, at least about 15 Gy, at least about 20 Gy, at least about 25 Gy, at least about 30 Gy, at least about 35 Gy, at least about 40 Gy, at least about 45 Gy or at least about 50 Gy.

[0217] In any embodiment, the radiation exposure that, if untreated, would cause acute radiation syndrome (ARS) in the individual is at least 0.5 Gy, at least 0.6 Gy, at least 0.7 Gy, at least 0.8 Gy, at least 0.9 Gy, at least 1 Gy, at least 2 Gy, at least 3 Gy, at least 4 Gy, at least 5 Gy, at least 10 Gy, at least 15 Gy, at least 20 Gy, at least 25 Gy, at least 30 Gy, at least 35 Gy, at least 40 Gy, at least 45 Gy or at least 50 Gy.

[0218] Acute radiation syndrome usually progresses with four clinical stages: prodromal phase, latent phase, manifest phase, and recovery or death. Depending on the amount of radiation absorbed, the symptoms may appear within hours to weeks. The prodromal phase usually starts within 48 hours after radiation exposure, but may last up to 6 days after the exposure and symptoms may include nausea, vomiting, fatigue, autonomic nerve anxiety and loss of consciousness. The latent phase may last from several days to several weeks depending on the amount of radiation exposure, and clinical symptoms may not appear partially or completely. However, at this stage, symptoms such as lymphocytopenia, granulocytopenia, and myelogenous deficiency may occur. Symptoms from the manifest phase may occur with several weeks delay. Symptoms from the manifest phase may include hematopoietic syndrome,

gastrointestinal syndrome, cardiovascular/central nervous system syndrome neurovascular syndrome depending on the amount of radiation exposure. Patients exposed to extreme amounts of radiation can experience all four of these steps within a few hours and die within a short period of time.

[0219] The cerebrovascular/central nervous system syndrome, the dominant manifestation of extremely high whole-body doses of radiation (> 30 Gy), is always fatal unless treated. The prodrome develops within minutes to 1 hour after exposure. There is little or no latent phase. Patients develop tremors, seizures, ataxia, and cerebral edema and die within hours to 1 or 2 days.

[0220] Accordingly, cerebrovascular/central nervous system ARS may be caused by radiation exposure at a dose of at least about 3 Gy, at least about 4 Gy, at least about 5 Gy, at least about 6 Gy, at least about 7 Gy, at least about 8 Gy, at least about 9 Gy, at least about 10 Gy, at least about 11 Gy, at least about 12 Gy, at least about 13 Gy, at least about 14 Gy, at least about 15 Gy, at least about 20 Gy, at least about 25 Gy, at least about 30 Gy, at least about 35 Gy, at least about 40 Gy, at least about 45 Gy or at least about 50 Gy. In any embodiment, the cerebrovascular/central nervous system ARS may be caused by radiation exposure at a dose of about 5 Gy to about 100 Gy, about 5 Gy to about 80 Gy, about 5 Gy to about 60 Gy, about 5 Gy to about 50 Gy, about 5 Gy to about 40 Gy, or about 5 Gy to about 30 Gy.

[0221] The cerebrovascular/central nervous system ARS may be caused by radiation exposure at a dose of at least 3 Gy, at least 4 Gy, at least 5 Gy, at least 6 Gy, at least 7 Gy, at least 8 Gy, at least 9 Gy, at least 10 Gy, at least 11 Gy, at least 12 Gy, at least 13 Gy, at least 14 Gy, at least 15 Gy, at least 20 Gy, at least 25 Gy, at least 30 Gy, at least 35 Gy, at least 40 Gy, at least 45 Gy or at least 50 Gy. In any embodiment, the cerebrovascular/central nervous system ARS may be caused by radiation exposure at a dose of 5 Gy to 100 Gy, 5 Gy to 80 Gy, 5 Gy to 60 Gy, 5 Gy to 50 Gy, 5 Gy to 40 Gy, or 5 Gy to 30 Gy.

[0222] The GI syndrome is the dominant manifestation after whole-body doses of about 6 to 30 Gy. Prodromal symptoms, often marked, develop within about 1 hour and resolve within 2 days. During the latent period of 4 to 5 days, GI mucosal cells die. Cell death is followed by intractable nausea, vomiting, and diarrhea, which lead to severe dehydration and electrolyte imbalances, diminished plasma volume, and vascular

collapse. Necrosis of intestine may also occur, predisposing to intestinal perforation, bacteremia, and sepsis. Patients receiving > 10 Gy may have cerebrovascular symptoms (suggesting a lethal dose). Survivors also have the hematopoietic syndrome.

[0223] Accordingly, GI ARS may be caused by radiation exposure at a dose of at least about 2 Gy, at least about 3 Gy, at least about 4 Gy, at least about 5 Gy, at least about 6 Gy, at least about 7 Gy, at least about 8 Gy, at least about 9 Gy, at least about 10 Gy, at least about 11 Gy, at least about 12 Gy, at least about 13 Gy, at least about 14 Gy, at least about 15 Gy, at least about 20 Gy, at least about 25 Gy, or at least about 30 Gy. In any embodiment, the GI ARS may be caused by radiation exposure at a dose of about 2 Gy to about 30 Gy, about 2 Gy to about 25 Gy, about 2 Gy to about 20 Gy, about 5 Gy to about 20 Gy, about 5 Gy to about 15 Gy, or about 5 Gy to about 10 Gy.

[0224] The GI ARS may be caused by radiation exposure at a dose of at least about 2 Gy, at least 3 Gy, at least 4 Gy, at least 5 Gy, at least 6 Gy, at least 7 Gy, at least 8 Gy, at least 9 Gy, at least 10 Gy, at least 11 Gy, at least 12 Gy, at least 13 Gy, at least 14 Gy, at least 15 Gy, at least 20 Gy, at least 25 Gy, or at least 30 Gy. In any embodiment, the GI ARS may be caused by radiation exposure at a dose of 2 Gy to 30 Gy, 2 Gy to 25 Gy, 2 Gy to 20 Gy, 5 Gy to 20 Gy, 5 Gy to 15 Gy, or 5 Gy to 10 Gy.

[0225] The hematopoietic syndrome is the dominant manifestation after whole-body doses of about 1 to 6 Gy and consists of a generalized pancytopenia. A mild prodrome may begin after 1 to 6 hours, lasting 24 to 48 hours. Bone marrow stem cells are significantly depleted, but mature blood cells in circulation are largely unaffected. Circulating lymphocytes are an exception, and lymphopenia may be evident within hours to days after exposure. As the cells in circulation die by senescence, they are not replaced in sufficient numbers, resulting in pancytopenia. Thus, patients remain asymptomatic during a latent period of up to 4.5 weeks after a 1-Gy dose as the impediment of hematopoiesis progresses. Risk of various infections is increased as a result of the neutropenia (most prominent at 2 to 4 weeks) and decreased antibody production. Petechiae and mucosal bleeding result from thrombocytopenia, which develops within 3 to 4 weeks and may persist for months. Anemia develops slowly, because preexisting red blood cells have a longer life span than white blood cells and platelets. Survivors have an increased incidence of radiation-induced cancer, including leukemia.

[0226] Accordingly, hematopoietic ARS may be caused by radiation exposure at a dose of at least about 0.5 Gy, at least about 0.6 Gy, at least about 0.7 Gy, at least about 0.8 Gy, at least about 0.9 Gy, at least about 1 Gy, at least about 1.5 Gy, at least about 2 Gy, at least about 2.5 Gy, at least about 3 Gy, at least about 3.5 Gy, at least about 4 Gy, at least about 4.5 Gy, at least about 5 Gy, at least about 6 Gy, at least about 7 Gy, at least about 8, at least about 9, or at least about 10 Gy. In any embodiment, the hematopoietic ARS may be caused by radiation exposure at a dose of about 0.5 Gy to about 10 Gy, about 0.5 Gy to about 8 Gy, about 0.5 Gy to about 6 Gy, about 0.5 Gy to about 4 Gy, about 0.5 Gy to about 2 Gy, 0.7 Gy to about 10 Gy, about 0.7 Gy to about 8 Gy, about 0.7 Gy to about 6 Gy, about 0.7 Gy to about 4 Gy, or about 0.7 Gy to about 2 Gy.

[0227] The GI ARS may be caused by radiation exposure at a dose of at least 0.5 Gy, at least 0.6 Gy, at least 0.7 Gy, at least 0.8 Gy, at least 0.9 Gy, at least 1 Gy, at least 1.5 Gy, at least 2 Gy, at least 2.5 Gy, at least 3 Gy, at least 3.5 Gy, at least 4 Gy, at least 4.5 Gy, at least 5 Gy, at least 6 Gy, at least 7 Gy, at least 8, at least 9, or at least 10 Gy. In any embodiment, the hematopoietic ARS may be caused by radiation exposure at a dose of 0.5 Gy to 10 Gy, 0.5 Gy to 8 Gy, 0.5 Gy to 6 Gy, 0.5 Gy to 4 Gy, 0.5 Gy to 2 Gy, 0.7 Gy to 10 Gy, 0.7 Gy to 8 Gy, 0.7 Gy to 6 Gy, 0.7 Gy to 4 Gy, or 0.7 Gy to 2 Gy.

Delayed effects of acute radiation exposure (DEARE)

[0228] Survivors of ARS may be at risk for developing the delayed effects of acute radiation exposure (DEARE), a spectrum of chronic illnesses involving multiple organ systems and occurring months to years after radiation exposure. In general, cells that are undifferentiated and those that have high mitotic rates (eg, stem cells, cancer cells) are particularly vulnerable to radiation. Because radiation preferentially depletes rapidly dividing stem cells over the more resistant mature cells, there is typically a latent period between radiation exposure and overt radiation injury. Injury does not manifest until a significant fraction of the mature cells die of natural senescence and, due to loss of stem cells, are not replaced. Without being bound by theory, DEARE is believed to result from some combination of oxidative stress, inflammation, senescence, fibrosis, and loss of stem cell self-renewal potential in the ARS survivors.

[0229] Symptoms of DEARE include, but are not limited to, symptomatic pneumonitis, radiation-induced pneumonitis, pulmonary fibrosis, neurocognitive deficits, cancer such as hematopoietic malignancies, radiation cystitis, mucocitis, proctitis, cataracts, and myelodysplastic syndromes.

[0230] DEARE on the hematopoietic system are known as residual bone marrow damage (RBMD), a latent condition characterized by prolonged inhibition of hematopoiesis leading to immunosuppression and increased infection risk.

[0231] In any embodiment, an individual may develop one or more symptoms of DEARE at least about 1 month, at least about 2 months, at least about 3 months, at least about 4 months, at least about 5 months, at least about 6 months, at least about 1 year, at least about 2 years, at least about 3 years, at least about 4 years, at least about 5 years, at least about 6 years, at least about 7 years, at least about 8 years, at least about 9 days and more than 10 years after radiation exposure.

[0232] In any embodiment, an individual may develop one or more symptoms of DEARE at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 1 year, at least 2 years, at least 3 years, at least 4 years, at least 5 years, at least 6 years, at least 7 years, at least 8 years, at least 9 days and more than 10 years after radiation exposure.

