

Radiotherapeutic particles and suspensions**FIELD OF THE INVENTION**

The present invention relates to a particle or pharmaceutical composition comprising one, more particles or a suspension of same or different particles comprising a degradable compound and an alpha emitting radionuclide and/or a radionuclide generating alpha emitting daughter. The particles are beneficial for use in the treatment of cancer.

BACKGROUND OF THE INVENTION

Beta-emitting radiocolloids and microparticles were used for several years with some success against peritoneal ascites and microscopic tumor seeds. However, late effects and morbidity due to intestinal toxicity have made these treatments obsolete and chemotherapy has become the standard adjuvant therapy in *e.g.* ovarian cancer. There still exists a considerable medical need for new modalities against intracavitary cancer.

Alpha emitters have previously been proposed as a treatment for intraperitoneal cancer. Two types of chemical classes have been proposed, (1) radioimmunoconjugates and (2) micro- or nano-sized particular suspensions. The advantage with the radioimmunoconjugates is the potential for cell specific targeting and the disadvantage is the substantial leakage of product into the bloodstream causing potential systemic toxicity.

The advantage with micro/nano particles and colloids is the potential for improved local retention reducing distant toxicity. On the down side is the potential for inhomogenous dose deposition and radiation hot spots and also whether the particle itself can cause irritation because of inertness to degradation etc.

If microparticles and/or nanoparticles are to be used the choice is if they should be completely stable or slowly degradable.

By using completely stable particles the advantages include low risk of systemic toxicity. Disadvantages include potentially more heterogenous radiation dose distribution and some risk of local toxicity from "hot spots". Stable radiotherapeutic particles have been used for radioembolization using the high energetic beta emitter ⁹⁰Y stably labeled to non-degradable glass spheres (TheraSphere™) or resin based

spheres (SIR-Spheres™) for treating primary tumors and metastases to the liver. The liver tissue will in this instance shield against toxic radiation to intestines etc.

A second approach would be to use degradable particles slowly releasing some of the radionuclides: Possible advantages includes a more homogenous radiation dose distribution due to improved diffusion of mother nuclides and or short lived daughter nuclides and less tendency for "hot spots" causing local toxicity. Possible disadvantages include potential for systemic toxicity due to possible transport of released radionuclide into the blood and further redistribution. Degradable particles are mostly used for other cytotoxic compounds like chemotherapeutics and not for radionuclides at the moment.

Thus there is a need for an improved delivery system for alpha particle radiation against intracavitary cancers.

SUMMARY OF THE INVENTION

An object of the present invention relates to a particle comprising a degradable compound and an alpha emitting radionuclide and/or a radionuclide generating alpha emitting daughter.

In one embodiment of the present invention is the radionuclide selected from the group consisting of ^{224}Ra , ^{212}Bi , ^{212}Pb , ^{223}Ra , ^{225}Ra , ^{225}Ac , ^{213}Bi , ^{211}At , ^{227}Th .

In another embodiment of the present invention is the degradable compound selected from the group consisting of CaCO_3 , PEG modified CaCO_3 , protein modified CaCO_3 , carbohydrate modified CaCO_3 , lipid modified CaCO_3 , vitamin modified CaCO_3 , organic compound modified CaCO_3 , polymer modified CaCO_3 and/or inorganic crystal modified CaCO_3 .

In a further embodiment of the present invention is the size of the particle from 1 nm to 500 μm .

In another embodiment of the present invention, the particle comprises one or more compounds selected from the group consisting of a monoclonal antibody, a polyclonal antibody, a radioimmunoconjugate, an immunoconjugate, a chelate antibody conjugate, vitamins including folate and folate derivatives, peptides, minibodies, and affibodies.

In a further aspect of the present invention relates to a pharmaceutical composition comprising one or more particles according to the invention and a diluent, carrier, surfactant, and/or excipient.

In another embodiment of the present invention is the pharmaceutical composition prepared with an amount of radionuclide that is 1kBq to 10GBq per dosing.

In another embodiment of the present invention is the pharmaceutical composition prepared with an amount of radionuclide that is 50 MBq to 100 GBq suitable for multidose industrial scale production. For instance if 100 patient doses is produced in one batch per day this could be made up of a total of 1-10 GBq divided into 100 single dosing vials or ready to use syringes.

In another embodiment of the present invention is the pharmaceutical composition a particle suspension comprising monodisperse or polydisperse particles labeled with an alpha emitting radionuclide and/or a radionuclide generating alpha emitting daughter.

In another embodiment of the present invention is the pharmaceutical composition suitable for intravenous or intracavitary injection.

Another aspect of the present invention relates to a particle or pharmaceutical composition of the present invention for use as a medicament.

In an aspect of the present invention is the particle according to the present invention a medical device or is comprised in a medical device.

A further aspect of the present invention relates to a particle or pharmaceutical composition of the present invention for use in intracavitary therapy, radioembolization or radiosynovectomy.

Another aspect of the present invention relates to a particle or pharmaceutical composition of the present invention for use in the treatment of cancer.

In one embodiment of the present invention is the cancer selected from the group consisting of intraperitoneal cancers, intracranial cancers, pleural cancers, bladder cancers, cardiac cancers, and cancers in the subarachnoid cavity.

Another aspect of the present invention relates to a method of treatment or amelioration comprising administration of the particles or the pharmaceutical composition of the present invention to an individual in need thereof.

Another aspect of the present invention relates to a method for preparing a particle of the present invention, the method comprising bringing an alpha emitting radionuclide and a biodegradable compound in contact with each other with or without using a carrier for the radionuclide.

Another aspect of the present invention relates to a kit comprising a nano or micro particle according to the present invention, an alpha emitting radionuclide or a radionuclide generating an alpha emitting daughter, a carrier, diluent and/or excipient, and optionally instructions to use the kit.

Another aspect of the present invention relates to a kit comprising a nano or micro particles according to the present invention, an alpha emitting radionuclide or a radionuclide generating an alpha emitting daughter, a carrier, diluent and/or excipient, and optionally instructions to use the kit to prepare a bifunctional pharmaceutical solution comprising particles suspension and radioimmunoconjugate solution.

In one embodiment of the present invention, the kit comprises a chelator-conjugated molecule, including monoclonal antibody.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 Tissue distribution 20 hours, 4 days and 7 days after intraperitoneal injection of ^{224}Ra -labeled CaCO_3 microparticles (A) and dissolved $^{224}\text{RaCl}_2$ (B) in nude mice. The radioactivity measurements are performed minimum 3 days after sacrificing the animals, *i.e.* allowing time for daughter nuclides to be in equilibrium with ^{224}Ra .

Figure 2 Weight of intraperitoneal SKOV-3 tumors treated with saline, cold particles or ^{224}Ra -labeled calcium carbonate microparticles on day 44 and 45 after treatment start.

Figure 3 Survival of animals with intraperitoneal ES-2 ascites cancer treated with saline or ^{224}Ra -labeled calcium carbonate microparticles.

Figure 4: Bar graph illustrating the ^{224}Ra and daughter ^{212}Pb labeling efficiencies for CaCO_3 microparticles of 1.1 and 8.9 μm median size. The bars represent the mean value of 14 and 12 individual experiments for the small and large particles respectively, and the error bars represent the standard deviation.

Figure 5: Bar graph illustrating the percent of ^{224}Ra activity which is retained on CaCO_3 microparticles of 1.1 and 8.9 μm median size at different time points. The bars represent the mean values of 5 and 4 individual experiments for small and large particles respectively, and the error bars represent the standard deviation.

Figure 6: Survival of mice injected intraperitoneally with $1 \cdot 10^6$ ES-2 cells and treated 22 hours later with saline or ^{224}Ra -labeled CaCO_3 microparticles.

Figure 7: Effect of treatment with ^{224}Ra -labeled CaCO_3 microparticles on hematological parameters: white blood cells (WBC), red blood cells (RBC), and platelets (PLT) as a function of time after treatment start. From 3-5 mice in each group were sampled at each time point. The plot shows individual data points for each mouse together with the mean value for each group represented by a horizontal bar. The error bars correspond to the standard deviation.

Figure 8: Survival of mice with intraperitoneal ES-2 ovarian cancer ascites model treated with saline or ^{224}Ra -labeled CaCO_3 microparticles on different times after cell inoculation.

Figure 9: Survival of mice injected intraperitoneally with $1 \cdot 10^5$ ES-2 cells and treated 1 hour later with ^{224}Ra -labeled CaCO_3 microparticles of two different sizes compared to the saline control group.

Figure 10: Biodistribution presented as mean ^{224}Ra activity in Bq per gram tissue 20 hours, 4 days and 7 days after intraperitoneal injection of ^{224}Ra -labeled CaCO_3 microparticles (A) and free ^{224}Ra solution (B) in nude mice. Injected activity is normalized to 10 kBq/mouse. Error bars represent standard deviation.

Figure 11: Biodistribution of daughter ^{212}Pb presented as mean activity in Bq per gram tissue 20 hours, 4 days and 7 days after intraperitoneal injection of ^{224}Ra -labeled CaCO_3 microparticles (A) and free ^{224}Ra solution (B) in nude mice. Injected activity is normalized to 10 kBq/mouse. Error bars represent standard deviation.

Figure 12: Survival of mice injected intraperitoneally with $10 \cdot 10^6$ ES-2 cells and treated 25 hours later with saline or ^{224}Ra -labeled CaCO_3 microparticles.

DETAILED DESCRIPTION OF THE INVENTION

The present inventors have identified a treatment of cancer with less risk for intestinal toxicity based on short ranging alpha emitters.

The current invention is based on slowly degradable nano-or microparticles comprising an alpha emitting radionuclide and/or a radionuclide generating alpha emitting daughter, *e.g.* ^{224}Ra .

Thus, one object of the present invention relates to a particle comprising a degradable compound and an alpha emitting radionuclide and/or a radionuclide generating alpha emitting daughter.

An aspect of the present invention relates to a particle comprising; CaCO_3 , the alpha emitting radionuclide ^{224}Ra , and the ^{224}Ra radionuclide progeny selected from the group consisting of ^{220}Rn , ^{216}Po , ^{212}Pb , and ^{212}Bi . ^{220}Rn is a daughter radionuclide of ^{224}Ra , ^{216}Po is a granddaughter radionuclide of ^{224}Ra , and ^{212}Pb is a great granddaughter radionuclide of ^{224}Ra etc.

Another aspect of the present invention relates to a particle comprising; CaCO_3 , the alpha emitting radionuclide ^{224}Ra , and the ^{224}Ra radionuclide progeny selected from the group consisting of ^{220}Rn , ^{216}Po , and ^{212}Pb . ^{220}Rn is a daughter radionuclide of ^{224}Ra , ^{216}Po is a granddaughter radionuclide of ^{224}Ra , and ^{212}Pb is a great granddaughter radionuclide of ^{224}Ra .

Radionuclides

The radionuclides of the present invention can be any alpha emitting radionuclide and/or a radionuclide generating alpha emitting daughter.

