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Radium 223-Mediated Zonal Cytotoxicity of Prostate Cancer in Bone

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Abstract

Background: Bone-targeting radiotherapy with Radium-223 (Rad-223), a radioisotope emitting genotoxic alpha-radiation with limited tissue penetrance ($\sim 100\ \mu\text{m}$), prolongs the survival of patients with metastatic prostate cancer (PCa). Confoundingly, the clinical response to Rad-223 is often followed by detrimental relapse and progression, and whether Rad-223 causes tumor-cell directed cytotoxicity in vivo remains unclear. We hypothesized that limited radiation penetrance in situ defines outcome.

Methods: We tested Rad-223 overall response by PC3 and C4-2B human PCa cell lines in mouse bones ($n = 5\text{--}18$ tibiae per group). Rad-223 efficacy at subcellular resolution was determined by intravital microscopy analysis of dual-color fluorescent PC3 cells ($n = 3\text{--}4$ mice per group) in tissue-engineered bone constructs. In vivo data were fed into an in silico model to predict Rad-223 effectiveness in lesions of different sizes (1-27, 306 initial cells; $n = 10\text{--}100$ simulations) and the predictions validated in vivo by treating PCa tumors of varying sizes in bones ($n = 10\text{--}14$ tibiae per group). Statistical tests were performed by two-sided Student *t* test or by one-way ANOVA followed by Tukey's post-hoc test.

Results: Rad-223 (385 kBq/kg) delayed the growth (means [SD]; comparison with control-treated mice) of PC3 (6.7×10^5 [4.2×10^5] vs 2.8×10^6 [2.2×10^6], $P = .01$) and C4-2B tumors in bone (7.7×10^5 [4.0×10^5] vs 3.5×10^6 [1.3×10^6], $P < .001$). Cancer cell lethality in response to Rad-223 (385 kBq/kg) was profound but zonally confined along the bone interface compared with the more distant tumor core, which remained unperturbed (day 4; 13.1 [2.3%] apoptotic cells, 0-100 μm distance from bone vs 3.6 [0.2%], $>300\ \mu\text{m}$ distance; $P = .01$). In silico simulations predicted greater efficacy of Rad-223 on single-cell lesions (eradication rate: 88.0%) and minimal effects on larger tumors (no eradication, 16.2% growth reduction in tumors of 27 306 cells), as further confirmed in vivo for PC3 and C4-2B tumors.

Conclusions: Micro-tumors showed severe growth delay or eradication in response to Rad-223, whereas macro-tumors persisted and expanded. The relative inefficacy in controlling large tumors points to application of Rad-223 in secondary prevention of early bone-metastatic disease and regimens co-targeting the tumor core.

Bone metastases cause major complications and reduce life expectancy in patients with advanced prostate cancer (PCa) because they resist conventional or molecular-targeted therapy (1). Radiotherapy, either as external beam radiation or as systemically applied beta-emitting radiopharmaceuticals strontium and samarium, has been used for palliative treatment of symptomatic PCa bone lesions without life-prolonging effects

(2,3). Instead, Radium-223 (Rad-223), a rare earth metal radioisotope, emerged as the first targeted ionizing radiation therapy that improves symptom-free and overall survival in patients with advanced metastatic PCa (4). Rad-223 displays chemical properties similar to calcium, becomes enriched in bone after in vivo administration, and emits alpha particles with locally high energy but limited tissue penetrance ($<100\ \mu\text{m}$) (3,5). As a

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consequence, alpha emission induces toxicity and mutagenesis in directly exposed cells but causes only negligible toxicity systemically and toward the bone marrow compared with beta-emitting isotopes (3,4).

The efficacy of Rad-223 in patients correlates with a sustained decline of osteoblast-derived alkaline phosphatase in serum, denoting reduced bone turnover, whereas levels of tumor cell-derived prostate-specific antigen are variable without predicting outcome (4,6). We and others have speculated that these clinical results may be attributable to a primary effect of Rad-223 on the tumor-associated microenvironment in bone (7,8), and such stromal reprogramming might de-escalate the microenvironmental support for PCa growth and hence delay disease progression. Direct toxicity of Rad-223 on cancer cells has been detected after high-dose application *in vitro* (9); however, the relevance of this effect *in vivo* remains unclear given the low tissue penetration of alpha particles and divergent clinical prostate-specific antigen responses (3,5).

We hypothesized that limited radiation penetrance affects the therapeutic effects of Rad-223 on metastatic PCa to bone. In this work we evaluated the role of Rad-223 physical and mechanical determinants of response on the growth of PCa in bone and addressed the principles discriminating efficacy from failure. We determined the outcome of Rad-223 treatment by combining 3D intravital microscopy, *in silico* modeling, and end-point analysis in preclinical PCa models in bone.