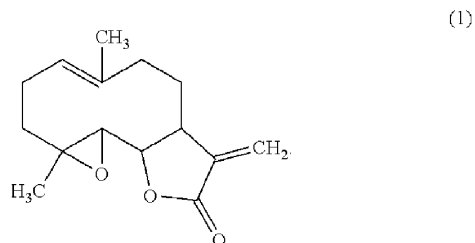
Sesquiterpene lactones

[0233] Sesquiterpene lactones are a group of secondary plant metabolites consisting of a 15-carbon structure, preferably containing an α -methylene- γ -butyrolactone moiety. Sesquiterpene-lactone containing extracts may be obtained by mixing dried, pulverized *Tanacetum parthenium* (also known as feverfew leaves) with the non-polar solvent and leaving the mixture to stand to allow the extraction to take place. Alternatively, the dry powder may be exhaustively extracted with the non-polar organic solvent, for example in a Soxhlet apparatus.

[0234] Classes of sesquiterpene lactones include germacranolides and guaianolides, including pseudoguaianolides, in particular those sesquiterpene lactones having an α -methylene substituent in the lactone ring. An extract from feverfew leaves may contain a plurality of sesquiterpene lactones as described herein.

[0235] Parthenolide is a Germacrane sesquiterpene lactone with a unique structure. The active component is parthenolide which is a lipophilic, neutral lactone. Parthenolide is typically extracted from feverfew. By “extracts of feverfew,” it is meant extracts of the plant “*Tanacetum parthenium*”. Parthenolide can also be synthesized using various chemical methods, including as described in Long et al. *J. Med. Chem.* 2014, 57, 16, 7098–7112.

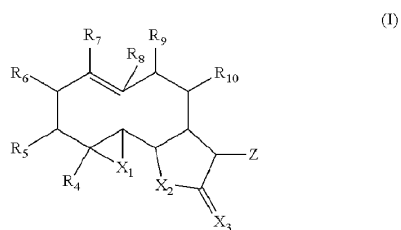
[0236] The chemical formula (1) of parthenolide is given below:



[0237] Parthenolide comprises chiral centres and thus its racemates, enantiomers or stereoisomers are possible and also usable.

[0238] The parthenolide (1) molecule comprises an α -methylene- γ -butyrolactone moiety that can be structurally modified to synthesise parthenolide based derivatives or analogs, for example a compound of formula (I) below.

[0239] In any embodiment, the sesquiterpene lactone is of formula (I):



wherein:

X₁, X₂ and X₃ are heteroatoms;

R₄, R₅, R₆, R₇, R₈, R₉ and R₁₀ are independently selected from H, halo, —OH, —NO₂, —CN and optionally substituted aliphatic, cycloalkyl, heterocycloalkyl, aryl or heteroaryl;
and

Z is optionally substituted C₁₋₈ straight-chained or branched aliphatic, optionally containing 1 or more double or triple bonds, wherein one or more carbons are optionally replaced by R* wherein R* is optionally substituted cycloalkyl, heterocycloalkyl, aryl or heteroaryl; an amino acid residue, H, —CN, —C(O)—, —C(O)C(O)—, —C(O)NR¹—, —C(O)NR¹NR²—, —C(O)O—, —OC(O)—, —NR¹CO₂—, —O—, —NR¹C(O)NR²—, —OC(O)NR¹—, —NR¹C(O)—, —S—, —SO—, —SO₂—, —NR¹—, —SO₂NR¹—, —NR¹R², or —NR¹SO₂—, wherein R¹ and R² are independently selected from H and optionally substituted aliphatic, cycloalkyl, heterocycloalkyl, aryl or heteroaryl; or where R* is NR¹R², R¹ and R² optionally together with the nitrogen atom form an optionally substituted 5-12 membered ring, said ring optionally comprising 1 or more heteroatoms or a group selected from —CO—, —SO—, —SO₂— and —PO—; or

a pharmaceutically acceptable salt, solvate, tautomer, N-oxide, stereoisomer and/or prodrug thereof.

[0240] The phrase “optionally substituted” is used interchangeably with the phrase “substituted or unsubstituted.” Unless otherwise indicated, an optionally substituted group may have a substituent at each substitutable position of the group, and each substitution is independent of any other. Also, combinations of substituents or variables are permissible only if such combinations result in stable compounds. In addition, unless otherwise indicated, functional group radicals are independently selected. Where “optionally substituted” modifies a series of groups separated by commas (e.g., “optionally substituted A, B or C”; or “A, B or C optionally substituted with”), it is intended that each of the groups (e.g., A, B and C) is optionally substituted.

[0241] The term “aliphatic” or “aliphatic group” as used herein means a straight-chain or branched C₁₋₁₂ hydrocarbon chain that is completely saturated or that contains one or more units of unsaturation, or a monocyclic C₃₋₈ hydrocarbon or bicyclic C₈₋₁₂ hydrocarbon that is completely saturated or that contains one or more units of unsaturation, but which is not aromatic (also referred to herein as “carbocycle” or “cycloalkyl”), that has a single point of attachment to the rest of the molecule wherein any individual ring in said bicyclic ring system has 3-7 members. For example, suitable aliphatic groups include, but are not limited to, linear or branched or alkyl, alkenyl, alkynyl groups and hybrids thereof such as (cycloalkyl)alkyl, (cycloalkenyl)alkyl or (cycloalkyl)alkenyl.

[02442] The terms “alkyl,” “alkoxy,” “hydroxyalkyl,” “alkoxyalkyl” and “alkoxycarbonyl,” used alone or as part of a larger moiety include both straight and branched chains containing one to twelve carbon atoms. The terms “alkenyl” and “alkynyl” used alone or as part of a larger moiety shall include both straight and branched chains containing two to twelve carbon atoms.

[02443] The terms “haloalkyl,” “haloalkenyl” and “haloalkoxy” means alkyl, alkenyl or alkoxy, as the case may be, substituted with one or more halogen atoms. The term “halogen” or “halo” means F, Cl, Br or I.

[02444] The term “heteroatom” means nitrogen, oxygen, or sulfur and includes any oxidized form of nitrogen and sulfur, and the quaternized form of any basic nitrogen. Heteroatom further includes Se, Si and P.

[02445] The term “aryl” used alone or in combination with other terms, refers to monocyclic, bicyclic or tricyclic carbocyclic ring systems having a total of five to fourteen ring members, wherein at least one ring in the system is aromatic and wherein each ring in the system contains 3 to 8 ring members. The term “aryl” may be used interchangeably with the term “aryl ring”. The term “aralkyl” refers to an alkyl group substituted by an aryl. The term “aralkoxy” refers to an alkoxy group substituted by an aryl.

[02446] The term “heterocycloalkyl,” “heterocycle,” “heterocyclyl” or “heterocyclic” as used herein means monocyclic, bicyclic or tricyclic ring systems having five to fourteen ring members in which one or more ring members is a heteroatom, wherein each ring in the system contains 3 to 7 ring members and is non-aromatic.

[02447] The term “heteroaryl,” used alone or in combination with other terms, refers to monocyclic, bicyclic and tricyclic ring systems having a total of five to fourteen ring members, and wherein: 1) at least one ring in the system is aromatic; 2) at least one ring in the system contains one or more heteroatoms; and 3) each ring in the system contains 3 to 7 ring members. The term “heteroaryl” may be used interchangeably with the term “heteroaryl ring” or the term “heteroaromatic”. Examples of heteroaryl rings include 2-furanyl, 3-furanyl, N-imidazolyl, 2-imidazolyl, 4-imidazolyl, 5-imidazolyl, 3-isoxazolyl, 4-isoxazolyl, 5-isoxazolyl, 2-oxadiazolyl, 5-oxadiazolyl, 2-oxazolyl, 4-oxazolyl, 5-oxazolyl, 1-pyrrolyl, 2-pyrrolyl, 3-pyrrolyl, 1-pyrazolyl, 3-pyrazolyl, 4-

pyrazolyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, 2-pyrimidyl, 4-pyrimidyl, 5-pyrimidyl, 3-pyridazinyl, 2-thiazolyl, 4-thiazolyl, 5-thiazolyl, 5-tetrazolyl, 2-triazolyl, 5-triazolyl, 2-thienyl, 3-thienyl, carbazolyl, benzimidazolyl, benzothienyl, benzofuranyl, indolyl, quinolinyl, benzotriazolyl, benzothiazolyl, benzooxazolyl, benzimidazolyl, isoquinolinyl, indazolyl, isoindolyl, acridinyl, and benzoisoxazolyl. The term "heteroaralkyl" refers to an alkyl group substituted by a heteroaryl. The term "heteroarylalkoxy" refers to an alkoxy group substituted by a heteroaryl.

[0248] An aryl (including aralkyl, aralkoxy, aryloxyalkyl and the like) or heteroaryl (including heteroaralkyl, heteroarylalkoxy and the like) group may contain one or more substituents. Suitable substituents on an unsaturated carbon atom of an aryl, heteroaryl, aralkyl or heteroaralkyl group are selected from halogen; haloalkyl; $-\text{CF}_3$; $-\text{R}$; $-\text{OR}$; $-\text{SR}$; 1,2-methylenedioxy; 1,2-ethylenedioxy; protected OH (such as acyloxy); phenyl (Ph); Ph substituted with R; $-\text{O}(\text{Ph})$; $-\text{O}(\text{Ph})$ substituted with R; $-\text{CH}_2(\text{Ph})$; $-\text{CH}_2(\text{Ph})$ substituted with R; $-\text{CH}_2\text{CH}_2(\text{Ph})$; $-\text{CH}_2\text{CH}_2(\text{Ph})$ substituted with R; $-\text{NO}_2$; $-\text{CN}$; $-\text{N}(\text{R})_2$; $-\text{NRC}(\text{O})\text{R}$; $-\text{NRC}(\text{O})\text{N}(\text{R})_2$; $-\text{NRCO}_2\text{R}$; $-\text{NRNRC}(\text{O})\text{R}$; $-\text{NR}-\text{NRC}(\text{O})\text{N}(\text{R})_2$; $-\text{NRNRCO}_2\text{R}$; $-\text{C}(\text{O})\text{C}(\text{O})\text{R}$; $-\text{C}(\text{O})\text{CH}_2\text{C}(\text{O})\text{R}$; $-\text{CO}_2\text{R}$; $-\text{C}(\text{O})\text{R}$; $-\text{C}(\text{O})\text{N}(\text{R})_2$; $-\text{OC}(\text{O})\text{N}(\text{R})_2$; $-\text{S}(\text{O})_2\text{R}$; $-\text{SO}_2\text{N}(\text{R})_2$; $-\text{S}(\text{O})\text{R}$; $-\text{NRSO}_2\text{N}(\text{R})_2$; $-\text{NRSO}_2\text{R}$; $-\text{C}(=\text{S})\text{N}(\text{R})_2$; $-\text{C}(=\text{NH})-\text{N}(\text{R})_2$; $-(\text{CH}_2)_3$, $\text{NHC}(\text{O})\text{R}$; $-(\text{CH}_2)_y\text{R}$; $-(\text{CH}_2)_y\text{NHC}(\text{O})\text{NHR}$; $-(\text{CH}_2)_y\text{NHC}(\text{O})\text{OR}$; $-(\text{CH}_2)_y\text{NHS}(\text{O})\text{R}$; $-(\text{CH}_2)_y\text{NHSO}_2\text{R}$; or $-(\text{CH}_2)_y\text{NHC}(\text{O})\text{CH}((\text{V})_z-\text{R})(\text{R})$ wherein each R is independently selected from hydrogen, optionally substituted C1-6 aliphatic, an unsubstituted 5-6 membered heteroaryl or heterocyclic ring, phenyl (Ph), $-\text{O}(\text{Ph})$, or $-\text{CH}_2(\text{Ph})-\text{CH}_2(\text{Ph})$, wherein y is 0-6; z is 0-1; and V is a linker group. When R is C1-6 aliphatic, it may be substituted with one or more substituents selected from $-\text{NH}_2$, $-\text{NH}(\text{C1-4 aliphatic})$, $-\text{N}(\text{C1-4 aliphatic})_2$, $-\text{S}(\text{O})(\text{C1-4 aliphatic})$, $-\text{SO}_2(\text{C1-4 aliphatic})$, halogen, (C1-4 aliphatic), $-\text{OH}$, $-\text{O}(\text{C1-4 aliphatic})$, $-\text{NO}_2$, $-\text{CN}$, $-\text{CO}_2\text{H}$, $-\text{CO}_2(\text{C1-4 aliphatic})$, $-\text{O}(\text{halo C1-4 aliphatic})$, or $-\text{halo}(\text{C1-4 aliphatic})$; wherein each C1-4 aliphatic is optionally.

