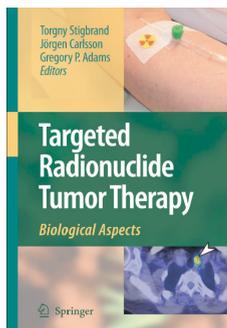


# Chapter 10

## Targeted High-LET Therapy of Bone Metastases

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### Introduction

The clinical implications of skeletal metastases such as pain, pathological fractures, nerve entrapment/spinal cord compression and bone marrow insufficiency have a devastating impact on patients' quality of life [1–4]. External beam radiotherapy effectively relieves pain from localized sites of skeletal metastases [5–9], but the lack of tumor cell selectivity limits its clinical usefulness since normal cells within the target volume receive the same radiation dose as the tumor cells. Furthermore, since skeletal metastases usually are multiple and distributed throughout the axial skeleton [2–4], larger or multiple fields of irradiation are often necessary. However, external beam radiotherapy may further reduce the patient's haematopoietic capacity, already compromised due to bone marrow infiltration of metastases, and, thus, reduce the subsequent tolerance for chemotherapy.

A single fraction of external beam irradiation (8.0 Gy) should be offered to most patients when the clinical indication is pain relief [10–13]. Patients not responding, or those with new pain arising at a previously irradiated site, should be given re-treatment [6–9, 14–17]. In contrast, when the therapeutic aim is local tumor

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control, such as in patients with solitary bony metastases and long life expectancy, or when medullar compression or imminent fractures are present, fractionated radiotherapy is advisable ( $3.0\text{ Gy} \times 10$  or higher) in selected cases [7, 18].

Treatment with bone-seeking radiopharmaceuticals is an intriguing alternative that will target multiple metastases simultaneously – symptomatic as well as asymptomatic foci [19]. Following i.v. injection a selective delivery of ionizing radiation to targeted areas of amplified osteoblastic activity can be obtained. The target is Ca-hydroxy-apatite in the metastasis, particularly abundant in sclerotic metastases from prostate cancer, and also present, although more heterogeneously distributed, in mixed sclerotic/osteolytic metastases from breast cancer. This is evident from a biodistribution image common to all bone-seeking radiopharmaceuticals – exemplified as “hot-spots” visualized on a routine diagnostic bone-scan (by  $^{99\text{m}}\text{Tc-MDP}$ , a radiolabelled bisphosphonate). The clinical experiences using bone-seeking radiopharmaceuticals to relieve pain have been thoroughly reviewed [19–23]. In the commercially available formulations, the radioisotopes involved are beta-emitters: Strontium-89 dichloride (Metastron, GE Healthcare, Chalfont St. Giles, UK) and  $^{153}\text{Sm}$  in a complex with EDTMP (Quadramet, Schering AG, Berlin, Germany, and Cytogen Co., Princeton, NJ, USA).

Published data indicate that lower dosages aimed for pain palliation result in relatively few complications in patients with sufficient bone marrow function. Following i.v. injection, the bone-marrow is, however, an innocent bystander and the dose-limiting organ, and the cross-irradiation of the bone marrow due to the millimeter range of the emitted electrons, represents an ever-present concern with beta-emitting bone-seekers. Furthermore, disease-associated bone marrow suppression already present in these patients may often result in delayed and unpredictable recovery. This severely limits the usefulness of beta-emitting radiopharmaceuticals, especially when dosages are increased to deliver potential antitumor radiation levels [22, 24] and/or repeated treatments are attempted. Only a few clinical studies have so far reported on the feasibility of combining bone-seeking radiopharmaceuticals and chemotherapy [25–30].

## High-LET Radiopharmaceuticals

Dosimetric modeling and preclinical studies have indicated that alpha-emitting radionuclides could be a promising alternative to beta-emitters in the treatment of minimal residual disease by radioimmunotherapy, and there is an increasing interest to apply alpha emitters in cancer therapy [31–35]. The ranges of alpha-emitters are typically between 40 and  $100\mu\text{m}$  in tissue. These ranges are well matched with the size of micrometastases, indicating the potential for a more tumor selective irradiation [36].

