EP-0788 Alpha-Emitting Radiopharmaceutical for IP Therapy of Peritoneal Metastases: In Vitro Evaluation and Therapeutic Effects in a Murine Model

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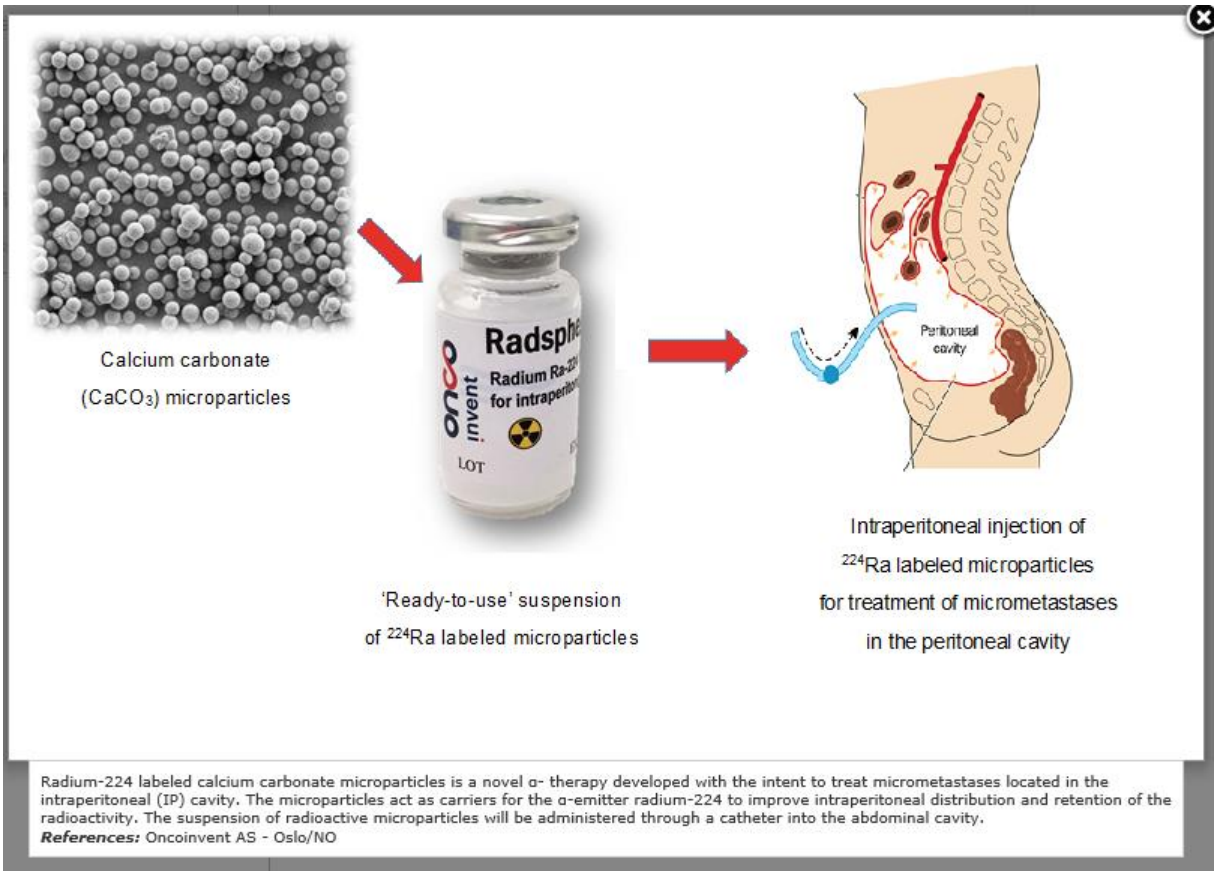


Radium-224 labeled calcium carbonate microparticles is a novel α- therapy developed with the intent to treat micrometastases located in the intraperitoneal (IP) cavity. The microparticles act as carriers for the α-emitter radium-224 to improve intraperitoneal distribution and retention of the radioactivity. The suspension of radioactive microparticles will be administered through a catheter into the abdominal cavity.