[0249] An aliphatic group or a non-aromatic heterocyclic ring may contain one or more substituents. Suitable substituents on a saturated carbon of an aliphatic group or of a non-aromatic heterocyclic ring are selected from those listed above for the unsaturated carbon of an aryl or heteroaryl group and the following: $=\text{O}$, $=\text{S}$, $=\text{NNHR}$, $=\text{NN}(\text{R})_2$, $=\text{N}-$, $=\text{NNHC}(\text{O})\text{R}$, $=\text{NNHCO}_2(\text{alkyl})$, $=\text{NNHSO}_2(\text{alkyl})$, or $=\text{NR}$, where each R is independently selected from hydrogen or an optionally substituted C1-6 aliphatic.

When R is C1-6 aliphatic, it may be substituted with one or more substituents selected from —NH_2 , $\text{—NH(C1-4 aliphatic)}$, $\text{—N(C1-4 aliphatic)}_2$, halogen, —OH , $\text{—O—(C1-4 aliphatic)}$, —NO_2 , —CN , $\text{—CO}_2\text{H}$, $\text{—CO}_2\text{(C1-4 aliphatic)}$, $\text{—O(halo C1-4 aliphatic)}$, or $\text{—halo(C1-4 aliphatic)}$; wherein each C1-4 aliphatic is optionally substituted.

[0250] Substituents on a nitrogen of a non-aromatic heterocyclic ring are selected from —R , —N(R)_2 , —C(O)R , —C(O)OR , —C(O)C(O)R , $\text{—C(O)CH}_2\text{C(O)R}$, $\text{—SO}_2\text{R}$, $\text{—SO}_2\text{N(R)}_2$, —C(=S)N(R)_2 , —C(=NH)—N(R)_2 or $\text{—NRSO}_2\text{R}$; wherein each R is independently selected from hydrogen, an optionally substituted C1-6 aliphatic, optionally substituted phenyl (Ph), optionally substituted —O(Ph) , optionally substituted $\text{—CH}_2\text{(Ph)}$, optionally substituted $\text{—CH}_2\text{CH}_2\text{(Ph)}$, or an unsubstituted 5-6 membered heteroaryl or heterocyclic ring. When R is a C1-6 aliphatic group or a phenyl ring, it may be substituted with one or more substituents selected from —NH_2 , $\text{—NH(C1-4 aliphatic)}$, $\text{—N(C1-4 aliphatic)}_2$, halogen, —(C1-4 aliphatic) , —OH , $\text{—O—(C1-4 aliphatic)}$, —NO_2 , —CN , $\text{—CO}_2\text{H}$, $\text{—CO}_2\text{(C1-4 aliphatic)}$, $\text{—O(halo C1-4 aliphatic)}$ or $\text{—halo(C1-4 aliphatic)}$; wherein each C1-4 aliphatic is optionally substituted.

[0251] The term “linker group” or “linker” means an organic moiety that connects two parts of a compound. Linkers include alkylidene chain that is a saturated or unsaturated, straight or branched, C1-8 carbon chain which is optionally substituted, and wherein up to two non-adjacent saturated carbons of the chain are optionally replaced by R^* wherein R^* is —C(O)— , —C(O)C(O)— , —C(O)NR— , —C(O)NRNR— , —C(O)O— , —OC(O)— , $\text{—NRCO}_2\text{—}$, —O— , —NRC(O)NR— , —OC(O)NR— , —NRNR— , —NRC(O)— , —S— , —SO— , $\text{—SO}_2\text{—}$, —NR— , $\text{—SO}_2\text{NR—}$, or $\text{—NRSO}_2\text{—}$; wherein R is selected from hydrogen or optionally substituted aliphatic, cycloalkyl, heterocycloalkyl, aryl or heteroaryl, and preferably H or optionally substituted C1-4 aliphatic. Optional substituents on the alkylidene chain are as described above for an aliphatic group. Alternatively, the linker group is R^* .

[0252] The term “pharmaceutically acceptable salts” includes, but is not limited to, salts well known to those skilled in the art, for example, mono-salts (e.g. alkali metal and ammonium salts) and poly salts (e.g. di- or tri-salts,) of the compounds of the invention. Pharmaceutically acceptable salts of compounds of formulas (I), (II), (III), or (IV) are where, for example, an exchangeable group, such as hydrogen in —OH , —NH— , or —P(=O)(OH)— , is replaced with a pharmaceutically acceptable cation (e.g. a sodium, potassium, or ammonium ion) and can be conveniently be prepared from a

corresponding compound of formula (I) by, for example, reaction with a suitable base. In cases where compounds are sufficiently basic or acidic to form stable nontoxic acid or base salts, administration of the compounds as salts may be appropriate. Examples of pharmaceutically acceptable salts are organic acid addition salts formed with acids that form a physiological acceptable anion, for example, tosylate, methanesulfonate, acetate, citrate, malonate, tartarate, succinate, benzoate, ascorbate, α -ketoglutarate, and α -glycerophosphate. Suitable inorganic salts may also be formed, including hydrochloride, sulfate, nitrate, bicarbonate, and carbonate salts.

[0253] Pharmaceutically acceptable salts include quaternary ammonium salts formed with R'Y; where Y is selected from halogen, tosylate, methanesulfonate, benzenesulfonate, trifluoromethanesulfonate and the like; and R' is selected from an optionally substituted cycloalkyl, heterocycloalkyl, aryl or heteroaryl.

[0254] Suitable acids include hydrofluoric acid, hydrochloric acid, hydrobromic acid, hydriodic acid, sulfuric acid, nitric acid, phosphoric acid, carbonic acid, boric acid, selenious acid, hydrogen sulfide, phosphomolybdic acid, phosphorous acid, sulfurous acid, citric acid, maleic acid, D-malic acid, L-lactic acid, D-lactic acid, DL-lactic acid, oxalic acid, methanesulfonic acid, valeric acid, oleic acid, lauric acid, para-toluenesulfonic acid, 1-naphthalensulfonic acid, 2-naphthalensulfonic acid, phthalic acid, tartaric acid, L-malic acid, DL-malic acid, malonic acid, succinic acid, fumaric acid, glycolic acid, thioglycolic acid, glycine, sarcosine, sulfonic acid, nicotinic acid, picolinic acid, isonicotinic acid, benzoic acid and substituted benzoic acid where benzene ring bears one or more substituents.

[0255] Pharmaceutically acceptable salts may be obtained using standard procedures well known in the art, for example, by reacting a sufficiently basic compound such as an amine with a suitable acid affording a physiologically acceptable anion. Alkali metal (for example, sodium, potassium or lithium) or alkaline earth metal (for example, calcium) salts of carboxylic acids can also be made.

[0256] The term "prodrug" or "prodrugs" is used in its ordinary meaning in the art and means a compound of the invention that has its charged moieties masked or protected by another moiety that is designed to be cleaved under particular physiological conditions, leaving the deprotected or unmasked compound of the invention. The use of masking agents is common and well-known in the art and, in particular, masking

phosphate or phosphonate groups. All such masking agents are suitable and can be used with the compounds of the invention. Various agents such as acyloxy alkyl esters are described by Srivasta et al., (1984 Bioorganic Chemistry 12, 118-12), and by Freeman et al. (1997 Progress in Medicinal Chemistry 34:112-147) which are each incorporated in their entirety herein by reference; and 3-phthalidyl phosphonate esters are described by Dang Q., et al., (1999 Bioorganic & Med. Chem Letters, 9:1505-1510), which is incorporated in its entirety herein by reference. For example, and not by way of limitation, Srivasta et al. also describe acetoxymethyl, isobutyloxymethyl, and pivaloxymethyl as masking agents. Other suitable masking groups comprising pivaloxyalkyl, e.g., pivaloxymethyl, or a pivaloyloxy group as described by Farquhar D. et al., (1995 J. Med. Chem., 38:488-495) which is incorporated in its entirety herein by reference. Still other masking or protecting agents are described in U.S. Pat. Nos. 4,816,570 and 4,968,788 both of which are incorporated in their entirety herein by reference. Lipid prodrugs are also suitable for use with the compounds of the invention. By non-limiting example, certain lipid prodrugs are described in Hostetler et al., (1997 Biochem. Pharm. 53:1815-1822), and Hostetler et al., 1996 Antiviral Research 31:59-67), both of which are incorporated in their entirety herein by reference. Additional examples of suitable prodrug technology is described in WO 90/00555; WO 96/39831; WO 03/095665A2; U.S. Pat. Nos. 5,411,947; 5,463,092; 6,312,662; 6,716,825; and U.S. Published Patent Application Nos. 2003/0229225 and 2003/0225277 each of which is incorporated in their entirety herein by reference. Such prodrugs may also possess the ability to target the drug compound to a particular tissue within the patient, e.g., liver, as described by Erion et al., (2004 J. Am. Chem. Soc. 126:5154-5163; Erion et al., Am. Soc. Pharm. & Exper. Ther. DOI: 10.1124/jep.104.75903 (2004); WO 01/18013 A1; U.S. Pat. No. 6,752,981), each of which is incorporated in their entirety herein by reference. By way of non-limiting example, other prodrugs suitable for use with the compounds of the invention are described in WO 03/090690; and by Harris et al., (2002 Antiviral Chem & Chemo. 12: 293-300; Knaggs et al., 2000 Bioorganic & Med. Chem Letters 10: 2075-2078) each of which is incorporated in their entirety herein by reference.

[0257] Presently preferred compounds include compounds of formula (I) wherein R₅, R₆, R₇, R₉ and R₁₀ are independently selected from H, halo, —OH, —NO₂, —CN, —CH₃, —CF₃, —CH₂CH₃, —CH₂CF₃, —CH₂Cl, —CH₂OH, —CH₂CH₂OH and —CH₂NH₂.

Further preferred embodiments include compounds where R_5 , R_6 , R_7 , R_9 and R_{10} are each H.

[0258] Other preferred embodiments of the present invention include compounds where R_4 and R_8 are independently selected from optionally substituted C1-C4 alkyl. In one preferred embodiment, R_4 is $-\text{CH}_3$, and in another, R_4 and R_8 are each $-\text{CH}_3$.

[0259] In one embodiment X_1 , X_2 and X_3 are heteroatoms independently selected from O, N and S, and in one particular embodiment X_1 , X_2 and X_3 are each O.