The main advantages of alpha particle emitting compounds in local therapy in *e.g.*, the intraperitoneal cavity is the shorter range, typically less than 0.1 mm for alphas compared with mm to cm ranges for beta-particles from medical beta emitters like ^{90}Y , ^{131}I and ^{32}P .

Use of alpha-emitters would in an intracavitary setting reduce risk for toxicity due to irradiation of deeper regions of internal organs like the radiosensitive intestinal crypt

cells in the case of *i.p.* use. Also is the high linear energy transfer of the emitted alpha particles advantageous since very few alpha hits are needed to kill a cell and cellular resistance mechanism like high repair capacity for DNA strand breaks is less of a problem because of the high probability of producing irreparable double strand breaks (Ritter *et al.*, 1977).

The high effect per decay means less radioactivity is needed reducing the need for shielding of hospital staff and relatives since most alpha- and beta emitters also emits some X-rays and gammas which needs to be shielded against.

Table 1 shows the main radiation properties of ^{224}Ra . The complete decay of ^{224}Ra and daughters produce in total 4 alpha-particles. An important aspect is the fate of the ^{220}Rn as this nuclide potentially could diffuse away from the mother nuclide as it is potentially chemical inert to bonding in crystals.

This means that the primary decays of ^{224}Ra into ^{220}Rn (the daughter radionuclide), then into ^{216}Po (the granddaughter radionuclide), and subsequently into the longer lived ^{212}Pb (the great granddaughter radionuclide) which again decays into ^{212}Bi .

Progeny is understood as the radionuclides that are the result of the decay of a parent radionuclide. Thus, when ^{224}Ra is the parent radionuclide will ^{220}Rn (the daughter radionuclide), ^{216}Po (the granddaughter radionuclide), and ^{212}Pb (the great granddaughter radionuclide) and all radionuclides listed in table 1 to be considered progeny radionuclides.

Thus, in one embodiment is the alpha-emitting radionuclide ^{224}Ra with the daughter radionuclide ^{220}Rn , the granddaughter radionuclide ^{216}Po , and the great granddaughter radionuclide ^{212}Pb . For the particle of the present invention will these all be comprised by the particle when ^{224}Ra is the alpha-emitting radionuclide.

In an aspect of the present invention is the particle according to the present invention a medical device or is comprised in a medical device.

A medical device is any instrument, apparatus, appliance, software, material or other article, whether used alone or in combination, including the software intended by its manufacturer to be used specifically for diagnostic and/or therapeutic purposes and

necessary for its proper application, intended by the manufacturer to be used for human beings for the purpose of: Diagnosis, prevention, monitoring, treatment or alleviation of disease; Diagnosis, monitoring, treatment, alleviation of or compensation for an injury or handicap; Investigation, replacement or modification of the anatomy or of a physiological process; Control of conception; and which does not achieve its principal intended action in or on the human body by pharmacological, immunological or metabolic means, but which may be assisted in its function by such means

Medical devices vary according to their intended use and indications. Examples range from simple devices such as tongue depressors, medical thermometers, and disposable gloves to advanced devices such as computers which assist in the conduct of medical testing, implants, and prostheses.

According to the FDA is medical device "an instrument, apparatus, implement, machine, contrivance, implant, in vitro reagent, or other similar or related article, including a component part, or accessory which is: recognized in the official National Formulary, or the United States Pharmacopoeia, or any supplement to them, intended for use in the diagnosis of disease or other conditions, or in the cure, mitigation, treatment, or prevention of disease, in man or other animals, or intended to affect the structure or any function of the body of man or other animals, and which does not achieve any of its primary intended purposes through chemical action within or on the body of man or other animals and which is not dependent upon being metabolized for the achievement of any of its primary intended purposes."

The present particles are not being metabolized nor do they have significant chemical action within the body. The particles are carriers of radioactivity that are designed not be metabolized or have any chemical action within the body, and this allows for radiotherapy with very limited unwanted side-effects, such as toxicity.

Thus, in one embodiment is the term "medical device" understood as FDAs definition above.

Table 1. Main radiation properties from the ^{224}Ra series.

Radionuclide (half life)	Alphas and betas (mean energy in MeV)	X-rays and gammas Energy and % abundance
^{224}Ra (3.6 days)	α 5.6	241 keV, 4.1%
^{220}Rn (55.6 s)	α 6.3	
^{216}Po (145 ms)	α 6.8	
^{212}Pb (10.6 h)	β 0.1	75 keV, 10.3% 77 keV, 17.1% 87 keV, 6.0% 90 keV, 1.5% 239 keV, 43.6% 300 keV, 3.3%
^{212}Bi (1.0 h)	α 6.1×0.36 (2.2 effective ¹) β 0.7×0.64 (0.4 effective)	727 keV, 6.7% (4.3% effective)
^{212}Po (299 ns) (64% branch)	α 8.8 (5.6 effective)	
^{208}Tl (3.1 min) (36% branch)	β 0.6 (0.2 effective)	75 keV, 3.4% (1.2% effective) 511 keV, 22.6% (8.1% effective) 583 keV, 85.0% (30.6% effective) 860 keV, 12.5% (4.5% effective) 2615 keV, 99.8% (35.9% effective)

¹Average per ^{224}Ra transformation due to branching. Only X-rays or gammas above 1% effective abundance accounted for. Adds up to a total effective energy of approximately 26.5 MeV of alpha of 0.7 MeV of beta per complete decay of ^{224}Ra and daughters.

Radium-224 is one preferred alpha-emitter, but others can also be applied to the present invention.

Thus, in one embodiment of the present invention is the radionuclide selected from the group consisting of ^{224}Ra , ^{212}Bi , ^{212}Pb , ^{223}Ra , ^{225}Ra , ^{225}Ac , ^{213}Bi , ^{211}At , ^{227}Th .

A very advantageous finding in the examples was that the amount of radioactivity needed for producing significant therapeutic effects was as low as 100 kBq per kg of body weight, - which is equivalent with only 2-2.5 kBq per mouse. This compares

favorably to the several hundred kBq per mouse of ^{211}At and ^{212}Pb needed in alpha-radioimmunotherapy against experimental peritoneal cancer in mice (Gustafsson *et al.*, 2012; Boudousq *et al.*, 2013). This property could strongly reduce exposure problems from X-rays and gammas during administration and use of the particles of the present invention, exemplified by $^{224}\text{Ra}-\text{CaCO}_3$.

The amount of ^{224}Ra used per patient dosage may be in the range of 1 kBq to 10 GBq more preferably 100 kBq to 100 MBq, event more preferably range is 0.5 MBq to 25 MBq.

Dosage will depend on the cancer type, and for example how aggressive the disease is. In one embodiment is the dosage 10-100 kBq/kg, such as 20-50 kBq/kg. In another embodiment is the dosage 10-1000 kBq/kg, such as 25-300 kBq/kg. In a further embodiment the is the dosage 100-500 kBq/kg, such as 150-300 kBq/kg

In one embodiment of the present invention is the pharmaceutical composition prepared with an amount of radionuclide that is 1 kBq to 10 GBq per dosing.

For instance, if 100 patient doses is produced in one batch per day this could be made up of a total of 1-10 GBq divided into 100 single dosing vials or ready to use syringes.

In another embodiment of the present invention is the pharmaceutical composition prepared with an amount of radionuclide that is suitable for multidose industrial scale production e.g., 50 MBq to 100 GBq.

Degradable compound

The degradable compound of the present invention can be any compound that can be degraded.

The degradation can be done by any route selected from the group consisting of high pH, low pH, proteases, enzymes, nucleases and/or by cellular processes like endocytosis, which also includes phagocytosis.

In one embodiment of the present invention is the degradable compound selected from the group consisting of CaCO_3 , PEG modified CaCO_3 , protein modified CaCO_3 , carbohydrate modified CaCO_3 , lipid modified CaCO_3 , vitamin modified CaCO_3 , organic

compound modified CaCO_3 , polymer modified CaCO_3 and/or inorganic crystal modified CaCO_3 .

In a preferred embodiment of the present invention is the degradable compound CaCO_3 (CC).

Calcium carbonate (CC) particles may be used as composites with other salts or proteins or peptides and subject to surface modification by surfactants like oleates and similar.

In a special embodiment is CC used with a compound selected from the group consisting of poly ethylene glycol modified particles of calcium carbonate or inorganic crystal modified CC.

In a special embodiment the CC particles is modified with functional receptor and or antigen binding groups, including monoclonal antibodies and derivatives and vitamins and derivatives allowing receptor or antigen binding of particle to individual target cells and diseased tissues. This means that modifications of the particles relate to the addition of compounds to CC. This can be done in various ways, and through interactions such as dipole-dipole interactions, ion-dipole and ion-induced dipole forces, hydrogen bonding, Van der Waals forces, and relative strength of forces.

When ^{224}Ra solution in equilibrium with daughter nuclides is used for labeling of particles a special embodiment is to firstly add to the solution a chelator for ^{212}Pb before contacting CC particles, thus creating a bifunctional radiotherapeutic mixture. The chelator is preferentially conjugated to a target affinic molecule, e.g., monoclonal or polyclonal antibody or derivatives of antibody, vitamins or derivatives of vitamins.

Characteristics

The particles can have a variety of characteristics.

The size of the particles can vary depending on the intended uses and applications.

The type of crystals may be any known form of CC and sizes varying from 1 nm to 500 μm may be used. More preferently the size is in the range of 100 nm to 50 μm and further preferably is size in the range of 1-10 μm .

In one preferred embodiment is the size 1-10 μm .

In one embodiment of the present invention is the size of the particle from 1 nm to 500 μm .

In mice, based on the peritoneal surfaces the amount of CC-particles should be in the range of 0.1 mg to 50 mg more beneficial probably 1 mg to 15 mg. In humans the amounts used should be multiplied by 10 to 10 000 compared with mice probably more beneficial with 0.1 – 10 g for *e.g.*, intraperitoneal therapy. For other cavities the amounts may be adjusted according to relative surface area or to the volume of fluid present.

In the examples of the current invention it was found that ^{224}Ra could be used for radiolabeling of degradable calcium carbonate. Calcium carbonate has about 14 % lower density than calcium hydroxyapatite and may be easier to keep in suspension without sedimenting vs. calcium hydroxyapatite particles of same size. Calcium carbonate was used as main ingredient with or without the addition of small amounts of co-precipitate *e.g.*, barium sulphate, as carrier for the ^{224}Ra .

Thus, in one embodiment are co-precipitate added. These are selected from the group consisting of barium sulphate, strontium sulphate, and barium chromate. The amount ranging typically from 0.01% to 10% vs. calcium carbonate, and preferably 0.1-1% vs. calcium carbonate.

The total amount of calcium carbonate in the particle, before addition of radionuclide can vary depending on whether for example a co-precipitate is added. In an embodiment is the amount of calcium carbonate more than 90%. The range can be 90-95%, or 90-99%. The amount can also be more than 98 or more than 99%.

Additional compounds in the particle

The degradable particle can comprise many different additional compounds. These can serve various purposes included targeting, stability, solubility and rate of degradation.