Methods

Rad-223 Therapy

Animal studies were approved by the Institutional Animal Care and Use Committee of The University of Texas MD Anderson Cancer Center and performed according to the institutional guidelines for animal care and handling (more details are provided in the [Supplementary Methods](#), available online). Mice bearing PC3 or C4-2B luciferase-expressing tumors ($n = 5-7$ or 18 tibiae per group, respectively) were randomized and received a single dose of Rad-223 (0–770 or 385 kBq/kg, respectively; Xofigo, Bayer, Leverkusen, Germany) intravenously 5 or 11 days after intra-tibia injection. Tumor growth was monitored by macroscopic bioluminescence imaging using an IVIS 200 imaging system (Perkin Elmer, Waltham, MA). For intravital microscopy experiments, mice bearing PC3 dual-color tumors in the miniaturized tissue-engineered bone construct (mTEBC) were treated with a single dose of Rad-223 (385 kBq/kg) the day following the onplantation of the dorsal skin fold chamber ($n = 3-4$ mice per group). Onplantation occurred when a micro-osteolytic lesion was identifiable within the mTEBC, about 7–10 days after PC3 cell administration. For experiments on the role of tumor size in Rad-223 response, $n = 10-14$ tibiae were implanted with PC3 or C4-2B Luc cells.

Multi-Parameter Intravital Microscopy, Digital Image Processing, Segmentation, and Quantitative Analysis

Tumors in the mTEBC were monitored by combined near-infrared and infrared intravital multiphoton microscopy (Trimscope II, LaVision BioTech) using a long-working distance $25\times$ NA 1.05 oil/water objective (Olympus) through the imaging window stably mounted onto a temperature-controlled platform (37°C) (10). The microscope was equipped with three Titanium (Ti): Sapphire lasers (Chameleon-XR, Coherent) and

two Optical Parametric Oscillators (APE/Coherent), resulting in a tunable excitation range from 800 to 1300 nm (11). Detection of bone and cancer cells was achieved by spectral separation of eGFP (525/50, 920 nm), DsRed2 (595/40, 1090 nm), and second harmonic generation (525/50, 1090 nm), and 3D stacks were obtained for up to 300 μm depth at 10 μm step-size.

Images from individual 3D stacks were reconstructed and analyzed using FIJI (12). Adjacent 3D scan volumes were stitched to generate large-field overviews. The replicative and apoptotic status of PC3 dual-color cells at different distances from the bone interface in the mTEBC was detected as follows. Single slices were segmented in equidistant corridors parallel to the bone interface (0–100, 100–200, 200–300, $>300\mu\text{m}$ in x-y) delineated by the shape of the bone within osteolytic lesions. The number of mitotic, apoptotic, or nondividing cells contained in each area was counted manually from the H2B/eGFP signal of the tumor cell nuclei based on nuclear shape, as follows: presence of condensed chromosomes, chromosomal alignment on the metaphase plate, or ongoing anaphase/telophase for mitotic cells; nuclear fragmentation for apoptotic cells; or round, intact nucleus for interphase. Cell feature analysis was performed by a single operator and validated by blinded analysis by an independent operator.

Further experimental methods are detailed in the [Supplementary Methods](#) (available online).

Statistical Analysis

Statistical analysis was performed using GraphPad Prism 7.0 (GraphPad Software, San Diego, CA) To test differences between two populations the unpaired two-sided Student *t* test was applied. To test the differences among more than two populations, one-way ANOVA was performed followed by Tukey's honestly significant difference (HSD) post-hoc test. All statistical tests were two-sided, and a *P* value of less than .05 was considered statistically significant.