[0260] According to one embodiment, it is preferred that where R_5 , R_6 , R_7 , R_9 and R_{10} are H, R_4 and R_8 are each $-\text{CH}_3$, and X_1 , X_2 and X_3 are each O; Z is not $=\text{CH}_2$.

[0261] According to a further embodiment of the invention, Z is $-(\text{CH}_2)_m-\text{NR}^1\text{R}^2$ where m is an integer from 0 to 4, where preferably, R_5 , R_6 , R_7 , R_9 , and R_{10} are H; and R_4 and R_8 are each $-\text{CH}_3$. In one particular embodiment, m is 1. In other embodiments, R_1 and R_2 are independently selected from hydrogen, $-\text{CN}$ or optionally substituted C1-C4 alkyl. In particular embodiments, R_1 and R_2 are independently selected from $-\text{NO}_2$, $-\text{CN}$, $-\text{CH}_3$, $-\text{CF}_3$, $-\text{CH}_2\text{CH}_3$, $-\text{CH}_2\text{CF}_3$, $-\text{CH}_2\text{Cl}$, $-\text{CH}_2\text{OH}$, $-\text{CH}_2\text{CH}_2\text{OH}$ and $-\text{CH}_2\text{NH}_2$.

[0262] In yet a further embodiment, R_1 and R_2 together with N form an optionally substituted ring. The ring is a monocyclic, bicyclic or tricyclic aliphatic or aryl ring system, where the ring system is optionally substituted and optionally comprises one or more heteroatoms or a group selected from $-\text{CO}-$, $-\text{SO}-$, $-\text{SO}_2-$ and $-\text{PO}-$. In one particular embodiment, R_1 and R_2 are $-\text{CH}_2(\text{CH}_2)_n\text{CH}_2\text{Y}-$, where Y is a heteroatom or a group selected from $-\text{CO}-$, $-\text{SO}-$, $-\text{SO}_2-$ and $-\text{PO}-$; n is an integer 0 to 5; and together with N form an optionally substituted ring, which may be additionally fused to a cycloalkyl or aryl group to form a bicyclic or tricyclic ring system, where the system is optionally substituted and optionally comprises one or more heteroatoms. Alternatively, R_1 and R_2 are $-(\text{CH}_2)_a-\text{Y}-(\text{CH}_2)_b-$, where Y is a heteroatom or a group selected from $-\text{CO}-$, $-\text{SO}-$, $-\text{SO}_2-$ and $-\text{PO}-$; a is an integer 0 to 5; b is an integer 0 to 5; where the sum of a and b is 0 to 5; and together with N form an optionally substituted ring, the ring being optionally fused to a cycloalkyl or aryl group to form a bicyclic or tricyclic ring system, where the system is optionally substituted and optionally comprises one or more heteroatoms.

[0263] Examples of ring systems include an optionally substituted uracil ring or a derivative thereof. Other examples include optionally substituted pyrrole, imidazole, purine and pyrazole and derivative thereof. Examples of fused ring systems include optionally substituted aziridin-1-yl, azetidin-1-yl, pyrrolidin-1-yl, piperidin-1-yl, homopiperidin-1-yl and heptamethyleneimin-1-yl.

[0264] According to a further embodiment of the invention, Z is $-(CH_2)_m-S(O)_n-R^1$ where m is an integer from 0 to 4, n is an integer from 0 to 2, where preferably, R₅, R₆, R₇, R₉, and R₁₀ are H; and R₄ and R₈ are each $-CH_3$. In one particular embodiment, m is 1. In another embodiment n is 0. In other embodiments, R₁ is independently selected from hydrogen, $-CN$, optionally substituted C₁-C₄ alkyl or aryl. In particular embodiments, R₁ is selected from $-C_6H_5$, $-CH_2CH(CO_2H)(NH_2)$, and $-CH_2CO_2H$.

[0265] Exemplary compounds of the present invention include the parthenolide based derivatives:

- 11S,11,13-Dihydro,13-dimethylaminoparthenolide (DMAPT);
- 11S, 11,13-Dihydro,13-diethylaminoparthenolide;
- 11S, 11,13-Dihydro,13-(tert-butylamino)parthenolide;
- 11S, 11,13-Dihydro,13-(pyrrolidin-1-yl)parthenolide;
- 11S, 11,13-Dihydro,13-(piperidin-1-yl)parthenolide (PIPT);
- 11S, 11,13-Dihydro,13-(morpholin-1-yl)parthenolide;
- 11S, 11,13-Dihydro,13-(4-methylpiperidin-1-yl)parthenolide (4MEPT);
- 11S, 11,13-Dihydro,13-(4-methylpiperazin-1-yl)parthenolide;
- 11S, 11,13-Dihydro,13-(homopiperidin-1-yl)parthenolide;
- 11S, 11,13-Dihydro,13-(heptamethyleneimin-1-yl)parthenolide;
- 11S, 11,13-Dihydro,13-(azetidin-1-yl)parthenolide;
- 11S, 11,13-Dihydro,13-methylbutyl aminoparthenolide;
- 11S, 11,13-Dihydro,13-methyl pentyl aminoparthenolide;

- 11S, 11,13-Dihydro,13-ethylaminoparthenolide;
- 11S, 11,13-Dihydro,13-methylaminoparthenolide;
- 11S, 11,13-Dihydro,13-cyclopropylaminoparthenolide;
- 11S, 11,13-Dihydro,13-propargylaminoparthenolide;
- 11S, 11,13-Dihydro,13-(N-benzyl-N-ethylamine)parthenolide;
- 11S, 11,13-Dihydro,13-(N-propyl)parthenolide;
- 11S, 11,13-Dihydro,13-(S-thiophenolyl)parthenolide;
- 11S, 11,13-Dihydro,13-(N,N-diethanolamine)parthenolide
- 11S, 11,13-Dihydro,13-(thiomorpholin-4-yl)parthenolide;
- 11S, 11,13-Dihydro,13-(4-hydroxypiperidin-1-yl)parthenolide;
- 11S, 11,13-Dihydro,13-(1-methylhomopiperizin-4-yl)parthenolide;
- 11S, 11,13-Dihydro,13-(S-mercaptoacetyl)parthenolide;
- 11S, 11,13-Dihydro,13-(4-(2'-hydroxyethyl)piperidin-1-yl)parthenolide;
- 11S, 11,13-Dihydro,13-(piperazin-1-yl-4-carboxaldehyde)parthenolide;
- 11S, 11,13-Dihydro,13-(4-benzylpiperidin-1-yl)parthenolide;
- 11S, 11,13-Dihydro,13-(piperidin-1-yl-4-carboxylic acid)parthenolide;
- 11S, 11,13-Dihydro,13-(azetidin-1-yl-3-carboxylic acid)parthenolide;
- 11S, 11,13-Dihydro,13-(S-cysteinyl)parthenolide;
- 11S, 11,13-Dihydro,13-(4-(piperidin-1'-yl)piperidin-1-yl))parthenolide; and
- 11S, 11,13-Dihydro,13-diallylaminoparthenolide.

[0266] Those of skill in the art will recognize that the invention comprises compounds that may contain one or more chiral centers on, for example, the parthenolide C-11 and thus can exist as racemic mixtures as pure diastereomers, or as pure enantiomers. For

many applications, it is preferred to carry out stereoselective synthesis and/or to subject the reaction product to appropriate purification steps so as to produce substantially stereochemically pure or optically pure materials. Suitable stereoselective synthetic procedures for producing stereochemically pure or optically pure materials are well known in the art, as are procedures for resolving racemic mixtures into their optically pure enantiomers.

[0267] The methods of the present invention further provides for compounds having formula (I), or a pharmaceutically acceptable salt thereof, wherein Z is a group that is converted to =CH₂ under physiological conditions during or after administration to a mammalian patient, thereby yielding a methylene group. In particular embodiments X₁, X₂ and X₃ are O; R₅, R₆, R₇, R₉, and R₁₀ are H; R₄ and R₈ are —CH₃; and Z is optionally substituted C1-8 straight-chained or branched aliphatic, optionally containing 1 or more double or triple bonds, wherein one or more carbons are optionally replaced by R* wherein R* is optionally substituted cycloalkyl, heterocycloalkyl, aryl or heteroaryl; an amino acid residue, H, —CN, —C(O)—, —C(O)C(O)—, —C(O)NR'—, —C(O)NR₁NR₂—, —C(O)O—, —OC(O)—, —NR₁CO₂—, —O—, —NR₁C(O)NR₂—, —OC(O)NR₁—, —NR₁NR₂—, —NR₁C(O)—, —S—, —SO—, —SO₂—, —NR₁—, —SO₂NR₁—, —NR₁R₂ or —NR₁SO₂—, wherein R₁ and R₂ are independently selected from H and optionally substituted aliphatic, cycloalkyl, heterocycloalkyl, aryl or heteroaryl. In preferred embodiments Z is —CH₂N(CH₃)₂, —CH₂N(H)(C(CH₃)₃), —CH₂N(CH₂CH₃)₂, —CH₂N(CH₂CH=CH₂)₂, —CH₂-azetidine, —CH₂-pyrrolidine, —CH₂-piperidine, —CH₂-homopiperidine, —CH₂-heptamethyleneimine, —CH₂-4-methylpiperidine, —CH₂-morpholine, —CH₂-pyrrolidine, —CH₂-proline, —CH₂-thiophenol, —CH₂-diethanolamine, —CH₂-hydroxypiperidine, —CH₂-methylhomopiperazine, —CH₂-thiomorpholine, —CH₂-mercaptoacetic acid, —CH₂-benzylpiperidine, —CH₂-piperidine-4-carboxylic acid, —CH₂-azetidine-3-carboxylic acid, —CH₂-piperidinylpiperidine, or —CH₂-cysteine.

[0268] The methods of the present invention further provides for compounds having formula (I), or a pharmaceutically acceptable salt thereof, wherein Z is CH₂N(CH₃)₂ which under physiological conditions during or after administration to a mammalian patient, undergoes mono- or di-demethylation; conversion to =CH₂, or cysteine or protein conjugation. In particular embodiments, X₁, X₂ and X₃ are O; R₅, R₆, R₇, R₉, and R₁₀ are H; R₄ and R₈ are —CH₃

[0269] The methods of the present invention further provides analogues of compounds of formula (I). Examples are described below and include costunolide, dehydrocostuslactone, alantolactone, isoalantolactone, amino-3-oxo-isoalantolactone, helenalin, 11,13-dihydrohelenalin, aminocyanaropicrin, aminodesacylcyanaropicrin, (+)-aminoreynosin, aminosantamarin, aminosoulangianolide and aminoisotelekin. In addition, the present invention provides compounds of the analogues below, amino-3-oxo-isoalantolactone, aminocyanaropicrin, aminodesacylcyanaropicrin, (+)-aminoreynosin, aminosantamarin, aminosoulangianolide and aminoisotelekin wherein ($-\text{CH}_2-\text{NR}_1\text{R}_2$) is replaced by Z, where Z is as defined herein.

[0270] In other embodiments, Z is hydroxylamine, a hydroxyalkylamino compound, a thioalkylamino compound, a diaminoalkane. Examples include ethylenediamine, piperazine, triaminoalkanes, polyamines, polylysine, putrescine, spermine, spermidine, aminoguanidines and agmatine. In other embodiments, Z is an amino acid. For example glycine, serine, hydroxyproline, β -alanine, cysteine, homocysteine, arginine, lysine, glutamic acid, ornithine, aspartic acid, γ -aminobutyric acid, or taurine. In other embodiments, Z is an amino sugar; for example glucosamine. In other embodiments, Z is a polyoxyethylene glycol of various molecular weights, each of which terminate in an amino functionality that will form an adduct with the appropriate sesquiterpene.