Section: Aim

Radium-224 (^{224}Ra) labeled calcium carbonate ($CaCO_3$) microparticles have been developed with the intent to treat micrometastases located in the abdominal cavity. The microparticles act as carriers for the α-emitter ^{224}Ra to ensure high intraperitoneal (IP) retention of the radioactivity without cellular targeting. This novel α- therapy has a short range of action in tissue and is designed to confine the radiation exposure to the IP cavity, treating both linings of the peritoneal surfaces and liquid volumes. The main function of the microparticle component of ^{224}Ra-$CaCO_3$ is to retain the radionuclides in the peritoneal cavity. The particles themselves are too big to penetrate the peritoneum, while free radionuclides will leak through the membrane and distribute to extraperitoneal tissues. Due to the bone-seeking property of ^{224}Ra, the level of release of ^{224}Ra from the radiolabeled particles *in vivo* can be determined by measurement of the skeletal uptake of ^{224}Ra. Here we compare data from an *in vitro* model of the *in vivo* retention of radionuclides across the peritoneum with biodistribution data from femur of mice. The effect of varying the microparticle amount on ^{224}Ra retention has been examined. Furthermore, the therapeutic efficacy of IP injected ^{224}Ra-microparticles was compared to that of injection of a solution of $^{224}RaCl_2$ in a murine xenograft of an ascites presenting human cell line.

Section: Materials & Methods

Preparation of $CaCO_3$ microparticles and ^{224}Ra labeling of the microparticles is described in Westrøm et al., 2018a.

The *in vitro* test model for mimicking release of free ^{224}Ra from the peritoneal cavity after IP injection of ^{224}Ra treatments was based on Dialysis Cassettes (Slide-A-Lyzer™ G2, ThermoFisher Scientific) submerged in vials with physiological saline solution with 25% Fetal Bovine Serum. Samples of 0.4 ml ^{224}Ra solution or ^{224}Ra microparticle suspension were injected into the dialysis cassettes and submerged into the vials containing saline before incubation on a shaker at 37 °C. Controls were made by diluting 0.4 ml aliquots of the different ^{224}Ra samples directly into vials with saline. All samples were prepared in duplicate for each particle concentration. After 24 hours, aliquots were drawn from both vials with cassettes and the controls before measurement on a Hidex Automatic Gamma Counter. Released activity from the dialysis cassettes was determined as a percentage of the activity in the control samples.

Institutionally bred, female Athymic nude $Foxn^{nu}$ mice were used. Biodistribution and therapy studies were performed as described in Westrøm et al., 2018a and Westrøm et al., 2018b, respectively. Mice without tumor cell inoculation were used in the biodistribution study. Efficacy studies were performed with mice inoculated IP with the ES-2 cell line representing human ovarian epithelial adenocarcinoma. Inoculation with the ES-2 cell line leads to aggressive development of ascites. The dosages of microparticles and ^{224}Ra activity are

described in Figure 3. For the therapy studies the treatment was injected 1 day after cell inoculation.

Radioactive samples were measured in the window 65 to 345 keV on the Hidex AMG. In this window the most abundant X- and γ-radiation is from ^{212}Pb, a progeny of ^{224}Ra, with minimal contribution from other nuclides in the series. Since ^{224}Ra results in modest emission in the 65 to 345 keV region, the ^{224}Ra activity was determined indirectly based on the counts in this window. This was carried out by re-measuring the samples between 1 and 2 days after the first measurement, when the initial ^{212}Pb present in the sample had decayed and equilibrium between ^{224}Ra and newly produced ^{212}Pb had been established.