In one embodiment of the present invention, the particle comprises one or more compounds selected from the group consisting of a monoclonal antibody, a polyclonal

antibody, a radioimmunoconjugate, an immunoconjugate, a chelate antibody conjugate, vitamins including folate and folate derivatives, peptides, minibodies, and affibodies.

In one embodiment of the present invention the antibody is selected from one or more of the group consisting of trastuzumab, rituximab, HH1, cetuximab, bevacizumab, daratumumab, alemtuzumab, Pembrolizumab, Epratuzumab, L19, F8, F16, Galiximab, Toralizumab, Alemtuzumab, Ofatumumab, Veltuzumab, Afutuzumab, Tositumomab, Reditux and Ibritumomab.

In another embodiment of the present invention the compound is specific for a target selected from the group consisting of CD19, CD20, CD22, CD33, CD37, CD38, CD45, CD74, CD138, PSMA, HER-2, EGFR, MUC-1, MUC-18, CEA, FBP, NG2, EPCAM, Syndecan-1, Ca-125, LK-26, HMFG, CS-1, and BCMA.

In a special embodiment a pharmaceutical suspension of ^{224}Ra -labeled includes a ^{212}Pb -labeled antibody, antibody fragment or protein or peptide or vitamin derivative (targeting conjugate) with affinity for receptors including antigens on the tumor cells whereby the ^{224}Ra -labeled particles will give a general alpha particle radiation field on the intraperitoneal surfaces including on the surfaces of intraperitoneal organs, and the ^{212}Pb -labeled antibody or similar gives a specific alpha particle dose to the tumor cells by receptor or antigen binding.

The radionuclides in the present invention can be conjugated to a targeting molecule by using bifunctional chelators.

These could be cyclic, linear or branched chelators. Particular reference may be made to the polyaminopolyacid chelators which comprise a linear, cyclic or branched polyazaalkane backbone with acidic (e.g. carboxyalkyl) groups attached at backbone nitrogens.

Examples of suitable chelators include DOTA derivatives such as p-isothiocyanatobenzyl-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (p-SCN-Bz-DOTA) and the tetra primary amide variant of this DOTA compound, termed TCMC, and DTPA derivatives such as p-isothiocyanatobenzyl-diethylenetriaminepenta-

acetic acid (p-SCN-Bz-DTPA), the first being cyclic chelators, the latter linear chelators.

Metallation of the complexing moiety may be performed before or after conjugation of the complexing moiety to the targeting moiety.

The radiolabeling procedure will in general be more convenient in terms of time used etc if the chelator is conjugated to the antibody before the radiolabeling takes place. The principles of preparing radiolabeled conjugates using chelators attached to antibodies are described broader in *e.g.* Liu, 2008.

Pharmaceutical composition and compositions

An aspect relates to a composition comprising a particle according to the present invention. The composition may be a particle suspension comprising monodisperse or polydisperse particles labeled with ^{224}Ra and/or a radionuclide progeny.

The composition is preferably an aqueous composition.

In a further aspect of the present invention relates to a composition or a pharmaceutical composition comprising one or more particles according to the invention and a diluent, carrier, surfactant, deflocculant and/or excipient.

Acceptable carriers and pharmaceutical carriers include but are not limited to non-toxic buffers, fillers, isotonic solutions, solvents and co-solvents, anti-microbial preservatives, anti-oxidants, wetting agents, antifoaming agents and thickening agents etc. More specifically, the pharmaceutical carrier can be but are not limited to normal saline (0.9 %), half-normal saline, Ringer's lactate, dissolved sucrose, dextrose, *e.g.* 3.3 % Dextrose/0.3 % Saline. The physiologically acceptable carrier can contain a radiolytic stabilizer, *e.g.* ascorbic acid, human serum albumin, which protect the integrity of the radiopharmaceutical during storage and shipment.

The pharmaceutical compositions can comprise a multitude of particles. These can be the same or different.

Thus, in another embodiment of the present invention is the pharmaceutical composition a particle suspension comprising monodisperse or polydisperse particles

labeled with an alpha emitting radionuclide and/or a radionuclide generating alpha emitting daughter.

Administration

In another embodiment of the present invention is the pharmaceutical composition suitable for intravenous, intratumor or intracavitary injection.

Applications

The use of alpha emitting microparticles against *i.p.* cancers have been suggested previously. Archer *et al* (US 4970062 A) suggested to use ferric hydroxide colloid as carrier for alpha emitters, with emphasis of ^{212}Pb but listing several other potential useful alpha-emitters including ^{224}Ra . Bloomer *et al* (1981) suggested to use ^{211}At labeled tellurium colloid, while Vergote *et al* (1992) suggested to use ^{211}At -labeled monodisperse polymer particles. Larsen and Salberg (US 8142758 B2) suggested to use hydroxyapatite particles labeled with ^{223}Ra or other alpha emitters, including ^{224}Ra . A problem with these are in the case of Archer *et al.*, that hydroxide may not be good for preparing radium labeled particle since hydroxide of alkaline earth and particularly radium has a relatively high solubility in water (Kirby *et al.*, 1964).

Astatine-211 tellurium colloid was found to be unstable causing exposure to thyroidea (Vergote *et al.*, 1992) and that ^{211}At -labeled polymer particles are not biodegradable and because of short half-life and limited existing production capacity for ^{211}At would be expensive and impractical in large scale clinical use. Also because of the chemical inertness and low complexability of cationic radium the use of tellurium colloids or polymer particles was not considered as carrier for radium. The use of hydroxyapatite as carrier for radium gives a good labeling yield but the calcium hydroxyapatite has a high density which could cause a more rapid sedimentation and less ideal dose distribution of the radiation when used in cavitary therapy as microparticulate suspension.

The testing and research related to the novel particles, exemplified by ^{224}Ra -labeled calcium carbonate (CC) particles presented herein had some unexpected findings: It was possible to obtain high labeling yield and relevant stability of the product *in vitro*, good *i.p.* retention compatible with ^{224}Ra half-life, slow release of ^{224}Ra *in vivo*, good tolerance for particles in mice and significant antitumor activity in tumor models in mice. A particularly interesting and unexpected finding was the good uptake in *i.p.* fat

which is of importance since *i.p.* fat including omentum is ground for metastatic tumor growth (Gerber *et al.*, 2006). One would assume a more lipophilic structure would be required for *i.p.* fat uptake it was thus a surprise that the calcium carbonate particles used herein would show such a substantial uptake.

Another aspect of the present invention relates to a particle or pharmaceutical composition of the present invention for use as a medicament.

The particles and compositions of the present invention can be used as radiotherapeutic compounds and/or radiotherapeutic mixtures.

Medical uses of the particles of the present invention includes human or veterinary use in (1) Intracavitary therapy (2) radioembolization (3) radiosynovectomy.

Intracavitary therapy may include treatment of *e.g.*, intraperitoneal cancers, intracranial cancers, pleural cancers, bladder cancers, cardiac cancers, cancers in the subarachnoid cavity. Examples of cavities where the particles may be used is cranial cavity, thoracic cavity, lung cavity, spinal cavity, pelvic cavity, pericardium, pleural cavity, bladder cavity or a combination of these including cancers spreading on the peritoneum or meninges and organs within any of these cavities.

In a special embodiment for the use of the particles of the present is treatment or amelioration of an intracavitary disease which is an infection or inflammation rather than or in combination with cancer.

In one embodiment of the present invention is the infection selected from the group consisting of a bacterial infection and viral infection.

Radioembolization may include treatment of primary or metastatic cancer in an organ *e.g.*, the liver by administering the particles of the present invention to a blood vessel leading to a tumor in the liver or another solid organ infiltrated by tumor tissue.

Radiosynovectomy for joint disorders including chronic inflammations is targeted radiation treatment for painful joint diseases using radioactive substances. Its use includes treatment of hemophilic arthritis.

Today it is based on beta-particle emitting compounds used for inflammatory or rheumatoid diseases, or synovial arthrosis of various joints, in particular of the knee, hand and ankle. The ^{224}Ra -CC particles described herein which are degradable could be very useful in radiosynovectomy.

The particles are preferably administered by local injection, *e.g.* intracavitary.

In a special embodiment the particles are injected directly into a tumor.

The articles may be dispersed in various buffers compatible with medical injections, *e.g.*, dissolved salts and/or proteins and/or lipids and or sugars.

A further aspect of the present invention relates to a particle or pharmaceutical composition of the present invention for use in intracavitary therapy, radioembolization or radiosynovectomy.

Another aspect of the present invention relates to a particle or pharmaceutical composition of the present invention for use in the treatment of cancer.

In one embodiment of the present invention is the cancer selected from the group consisting of intreaperitoneal cancers, intracranial cancers, pleural cancers, bladder cancers, cardiac cancers, and cancers in the subarachnoid cavity.

In one embodiment of the present invention is the cancer selected from the group consisting of metastatic cancer, lung cancer, ovarian cancer, colorectal cancer, stomach cancer, pancreatic cancer, breast cancer, neoplastic meningitis, peritoneal cancer, pleural effusion, malignant mesothelioma, breast cancer, sarcomas, brain cancers like glioblastoma and astrocytoma, bladder cancer, and liver cancer.

Another aspect of the present invention relates to a method of treatment or amelioration comprising administration of the particles or the pharmaceutical composition of the present invention to an individual in need thereof.

Methods for preparations and kits

Another aspect of the present invention relates to a method for preparing a particle of the present invention, the method comprising bringing an alpha emitting radionuclide

and a biodegradable compound in contact with each other with or without using a carrier for the radionuclide.

A solution or composition comprising an alpha emitter, *i.e.* a ^{224}Ra solution or composition with progeny ^{212}Pb in mixture could be pretreated with chelate-antibody conjugate to complex ^{212}Pb prior to particle labeling to produce a two-component therapeutic system containing a radioimmunoconjugate for ^{212}Pb antigen-specific treatment and alpha emitter, *e.g.* ^{224}Ra -particles for a general cavity treatment.

An embodiment relates to a two-component system or kit comprising a radioimmunoconjugate for ^{212}Pb antigen-specific treatment, and a particle according to the present invention.

The preferable way to use this would be by a kit containing a vial A with chelate-conjugated antibody and a vial B with alpha emitter, *e.g.* ^{224}Ra in equilibrium with daughter nuclides, and a vial C with microparticles, whereby the content of A is added to vial B, or *vice versa*, and incubated from a few minutes to a few hours before the mixture is transferred to vial C for further incubation for a few minutes to a few hours before transferring to a syringe and injected into the patient.

This principle could significantly reduce the level of ^{212}Pb -radioimmunoconjugate needed for therapy since ^{224}Ra -CC-particles is expected to contribute strongly to the antitumor activity in such a system.

Another aspect of the present invention relates to a kit comprising a nano or micro particle according to the present invention, an alpha emitting radionuclide or a radionuclide generating an alpha emitting daughter, a carrier, diluent and/or excipient, and optionally instructions to use the kit.

Another aspect of the present invention relates to a kit comprising a composition comprising CaCO_3 , a composition comprising the alpha emitting radionuclide ^{224}Ra , and optionally instructions to use the kit.