[0271] Modification of the sesquiterpene molecules by these methodologies, affords adducts that can easily be obtained as different inorganic or organic salts to further increase water solubility.

[0272] Methods for synthesising and preparing sesquiterpene lactones as described herein, and others suitable for use in the methods of the present invention are known in the art and are also described in US Pat No. 7,678,904.

G-CSF

[0273] Exposure to ionizing radiation may lead to decreases in WBC count, which manifests as neutropenia (reduction in neutrophils/granulocytes) and lymphopenia (reduction in lymphocytes).

[0274] Various cytokines are involved in hematopoiesis. Granulocyte-colony stimulating factor (G-CSF or GCSF), also known as colony-stimulating factor 3 (CSF 3), is a glycoprotein that stimulates the hematopoietic precursor cells in the bone marrow to

proliferate and differentiate into mature granulocytes and stem cells and release them into the bloodstream. It also induces release of hematopoietic stem cells (HSCs) from the bone marrow into the blood stream, although it does not specifically stimulate these cells. In humans, it exists in two active forms, the more abundant of which is 174 amino acids long; the other is 177 amino acids long.

[0275] The pharmaceutical analogs of naturally occurring G-CSF are recombinant forms of the human 174-amino acid peptide (rhG-CSF), and include filgrastim (e.g. Neupogen® from Amgen, Nivestym® from Pfizer, Releuko® from Amneal, Zario® from Sandoz), which made in *E. coli*, having the same activity, but differing from the natural glycoprotein in having an N-terminal methionine residue and lacking glycosylation; lenograstim (e.g., Granocyte® from Chugai), which is made in mammalian cells (Chinese Hamster Ovary (CHO) cells), and so is essentially indistinguishable from human G-CSF; pegfilgrastim, a PEGylated form of filgrastim, (e.g., Neulasta® from Amgen and Neulastim® from Roche), having a 20 kD monomethoxypolyethylene glycol moiety covalently bound to the N-terminal methionyl residue of filgrastim, which increases solubility and duration of action compared to filgrastim.

[0276] G-CSF has been approved as a drug by the FDA for treating radiation-induced hematopoietic damage. It has also been approved by the Centers for Disease Control and Prevention for administration to victims exposed to a radiological nuclear incident. The recommended dosage of commercial G-CSF (Filgrastim, Neupogen®) is generally given at doses of 4 to 8 mcg/kg/day, up to 10 mcg/kg/day via subcutaneous injection or intravenous infusion. Higher doses, up to 138 mcg/kg/day have been administered without toxic effects, but there is a flattening of the dose response curve above daily doses of greater than 10 mcg/kg/day. Side effects of Neupogen® and other forms of G-CSF may include mild-to-moderate bone pain after repeated administration, local skin reactions at the site of injection, allergic reactions, enlarged or ruptured spleen, alveolar hemorrhage, acute respiratory distress syndrome (ARDS), hemoptysis, and (in patients with pre-existing sickle cell disorders) sickle cell crises.

Administration, dosage and formulations

[0277] In any aspect, the methods of the present invention may be used to minimize the effects of radiation exposure in an individual.

[0278] Minimising the effects of radiation exposure may refer to the treatment or prevention of radiation-induced injury or symptoms, including complications such as organ damage and cancer. The effects of non-targeted and/or non-therapeutic radiation exposure include any symptoms, conditions, syndromes or diseases as described elsewhere herein.

[0279] The existence of, improvement in, treatment of or prevention non-targeted and/or non-therapeutic radiation exposure as described herein may be determined by clinically or biochemically, for example by assessing an individual's history of exposure, symptoms and signs, and conducting laboratory testing. The onset, time course, and severity of symptoms can help determine dose. Laboratory tests that may be conducted to determine whether an individual has been exposed to radiation include, but are not limited to, measuring an individual C-reactive protein (CRP) level, Blood citrulline level, Blood fms-related tyrosine kinase-3 (FLT-3) ligand level, Interleukin-6 (IL-6) level, granulocyte colony-stimulating factor (G-CSF) level, and conducting Cytogenetic studies with overdispersion index.

[0280] As used herein, "preventing" or "prevention" is intended to refer to at least the reduction of likelihood of the risk of (or susceptibility to) acquiring a disease or disorder (i.e., causing at least one of the clinical symptoms of the disease not to develop in a patient that may be exposed to or predisposed to the disease but does not yet experience or display symptoms of the disease). Biological and physiological parameters for identifying such patients are provided herein and are also well known by physicians. Accordingly, to prevent the effects of non-targeted and/or non-therapeutic radiation exposure, an individual may be administered sesquiterpene lactone before, during and/or after exposure to radiation.

[0281] The terms "treatment" or "treating" of a subject includes the application or administration of a sesquiterpene lactone as described herein with the purpose of delaying, slowing, stabilizing, curing, healing, alleviating, relieving, altering, remedying, less worsening, ameliorating, improving, or affecting the disease or condition, the symptom of the disease or condition, or the risk of (or susceptibility to) the disease or condition. The term "treating" refers to any indication of success in the treatment or amelioration of an injury, pathology or condition, including any objective or subjective parameter such as abatement; remission; lessening of the rate of worsening; lessening severity of the disease; stabilization, diminishing of symptoms or making the injury,

pathology or condition more tolerable to the subject; slowing in the rate of degeneration or decline; or making the final point of degeneration less debilitating. Accordingly, to treat the effects of non-targeted and/or non-therapeutic radiation exposure, an individual may be administered sesquiterpene lactone during, and/or after exposure to radiation.

[0282] The term "ameliorate" or "amelioration" refers to a decrease, reduction or elimination of a condition, disease, disorder, or phenotype, including an abnormality or symptom. A subject in need of treatment may already have the condition, or may be prone to have the condition or may be one in whom the condition is to be prevented.

[0283] The sesquiterpene lactones as described herein may be adapted to aid in the prophylactic or therapeutic treatment, prevention or amelioration of symptoms of radiation exposure in a form suitable for delivery to a patient.

[0284] The sesquiterpene lactone as described herein may be administered within about 1 hour, within about 2 hours, within about 3 hours, within about 4 hours, within about 5 hours, within about 6 hours, within about 7 hours, within about 8 hours, within about 9 hours, within about 10 hours, within about 12 hours, within about 16 hours, within about 20 hours, within about 30 hours, within about 40 hours, or within about 48 hours or greater than about 48 hours after the radiation exposure.

[0285] Alternatively, the sesquiterpene lactone as described herein may also be administered within 1 hour, within 2 hours, within 3 hours, within 4 hours, within 5 hours, within about 6 hours, within 7 hours, within 8 hours, within 9 hours, within 10 hours, within 12 hours, within 16 hours, within 20 hours, within 30 hours, within 40 hours, or within 48 hours or greater than 48 hours after the radiation exposure.

[0286] In any embodiment, the sesquiterpene lactone may be administered as an individual therapeutic agent or may be administered in combination with another drug. In particular the drug may have known efficacy for treating or preventing radiation injury. For example, the sesquiterpene lactone may be administered with one or more of therapeutic agents including proteins, small molecule drugs, nucleic acids or the like. Preferably, the sesquiterpene lactone is administered with a therapeutic agent including granulocyte-colony stimulating factor (G-CSF). The individual may be administered the sesquiterpene lactone and the G-CSF concurrently or sequentially in any order.

[0287] The amount and duration of administration of the sesquiterpene employed in the methods of the present invention to be used varies according to the degree of the exposure to ionizing radiation encountered, and the stages of any radiation-induced damage. A suitable dosage is that which will result in concentration of the sesquiterpene lactone (in blood and/or tissues) sufficient to protect or mitigate the effects of the radiation exposure. The preferred dosage is that amount sufficient to render an individual asymptomatic after radiation exposure.

[0288] The selected dosage level will depend upon a variety of factors including the activity of the sesquiterpene lactone, or pharmaceutically acceptable salt, solvate, tautomer, N-oxide, stereoisomer and/or prodrug thereof, the route of administration, the time of administration, the rate of excretion of the compound, the duration of the treatment, other drugs such as G-CSF combination treatment, compounds and/or materials used in combination with the particular compound(s) employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

[0289] A physician or veterinarian having ordinary skill in the art can readily determine and prescribe the therapeutically effective amount of the pharmaceutical composition required. For example, the physician or veterinarian could start doses of the pharmaceutical composition or compound at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved. By "therapeutically effective amount" is meant the concentration of a compound that is sufficient to elicit the desired therapeutic effect. It is generally understood that the effective amount of the compound will vary according to the weight, sex, age, and medical history of the subject. Other factors which influence the effective amount may include, but are not limited to, the severity of the patient's condition, the disorder being treated, the stability of the compound, and, if desired, another type of therapeutic agent being administered with the compound of the invention. A larger total dose can be delivered by multiple administrations of the agent. Methods to determine efficacy and dosage are known to those skilled in the art (Isselbacher et al. (1996) Harrison's Principles of Internal Medicine 13 ed., 1814-1882, herein incorporated by reference).

[0290] In general, a suitable daily dose of an active compound used in the compositions and methods of the invention will be that amount of the compound that is

the lowest dose effective to produce a therapeutic effect. Such an effective dose will generally depend upon the factors described above.

[0291] Dosing can be single dosage or cumulative (serial dosing), and can be readily determined by one skilled in the art. For instance, treatment may comprise a one-time administration of an effective dose of a pharmaceutical composition disclosed herein.

[0292] Alternatively, treatment may comprise multiple administrations of an effective dose of a pharmaceutical composition carried out over a range of time periods, such as, e.g., once daily, twice daily, thrice daily, once every few days, or once weekly. The timing of administration can vary from individual to individual, depending upon such factors as the severity of an individual's symptoms. For example, an effective dose of a pharmaceutical composition disclosed herein can be administered to an individual once daily for an indefinite period of time, or until the individual no longer requires therapy. A person of ordinary skill in the art will recognize that the condition of the individual can be monitored throughout the course of treatment and that the effective amount of a pharmaceutical composition disclosed herein that is administered can be adjusted accordingly.

[0293] Pharmaceutical formulations as described herein may be suitable for oral, enteral (including gastric feeding) or parenteral (including intramuscular, subcutaneous and intravenous) administration. Forms suitable for parenteral administration also include forms suitable for administration by inhalation or insufflation or for nasal, or topical (including buccal, rectal, vaginal and sublingual) administration. The formulations may, where appropriate, be conveniently presented in discrete unit dosage forms and may be prepared by any of the methods well known in the art of pharmacy. Such methods include the step of bringing into association the active compound with liquid carriers, solid matrices, semi-solid carriers, finely divided solid carriers or combinations thereof, and then, if necessary, shaping the product into the desired delivery system.

[0294] Pharmaceutical formulations suitable for oral administration may be presented as discrete unit dosage forms such as hard or soft gelatin capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or as granules; as a solution, a suspension or as an emulsion; or in a chewable base such as a synthetic resin or chicle for ingestion of the agent from a chewing gum. The active ingredient may also be presented as a bolus, electuary or paste. Tablets and capsules

for oral administration may contain conventional excipients such as binding agents, fillers, lubricants, disintegrants, or wetting agents. The tablets may be coated according to methods well known in the art, i.e., with enteric coatings.