In contrast to the beta-emitters, the alpha-particle-emitters deliver a much more energetic and localized radiation, classified as high-linear energy-transfer (LET) radiation [37]. Alpha-particles are relatively heavy, charged particles (helium nuclei

with two positive charges) and produce densely ionizing tracks through tissue that induces predominantly non-reparable double DNA-strand breaks [38]. Patients with skeletal metastases often have chemoresistant disease and/or micrometastases with dormant clonogenic tumor cells residing in cell cycle growth phase  $G_0$ . High-LET irradiation from alpha-emitters will kill such cells at a lower dose/dose-rate than low-LET irradiation [37, 39].

Despite the fact that alpha-emitters are more toxic and mutagenic than beta-emitters, these adverse properties can be compensated for in targeted therapy because of the potential to irradiate much less volumes of normal cells when alpha-emitters are targeted against tumor cell clusters [40]. This feature helps treat skeletal metastases because the short alpha tracks would cause less dose delivered from the bone surfaces to the clonogenic bone marrow cells located within the center of bone marrow containing cavities [40]. Also the spatial distribution of the hydroxy-apatite target within an osteoblastic tumor would facilitate a volume distribution of the radionuclide and make it less likely that tumor cells evade the alpha-particles despite the limited track lengths [39].

The progress in the biomedical application of alpha emitters have been slowed down by the low availability of radionuclides with proper physical and chemical characteristics, supply limitations, and/or expenses for the most popular alpha-emitters,  $^{211}\text{At}$  ( $t_{1/2} = 7.2\text{ h}$ ),  $^{213}\text{Bi}$  ( $t_{1/2} = 46\text{ min}$ ) and  $^{225}\text{Ac}$  ( $t_{1/2} = 10\text{ days}$ ) [35, 41]. Also, because of limited chemical yields and/or short half lives, the production of a final product in clinically useful quantities has been expensive and challenging.

## Radium-223: From Bench to Bedside

Lately, a significant research activity has been conducted on alpha emitters that can be prepared in large quantities from long term operating generators [42, 43]. Examples of such alpha-emitters are  $^{223}\text{Ra}$  ( $t_{1/2} = 11.4\text{ days}$ ),  $^{224}\text{Ra}$  ( $t_{1/2} = 3.7\text{ days}$ ),  $^{227}\text{Th}$  ( $t_{1/2} = 18.7\text{ days}$ ) and the alpha-emitter generator  $^{212}\text{Pb}$  ( $t_{1/2} = 10.6\text{ h}$ ). The unavailability of suitable complexing agents for radium isotopes has prevented the exploration of  $^{223}\text{Ra}$  in radioimmunotherapy [44], but methods have recently been developed to stably encapsulate  $^{223}\text{Ra}$  and  $^{225}\text{Ac}$  into liposomes [45–47].

Technology related to these radionuclides has recently led to a significant commercial development (see [www.algeta.com](http://www.algeta.com)) and mature clinical stage development of a new therapy against bone metastases based on radium-223 – Alpharadin® [48–50].

Like strontium, radium is a natural bone seeker that has previously been used for targeting non-malignant skeletal diseases, such as the use of  $^{224}\text{Ra}$  for treating ankylosing spondylitis, characterized by elevated bone synthesis [51]. Radium-223 is, in our view, the most promising radium isotope, with favorable features for use in targeted radiotherapy. Radium-223 decays ( $t_{1/2} = 11.4\text{ days}$ ) via a chain of short-lived daughter radionuclides to stable lead, producing four alpha-particles (Table 10.1). In the decay of  $^{223}\text{Ra}$ , about 94% of the total decay energy is released

as alpha-particles. The noble gas first daughter  $^{219}\text{Rn}$  has a  $t_{1/2}$  of approximately 4 s, in contrast to the longer-lived radon-daughters from the other naturally occurring radium isotopes.

Radium-223 can be efficiently produced in large amounts from sources of the precursor  $^{227}\text{Ac}$  ( $t_{1/2} = 21.7$  years) in a long-term operating generator [42]. Moreover,  $^{223}\text{Ra}$ 's half-life provides sufficient time for its preparation, distribution (including long distance shipment), and administration to patients. Its low gamma-irradiation is favorable from the point of view of handling, radiation protection, and treatment on an outpatient basis.