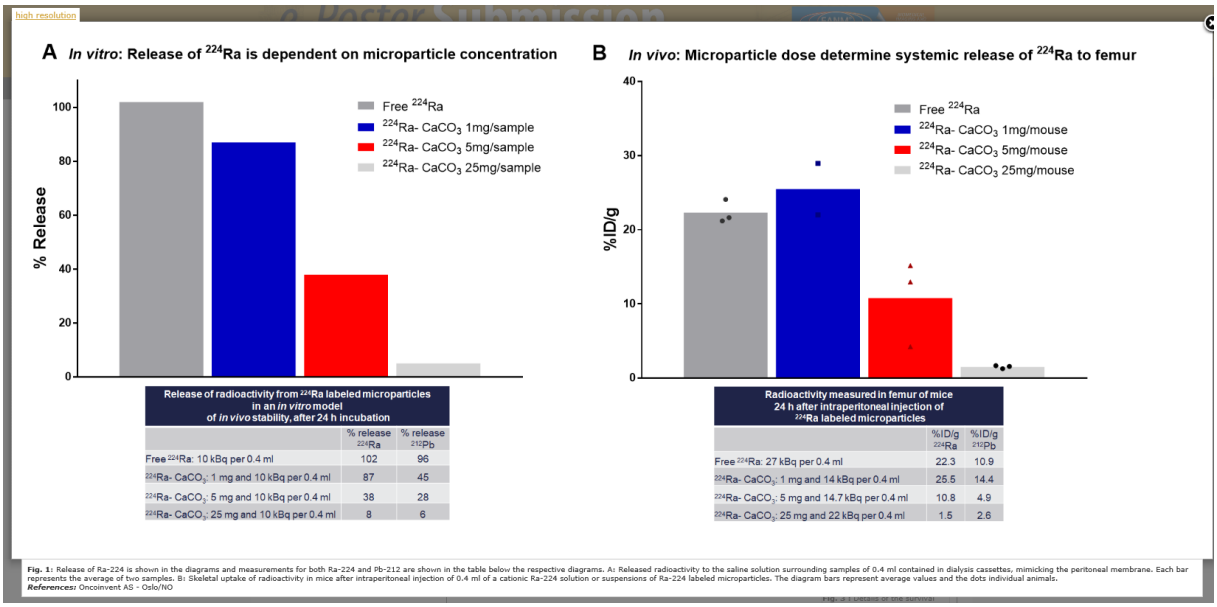
Section: Results

The retention of ^{224}Ra and its daughter ^{212}Pb on $CaCO_3$ microparticles increased with increasing amounts of microparticles. This was observed both *in vitro* and in biodistribution studies in mice (Figure 1).

Mice gained a survival benefit after IP treatment with ^{224}Ra labeled microparticles compared to IP administration of free cationic ^{224}Ra (given as $^{224}RaCl_2$ solution, Figure 2). Treatment with $^{224}RaCl_2$ also improved survival compared to the non-treated control, but the median survival was notable higher (29 days vs 46 days) when microparticles act as a carrier for ^{224}Ra to retain the radioactivity in the peritoneal cavity (Figure 2 and 3).

The survival performance of the studies presented here with activity dose per mouse of 10-24 kBq for 5 mg injected microparticles (TI: 2.0-2.9) is comparable to the study of first generation microparticles previously presented (TI: 2.0-2.7 at doses from 4 – 27 kBq/mouse, Westrøm et al., 2018b).

The highest microparticle amount tested, 25 mg per mice, showed slightly inferior survival (TI of 1.7) to that of 5 mg per mice in the same study (TI of 2.0), see Study 2 of Figure 3.



Release of radioactivity from ^{224}Ra labeled microparticles in an *in vitro* model of *in vivo* stability, after 24 h incubation

	% release ^{224}Ra	% release ^{212}Pb
Free ^{224}Ra: 10 kBq per 0.4 ml	102	96
^{224}Ra- $CaCO_3$: 1 mg and 10 kBq per 0.4 ml	87	45
^{224}Ra- $CaCO_3$: 5 mg and 10 kBq per 0.4 ml	38	28
^{224}Ra- $CaCO_3$: 25 mg and 10 kBq per 0.4 ml	8	6

Radioactivity measured in femur of mice 24 h after intraperitoneal injection of ^{224}Ra labeled microparticles

	%ID/g ^{224}Ra	%ID/g ^{212}Pb
Free ^{224}Ra: 27 kBq per 0.4 ml	22.3	10.9
^{224}Ra- $CaCO_3$: 1 mg and 14 kBq per 0.4 ml	25.5	14.4
^{224}Ra- $CaCO_3$: 5 mg and 14.7 kBq per 0.4 ml	10.8	4.9
^{224}Ra- $CaCO_3$: 25 mg and 22 kBq per 0.4 ml	1.5	2.6

Fig. 1: Release of Ra-224 is shown in the diagrams and measurements for both Ra-224 and Pb-212 are shown in the table below the respective diagrams. A: Released radioactivity to the saline solution surrounding samples of 0.4 ml contained in dialysis cassettes, mimicking the peritoneal membrane. Each bar represents the average of two samples. B: Skeletal uptake of radioactivity in mice after intraperitoneal injection of 0.4 ml of a cationic Ra-224 solution or suspensions of Ra-224 labeled microparticles. The diagram bars represent average values and the dots individual animals.
References: Oncoinvent AS - Oslo/NO