[0295] Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, emulsifying agents, non-aqueous vehicles (which may include edible oils), or preservatives.

[0296] The sesquiterpene lactones for use in the methods of the present invention may also be formulated for parenteral administration (e.g., by injection, for example, bolus injection or continuous infusion) and may be presented in unit dose form in ampules, pre-filled syringes, small volume infusion containers or in multi-dose containers with an added preservative. The compositions may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form, obtained by aseptic isolation of sterile solid or by lyophilization from solution, for constitution with a suitable vehicle, e.g., sterile, pyrogen-free water, before use.

[0297] For topical administration to the epidermis, the sesquiterpene lactones may be formulated as ointments, creams or lotions, or as the active ingredient of a transdermal patch. Suitable transdermal delivery systems are disclosed, for example, in A. Fisher et al. (U.S. Pat. No. 4,788,603), or R. Bawa et al. (U.S. Pat. Nos. 4,931,279; 4,668,506 and 4,713,224). Ointments and creams may, for example, be formulated with an aqueous or oily base with the addition of suitable thickening and/or gelling agents. Lotions may be formulated with an aqueous or oily base and will in general also contain one or more emulsifying agents, stabilizing agents, dispersing agents, suspending agents, thickening agents, or coloring agents.

[0298] Formulations suitable for topical administration in the mouth include unit dosage forms such as lozenges comprising active ingredient in a flavored base, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert

base such as gelatin and glycerin or sucrose and acacia; mucoadherent gels, and mouthwashes comprising the active ingredient in a suitable liquid carrier.

[0299] When desired, the above-described formulations can be adapted to give sustained release of the active ingredient employed, e.g., by combination with certain hydrophilic polymer matrices, e.g., comprising natural gels, synthetic polymer gels or mixtures thereof. The polymer matrix can be coated onto, or used to form, a medical prosthesis, such as a stent, valve, shunt, graft, or the like.

[0300] Pharmaceutical formulations suitable for rectal administration wherein the carrier is a solid are most preferably presented as unit dose suppositories. Suitable carriers include cocoa butter and other materials commonly used in the art, and the suppositories may be conveniently formed by admixture of the active compound with the softened or melted carrier(s) followed by chilling and shaping in moulds.

[0301] Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or sprays containing, in addition to the active ingredient, such carriers as are known in the art to be appropriate.

[0302] For administration by inhalation, the sesquiterpene lactones for use in the methods of the present invention are conveniently delivered from an insufflator, nebulizer or a pressurized pack or other convenient means of delivering an aerosol spray. Pressurized packs may comprise a suitable propellant such as dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount.

[0303] Alternatively, for administration by inhalation or insufflation, the sesquiterpene lactones for use in the methods of the present invention may take the form of a dry powder composition, for example, a powder mix of the compound and a suitable powder base such as lactose or starch. The powder composition may be presented in unit dosage form in, for example, capsules or cartridges or, e.g., gelatin or blister packs from which the powder may be administered with the aid of an inhalator or insufflator.

[0304] For intra-nasal administration, the sesquiterpene lactones for use in the methods of the present invention may be administered via a liquid spray, such as via a

plastic bottle atomizer. Typical of these are the Mistometer® (Wintrop) and the Medihaler® (Riker).

[0305] For topical administration to the eye, the sesquiterpene lactones can be administered as drops, gels (U.S. Pat. No. 4,255,415), gums (see U.S. Pat. No. 4,136,177) or via a prolonged-release ocular insert.

[0306] The exact amount required will vary from subject to subject, depending on the species, age and general condition of the subject, mode of administration and the like. Thus, it may not be possible to specify an exact "effective amount". However, an appropriate "effective amount" in any individual case may be determined by one of ordinary skill in the art using only routine experimentation. In one aspect, the dose administered to a subject is any dose that reduces viral load.

[0307] Any suitable route of administration may be employed for providing the patient with an effective dosage of a sesquiterpene lactone compound as described herein (e.g., oral, sublingual, rectal, intravenous, epidural, intrathecal, subcutaneous, transcutaneous, intramuscular, intraperitoneal, intracutaneous, inhalation, transdermal, nasal spray, nasal gel or drop, and the like). While it is possible that, for use in minimising non-targeted and/or non-therapeutic radiation exposure in an individual, the sesquiterpene lactone compounds may be administered as the pure chemicals, as by inhalation of a fine powder via an insufflator, it is preferable to present the active ingredient as a pharmaceutical formulation.

[0308] Although the methods of the present invention finds application in humans, the methods of the invention is also useful for therapeutic veterinary purposes. The invention is useful for domestic or farm animals such as cattle, sheep, horses and poultry; for companion animals such as cats and dogs; and for zoo animals.

[0309] Further, the individual whom has been exposed to radiation may not have been diagnosed with medical condition that is responsive to radiation therapy. For example, the individual may not have been diagnosed with cancer or has a detectable tumour that a physician may treat with radiotherapy.

Kits

[0310] Kits are also provided for use in the methods of the invention, in particular to minimize the effects of non-targeted and/or non-therapeutic radiation exposure, which kit comprises at least a sesquiterpene lactone as described herein together with instructions for its use, wherein the instructions for use describe a method of the invention.

[0311] The contents of the kit can be lyophilized and the kit can additionally contain a suitable solvent for reconstitution of the lyophilized components. Individual components of the kit would be packaged in separate containers and, associated with such containers, can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

[0312] As used herein the term “derived” and “derived from” shall be taken to indicate that a specific integer may be obtained from a particular source albeit not necessarily directly from that source.

[0313] As used herein, the singular forms “a,” “an” and “the” include plural references unless the context clearly dictates otherwise.

[0314] Other than in the operating example, or where otherwise indicated, all numbers expressing quantities of ingredients, reaction conditions, and so forth used in the specification and claims are to be understood as being modified in all instances by the term “about”. Accordingly, unless indicated to the contrary, the numerical parameters set forth in the specification and claims are approximations that may vary depending upon the desired properties sought to be obtained by the methods of the present invention. Hence “about 80 %” means “about 80 %” and also “80 %”. At the very least, each numerical parameter should be construed in light of the number of significant digits and ordinary rounding approaches.

[0315] Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the methods of the present invention are approximations, the numerical values set forth in the specific examples are reported as precisely as possible. Any numerical value; however, inherently contains certain errors necessarily resulting from the standard deviation found in their respective testing measurements.

[0316] Other definitions for selected terms used herein may be found within the detailed description of the invention and apply throughout. Unless otherwise defined, all other scientific and technical terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which the invention belongs.

Examples

Example 1 - DMAPT reduces normal tissue damage following a lethal dose of radiation.

[0317] To examine the ability of DMAPT to protect healthy tissues from damage, the inventors initiated an in vivo experiment where 10-week-old male and female C57BL/6J mice were exposed to a single LD50 whole body X-ray dose. Mice were treated with DMAPT (100mg/kg) or vehicle by oral gavage 1hr prior to irradiation and then daily over the following three days. Tissues were collected for analysis 10.9 weeks-post irradiation. Even with this short DMAPT treatment regime following radiation exposure, the inventors observed that DMAPT treatment was able to reduce the early signs of radiation-induced fibrosis in bladder (42% reduction, $p=0.048$; Fig. 1) and lung (24.3% reduction; data not shown) tissues. In addition, a proportion of mice treated with DMAPT did not exhibit observable radiation-induced lung injury, as measured by mean lung air space expansion (data not shown). A regime of four doses of DMAPT was unable to prevent gross tubule damage in the testes of male mice following LD50 radiation exposure (data not shown). However, closer examination of the type of damage occurring in the tubules, revealed that DMAPT treated mice had a significantly lower proportion of hypospermia compared with vehicle treated mice (Fig. 2).

[0318] These results indicate that DMAPT is able to provide protection of male fertility following exposure to a lethal dose of radiation.

Example 2 – Radiation Mitigation Models

H-ARS murine model

[0319] This experiment uses a total body irradiation (TBI) murine model of H-ARS in accordance with the FDA's Animal Rule (2002) as described in Plett PA et al., *Health Phys*, 2012; 103:343-55.

Experimental design

[0320] All groups consist of 50% males and 50% females. Mice are first be exposed to their age- and sex-specific LD50/30, treated with DMAPT, G-CSF, or respective vehicles for DMAPT and G-CSF (Table 1). G-CSF is administered using the regimen shown to be most effective: 3 daily subcutaneous (sc) doses of 0.17 mg/kg beginning on day 1 post-IRR according to according to method described in Satyamitra et. al., *Radiat Res*; 2017; 187:659-671. Vehicle controls for DMAPT and G-CSF are administered so that all mice receive the same number and timing of injections to avoid differential stress effects. Mice are monitored for morbidity and mortality for 30 days. LD50/30 radiation doses and LD70/30 radiation are assessed.

[0321] Complete blood counts (CBC) are determined on days 10, 19, 23, and 30 (6 mice / group). Day 10 represents the nadir for CBC and is generally insensitive to medical countermeasures (MedCM) that enhance survival. Each mouse is bled a maximum of two times during the study at least 14 days apart to avoid stress effects due to handling.

Table 1. Experimental design for combinatorial studies of DMAPT as a protector and mitigator + G-CSF as a mitigator

Groups	DMAPT-Post + G-CSF-Post Dosing Plan	No. of Mice/group
Vehicle	Vehicle _{DMAPT} -2 hours pre XRT, and 24 hours and daily for 14 days oral or IV post XRT + Vehicle _{GCSF} +24, 48, and +72 hrs sc	20
DAMPT	DMAPT -2 hours pre XRT, and 24 hours and daily for 14 days oral or IV post XRT + Vehicle _{GCSF} +24, +48, and +72 hrs sc	20
G-CSF	Vehicle _{DMAPT} -2 hours pre XRT, and 24 hours and daily for 14 days oral or IV post XRT +G-CSF +24, +48, and +72 hrs sc	20
DMAPT + G-CSF	DMAPT -2 hours pre XRT, and 24 hours and daily for 14 days oral or IV post XRT +G-CSF +24, +48, and +72 hrs sc	20

Statistical analyses

[0322] Statistical analyses will be performed as previously described in similar studies (Plett PA et al., *Health Phys*, 2012; 103:343-55; Chua HL et al., *Health Phys*, 2014; 106:21-38; Plett PA el al., *Health Phys*, 2014; 106:7-20). The logistic regression and proportional hazards models will include effects for the two drugs and their interaction. Gender will also be included in the models as a covariate. Focusing first on the LD50/30 dosing, the study design allows for 80% power to detect a difference of 31% (i.e.

improve 30-day survival from 50% to 81% for the main effect of each drug (DMAPT and G-CSF alone) with a two-tailed significance level of 5%. CBC will be analyzed using ANOVA to compare groups at each time point. Prior to testing, the data will be examined to see if a transformation is necessary to satisfy the assumptions of the ANOVA model. In addition, data for multiple time points will be examined together in a repeated measures ANOVA.

Conclusions

[0323] The inventors believe that DMAPT administration will improve mouse survival and lessen A-HARS when given as monotherapy as a radiomitigator. The inventors also believe that administration of DMAPT with G-CSF will have positive on mouse survival and neutrophil recovery.

Example 3 – Determine the ability of DMAPT to mitigate the DEARE.