Alpha-particles from the first three nuclides in the decay chain are emitted almost instantaneously (Table 10.1). They are therefore likely to contribute to the radiation dose in the vicinity of the site of  $^{223}\text{Ra}$  decay. Hence,  $^{223}\text{Ra}$  has the potential to deliver a therapeutically relevant tumor dose from a relatively small amount of administered activity without causing unacceptable doses to non-target tissue.

**Preclinical studies with  $^{223}\text{Ra}$ .** Animal data and dosimetric studies have indicated that bone-targeted alpha-emitters can deliver therapeutically relevant radiation doses to bone surfaces and skeletal metastases, at activity levels that are acceptable in terms of bone marrow radiation exposure [52]. In a comparative study of  $^{223}\text{Ra}$  and the beta-emitter  $^{89}\text{Sr}$  we found that  $^{223}\text{Ra}$  and  $^{89}\text{Sr}$  had similar bone uptake, and estimates of dose deposition in bone marrow demonstrated a clear advantage of alpha-particle emitters being bone marrow sparing [40].

A therapeutic study of  $^{223}\text{Ra}$  in a nude rat skeletal metastases model showed a significant antitumor activity [32]. In this model, the tumor cells were resistant to

**Table 10.1** Summary of effective energy and dose constants for  $^{227}\text{Ac}$  and progeny

Nuclide	Effective energy <sup>a</sup> (MeV)	Dose constant $\Delta$ (Gy kg Bq <sup>-1</sup> s <sup>-1</sup> )
$^{227}\text{Ac}$ (21.77 years)	0.079	$1.28 \times 10^{-14}$
$^{227}\text{Th}$ (18.68 days)	6.07	$9.73 \times 10^{-13}$
	5.86 <sup>b</sup>	$9.39 \times 10^{-13}$
$^{223}\text{Ra}$ (11.43 days)	5.85	$9.37 \times 10^{-13}$
	5.65 <sup>b</sup>	$9.05 \times 10^{-13}$
$^{219}\text{Rn}$ (3.96 s)	6.81	$1.09 \times 10^{-12}$
	6.75 <sup>b</sup>	$1.08 \times 10^{-12}$
$^{215}\text{Po}$ (1.78 ms)	7.53	$1.21 \times 10^{-12}$
	7.53 <sup>b</sup>	$1.21 \times 10^{-12}$
$^{211}\text{Pb}$ (36.1 min)	0.512	$8.20 \times 10^{-14}$
$^{211}\text{Bi}$ (2.14 min)	6.73	$1.08 \times 10^{-12}$
	6.67 <sup>b</sup>	$1.07 \times 10^{-12}$
$^{207}\text{Tl}$ (4.77 min)	0.498	$7.98 \times 10^{-14}$

Schematic summary of decay data extracted from the MIRD data base (<http://www.nndc.bnl.gov/mird>). Database version of July 2, 2007.

<sup>a</sup>Includes alpha, beta, photon, X-ray, and electron energies.

<sup>b</sup>Includes only alpha particle energies. Branching of less than 1% is not considered.

high doses of cisplatin, doxorubicin and an immunotoxin, as well as to both pamidronate (Aredia) and  $^{131}\text{I}$ -labeled bisphosphonate treatment, suggesting that  $^{223}\text{Ra}$  is therapeutically more effective and could be beneficial in the treatment-resistant skeletal metastases [33].

**Clinical studies with  $^{223}\text{Ra}$ .** A clinical development program for  $^{223}\text{RaCl}_2$  was initiated, based on these results and on approval obtained from the institutional review boards and regulatory authorities.

**Phase 1A.** In a phase 1 study of single-dosage administration of escalating amounts of  $^{223}\text{Ra}$  (46, 93, 163, 213, or 250 kBq/kg) in 25 patients with bone metastases from breast and prostate cancer [49], dose-limiting hematological toxicity was not observed. Mild and reversible myelosuppression occurred, with only grade 1 toxicity for thrombocytes at the two highest dose levels. Quality of life was evaluated at baseline and at 1, 4, and 8 weeks after injection, and pain relief was observed for all time points in more than 50% of the patients [49]. Furthermore, a decline in total serum alkaline phosphatase greater than 50%, increasingly used as a prognostic marker in metastatic prostate cancer, was observed among patients with elevated pretreatment values. Radium-223 was rapidly cleared from the blood with only 12% of its initial value at 10 min after injection. It was further reduced to 6% at 1 h and to less than 1% at 24 h after infusion. In patients where gamma-camera scintigraphy was performed,  $^{223}\text{Ra}$  accumulated in skeletal lesions similar to patterns observed in diagnostic bone scans with  $^{99\text{m}}\text{Tc-MDP}$  [49], and a predominantly intestinal clearance was demonstrated.