Another aspect of the present invention relates to a kit comprising a nano or micro particle according to the present invention, an alpha emitting radionuclide or a radionuclide generating an alpha emitting daughter, a carrier, diluent and/or

excipient, and optionally instructions to use the kit to prepare a bifunctional pharmaceutical solution comprising particles suspension and radioimmunoconjugate solution.

In one embodiment of the present invention, the kit comprises a chelator-conjugated molecule, including monoclonal antibody.

The current methods and product allow for centralized production and shipment to the end user since the radionuclide has several days half-life. Another aspect of the presented invention is the use of a biodegradable particle that slowly dissolves into calcium and carbonate thereby producing small amounts of products that are already abundantly present in the body. It is also noteworthy of the following feature: When alpha emitter, *e.g.* ^{224}Ra is absorbed on the surface of the calcium carbonate particles, there is a significant release of short living ^{220}Rn ($t_{1/2} = 56 \text{ s}$) which will together with the ultra-short lived ^{216}Po ($t_{1/2} = 0.16 \text{ s}$) produce two alpha particles before decaying to the longer lived beta emitter ^{212}Pb ($t_{1/2} = 10.6 \text{ h}$). Lead has a very high precipitability with calcium carbonate so the ^{212}Pb in the *i.p.* fluid will tend to re-associate to the particles diminishing leakage of ^{212}Pb into the systemic circulation.

It is therefore a very special technical feature that ^{224}Ra decays into a gas that can diffuse out of the particle and afterwards decay further into ^{212}Pb that precipitates with calcium carbonate.

It may be of benefit that the ^{220}Rn , if released from micro particles, is highly lipophilic as *e.g.*, intraperitoneal cancer to a significant degree tends to grow in the omentum, a large fatty pad of tissue that drapes over the intestines in the abdomen (Gerber *et al.*, 2006).

Pre-produced particles and subsequent surface sedimentation or radionuclide co-sedimentation for deeper inclusion of radionuclide are two methods useful for producing a therapeutic product. The first method will allow some release of daughter nuclide ^{220}Rn which could reduce dose inhomogeneity from inhomogenous particle distribution. Because of the short half-life (56 s.) of ^{220}Rn it will not significantly redistribute from the cavity and not diffuse into deeper layers of the tissue surfaces. Also, the amount of radionuclides is too small to cause any significant physical or chemical effects, *e.g.*, gas pressure, from radon production in the cavity. To some

extent it would be beneficial to use larger amounts of particles *i.e.*, a reduced specific activity to improve surface distribution of the radionuclides in the ^{224}Ra series.

A bifunctional suspension can be made *e.g.*, by the following a ^{224}Ra solution in pH 5-6 buffer is added TCMC-labeled antibody to 1 mg/ml and incubated from 2 minutes to several hours whereafter the solution is added to a vial with calcium carbonate (CC) particles and incubated for 2 minutes to several hours. The mixture should be administered as soon as possible to avoid reduction of the specific activity of the ^{212}Pb -labeled product. This will probably best be used as a kit system whereby ^{224}Ra is in vial A, the chelator conjugated protein is in vial B and CC particles are in vial C.

It may also be possible to add ^{212}Pb to give an extra strength targeting conjugate in the mixture with ^{224}Ra -CC particles. Usually, the ratio between ^{224}Ra and ^{212}Pb in such a system may be close to 1:1 but in some treatment situations it may be beneficial to increase the amount of ^{212}Pb -conjugate vs. ^{224}Ra particles to as much as 10:1 or higher. In the last case it would be required to either add extra ^{212}Pb before preparation of the targeting conjugate or withdraw some of the ^{224}Ra -CC particles before the administration of the therapeutic mixture.

The present invention relates to novel radiotherapeutic compounds based on alpha-emitters like ^{224}Ra with daughter radionuclides. Radium-224 is absorbed onto surfaces of calcium carbonate particles or can be co-sedimented during preparation using carriers *e.g.*, traces of barium sulphate.

In a special embodiment the ^{224}Ra may be co-crystallized with calcium to form carbonate crystals whereby the ^{224}Ra is inside the crystals and not on the surface to avoid escape of daughter nuclides.

However, in some settings, a partial slow release of radionuclides may be beneficial as this may effect a better dose homogeneity, *e.g.*, at the surfaces of peritoneum, and the diminishing of radiation "hot spots" from local aggregates of crystal particles.

The radiation range of the major dose component of ^{224}Ra series, the alpha particles, is typically less than 0.1 mm in tissue allowing the delivery of therapeutically relevant radiation dose levels to the surfaces of the peritoneum and the organs present in the cavity without causing damage to deeper regions of the tissues and peritoneum. It is

known from older studies that beta-emitting colloids and particles can show some antitumor activity when used as adjuvant in intraperitoneal therapy, but late effects due to radiation of intestines etc. have made these products cost-benefit ratio unfavorable.

The main reason for the side effects is the penetration of radiation in to deeper regions of the intestines due to radiation ranges of several mm. By switching to alpha emitters the problem of irradiating deep below tissue surfaces can be avoided. Another aspect in favor of alpha particles is the high linear energy transfer of the alphas causing a high fraction of lethal double strand breaks in the cells and reducing the effect of oxygen status for cell to survive the treatment. Also the relative biological effectiveness is usually considerably higher for alphas vs. betas.

The current invention is different from previous described alpha-emitting colloids in several ways, (1) it has a slow release of ^{224}Ra and the daughter nuclide, which may have a dose "smoothing" effect reducing the problems of inhomogeneous distribution of alpha particles in the area of administration. (2) The ^{212}Pb , which is the longer lived daughter ($t_{1/2} = 10.6 \text{ h.}$) following the decay of the short lived ^{220}Rn ($t_{1/2} = 56 \text{ s.}$) and ^{216}Po ($t_{1/2} = 0.15 \text{ s.}$) decay, is easily reabsorbed by the tested particles, which could reduce the leakage of ^{212}Pb into systemic circulation. Thus it was found that calcium carbonate particles are particularly suitable as carrier for ^{224}Ra . (3) the particle material itself is non-toxic at the levels used and the particles are slowly degradable to non-toxic ions, thereby highly biocompatible.

The particles may be produced in sizes from nanometers to several tens of micrometers and radiolabeled with high labeling yields and can be stored for several days which is important since it allows centralized production and shipment to the hospitals of ready to use particle suspensions. Several different classes of CC crystals may be used including hexagonal $\beta\text{-CaCO}_3$, orthorhombic $\lambda\text{-CaCO}_3$.

General

It should be understood that any feature and/or aspect discussed above in connections with the compounds and particles according to the invention apply by analogy to the methods and applications described herein.

The following figures and examples are provided below to illustrate the present invention. They are intended to be illustrative and are not to be construed as limiting in any way.

EXAMPLES

Example 1 Production of ^{224}Ra

All work with the concentrated radioactive preparations including evaporation of solvent etc was performed in a glove-box. A source of ^{228}Th in 1 M HNO_3 was acquired from a commercial supplier. Ac-resin was obtained from Eichrom Technologies LLC (Lisle, IL, USA) in the form of a pre-packed cartridge.

To use smaller volume of solvent, about thirty percent of the materials in a cartridge (Cartridge 1) was extracted and repacked in a smaller column (Cartridge 2) made by a 1 ml filtration column (Isolute SPE, Biotage AB, Uppsala, Sweden). A slurry representing 20% of the original cartridge content was used for immobilizing of ^{228}Th in 500 microliter 1 M HNO_3 which was added 500 microliter of 1 M HCl and incubated by shaking the vial (4 ml vial, E-C sample, Wheaton, Millville, NJ, USA) for at least 4 hours. Cartridge 2 was added a small amount (about 0.1 ml) of the Ac-resin. Thereafter, the slurry was added to cartridge 2 using the prefilled material as a catcher layer. Radium could be eluted from the Cartridge 2 in 2 ml of 1 M HCl . The 2 ml radium solution was evaporated to dryness, using a heater block and flushing the vial with N_2 gas through a Teflon tube inlet and outlet in the rubber/Teflon septum on the vial and by leading the acid vapor into a beaker of saturated NaOH by a stream of N_2 -gas.

The residue was resolved in 0.5 ml 1 M HNO_3 and loaded onto a cartridge 3 consisting of a 1 ml Isolute column packed with about 250 mg Dowex anion exchanger. Cartridge 3 was washed with 7 ml 1 M HNO_3 , which removed ^{212}Pb , and finally with 3-4 ml 8 M HNO_3 to elute ^{224}Ra . The ^{224}Ra eluate was evaporated to dryness, using the heater block and a flow of N_2 -gas, and the residue could be dissolved in 0.1 M HCl . Typically, more than 70% of the ^{224}Ra present in the ^{228}Th source could be extracted and purified using the described methods.

Later the anion exchange step was abandoned and the 2 ml crude 1 M HCl was used without evaporation and loaded onto a second Ac resin cartridge which was washed with additional 0.5 ml HCl to produce an eluate of 2.5 ml containing the ^{224}Ra . This was evaporated into dryness and dissolved in 0.2 ml or more of 0.1 M HCl . Before used in labeling of particles the ^{224}Ra solution was added an amount corresponding to 10% of the volume with 5 M ammonium acetate to adjust the pH to 5-6.

Example 2 Measurement of radioactive samples

Radioactive samples were counted on a Cobra II Autogamma counter (Packard Instruments, Downer Grove, IL, USA) or a Hidex Automatic Gamma Counter (Hidex, Turku, Finland). During extraction of ^{224}Ra from the ^{228}Th source, a CRC-25R dose calibrator (Capintec Inc., Ramsey, NJ, USA) was used.

To determine distribution of ^{224}Ra , ^{212}Pb and ^{212}Bi in real time in samples, a liquid nitrogen cooled high purity germanium (HPGe) detector (GWC6021, Canberra Industries, Meriden CT, USA) was used. This was combined with a DSA 1000 digital signal analyzer and the Genie 2000 software (Canberra).

Example 3 Preparation of microparticles

Calcium carbonate microparticles were prepared by a spontaneous precipitation method. A 0.33 M Na_2CO_3 (Merck, Germany) solution was rapidly poured into an equal volume of 0.33 M CaCl_2 (Merck, Germany). After intense vortexing for 30 seconds, the particle suspension was left for 5 minutes. The particles were filtered off on a filter paper, washed with approximately 30 ml water and dried overnight at room temperature. The filtration and washing was performed in a glass vacuum filtration device (Whatman) with a 0.45 μm nitrocellulose filter (Whatman). Dry microparticles were stored at room temperature. The obtained microparticles were spherical in shape with diameters within 1-10 μm and median 3-5 μm as indicated by microscopy supported by analysis in a Countess™ Automated Cell Counter (Invitrogen).