Mouse model of residual bone marrow damage (RBMD) (hematopoietic DEARE)

[0324] This experiment uses a murine model of RBMD/DEARE as described in Chua HL et al., *Health Phys*, 2012; 103:356-66 and Chua HL et al., *Health Phys*, 2014; 106:21-38.

Experimental design

[0325] The DMAPT dosing regimen (with and without G-CSF mitigation) from Example 1 is used to evaluate for alleviation of DEARE.

[0326] The large number of animals at the start of this experiment (Tables 2 and 3) accounts for the expected shortened lifespan of IRR mice. Animals are followed closely for signs of morbidity and humanely euthanized if death appears imminent. Necropsies are performed to identify cause of morbidity. Monthly body weight and CBC are determined. DEARE analyses is conducted at month 6 and month 18 post-IRR.

[0327] Ultimately there will be 5 males and 5 females per group euthanized for DEARE studies at 6 months and separate set of 5 males and 5 females per group euthanized for DEARE studies at 18 months.

- There are four groups for the 6 month (Table 1) and 18 month (Table 2) DEARE studies with ultimate dosing based on design from Example 1: There

are vehicle controls for DMAPT and G-CSF, DMAPT monotherapy with G-CSF vehicle control, G-CSF monotherapy with DMAPT vehicle control and DMAPT plus G-CSF combination.

Table 2. DEARE at 6 month

Group	Sex	No. of mice at day 0	^a % day 30 survival at LD50/30	No. of mice at day 30	^a % survival from day 30 to 18 months	No. of mice at 18 months
Veh _d / Veh _G	Male	20	50%	10	88%	8
	Female	20	50%	10	91%	9
DMAPT / Veh _G	Male	16	70%	11	88%	9
	Female	15	70%	10	91%	9
Veh _D / G-CSF	Male	16	70%	11	88%	9
	Female	15	70%	10	91%	9
DMAPT / G-CSF	Male	16	70%	11	88%	9
	Female	15	70%	10	91%	9

^a Estimate of survival.

Table 3. DEARE at 18 month

Group	Sex	No. of mice at day 0	^a % day 30 survival at LD50/30	No. of mice at day 30	^a % survival from day 30 to 18 months	No. of mice at 18 months
Veh _d / Veh _G	Male	52	50%	26	31%	8
	Female	134	50%	67	12%	8
DMAPT / Veh _G	Male	38	70%	26	31%	8
	Female	96	70%	67	12%	8
Veh _D / G-CSF	Male	38	70%	26	31%	8
	Female	96	70%	67	12%	8
DMAPT / G-CSF	Male	38	70%	26	31%	8
	Female	96	70%	67	12%	8

^a Estimate of survival.

[0328] Hematopoietic assays are conducted at month 6 post-IRR and verified at month 18 only in groups that demonstrated alleviation of hematopoietic DEARE at month 6.

[0329] Cardiac and renal tissues will be collected and assessed for pathology associated with DEARE with a focus on fibrosis, vascular injury, and oxidative stress / inflammation. Specific analyses include: (1) histological examination for fibrosis / collagen and hemosiderin deposition and organ-specific function-related parameters including renal tubules, pericardial thickening, EC density and arteriolar rarefaction (as described in Unthank JL et al., *Health Phys*, 2015; 109:511-21) (2) immunohistochemical (IHC) evaluation of local inflammation [macrophages by F4-80 &

nuclear localization of NF- κ B (p65)], oxidant damage (4-hydroxy-2-nonenal (4-HNE) and/or 8-hydroxy-2'-deoxyguanosine), EC activation (ICAM-1 or VCAM-1, p65), and vascular smooth muscle degradation (via smooth muscle α -actin), (3) biochemical analyses for more robust assessment and confirmation of IHC data (collagen assay, p16INK4a PCR for early senescence, and Nox4 or p47phox PCR for oxidant stress), (4) evaluation of systemic inflammation /oxidative stress (8-hydroxy-2'-deoxyguanosine and/or 4-hydroxy-2-nonenal), and (5) renal function via BUN. Cardiovascular tissues include heart and conduit arteries (thoracic aorta and/or carotid artery) and smaller coronary arteries.

[0330] Tissues are harvested in a manner that preserves the tissues for the planned analyses (e.g. histological, mRNA, protein, and hematopoietic cells) and maximizes information that can be obtained from a single mouse, as described in Unthank JL et al., *Health Phys*, 2015; 109:511-21.

Statistical analyses

[0331] Results for each of the DEARE outcome measures is compared among treatments using full factorial three-way ANOVA with the treatments and their interactions plus time point. This initial analysis will involve IRR groups only. To account for individual mice contributing multiple data points, a random mouse effect is included. Data is examined before the analyses are performed to determine if a transformation is needed to meet the assumptions of the ANOVA model. Pairwise follow-up comparisons of each treatment to the IRR control is also be performed. A second set of ANOVA models is also be implemented for all groups and time, allowing for comparison of each group to the NI control. Ten mice per group will allow detection of a treatment effect to reduce radiation effect by 50% or more (80% power, 5% significance level). Gender will also be included in the models as a covariate.

Example 4 – DMAPT reduces radiation-induced senescence in colorectal tissue and testis

[0332] To examine the ability of DMAPT to protect cells from radiation-induced senescence due to the inflammatory changes causing tissue injury from acute radiation exposure which later cause DEARE, the inventors initiated an *in vivo* experiment where mice were treated with DMAPT (100mg/kg) or vehicle by oral gavage prior, during and

after 2Gy daily radiation therapy to the lower-body for 10 doses, total 20Gy radiation (Figure 3A and Figure 4A). Tissues were collected for analysis on day 62 post-initial DMAPT administration.

[0333] Mice treated with DMAPT prior to lower-body radiation were shown to have significantly reduced cell senescence markers in both colorectal tissue and testis compared to irradiated mice administered vehicle (Figure 3B and Figure 4B).

[0334] These results indicate that DMAPT is able to provide protection from radiation-induced cell senescence.

Example 5 - DMAPT reduces markers of ARS in lung and bladder

[0335] Next the inventors investigated the ability of DMAPT to protect cells from radiation-induced fibrosis. Mice were whole body irradiated with a single dose of 8Gy on day 1, and treated with concomitant and adjuvant DMAPT (100mg/kg) or vehicle by oral gavage for 4 days (Figure 5A and Figure 6A). Lung and bladder tissue was collected on day 76 for analysis.

[0336] Mice treated with DMAPT showed reduced markers associated with acute radiation (*Wnt3* and *Ddb2*) in both lung and bladder tissue compared to irradiated mice administered vehicle (Figure 5B and Figure 6B).

[0337] Together, these results indicate that DMAPT is able to provide protection from acute radiation syndrome, which may reduce radiation-induced fibrosis.

Example 6 – DMAPT topical composition for the prevention and treatment of radiation-induced skin injury

[0338] A patient was administered 70Gy radiation therapy over thirty-fix session for treatment of a head and neck cancer. Radiation was administered on a daily basis (Monday through Friday).

[0339] Concomitant with radiation therapy, the patient applied a daily topical DMAPT composition to the skin of the neck region exposed to the radiation therapy. This topical composition was applied 7 days a week, and on radiation days, was applied prior to radiation therapy.

[0340] Over a period of 35 days post-commencement of radiation therapy, the subject had no evident skin breakdown. However, on day 36 the subject failed to replenish and apply the topical composition over a 24 hour period resulting in visible skin breakdown over the irradiated neck region within one day (data not shown). Upon restarting daily application of the topical composition, the patients skin wounds repaired over a ten day period, even with continual irradiation for an addition five treatment sessions. On the last day of radiation therapy, there was minimal skin change to the irradiated neck region of the subject compared to pre-irradiated skin (Figure 7).

[0341] The outcomes of this treatment plan suggest that topical DMAPT application can both prevent and treat acute radiation-induced skin injury, a component of acute radiation syndrome.

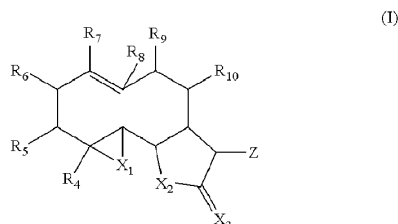
CLAIMS

1. A method of minimising the effects of non-targeted and/or non-therapeutic radiation exposure in an individual, the method comprising administering a sesquiterpene lactone to the individual, thereby minimising the effects of non-targeted and/or non-therapeutic radiation exposure in the individual.
2. A method of claim 1, wherein the non-targeted and/or non-therapeutic radiation exposure is at least 10% of the surface-area of the individual that is exposed to the radiation.
3. A method of claim 1 or 2, wherein the non-targeted and/or non-therapeutic radiation exposure is at least 25% of the surface-area of the individual that is exposed to the radiation.
4. A method of any one of claims 1 to 3, wherein the non-targeted and/or non-therapeutic radiation exposure is at least 75% of the surface-area of the individual that is exposed to the radiation.
5. A method of any one of claims 1 to 4, wherein the method comprises non-targeted and/or non-therapeutic radiation exposure to one or more organs selected from: brain, thyroid, breast, heart, liver, pancreas, bladder, ovaries, testes, bone, salivary glands, lung, stomach, intestines, kidneys, bladder, uterus, cervix, penis, inner ear, vagina, heart, skeletal muscles, thymus, spleen bone marrow, skin and hair.
6. A method of any one of claims 1 to 5, wherein the non-targeted and/or non-therapeutic radiation exposure is total body exposure.
7. A method of any one of claims 1 to 6, wherein the radiation exposure is a dose that, if untreated, would cause acute radiation syndrome (ARS) in the individual.
8. A method of any one of claims 1 to 7, wherein the radiation exposure is a dose that, if untreated, would cause delayed effects of acute radiation exposure (DEARE) in the individual to any organ.

9. A method according to claim 7, wherein the ARS is hematopoietic (H), gastrointestinal, pulmonary, genitourinary (including reproductive) or cardiovascular/central nervous system.
10. A method according to claim 7, wherein the acute radiation syndrome comprises acute radiation injury to one or more of the skin, testes, bladder and lung.
11. A method of any one of claims 1 to 10, wherein the radiation exposure is a dose of radiation expected to cause death to 50 percent of an exposed population within 30 days (LD 50/30).
12. A method of any one of claims 1 to 11, wherein the radiation exposure is a dose of radiation expected to cause death to 50 percent of an exposed population within 60 days (LD 50/60).
13. A method of any one of claims 1 to 12, wherein the radiation exposure is a dose of radiation expected to cause death to 70 percent of an exposed population within 30 days (LD 70/30).
14. A method of claim 12, wherein the radiation exposure is about equal to, or greater than, about 3 Gy.
15. A method of claim 12, wherein the radiation exposure is about equal to, or greater than, about 6 Gy.
16. A method of claim 12, wherein the radiation exposure is about equal to, or greater than, about 10 Gy.
17. A method of any one of claims 1 to 13, wherein the radiation exposure is acute exposure.
18. A method of any one of claims 1 to 13, wherein the radiation exposure is chronic exposure.
19. A method of any one of claims 1 to 18, wherein the radiation is ionising radiation.
20. A method of claim 19, wherein the ionising radiation is electromagnetic radiation.