**Phase 1B.** A small phase 1B feasibility study involving six patients with advanced prostate cancer was then performed [48] with the objective to evaluate the safety profile of repeated  $^{223}\text{Ra}$  injections. Six prostate cancer patients were administered a total dosage of up to 250 kBq  $\text{kg}^{-1}$  body weight, either as a fractionated regimen of two injections of 125 kBq  $\text{kg}^{-1}$  bodyweight with a 6-week interval (three patients) or 50 kBq  $\text{kg}^{-1}$  body weight dosages given five times with a 3-week interval (three patients). The patients in the 50 kBq  $\text{kg}^{-1} \times 5$  group did not experience any additional toxic effects compared with the single-injection phase 1A study related to repeated treatment. It appeared that the hematological profiles were smoothed out because of the fractionation schedule compared with a single dosage totaling the same as the five fractions combined. Because of non-skeletal disease progression, only one of the patients in the 125 kBq  $\text{kg}^{-1} \times 2$  group actually got the second dosage. Of the two patients not given the 125 kBq  $\text{kg}^{-1}$  follow-up dosage, one died due to progression of liver metastases, and the other was deemed unfit for further treatment due to recurrence of a previous heart condition. Mild and reversible myelosuppression occurred, with nadir 2 to 3 weeks after injection and complete recovery during the follow-up period. The thrombocytes revealed only grade 1 toxicity, whereas neutropenia of maximum grade 3 occurred in one of the patients. Few other adverse events were seen [39, 48].

The main experience from this small phase 1B study was that repeated administration of  $^{223}\text{Ra}$  was well tolerated, and that the time span between injections should be scheduled according to the dosages given; i.e. so that the blood cell count could normalize before a new injection was administered.

**Phase 2.** Mature data from a phase 2 randomized trial, of external beam radiation plus either saline injections (four times with 4-week intervals) or four times repeated  $^{223}\text{Ra}$  (50 kBq/kg given at 4-week intervals), has recently been published [50]. Adjuvant  $^{223}\text{Ra}$  treatment resulted in a statistically significant decrease in bone alkaline phosphatase from baseline compared with placebo showing a particularly strong decrease in patients with elevated pre-treatment levels [50]. The median relative change during treatment for the external radiation plus  $^{223}\text{Ra}$  group (33 patients) was  $-65.6\%$  vs.  $+9.3\%$  in the external beam radiation plus saline group (31 patients). This observation showed that the areas mostly affected by  $^{223}\text{Ra}$  were the regions with an elevated bone metabolism [39]. In the external radiation plus  $^{223}\text{Ra}$  group, 15 of 31 patients had a prostate-specific antigen decrease of more than 50% from baseline compared with only 5 of 28 patients in the group receiving external radiation plus saline. The median time to PSA progression was 26 weeks in the  $^{223}\text{Ra}$  group and 8 weeks in the placebo group [50].

A favorable adverse event profile was confirmed with minimal bone marrow toxicity for patients who received  $^{223}\text{Ra}$  [50]. The myelosuppression observed after  $^{223}\text{Ra}$  treatment was minimal and seems different from that observed with the beta-emitting nuclides [19, 22, 50]. With  $^{223}\text{Ra}$ , the neutrophils decreased more than thrombocytes, whereas for beta-emitters, thrombocytopenia are commonly dose limiting. It seems that with alpha-emitters, the endosteal bone surface receives high radiation doses, whereas fractions of the bone-marrow are spared.