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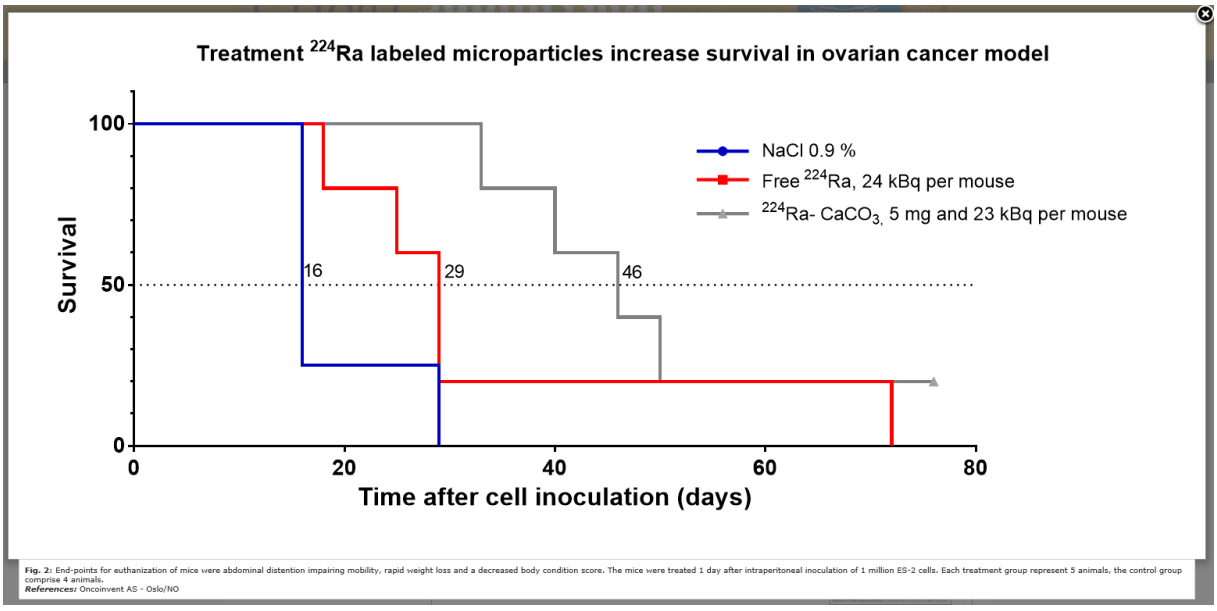

Fig. 2: End-points for euthanization of mice were abdominal distention impairing mobility, rapid weight loss and a decreased body condition score. The mice were treated 1 day after intraperitoneal inoculation of 1 million ES-2 cells. Each treatment group represent 5 animals, the control group comprise 4 animals.
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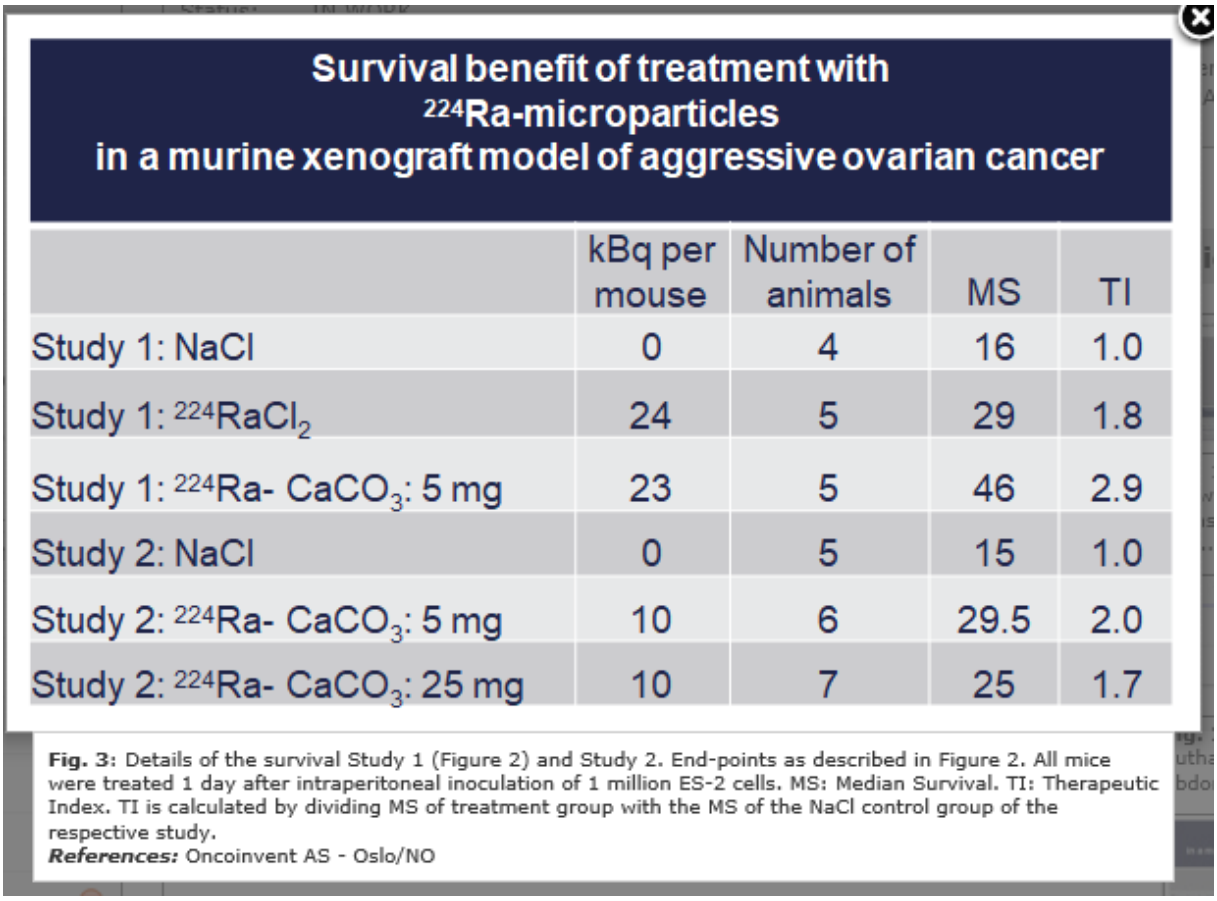