21. A method of claim 19, wherein the ionising radiation is particle radiation, preferably arising from a nuclear accident or attack.
22. A method of claim 21, wherein the particle radiation is selected from alpha particles, beta particles and neutrons.
23. A method of any one of claims 1 to 22, wherein the radiation exposure is irradiation.
24. A method of any one of claims 1 to 22, wherein the radiation exposure is contamination.
25. A method of claim 24, wherein the contamination is external.
26. A method of claim 24, wherein the contamination is internal.
27. A method of claim 26, wherein the internal contamination is via ingestion, inhalation, or breaks in the skin.
28. A method of any one of claims 1 to 27, wherein the radiation exposure is cosmic radiation exposure.
29. A method of any one of claims 1 to 28, wherein the individual is operating in a radiological or nuclear environment.
30. A method of claim 28 or 29, wherein the radiation exposure of the individual is continuous for greater than 12 hours, 24 hours, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 2 weeks, 1 month, 2 months, 3 months or 6 months.
31. A method of any one of claims 1 to 30, wherein the individual has not been diagnosed with cancer.
32. A method of any one of claims 1 to 31, wherein the individual does not have a detectable tumour.
33. A method of any one of claims 1 to 32, wherein the individual is human.
34. A method according to any one of claims 1 to 33, wherein the sesquiterpene lactone is administered after the radiation exposure.

35. A method according to any one of claims 1 to 33, wherein the sesquiterpene lactone is administered during and after exposure to radiation.
36. A method according to any one of claims 1 to 33, wherein the sesquiterpene lactone is administered after exposure to radiation and not before or during exposure.
37. A method according to any one of claims 1 to 33, wherein the sesquiterpene lactone is administered during and after exposure to radiation, and not before exposure.
38. A method according to any one of claims 1 to 33, wherein the sesquiterpene lactone is administered before, during and after exposure to radiation.
39. A method according to any one of claims 1 to 38, wherein the method minimises the effects of:
 - non-targeted and/or non-therapeutic radiation-induced cell senescence in the individual;
 - non-targeted and/or non-therapeutic radiation-induced fibrosis in the individual;
 - non-targeted and/or non-therapeutic radiation-induced inflammation in the individual;
 - non-targeted and/or non-therapeutic radiation-induced skin injury in the individual; or
 - non-targeted and/or non-therapeutic radiation-induced hypospermia in the individual.
40. A method according to any one of claims 1 to 39, wherein the sesquiterpene lactone is parthenolide or a pharmaceutically acceptable salt, solvate, tautomer, N-oxide, stereoisomer and/or prodrug thereof.
41. A method according to any one of claims 1 to 40, wherein the sesquiterpene lactone has formula (I):



wherein:

X₁, X₂ and X₃ are heteroatoms;

R₄, R₅, R₆, R₇, R₈, R₉ and R₁₀ are independently selected from H, halo, —OH, —NO₂, —CN and optionally substituted aliphatic, cycloalkyl, heterocycloalkyl, aryl or heteroaryl; and

Z is optionally substituted C₁₋₈ straight-chained or branched aliphatic, optionally containing 1 or more double or triple bonds, wherein one or more carbons are optionally replaced by R* wherein R* is optionally substituted cycloalkyl, heterocycloalkyl, aryl or heteroaryl; an amino acid residue, H, —CN, —C(O)—, —C(O)C(O)—, —C(O)NR¹—, —C(O)NR¹NR²—, —C(O)O—, —OC(O)—, —NR¹CO₂—, —O—, —NR¹C(O)NR²—, —OC(O)NR¹—, —NR¹C(O)—, —S—, —SO—, —SO₂—, —NR¹—, —SO₂NR¹—, —NR¹R², or —NR¹SO₂—, wherein R¹ and R² are independently selected from H and optionally substituted aliphatic, cycloalkyl, heterocycloalkyl, aryl or heteroaryl; or where R* is NR¹R², R¹ and R² optionally together with the nitrogen atom form an optionally substituted 5-12 membered ring, said ring optionally comprising 1 or more heteroatoms or a group selected from —CO—, —SO—, —SO₂— and —PO—; or

a pharmaceutically acceptable salt, solvate, tautomer, N-oxide, stereoisomer and/or prodrug thereof.

42. A method according to any one of claims 1 to 39, wherein the sesquiterpene lactone is selected from:

- 11S,11,13-Dihydro,13-dimethylaminoparthenolide (DMAPT);
- 11S, 11,13-Dihydro,13-diethylaminoparthenolide;
- 11S, 11,13-Dihydro,13-(tert-butylamino)parthenolide;

- 11S, 11,13-Dihydro,13-(pyrrolidin-1-yl)parthenolide;
- 11S, 11,13-Dihydro,13-(piperidin-1-yl)parthenolide (PIPT);
- 11S, 11,13-Dihydro,13-(morpholin-1-yl)parthenolide;
- 11S, 11,13-Dihydro,13-(4-methylpiperidin-1-yl)parthenolide (4MEPT);
- 11S, 11,13-Dihydro,13-(4-methylpiperazin-1-yl)parthenolide;
- 11S, 11,13-Dihydro,13-(homopiperidin-1-yl)parthenolide;
- 11S, 11,13-Dihydro,13-(heptamethyleneimin-1-yl)parthenolide;
- 11S, 11,13-Dihydro,13-(azetidin-1-yl)parthenolide;
- 11S, 11,13-Dihydro,13-methylbutyl aminoparthenolide;
- 11S, 11,13-Dihydro,13-methyl pentyl aminoparthenolide;
- 11S, 11,13-Dihydro,13-ethylaminoparthenolide;
- 11S, 11,13-Dihydro,13-methylaminoparthenolide;
- 11S, 11,13-Dihydro,13-cyclopropylaminoparthenolide;
- 11S, 11,13-Dihydro,13-propargylaminoparthenolide;
- 11S, 11,13-Dihydro,13-(N-benzyl-N-ethylamine)parthenolide;
- 11S, 11,13-Dihydro,13-(N-propyl)parthenolide;
- 11S, 11,13-Dihydro,13-(S-thiophenolyl)parthenolide;
- 11S, 11,13-Dihydro,13-(N,N-diethanolamine)parthenolide
- 11S, 11,13-Dihydro,13-(thiomorpholin-4-yl)parthenolide;
- 11S, 11,13-Dihydro,13-(4-hydroxypiperidin-1-yl)parthenolide;
- 11S, 11,13-Dihydro,13-(1-methylhomopiperizin-4-yl)parthenolide;
- 11S, 11,13-Dihydro,13-(S-mercaptoacetyl)parthenolide;

- 11S, 11,13-Dihydro,13-(4-(2'-hydroxyethyl)piperidin-1-yl)parthenolide;
- 11S, 11,13-Dihydro,13-(piperazin-1-yl-4-carboxaldehyde)parthenolide;
- 11S, 11,13-Dihydro,13-(4-benzylpiperidin-1-yl)parthenolide;
- 11S, 11,13-Dihydro,13-(piperidin-1-yl-4-carboxylic acid)parthenolide;
- 11S, 11,13-Dihydro,13-(azetidin-1-yl-3-carboxylic acid)parthenolide;
- 11S, 11,13-Dihydro,13-(S-cysteinyl)parthenolide;
- 11S, 11,13-Dihydro,13-(4-(piperidin-1'-yl)piperidin-1-yl))parthenolide; and
- 11S, 11,13-Dihydro,13-diallylaminoparthenolide,

or a pharmaceutical acceptable salt, solvate, tautomer, N-oxide, stereoisomer and/or prodrug thereof.

43. A method according to any one of claims 1 to 39, wherein the sesquiterpene lactone is 11S,11,13-Dihydro,13-dimethylaminoparthenolide (DMAPT) or a pharmaceutically acceptable salt, solvate, tautomer, N-oxide, stereoisomer and/or prodrug thereof.
44. A method according to any one of claims 1 to 39, wherein the sesquiterpene lactone is 11S,11,13-Dihydro,13-dimethylaminoparthenolide (DMAPT) or a pharmaceutically acceptable salt thereof.
45. A method according to claim 44, wherein the pharmaceutically acceptable salt of DMAPT is a fumarate salt.
46. A method according to any one of claims 1 to 45, wherein the individual is receiving or has received G-CSF or analog or derivative thereof, or a composition comprising G-CSF or analog or derivative thereof.
47. A method according to any one of claims 1 to 45, the method further comprising administering G-CSF or analog or derivative thereof, or a composition comprising G-CSF or analog or derivative thereof.

48. A method according to any one of claims 1 to 47, wherein the method minimises the effects of non-targeted and/or non-therapeutic radiation-induced inflammation in the individual.
49. A method of preventing or reducing the risk of a cancer, caused by non-targeted and/or non-therapeutic radiation exposure, in an individual, the method comprising administering a lactone sesquiterpene to the individual, thereby preventing or reducing the risk of a cancer caused by non-targeted and/or non-therapeutic radiation exposure.
50. A method of preventing or treating radiation-induced hypospermia in an individual, the method comprising administering a sesquiterpene lactone to the individual, thereby preventing or reducing radiation-induced hypospermia in the individual.
51. A method of preventing or treating radiation-induced cell senescence in an individual, the method comprising administering a sesquiterpene lactone to the individual, thereby preventing or reducing radiation-induced cell senescence in the individual.
52. A method according to claim 51, wherein the radiation-induced cell senescence is in the colorectum or testis.
53. A method of preventing or reducing radiation-induced fibrosis in an individual, the method comprising administering a sesquiterpene lactone to the individual, thereby preventing or treating radiation-induced fibrosis in the individual.
54. A method according to claim 53, wherein the radiation-induced fibrosis is in the lung or bladder.
55. A method of minimising the effects of radiation-induced injury to a second organ, wherein the radiation is applied to a first organ, the method comprising administering a sesquiterpene lactone to the second organ, thereby minimising the effects of radiation-induced injury to the second organ.
56. A method according to claim 55, wherein the second organ is skin and the first organ is not skin.

57. A method according to claim 56, wherein the radiation-induced the skin injury is one or more selected from: erythema, altered sensation, itching, edema, blistering, desquamation, ulcer, necrosis, hair loss and onycholysis
58. A method according to any one of claims 55 to 57, wherein the radiation applied to the first organ is for the treatment of a cancer.
59. A method according to claim 58, wherein the cancer is a head and neck cancer.
60. A method according to any one of claims 55 to 59, wherein the sesquiterpene lactone is administered topically.
61. A method according to claim 60, wherein the sesquiterpene lactone is topically administered in a composition.
62. A method of preventing or treating radiation-induced skin injury in an individual, the method comprising administering a sesquiterpene lactone topically to the individual, thereby preventing or treating radiation-induced skin injury in the individual.
63. A method according to claim 62, wherein the sesquiterpene lactone is administered topically, preferably the sesquiterpene lactone is topically administered in a composition in a composition.
64. A method according to claim 62 or 63, wherein the skin injury is one or more selected from: erythema, altered sensation, itching, edema, blistering, desquamation, ulcer, necrosis, hair loss and onycholysis.
65. A method according to any one of claim 49 to 64, wherein the radiation exposure is a dose that, if untreated, would cause delayed effects of acute radiation exposure (DEARE) in the individual to any organ.
66. A method according to any one of claims 49 to 64, wherein the radiation exposure is a dose that, if untreated, would cause acute radiation syndrome (ARS) in the individual.
67. A kit comprising (a) a sesquiterpene lactone according to any one of claims 40 to 45 and (b) G-CSF or analog or derivative thereof, or a composition comprising G-CSF or analog or derivative thereof.