Example 4 Radiolabeling of microparticles

A desired amount of CaCO_3 -particles were transferred to an Eppendorf tube and suspended in 1 ml of water. The particle suspension was sonicated in an ultrasound bath for 10-15 minutes, followed by 4 washing steps; first 2 times with 1 ml of water and then 2 times with 1 ml 0.1M Na_2SO_4 (Alfa Aesar, Germany). Particles were separated from the washing solution by centrifugation. After washing, the particles were suspended in DPBS (Gibco, Life Technologies, Carlsbad, CA, USA) supplemented with 0.5% Bovine Serum Albumin (0.1 ml per 15 mg of particles) and incubated on a HulaMixer (Invitrogen, Life Technologies, Carlsbad, CA, USA) for 30 minutes at room temperature. The mixing program was as follows: the orbital range of rotation was 14 rpm, the reciprocal range was 20° and the vibration range was 3°. A volume of a 0.1M Na_2SO_4 solution corresponding to 3 μg SO_4 per mg of particles (0.3%) was added to

the particle suspension. Further, ^{224}Ra -solution was transferred to the tube with the particle suspension, immediately followed by adding 0.07 M $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$ (Merck, Germany) solution corresponding to 3 μg Ba per mg of particles (0.3%). Between addition of the different solutions, the particle suspension was thoroughly mixed on a vortex mixer. If the volume to be added of the radioactive and/or $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$ solution exceeded 10 μl , it was added stepwise (5-10 μl at a time, with thorough vortexing in between). The total radiolabeling volume equaled 0.1 ml solution per 15 mg of particles, *i.e.* the volume of supernatant removed before adding SO_4 -solution was adjusted according to the volumes of the other solutions to be added. Particles in radiolabeling solution were incubated on a HulaMixer for minimum 1 hour and 30 minutes at room temperature, with the same mixing program as previously described. Finally, the particles were washed from 1-3 times with sucrose buffer. The sucrose buffer contained 94 mg/ml sucrose (Sigma Ultra, St. Louis, MO, USA) and 2.1 mg/ml Na_2SO_4 . Labeling efficiency was determined by measuring the particles and washing solution(s) with the HPGe detector.

Results: For eight individual experiments, with particles from three different particle batches, the labeling yields were as follows: ^{212}Pb $96.5 \pm 1.9 \%$, ^{212}Bi $96.7 \pm 2.1\%$, ^{224}Ra $95.5 \pm 3.2\%$ (Mean $\pm$ SD). The results show that ^{224}Ra with daughter nuclides are effectively absorbed by the microparticles. Calcium carbonate particles that were stored in powder form at room temperature for 2 months absorbed ^{224}Ra and its daughter nuclides with similar efficiency as freshly prepared particles.

Example 5 In vitro stability of radiolabeled microparticles

The *in vitro* stability of radiolabeled microparticles, prepared as described in Example 4, were studied in 2 different solutions. Particles were incubated in either 1-1.4 ml sucrose buffer at room temperature or 0.5 ml fetal calf serum at 37°C. At different time points, the suspensions were centrifuged and activities in the supernatant and pelleted particles were measured. Afterwards, if the stability study was to be continued to a later time point, the particle pellet was resuspended in a new aliquot of either sucrose buffer or fetal calf serum and incubated further.

Table 2. Retention of ^{224}Ra by calcium carbonate particles *in vitro*.

Solution	Time point	% released activity
		^{224}Ra

Fetal calf serum	22 hours	$4.13 \pm 3.01 \%$
	3 days	$1.18 \pm 0.69 \%$
	7 days	$1.76 \pm 0.34 \%$
Sucrose buffer	16 hours	1.07 %
	3 days	$1.70 \pm 1.81 \%$

The data shows that ^{224}Ra is well retained on the calcium carbonate particles for several days *in vitro* indicating promising properties for radiotherapeutic use. It also suggest that the product may have a shelf life of several days allowing centralized production and shipment to distant end users.

Example 6 Re-absorption/association of ^{212}Pb onto microparticles

CaCO_3 microparticles were prepared as described for the radiolabeling procedure, except no radioactive solution was added. Instead, the particles were incubated in a mix of 450 μl fetal calf serum and 50 μl ^{224}Ra -solution (pre-heated to 37°C), in order to measure the amount of ^{212}Pb that absorbed onto "cold" microparticles prepared under the same conditions as for radiolabeling. The particle suspension was incubated at 37°C with a rotation rate of 800 rpm. After 10 minutes, the particle suspension was spun down, 250 μl of the supernatant was transferred to an Eppendorf tube and the activity was measured. Afterwards, the particles were resuspended in the supernatant, and the study was extended with measurements after 1 hour and 24 hours. Table 3 presents the results of the study.

Table 3. Absorption of ^{212}Pb from solutions to calcium carbonate particles.

Time	% of total ^{212}Pb activity measured in supernatant
	Canberra germanium detector
0 minutes	100 %
10 minutes	25.4 %
1 hour	17.9 %
24 hours	29.0 %

The data shows that ^{212}Pb in the medium is significantly absorbed from the medium indicated that ^{220}Rn diffusion in the microenvironment of the calcium carbonate particles may be followed by a significant reabsorption of the daughter product ^{212}Pb . This could reduce systemic toxicity from the uptake of ^{212}Pb into the blood.

Example 7 In vivo biodistribution and stability of radiolabeled microparticles.

Background: To evaluate the usefulness of ^{224}Ra labeled calcium carbonate particles for intracavitary use a particle suspension were injected intraperitoneally in mice and the subsequent biodistribution of ^{224}Ra was measured. Methods: Radiolabeled microparticles were prepared as described in example 4. After washing, the particle pellet was resuspended in sucrose buffer at pH 7-7.5 to a particle concentration of approximately 13 mg/ml. Institutionally bred, 6-19 weeks old female Athymic Nude-*Foxn1*^{nu} mice with body weights of 17.1-28.3 g were used for the biodistribution studies. They were administered 0.4 ml particle suspension by intraperitoneal injection, containing 11-18 kBq ^{224}Ra bound to approximately 5 mg microparticles. The mice were sacrificed and different tissues harvested for radioactivity measurements 20 hours (n=2), 4 days (n=3) and 7 days (n=3) after injection. As a control, biodistribution experiments with free ^{224}Ra (dissolved RaCl_2) were performed, by administering 0.25 ml of 0.9% NaCl solution with approximately 12kBq ^{224}Ra intraperitoneally to each mouse. The $^{224}\text{RaCl}_2$ -solution had a pH of 5.5. For comparison, groups of 3 mice were sacrificed at the same time points after injection as for the biodistribution study with radiolabeled microparticles (Figure 1A).

Results: Figure 1 A and B show the biodistribution profiles of ^{224}Ra -labeled calcium carbonate and free ^{224}Ra respectively. Based on femur uptake the release of ^{224}Ra is slow from ^{224}Ra -labeled calcium carbonate with about one fifth after 20 hours increasing to approximately one third at 7 days following administration. This limited release of radionuclide may in one aspect be beneficial since it can reduce dose heterogeneity from the radiolabeled particles. It is noteworthy that there is a considerable uptake in *i.p.* fat which is promising considering the role of *i.p.* fat in intraperitoneal spread of cancer metastases. In conclusion, ^{224}Ra -labeled calcium carbonate has very promising distribution properties regarding intracavitary radiotherapy.

Example 8. Antitumor activity of ^{224}Ra -labeled microparticles in a nude mouse i.p. cancer model.

Background: To test therapeutic activity of ^{224}Ra -labeled calcium carbonate microparticles a nude mouse tumor model of intraperitoneal micrometastases was used. Materials and methods: SKOV-3-luc cells ($5 \cdot 10^6$ cells in 0.25 ml RPMI) were injected intraperitoneally in institutionally bred, 6 weeks old female Athymic Nude-*Foxn1*^{nu} mice with body weights of 17.7-23.6 g. Three days later, mice were treated with intraperitoneal injections of ^{224}Ra -labeled calcium carbonate microparticles in sucrose buffer with activities of 200 kBq/kg (0.25-0.3 ml), 600 kBq/kg (0.35-0.4 ml) or 3 injections of 200 kBq/kg (0.25-0.4 ml). The latter group had 48 hours between each injected fraction. Control animals received saline (0.4 ml) or 200 mg/kg (0.35-0.4 ml) non-labeled microparticles in sucrose buffer. The mice were randomized into treatment groups before cell inoculation, with each group consisting of 8 mice. At day 44 and 45 after treatment start all animals were euthanized by cervical dislocation. During dissection, the presence of macroscopic tumors was assessed by careful visual inspection of each animal and all visible tumors in the peritoneal cavity were removed and weighed.

Results: The data are shown in Figure 2. There was no significant difference between the average tumor weights of the two control groups receiving either saline or non-labeled calcium carbonate microparticles. All groups receiving ^{224}Ra -labeled microparticles had a strong suppression of tumor growth as shown by the strongly reduced tumor weights that was statistically significant compared to the controls. Although there was no statistically difference between the ^{224}Ra treatment groups, there was a tendency towards more tumor growth suppression with higher dosage of ^{224}Ra and fractionated treatment.

In conclusion, ^{224}Ra labeled calcium carbonate microparticles showed a strong and consistent antitumor activity in mice with intraperitoneal tumors.

Example 9 Therapeutic effects in an aggressive cancer ascites model.

Background: Human ovarian cancer often leads to intraperitoneal ascites. The human ovarian cancer cell line ES-2 produces aggressive tumor cell growth and cancerous ascites in nude mice.

Materials and methods: ES-2 cells ($10 \cdot 10^6$ cells in 0.3 ml RPMI) were injected intraperitoneally in institutionally bred, 6 weeks old female Athymic Nude-*Foxn1*^{nu} mice with body weights of 18.1-23.2 g. 25 hours later, mice were treated with intraperitoneal injections of ^{224}Ra -labeled calcium carbonate microparticles in sucrose buffer with activities of 100 kBq/kg (0.3 ml), 300 kBq/kg (0.3-0.35 ml) or 500 kBq/kg (0.3-0.35 ml). Control animals received 0.35 ml saline. The mice were randomized into treatment groups before cell inoculation, with each group consisting of 7-8 mice. Animals were weighed and monitored for disease progression minimum 3 times a week, and every day when they displayed clinical signs indicating the approach of final stage of disease. All mice were euthanized by cervical dislocation on the day they reached a loss-of-wellness endpoint, taking into account abdominal distensions that impairs mobility or respiration, rapid loss or gain of body weight together with general animal appearance and behavior. Following euthanasia mice were necropsied for gross pathological examination.

Results: Survival times were recorded as days after tumor cell inoculation, and a preliminary survival curve including data until follow-up day 20 is presented (Figure 3). At day 19 after tumor cell inoculation, all mice in the saline and lowest dose group (100 kBq/kg) had been euthanized, whereas 86 % of the mice (6/7) in the medium (300 kBq/kg) and high (500 kBq/kg) dose group had not reached the study endpoint. These remaining animals were censored at day 20. The median survival of each group is presented in Table 4.

Table 4. Median survival of mice with intraperitoneal ES-2 cancer ascites treated with saline or ^{224}Ra -labeled calcium carbonate.