Importantly, survival analyzes from this Phase 2 trial showed a significant overall survival benefit [50]. The hazard ratio for overall survival, adjusted for baseline covariates was 2.12 ( $p = 0.020$ , Cox regression). This finding suggests that  $^{223}\text{Ra}$ , alone or in combined treatment strategies, should be further evaluated in future therapeutic studies aiming at further delaying disease progression and improving survival in patients with skeletal metastases from hormone-refractory prostate cancer.

## Radioimmunotherapy

Actinium-227 has several attractive features as source material not only for  $^{223}\text{Ra}$  but also for the alpha emitting radionuclide  $^{227}\text{Th}$ . Actinium-227 can be produced relatively easily in large amounts by neutron irradiation of  $^{226}\text{Ra}$  in reactors [53]. Its half life of 21.7 years is suitable for a long term operated generator.

Thorium is classified as an actinide although its chemical properties are slightly different from that of actinium. In aqueous solution Th exists as 4+ while Ac is present as 3+, suggesting some differences in the reactivity and stability with various complexing agents. Previously McDevitt et al. have found that DOTA was useful as chelator for  $^{225}\text{Ac}$  giving conjugates with monoclonal antibodies, but they required a change in standard reaction conditions compared with e.g.  $^{90}\text{Y}$  conjugates [54]. A two step reaction sequence including heating of the Ac-DOTA complex followed by cooling prior to antibody conjugation was required to obtain

sufficient stability of the radioimmunoconjugate. A similar two-step reaction sequence would also conjugate  $^{227}\text{Th}$  to antibodies [53].

As mentioned above, the mother nuclide for  $^{223}\text{Ra}$  is  $^{227}\text{Th}$ . This is also an alpha emitter with a half life of 18.7 days. Thus, relevant in vitro and in vivo properties have been demonstrated for monoclonal antibodies labeled with  $^{227}\text{Th}$  via the chelator p-SCN-benzyl-DOTA [53, 55, 56]. Recently, novel translational studies in CD-20 expressing human xenografts indicating a therapeutic potential of  $^{227}\text{Th}$ -Mabthera have recently been published [57].

### ***A Pilot Experiment with $^{227}\text{Th}$ -Labeled Herceptin***

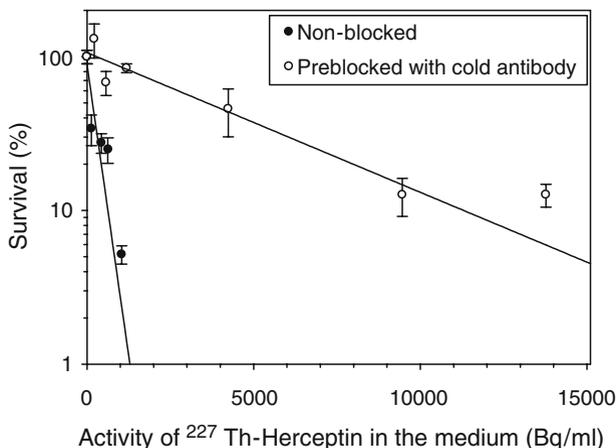
Based on these observations, a pilot experiment was therefore conducted with Her-2 receptor positive BT-474 breast cancer cells. Tumor cells growing as monolayer in culture flasks, were trypsinized and diluted in growth medium (RPMI 1640, 10% FCS supplied with glutamine, streptomycin and penicillin) to about one million cells per milliliter. Ten milliliter reaction tubes were added 0.5 ml of the cell suspension and half of the tubes were added 25  $\mu\text{g}$  unlabeled Herceptin and incubated for 5 min at room temperature to block the antigens and act as nonbinding control cells. Thereafter antibody-blocked, as well as non-blocked cells were incubated with various amounts of  $^{227}\text{Th}$ -radiolabeled Herceptin. After 1 h of incubation at 37 °C, the cell suspensions were diluted 1,000–5,000 times and plated into culture flasks supplied with growth medium. After 2–3 weeks colonies were fixed with ethanol, stained with methylene blue and counted using a magnifying glass and a phase contrast microscope. Colonies of more than 30 cells were counted.

Cell survival is presented in Fig. 10.1. Figure 10.2 demonstrates binding of  $^{227}\text{Th}$ -Herceptin to BT-474 cells. The tracks made by single alpha-particles emitted from the cell surfaces and from  $^{223}\text{Ra}$  and daughters in the medium are visualized by micro-autoradiography.