Survival benefit of treatment with ^{224}Ra-microparticles in a murine xenograft model of aggressive ovarian cancer

	kBq per mouse	Number of animals	MS	TI
Study 1: NaCl	0	4	16	1.0
Study 1: $^{224}RaCl_2$	24	5	29	1.8
Study 1: ^{224}Ra- $CaCO_3$: 5 mg	23	5	46	2.9
Study 2: NaCl	0	5	15	1.0
Study 2: ^{224}Ra- $CaCO_3$: 5 mg	10	6	29.5	2.0
Study 2: ^{224}Ra- $CaCO_3$: 25 mg	10	7	25	1.7

Fig. 3: Details of the survival Study 1 (Figure 2) and Study 2. End-points as described in Figure 2. All mice were treated 1 day after intraperitoneal inoculation of 1 million ES-2 cells. MS: Median Survival. TI: Therapeutic Index. TI is calculated by dividing MS of treatment group with the MS of the NaCl control group of the respective study.
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Details of the survival Study 1 (Figure 2) and Study 2. End-points as described in Figure 2. All mice were treated 1 day after intraperitoneal inoculation of 1 million ES-2 cells. MS: Median Survival. TI: Therapeutic Index. TI is calculated by dividing MS of treatment group with the MS of the NaCl control group of the respective study.

Section: Conclusion

High retention of the radioactivity on ^{224}Ra- $CaCO_3$ microparticles is obtained both *in vitro* and *in vivo*. The studies presented show that the high retention is strongly dependent on amount of microparticles per sample volume and per kBq. The *in vitro* model designed to simulate *in vivo* retention of radioactivity seems to be relevant for this type of microparticle suspension as there is a good agreement between the *in vitro* measurements and the biodistribution data in terms of indicated release of radionuclide. Radioactivity measured in bone, is a strong indicator for the release of ^{224}Ra from the peritoneal cavity. Therapeutic studies showed superior survival when treating with IP injection of ^{224}Ra-$CaCO_3$ microparticle suspension compared to IP injection of free cationic ^{224}Ra solution. The data also indicate that high concentrations of particles may potentially reduce the antitumor activity of the ^{224}Ra-$CaCO_3$-suspensions. The results from the biodistribution and therapy studies together suggest that some release of ^{224}Ra and progeny ^{212}Pb from the surface-labeled

microparticles can contribute to a "dose smoothening" effect, where a low level of release of radioactivity from ^{224}Ra- microparticles can have a therapeutic benefit.

Section: References

Westrøm et al., 2018a. Ra-224 labeling of calcium carbonate microparticles for internal α-therapy: Preparation, stability, and biodistribution in mice. J Labelled Comp Radiopharm. 2018 Jan 29.

Westrøm et al., 2018b. Therapeutic Effect of α-Emitting (224)Ra-Labeled Calcium Carbonate Microparticles in Mice with Intraperitoneal Ovarian Cancer. Transl Oncol. 2018 Apr;11(2):259-267.