Treatment group	Number of mice per group	Median survival time after cell inoculation
NaCl	8	12 days
100 kBq/kg ^{224}Ra -CaCO ₃ microparticles	8	13 days
300 kBq/kg ^{224}Ra -CaCO ₃ microparticles	7	More than 20 days

500 kBq/kg ^{224}Ra - CaCO_3 microparticles	7	More than 20 days
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In conclusion: Considerable disease free life extension was obtained with ^{224}Ra -labeled calcium carbonate microparticles indicating a significant potential for intracavitary ascites.

Example 10 A Preparation of a two-component radiotherapeutic mixture.

In some aspects it may be beneficial to combine ^{224}Ra -labeled calcium carbonate particles with a cell specific radiopharmaceutical. This is obtained when a ^{224}Ra solution in equilibrium with daughter nuclides is combined with a ^{212}Pb binding chelate conjugate prior to contacting the calcium carbonate particles.

Methods: A 0.2 ml 0.5 M ammonium acetate solution of ^{224}Ra in equilibrium with daughter nuclides was added 1 mg/ml of TCMC-labeled monoclonal antibody (mAb) (trastuzumab, cetuximab or OI-3) and incubated for 60 minutes. Thereafter the reaction mixture was added to 30 mg of calcium carbonate microparticles in 0.2 ml 1% bovine serum albumin and mixed for 30 minutes. The mixture was thereafter centrifuged and the supernatant and pellet was counted separately on a gamma counter and analysed with a germanium detector.

A radiotherapeutic mixture consisting of ^{212}Pb -labeled antibody and ^{224}Ra - CaCO_3 microparticles was prepared. For labeling antibody with ^{212}Pb , the antibody Cetuximab was first conjugated to a chelator, TCMC.

To prepare the radioimmunoconjugate, ^{224}Ra -solution with 0.5 M ammonium acetate (pH between 5 and 6) was mixed with TCMC-Cetuximab and reacted for 30 minutes at 37°C with a rotation rate of 350 rpm. The radiochemical purity of the resulting product was evaluated with chromatography strips (Biodex), and was found to be above 95% for ^{212}Pb . CaCO_3 microparticles were prepared as described for the radiolabeling procedure, except that the radioactivity added was the solution described above, containing both free ^{224}Ra and ^{212}Pb -labeled TCMC-Cetuximab. After 1.5 hours incubation at room temperature on a HulaMixer, the particles in radiolabeling solution were spun down and the supernatant and particle fraction separated. The activity distribution of ^{224}Ra and ^{212}Pb in the particle pellet and the

supernatant was determined with the HPGe detector. A radiochemical purity analysis was performed on an aliquot of the supernatant.

Data are presented in Tables 5 and 6. Table 6 shows that 66.39 % of the total ^{212}Pb activity was found in the supernatant, while 98.41 % of the ^{224}Ra was retained on the particles. Of the released ^{212}Pb at least 98 % was protein bound (Table 5), which represents the fraction of antibody bound ^{212}Pb before the antibody was mixed with the particles. In Table 6 the fraction of ^{212}Pb -antibody conjugate and ^{224}Ra in free circulation and bound to the calcium carbonate particles is presented. The data shows that ^{224}Ra binds to the particles while the major part of the ^{212}Pb -conjugate is free to circulate in the medium. Thus a bifunctional radiotherapeutic mixture suitable for injection was obtained.

In conclusion, ^{224}Ra solutions mixed with ^{212}Pb -TCMC-antibody conjugates can be used to prepare ^{224}Ra -CC microparticles yielding a two component therapeutic mixture with radioimmunoconjugate (RIC) and radiolabeled microparticles with antigen-targeting properties as well as microparticle radiotherapeutic properties. This may be advantageous in producing a combination of general cavity irradiation and a specific tumor cell targeting RIC treatment against cancer. The addition of RIC may enhance the microdistribution of alpha radiation to improve therapeutic effect on resistant cancer cells.

Table 5. Thin layer chromatography analyses of ^{212}Pb -TCMC-antibody conjugate before and after absorption to calcium carbonate particles.

RCP analyses of protein bound fraction						
	Time in formulation buffer	Canberra germanium detector gamma spectroscopy			Cobra II NaI gamma counter	
			^{212}Pb		70-80 keV	220-260 keV
Before mixing with particles	13 minutes		98,2 %		97,2 %	95,2 %
	20 minutes				99,6 %	98,1 %
	10 minutes				100,0 %	98,1 %

After particle labeling	20 minutes				98,2 %	100,0 %
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Table 6. Particle absorption of ^{224}Ra solution containing ^{212}Pb -TCMC-antibody

	% of total activity	
	^{212}Pb	^{224}Ra
Particles	33,61 %	98,41 %
Supernatant/antibody fraction	66,39 %	1,59 %

Example 10 B Description of a kit system for preparing a ^{212}Pb radioimmunoconjugate and ^{224}Ra microparticle radiotherapeutic mixture.

A vial (A) with solution of ^{224}Ra in an aqueous solution (e.g. 0.5 M ammonium acetate, pH 5-6) is left to decay for 1 day or more for producing ^{212}Pb . An aqueous solution (B) of TCMC-antibody conjugate or similar chelate conjugated antibody and a vial (C) with dry or aqueous calcium carbonate microparticles. The contents of vial A and B are mixed together in one of the vials and incubated for 1 min to 4 hours and thereafter mixed with vial C and incubated for 1 minute to 4 hours. After each steps of incubation a quality control may or may not be performed. Finally the combined mixture of A, B, and C is drawn into a syringe and administered to a patient.

Example 11 Radiolabeling of microparticles of different sizes and their retention of ^{224}Ra and daughter ^{212}Pb

The labeling efficiency and retention over time of ^{224}Ra and its daughter ^{212}Pb was investigated on radiolabeled CaCO_3 microparticles of 2 different sizes within the preferred size range of 1-10 μm . The number weighted median size of the particles were found to be 1.1 and 8.9 μm by single particle optical sensing, thus representing both extremes of the preferred size range for the particles. The largest particles were prepared as described in example 3 and the smaller particles were purchased from PlasmaChem GmbH, Berlin, Germany. The particles were labeled with ^{224}Ra as described in example 4, except that the sonication step and incubation in 0.5 % BSA in DPBS was omitted. After finalizing the labeling procedure, the particles and the wash solutions were measured for 1 minute in the Hidex gamma counter. Since ^{224}Ra decay results in

very little gamma emission, determination of the activity of ^{224}Ra is mainly carried out by the measurement of gamma emissions of its decay product ^{212}Pb . The counts from 65-345 keV, which include the gamma activity of ^{224}Ra and the gamma and X-ray activity of the daughter ^{212}Pb were selected and summarized for further calculations. The samples were allowed to decay for minimum 24 hours post labeling in order to establish equilibrium between ^{224}Ra and ^{212}Pb , before they were re-measured. Labeling efficiency was determined as bound activity: the percentage of the total activity added to microparticles that is bound to the particles after the labeling procedure.

$$\text{Labeling efficiency (\%)} = \frac{A_{\text{particles}}}{A_{\text{particles}} + W_{\text{solutions}}} \times 100$$

where $A_{\text{particles}}$ is the activity of the particle suspension after washing and $W_{\text{solutions}}$ is the total activity of the wash solutions. The measurements right after finalizing the particle labeling is used for estimating the ^{212}Pb labeling efficiency by assuming negligible contribution of the ^{224}Ra gamma emissions in the 65-345 keV window. Equilibrium between ^{224}Ra and the daughter ^{212}Pb is reached when the decay of ^{212}Pb is equal to its rate of production from ^{224}Ra . A pure source of ^{224}Ra reaches equilibrium conditions after approximately 2 days. It is assumed here that equilibrium between ^{224}Ra and ^{212}Pb in the samples are reached after 24 hours, since the samples are expected to have a relatively even distribution of the two nuclides after labeling, i.e. equilibrium will be reached faster than from a pure source of ^{224}Ra . Measurements of the samples at equilibrium are therefore used to estimate the ^{224}Ra labeling efficiency at time 0.

To determine the retention of ^{224}Ra and ^{212}Pb on the particles after labeling, the particles were incubated in 1 ml sucrose buffer (94 mg/ml sucrose, 2.1 mg/ml Na_2SO_4) at room temperature. After 24 hours, 3, 5 and 7 days, the particle suspension was centrifuged and activities in the supernatant and pelleted particles were measured. Afterwards, if the stability study was to be continued to a later time point, the particle pellet was resuspended in a new aliquot of sucrose buffer and incubated further. Retained activity was estimated as the percentage of the total activity of the sample at a given time that is not released from the particles after

$$\text{removal of the supernatant } (A_{\text{sup}}) \text{ Retained activity (\%)} = \frac{A_{\text{particles}}}{A_{\text{particles}} + A_{\text{sup}}} \times 100$$

The samples were stored for minimum 24 hours before they were re-measured, and like described above, the retained ^{212}Pb activity were estimated from measurements taken directly after removal of the supernatant whereas retained ^{224}Ra activity was calculated from the equilibrium measurements.

Results: For 12 (14) individual radiolabeling experiments with the 1.1 μm (8.9 μm) particles, the radiolabeling efficiencies were as follows (Mean $\pm$ Standard deviation): ^{224}Ra : $93.6 \pm 5.8 \%$ ($88.8 \pm 6.5 \%$) and ^{212}Pb : $87.6 \pm 7.7 \%$ ($84.3 \pm 7.7 \%$). The results are depicted in figure 4 and show that ^{224}Ra and daughter nuclide ^{212}Pb are absorbed in high yield by the microparticles, regardless their difference in size.

The results from the retention experiments (Figure 5) show that ^{224}Ra and daughter ^{212}Pb is well retained on the CaCO_3 particles for several days *in vitro* in sucrose buffer, which might be a relevant pharmaceutical carrier for the radiolabeled particles. The average retained activity is above 95 % for all time points for both particle sizes. This suggests that the product may have a shelf-life of several days allowing centralized production and shipment to distant end users.

Example 12 Therapeutic and hematological effect of ^{224}Ra -labeled CaCO_3 microparticles in mice with ascites

Background: Human ovarian cancer often leads to intraperitoneal ascites. The human ovarian cancer cell line ES-2 produces aggressive tumor cell growth and cancerous ascites in nude mice.

Methods: ES-2 cells ($1 \cdot 10^6$ cells in 0.35 ml RPMI) were injected intraperitoneally in institutionally bred, 5-6 weeks old female Athymic Nude-Foxn1nu mice with body weights of 17.0-23.9 g. Twenty-two hours later, mice were treated with intraperitoneal injections of ^{224}Ra -labeled CaCO_3 microparticles (8.9 μm median size determined by single particle optical sensing) in sucrose buffer with activities of 150 kBq/kg (0.25-0.35 ml), 300 kBq/kg (0.3-0.35 ml), 1000 kBq/kg (0.4 ml) or 2 injections of 150 kBq/kg (0.3-0.4 ml). The latter group had 1 week between each injected fraction. Control animals received 0.35 ml saline. The mice were randomized into treatment groups before cell inoculation, with each group consisting of 3-10 mice. Animals were euthanized by cervical dislocation when they reached a pre-determined endpoint, as described in Example 9. They were also monitored similarly as described in Example 9. In addition, maximum 100 μl blood was collected from the vena saphena lateralis 13 days prior to and 13 and 26 days after treatment start. From 3-5 mice were sampled from each group at every time point. Assessment of potential hematological toxicity was done by counting white blood cells, red blood cells and platelets in an automated veterinary hematology analyzer (scil Vet abc, ABX Diagnostics, Montpellier, France).