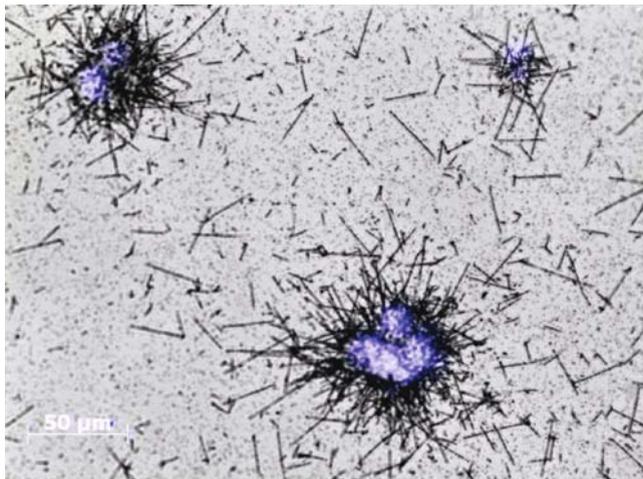
It is anticipated that similar results may be obtained by other monoclonal antibodies with specificity towards tumor-associated antigens (e.g. anti-PSMA against prostate cancer).

### **A Combined Treatment Strategy**

When a symptomatic skeletal metastasis is treated by external beam radiotherapy, new painful foci most often arise after a short time, indicating the existence of microscopic metastases alongside the macroscopic lesions. Bone-marrow micrometastases are also present in patients both with seemingly localized breast cancer [58] and prostate cancer [59]. They may later develop into skeletal metastases, and even act as a nidus for the subsequent growth of visceral metastasis [60].



**Fig. 10.1** Survival of HER-2 positive BT-474 cells treated with  $^{227}\text{Th}$ -Herceptin (closed circles). The BT-474 cells were incubated with  $^{227}\text{Th}$ -Herceptin for 1 h in suspension and seeded in flasks. During seeding the activity was diluted 1,000–5,000 times. The open circles represent experiments where binding of  $^{227}\text{Th}$ -Herceptin was blocked by pre-incubation of the cells with 50  $\mu\text{g}/\text{ml}$  cold Herceptin. Plating efficiency was determined using pre-blocked (open circles) or non-blocked (closed circles) cells. Treatment with 50  $\mu\text{g}/\text{ml}$  cold Herceptin resulted in 76% survival. The highest concentration of Herceptin used on the cells treated with only  $^{227}\text{Th}$ -Herceptin was 0.7  $\mu\text{g}/\text{ml}$  (1,000 Bq/ml). Saturated antigen:  $A_{10} = 11,290$  Bq/ml,  $A_{37} = 5,060$  Bq/ml. Unsaturated antigen:  $A_{10} = 620$  Bq/ml,  $A_{37} = 280$  Bq/ml