Results: Survival times were recorded as days after tumor cell inoculation and are presented as a survival curve in Figure 6 and as median survival times in table 7. All doses of ^{224}Ra -labeled CaCO_3 microparticles gave minimum a doubling in median survival compared to the saline control group. These results indicate that ^{224}Ra -labeled CaCO_3 microparticles, even in relatively low doses (2.6-3.6 kBq per mouse for the 150 kBq/kg group), have a significant potential for treatment of intracavitary ascites. The results from the hematological analyses (Figure 7) show no reduction in white blood cell counts compared to before treatment start. The increase in white blood cell counts of the control group at day 13 and the 150 kBq/kg group at day 26 compared to the earlier time points is probably due to an immune response to the build-up of ascites cancer cells in the abdomen, as the time points correlate with when the mice in these groups start experiencing a heavy ascites load and are close to reaching the end-point of the study. The mean platelet counts for all treatment groups are not significantly different from reference values ($1100 \pm 143 \times 10^9/\text{L}$) for this mouse strain provided by the breeder (Envigo). For red blood cells, no notable differences in counts were seen in any of the treatment groups compared to the saline control. These results suggest that the ^{224}Ra -labeled CaCO_3 microparticles are well tolerated and show no signs of causing hematological toxicity at the therapeutically effective dose levels tested in this study.

Conclusions: Considerable disease free life extension was obtained after treatment with ^{224}Ra -labeled CaCO_3 microparticles. No treatment related influence on hematological parameters were observed, altogether suggesting that the ^{224}Ra - CaCO_3 microparticle treatment is well tolerated at doses that resulted in significant therapeutic effect.

Table 7: Median survival times of mice injected intraperitoneally with $1 \cdot 10^6$ ES-2 cells and treated 22 hours later with saline or ^{224}Ra -labeled CaCO_3 microparticles.

Treatment group	Number of mice per group	Median survival in days after cell inoculation
NaCl	10	17
150 kBq/kg ^{224}Ra - CaCO_3 microparticles	9	34
300 kBq/kg ^{224}Ra - CaCO_3 microparticles	9	40
2×150 kBq/kg ^{224}Ra - CaCO_3 microparticles	9	36

1000 kBq/kg ^{224}Ra -CaCO ₃ microparticles	3	46
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Example 13 Treatment of mice with intraperitoneal ascites at different disease development stages

Background: Malignant ascites is common in advanced cancer in the peritoneal cavity and is associated with reduced life expectancy and quality of life for patients and there is a medical need for improved treatment strategies. The anti-ascites activity of ^{224}Ra -CaCO₃ microparticles was evaluated in the ES-2 ascites model in nude mice by giving the treatment on different days after cell inoculation.

Methods: ES-2 cells ($1 \cdot 10^6$ cells in 0.2-0.35 ml RPMI) were injected intraperitoneally in institutionally bred, 5-6 weeks old female Athymic Nude-Foxn1nu mice with body weights of 17.0-23.9 g at the time of inoculation. Randomized groups consisting of 5-10 mice were treated with intraperitoneal injections of ^{224}Ra -labeled CaCO₃ microparticles (8.9 μm median size determined by single particle optical sensing) in sucrose buffer 22 hours, 2, 5, 7 and 9 days after cell inoculation. All treatment groups received a dose of 700 kBq/kg (0.29-0.4 ml), except the group treated 22 hours after cell inoculation which was administered 300 kBq/kg (0.3-0.35 ml). Mice in the control groups received saline (0.35-0.4 ml) on day 1 (10 mice), 5 (5 mice) and 9 (10 mice) after cell inoculation. Animals were euthanized by cervical dislocation when they reached a pre-determined endpoint, as described in Example 9. They were also monitored similarly as described in Example 9.

Results: Survival times were recorded as days after tumor cell inoculation and are presented as a survival curve in Figure 8 and as median survival times in table 8. Increased median survival times compared to the saline control group is observed when the mice were treated with ^{224}Ra -labeled CaCO₃ microparticles from 1-7 days after cell inoculation. The therapeutic effect correlates with the treatment day, i.e. the most significant increase in survival is seen for treatment at the earliest time points after cell inoculation. As shown in table 8 there is also a reduction in mice having ascites at time of sacrifice in ^{224}Ra -CaCO₃ microparticles treated groups compared with control. This is despite that the treated groups had longer survival after tumor inoculation and, thus, more time to develop ascites.

Conclusions: Treatment with ^{224}Ra -labeled CaCO_3 microparticles had a notable anti-ascites effect in mice inoculated intraperitoneally with ES-2 cells.

Table 8: Median survival of mice with intraperitoneal ES-2 ovarian cancer ascites model treated with saline or ^{224}Ra -labeled CaCO_3 microparticles on different times after cell inoculation.

Treatment group	Median survival in days after cell inoculation	Number of mice sacrificed because of ascites	Number of mice per group	% mice sacrificed because of ascites
Day 1+5+9: NaCl	16	25	25	100 %
Day 1: 300 kBq/kg ^{224}Ra - CaCO_3 microparticles	40	4	9	44 %
Day 2: 700 kBq/kg ^{224}Ra - CaCO_3 microparticles	33	4	5	80 %
Day 5: 700 kBq/kg ^{224}Ra - CaCO_3 microparticles	26	1	5	20 %
Day 7: 700 kBq/kg ^{224}Ra - CaCO_3 microparticles	20	2	5	40 %
Day 9: 700 kBq/kg ^{224}Ra - CaCO_3 microparticles	16.5	7	10*	78 %

Example 14 Therapeutic effect of ^{224}Ra -labeled calcium carbonate microparticles in a mouse model of intraperitoneal micrometastases

Background: Patients with ovarian cancer treated with maximal cytoreductive surgery have a tendency to relapse due to remaining intraperitoneal tumor cells and micrometastases. To mimic the situation with residual intraperitoneal disease in patients, $1 \cdot 10^5$ cells of the ascites generating cell line ES-2 was inoculated in nude mice and treatment with ^{224}Ra -labeled CaCO_3 microparticles was given 1 hour after the cells.

Methods: ES-2 cells ($1 \cdot 10^5$ cells in 0.2 ml RPMI) were injected intraperitoneally in institutionally bred, 6-9 weeks old female Athymic Nude-Foxn1nu mice with body

weights of 18.5-28.8 g. One hour after cell inoculation, mice were treated with intraperitoneal injections of 750 kBq/kg ^{224}Ra -labeled CaCO_3 microparticles of 2 different sizes in sucrose buffer (0.31-0.5 ml). The control group received 0.4 ml saline. The number weighted median size of the particles were found to be 1.1 and 8.9 μm by single particle optical sensing, thus representing both extremes of the preferred size range for the particles. The largest particles were prepared as described in example 3 and the smaller particles were purchased from PlasmaChem GmbH. The mice were randomized into treatment groups before cell inoculation, with each group consisting of 12-13 mice. Animals were euthanized by cervical dislocation when they reached a pre-determined endpoint, as described in Example 9. They were also monitored similarly as described in Example 9.

Results: Survival times were recorded as days after tumor cell inoculation and are presented as a survival curve including data until follow-up day 80 in Figure 9 and as median survival times in table 9. Median survival time was extended by 42 days (3.2 fold) and 36 days (2.9 fold) compared to the saline control group for treatment with ^{224}Ra -labeled large and small CaCO_3 particles respectively. At day 80, 4/12 (33.3 %) and 3/12 (25 %) of the mice treated with 8.9 μm and 1.1 μm sized particles were alive compared to 1/13 (7.7 %) in the control group. There was no statistical significant difference between the survival curves of the two ^{224}Ra -treated groups.

Conclusion: The results show that ^{224}Ra -labeled CaCO_3 particles have a significant potential for treatment of residual intraperitoneal disease, with an approximate 3-fold increase in median survival compared to the saline control group and also producing several long term survivors. The results also indicate that particles in both extremes of the preferred size range of 1-10 μm have similar therapeutic efficacy in this model mimicking micrometastatic disease.

Table 9: Median survival times of mice injected intraperitoneally with $1 \cdot 10^5$ ES-2 cells and treated 1 hour later with ^{224}Ra -labeled CaCO_3 microparticles of two different sizes compared to the saline control group.

Treatment group	Number of mice per group	Median survival in days after cell inoculation	% survival at follow-up day 80
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NaCl	13	19	7.7 %
750 kBq/kg ²²⁴ Ra-CaCO ₃ microparticles (8.9 μm)	12	61	33.3 %
750 kBq/kg ²²⁴ Ra-CaCO ₃ microparticles (1.1 μm)	12	55	25.0 %

Example 15 Absorption of ²¹²Pb onto calcium carbonate microparticles

To determine the amount of released ²¹²Pb from ²²⁴Ra-labeled CaCO₃ microparticles that absorbed onto non-radioactive particles during 24 hours, a dialysis setup was used. CaCO₃ microparticles were labeled with ²²⁴Ra as described in example 4, except that the sonication step and incubation in 0.5 % BSA in DPBS was omitted. In addition, non-radioactive particles were prepared in the exact same way, excluding the step with adding radioactive solution. The non-radioactive particles (50 mg) were diluted in DPBS (pH 7) to a total volume of 14 ml in a conical 15 ml centrifuge tube. Approximately 5 mg radioactive particles in DPBS (0.15-0.40 ml) were added to the dialysis device (Slide-A-Lyzer MINI Dialysis Devices, 20 kDa MWCO, ThermoFisher) and placed in the tube containing the non-radioactive particles which functioned as the dialysis buffer. The tube with the inserted dialysis device was incubated for 24 hours with shaking at room temperature. After incubation, the 15 ml tube was spun down and the supernatant was removed. The activity in the dialysis device (A_D), the supernatant of the dialysis buffer (A_S), and the pelleted particles from the 15 ml tube (A_P) was measured in the Hidex gamma counter. The percentage of released ²¹²Pb during 24 hours was estimated as follows:

$$\text{Released activity (\%)} = \frac{A_S + A_P}{A_P + A_S + A_D} \times 100$$

The percentage of released ²¹²Pb activity that had absorbed onto the non-radioactive CaCO₃ particles was determined as follows:

$$\text{Activity absorbed on particles (\%)} = \frac{A_P}{A_P + A_S} \times 100$$

Table 10 presents the results of the study which shows that ²¹²Pb released to DPBS from radiolabeled particles is absorbed to a high degree onto CaCO₃ particles. More than half of the released ²¹²Pb activity tend to re-associate with the particles. This is an indication that if ²²⁴Ra daughters (²²⁰Rn, ²¹⁶Po or ²¹²Pb) are released from the particles, either because of recoil energy or because of ²²⁰Rn diffusion, it may be followed by a significant re-absorption of the daughter ²¹²Pb. This is a very beneficial concept as it can reduce

systemic toxicity from redistribution of the daughter nuclides from the intraperitoneal cavity, e.g. by preventing uptake of ^{212}Pb into the blood stream. In conclusion, calcium carbonate microparticles are particularly well suited for retaining ^{224}Ra and progeny as it can reabsorb free ^{212}Pb generated from the diffusion of ^{220}Rn from ^{224}Ra -labeled calcium carbonate particles.