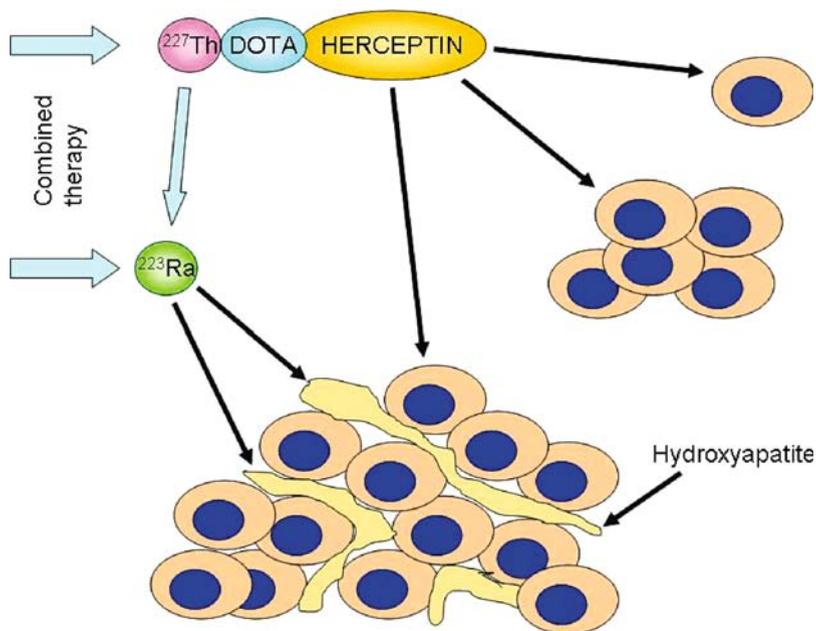


**Fig. 10.2** Microautoradiograph of individual alpha tracks from  $^{227}\text{Th}$ -Herceptin bound to BT-474 microcolonies; the lower comprising five tumor cells. The cells were seeded on slides and incubated with 10 kBq/ml  $^{227}\text{Th}$ -Herceptin for 4 h, washed with PBS with 1% BSA and fixed in 70% ethanol before dipping in autoradiographic emulsion (Hypercoat, Amersham Biosciences, Uppsala, Sweden). After 8 days of exposure the slides were processed according to the manufacturer's instructions. Subsequently, cells were stained with Hoechst 333258, which binds to DNA, and images were acquired using brightfield settings for the alpha-tracks and UV excitation for the nuclei

Because of the dynamic nature of the developing skeletal metastases, optimal therapy should effectively deliver radiation both to multiple macroscopic foci as well as to microscopic disease, including small tumor foci and single clonogenic tumor cells.

### ***Actinium-227 – Thorium-227 – Radium-223: A Novel Technology Platform***

Solid tumor deposits have barriers to the uptake of macromolecules, such as monoclonal antibodies [61, 62], whereas radium is a small cation that easily penetrates into a sclerotic metastasis. Based on the results presented above we here propose a strategy for how this might be accomplished. Depending on the biological half life of the antibody carrier, the  $^{227}\text{Th}$  will be an in vivo generator for the bone seeking  $^{223}\text{Ra}$ . Thus, if conjugated to an antibody with affinity for prostate or breast cancer cells,  $^{227}\text{Th}$ -immunoconjugates represent a dual action strategy for alpha emitter based targeted killing of bone metastases: First a cell



**Fig. 10.3** Dual action targeted strategy: Alpharadin<sup>®</sup> ( $^{223}\text{Ra}$ ) is a small molecule that rapidly targets hydroxyapatite in the sclerotic parts of the macroscopic skeletal metastasis. A macromolecule such as a monoclonal antibody will target single cells and may penetrate into small clusters of tumor cells – here exemplified by  $^{227}\text{Th}$ -Herceptin that binds to the cell surface of HER2-positive breast cancer cells and microcolonies. When  $^{227}\text{Th}$  decays,  $^{223}\text{Ra}$  is formed and will diffuse and bind to the calcified metastasis (yellow) and the treatment continues

surface antigen targeting by  $^{227}\text{Th}$  – then hydroxyapatite targeting by the daughter radionuclide  $^{223}\text{Ra}$ .

**Combined treatment**, with dual/plural modes of action, is a firm treatment principle in cancer therapy. We here propose to utilize two alpha-emitting radiopharmaceuticals (bone-seeking radium-223 and thorium-227 conjugated to a monoclonal antibody) targeting two different targets and stages in the development cascade of skeletal metastases (Fig. 10.3):

1. Targeting of hydroxyapatite producing macroscopic metastases by radium-223 (Alpharadin<sup>®</sup>).
2. Targeting of tumor single cell surface epitopes with thorium-227-labelled monoclonal antibodies which, due to their decay characteristics, will form radium-223 that is then partially trapped in the hydroxyapatite producing metastases.

**Repeated dosing** is the common way to use therapeutics in oncology. This is already shown to be feasible with bone-seeking radium-223 [50] and should be further exploited by two reasons. First the range of the radiation is short, and therefore repeating the treatment could improve dose homogeneity within the target. Second the bone metabolism in normal bone and calcified metastases is a dynamic process where the absorptive and resorptive zones change position over time, which would likely affect the microdistribution of the bone-seeking compound over time. Based on the low toxicity observed in Phase 1 and Phase 2 studies, the possibility seemingly exist to expand dosing further to at least six repeated monthly injections of Alpharadin.

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