Table 10 Amount of ^{212}Pb released from ^{224}Ra -labeled CaCO_3 microparticles and absorbed onto non-radioactive particles.

Particle size	% ^{212}Pb activity released	% of ^{212}Pb released activity absorbed on particles
1.1 μm	6.6 %	75.3 %
8.9 μm	5.9 %	56.7 %

Example 16 Biodistribution and in vivo stability of radiolabeled microparticles

In this example the data from example 7 is presented again as Bq per gram of the different tissues. The injected dose was normalized to 10 kBq per mouse. Measurements of the samples at approximately 2.5-3 hours after the mice were sacrificed were used to estimate the amount of ^{212}Pb , whereas re-measurements of the samples 3-4 days after sacrifice of the mice were used to determine the amount of ^{224}Ra . The data for ^{224}Ra were decay corrected to show the activity in the sample at time of sacrifice.

Results: The tissue distribution of ^{224}Ra -labeled CaCO_3 particles 20 hours, 4 and 7 days after injection compared to free ^{224}Ra solution is presented in figure 10 as mean activity per gram tissue. Figure 11 shows the biodistribution of daughter nuclide ^{212}Pb . When radiolabel is bound to the particle it shows a significant shift in tissue radiation exposure with strongly reduced exposure to skeleton and a high exposure, presumably to the surfaces, of intraperitoneal organs. In conclusion ^{224}Ra -labeled calcium carbonate microparticles show promising properties as local a radiotherapeutic in the peritoneal cavity.

Example 17 Therapeutic effect in an aggressive cancer ascites model

In this example the data from example 9 has been updated with extended follow-up time of the mice and adjusted dose levels after a re-calibration of the Capintec CRC-25R dose calibrator. The doses given to the mice have been adjusted to 35 kBq/kg, 100 kBq/kg and 165 kBq/kg.

Results: Survival times were recorded as days after tumor cell inoculation and are presented as a survival curve including data until follow-up day 30 in Figure 12 and as median survival times in table 11. The medium and high dose of ^{224}Ra - CaCO_3 particles gave almost a doubling in median survival times compared to the saline control in this aggressive ascites model. One mouse in the highest dose group lived for 138 days after cell inoculation and was sacrificed without showing any signs of disease.

Table 11: Median survival times of mice injected intraperitoneally with $10 \cdot 10^6$ ES-2 cells and treated 25 hours later with saline or ^{224}Ra -labeled CaCO_3 microparticles.

Treatment group	Number of mice per group	Median survival in days after cell inoculation
NaCl	8	12
35 kBq/kg ^{224}Ra - CaCO_3 microparticles	8	13
100 kBq/kg ^{224}Ra - CaCO_3 microparticles	7	21
165 kBq/kg ^{224}Ra - CaCO_3 microparticles	7	21

In conclusion, ^{224}Ra -labeled calcium carbonate microparticles show a substantial antitumor activity in mice with aggressive intraperitoneal cancer, but a dose higher than 35 kBq/kg is preferable to obtain significant antitumor effect against aggressive cancers.

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ITEMS OF THE INVENTION

1. A particle comprising a degradable compound and an alpha emitting radionuclide and/or a radionuclide generating alpha emitting daughter.
2. The particle according to item 1, wherein the radionuclide is selected from the group consisting of ^{224}Ra , ^{212}Bi , ^{212}Pb , ^{223}Ra , ^{225}Ra , ^{225}Ac , ^{213}Bi , ^{211}At , ^{227}Th .
3. The particle according to anyone of items 1-2, wherein the degradable compound is selected from the group consisting of CaCO_3 , PEG modified CaCO_3 , protein modified

CaCO₃, carbohydrate modified CaCO₃, lipid modified CaCO₃, vitamin modified CaCO₃, organic compound modified CaCO₃, polymer modified CaCO₃ and/or inorganic crystal modified CaCO₃.

4. The particle according to anyone of items 1-3, wherein size of the particle is from 1 nm to 500 μ m.

5. The particle according to anyone of items 1-4, further comprising one or more compounds selected from the group consisting of a monoclonal antibody, a polyclonal antibody, a radioimmunoconjugate, an immunoconjugate, a chelate antibody conjugate, vitamins including folate and folate derivatives, peptides, minibodies, and affibodies.

6. A pharmaceutical composition comprising one or more particles according to anyone of items 1-5 and a diluent, carrier, surfactant, and/or excipient.

7. The pharmaceutical composition according to item 6, prepared with an amount of radionuclide that is 1kBq to 10GBq per dosing.

8. The pharmaceutical composition according to anyone of items 6-7, prepared with an amount of radionuclide that is 50 MBq to 100 GBq suitable for multidose industrial scale production.

9. The pharmaceutical composition according to anyone of items 6-8, wherein the composition is a particle suspension comprising monodisperse or polydisperse particles labeled with an alpha emitting radionuclide and/or a radionuclide generating alpha emitting daughter.

10. The pharmaceutical composition according to anyone of items 6-9, which is suitable for intravenous or intracavitary injection.

11. The particle according to anyone of items 1-5 or the pharmaceutical composition according to items 6-9, for use as a medicament.

12. The particle according to anyone of items 1-5 or the pharmaceutical composition according to items 6-9, for use is intracavitary therapy, radioembolization or radiosynovectomy.

13. The particle according to anyone of items 1-5 or the pharmaceutical composition according to items 6-9, for use in the treatment of cancer.

14. The particle according to anyone of items 1-5 or the pharmaceutical composition according to items 6-9, for use according to item 12-13, wherein the cancer is selected from the group consisting of intreaperitoneal cancers, intracranial cancers, pleural cancers, bladder cancers, cardiac cancers, and cancers in the subarachnoid cavity.

15. A method of treatment or amelioration comprising administration of the particles according to anyone of items 1-5 or the pharmaceutical composition according to item 6-9 to an individual in need thereof using single treatment or repeated dosing.

16. A method for preparing a particle according to anyone of items 1-6, the method comprising bringing an alpha emitting radionuclide and a biodegradable compound in contact with each other with or without using a carrier for the radionuclide.

17. A kit comprising;

- a nano or micro particle according to anyone of items 1-6,
- an alpha emitting radionuclide or a radionuclide generating an alpha emitting daughter.
- a carrier, diluent and/or excipient, and
- optionally instructions to use the kit.

18. A kit comprising;

- a nano or micro particles according to anyone of items 1-6,
- an alpha emitting radionuclide or a radionuclide generating an alpha emitting daughter,
- a carrier, diluent and/or excipient, and
- optionally instructions to use the kit to prepare a bifunctional pharmaceutical solution comprising particles suspension and radioimmunoconjugate solution.

19. A kit according to item 18, further comprising a chelator-conjugated molecule, including monoclonal antibody.

CLAIMS

1. A particle comprising,
 - a degradable compound comprising CaCO_3 ,
 - the alpha emitting radionuclide ^{224}Ra , and
 - the ^{224}Ra radionuclide, progeny selected from the group consisting of ^{220}Rn , ^{216}Po , and ^{212}Pb .

2. The particle according to claim 1, wherein CaCO_3 is selected from the group consisting of CaCO_3 , PEG modified CaCO_3 , protein modified CaCO_3 , carbohydrate modified CaCO_3 , lipid modified CaCO_3 , vitamin modified CaCO_3 , organic compound modified CaCO_3 , polymer modified CaCO_3 and/or inorganic crystal modified CaCO_3 .

3. The particle according to any one of claims 1-2, wherein size of the particle is from 1 nm to 500 μm .

4. The particle according to any one of claims 1-3, further comprising one or more compounds selected from the group consisting of a monoclonal antibody, a polyclonal antibody, a radioimmunoconjugate, an immunoconjugate, a chelate antibody conjugate, vitamins including folate and folate derivatives, peptides, minibodies, and affibodies.

5. The particle according to claim 4, wherein the one or more compounds is/are targeting a target selected from the group consisting of CD19, CD20, CD22, CD33, CD37, CD38, CD45, CD74, CD138, PSMA, HER-2, EGFR, MUC-1, MUC-18, CEA, FBP, NG2, EPCAM, Syndecan-1, Ca-125, LK-26, HMFG, CS-1, and BCMA.

6. The particle according to any one of the claims 1-5, wherein the particle is a medical device or is comprised in a medical device.

7. A composition comprising a particle according to any one of claims 1-6.

8. A composition or pharmaceutical composition, comprising one or more particles according to any one of claims 1-6 and a diluent, carrier, surfactant, and/or excipient.

9. The pharmaceutical composition according to claim 8 or the composition according to claims 7-8, prepared with an amount of radionuclide that is 1kBq to 10GBq per dosing or with an amount of radionuclide that is 50 MBq to 100 GBq suitable for multidose industrial scale production.
10. The composition according to claims 7-8, or pharmaceutical composition according to anyone of claims 8-9 wherein the composition is a particle suspension comprising monodisperse or polydisperse particles according to claims 1-6.
11. The pharmaceutical composition according to any one of claims 8-10 or the composition according to claims 7-8, which is suitable for intravenous or intracavitary injection.
12. The particle according to any one of claims 1-6, or the pharmaceutical composition according to claims 8-11 or the composition according to claims 7-8, for use as a medicament.
13. The particle according to any one of claims 1-6, the composition according to claims 7-8, or the pharmaceutical composition according to claims 8-11, for use in intracavitary therapy, radioembolization or radiosynovectomy.
14. The particle according to any one of claims 1-6, the composition according to claims 7-8, or the pharmaceutical composition according to claims 8-11, for use in the treatment of cancer.
15. The particle according to any one of claims 1-6 or the composition according to claims 7-8, the pharmaceutical composition according to claims 8-11, or the particle for use according to claims 12-14, wherein the cancer is selected from the group consisting of intraperitoneal cancers, intracranial cancers, pleural cancers, bladder cancers, cardiac cancers, and cancers in the subarachnoid cavity.
16. A method for preparing a particle according to any one of claims 1-6, the method comprising bringing an alpha emitting radionuclide ^{224}Ra and a degradable compound comprising CaCO_3 in contact with each other with or without using a carrier for the radionuclide.

17. A kit comprising;

- a degradable compound comprising CaCO_3 ,
- a composition comprising the alpha emitting radionuclide ^{224}Ra , and the ^{224}Ra radionuclide progeny selected from the group consisting of ^{220}Rn , ^{216}Po , and ^{212}Pb , and
- optionally instructions to use the kit.

18. A kit according to claim 17, further comprising a chelator-conjugated molecule, including a monoclonal antibody.

19. A two-component kit comprising a radioimmunoconjugate for ^{212}Pb antigen-specific treatment, and a particle according to anyone of claims 1-6.