Results

Rad-223 Effects on PCa Cell Growth in the Tibia

To monitor Rad-223 effects on aggressive PCa tumors in bone, PC3 cells expressing luciferase were implanted into mouse tibiae ($n = 5-7$ per group) and treated with Rad-223 at different doses (0–770 kBq/kg), and growth response was monitored over time by macroscopic bioluminescent imaging ([Figure 1A](#)). Rad-223 at 192, 385, or 770 kBq/kg statistically significantly delayed tumor growth (means [SD]: 2.8×10^6 [2.2×10^6] in control-treated mice; 7×10^5 [7.4×10^5], 6.7×10^5 [4.2×10^5], or 7×10^5 [5.0×10^5] in 192, 385, or 770 kBq/kg-treated mice at day 14; $P = .009$, .01, and .01, respectively), whereas doses below 96 kBq/kg showed limited impact (means [SD]: 1.2×10^6 [7.1×10^6] at day 14; [Figure 1B](#)). Growth arrest induced by doses of 192 kBq/kg and beyond was transient and lasted for 11 days, followed by tumor regrowth ([Figure 1B](#)), in line with Rad-223 radioactive decay (11.4 days half-life) (3) and reduced radiotoxicity thereafter. To comply with previous preclinical dosing (300 kBq/kg) (9,13), we applied a comparable dose (385 kBq/kg) in subsequent experiments and detected a similar biphasic response to Rad-223 in bone metastasis-derived osteoblastic C4-2B tumors ($n = 18$ per group; means [SD]: 3.5×10^6 [1.3×10^6] in control-treated mice vs 7.7×10^5 [4.0×10^5] at day 15, $P < .001$), with transient growth arrest until day 11 followed by regrowth ([Figure 1C](#)). The transient

Figure 1. Macroscopic therapy response of prostate cancer tumors in bone to Radium-223 (Rad-223). **A)** Schematic representation of the experimental model. Tumors were implanted into the tibia, and the response to Rad-223 was monitored by bioluminescence. **B)** Rad-223 dose response of PC3 tumors in bone. Mice were randomized 5 days after tumor implantation (2.5×10^5 cells per tibia) and treated with Rad-223 at the indicated doses. Mean (SD), $n = 5-7$ tibiae per group. Grey box, first Rad-223 decay. P value by one-way ANOVA followed by Tukey's HSD post-hoc test. **C)** Response of C4-2B tumors in bone to Rad-223. Mice were randomized 11 days after tumor implantation (1×10^6 cells per tibia) and treated with Rad-223 at 385 kBq/kg. Mean (SD), $n = 18$ tibiae per group. Grey box, first Rad-223 decay. P value by Student t test, unpaired, two-sided.

Figure 2. Tissue-engineered bone model for intravital microscopy analysis. Schematic representation of the workflow for miniaturized tissue-engineered bone construct generation and application. impl = implantation; iMPM = intravital multiphoton microscopy; w = weeks.

growth delay in both PCa models in bone replicates the observed initial growth delay followed by progression during clinical Rad-223 therapy (4).

Longitudinal Intravital Microscopy of Rad-223-Treated PCa Tumors in Bone

To address the effects of Rad-223 on solid tumors in bone, we applied longitudinal intravital multiphoton microscopy and monitored the 3D niches of therapy response vs resistance (14). An mTEBC was generated in the flank of the mouse by co-implanting a calcium phosphate-coated medical-grade polycaprolactone scaffold, polymerized fibrin, and bone morphogenetic protein 7 (Figure 2). We previously demonstrated that the microanatomy and cellular composition of the mTEBC are very similar to normal mouse bones. We further showed that administration of PC3 PCa cells to the mTEBC induced osteoclast

recruitment, activation, and bone resorption and that pathological bone remodeling, halted by administration of zoledronic acid (a bisphosphonate that targets osteoclasts), could be monitored by intravital microscopy (14).

After maturation of the mTEBC, dual-color PC3 cells (which express nuclear H2B/eGFP and cytoplasmic DsRed2 to detect the chromatin organization during replication and apoptosis at the single-cell level) were implanted into the mTEBC (Figure 2; $n = 3-4$ mice per group), and their response to Rad-223 was visualized by intravital multiphoton microscopy (Figures 2 and 3A). Bone matrix was detected by second harmonic generation, a nonlinear scatter process elicited by its collagen content, as a topographic reference for the bone interface (Figure 3A).

We first tested which zones within the tumor responded preferentially to Rad-223 using 3D single-cell cytometry in situ. Images of osteolytic lesions were segmented within 100, 200,

Figure 3. Subregions of PC3 tumor response to Radium-223 (Rad-223) monitored by intravital multiphoton microscopy. **A**) Intravital multiphoton microscopy detection of PC3 cells adjacent to osteolytic zone in the miniaturized tissue-engineered bone construct 4 days post-Rad-223 treatment. Nucleus (H2B/eGFP, green); cytoplasm (DsRed2, red); collagen fibers, bone (second harmonic generation, cyan); boxes, magnification. Bar = 100 μ m. **B**) Magnification of PC3 cells. Asterisk, apoptotic nucleus; pound, mitotic nucleus. Bar = 20 μ m. **C**) Map of PC3 cell nuclear states 4 days post-Rad-223 treatment; dot, interphase (IP); green circle, mitosis; red circle, apoptosis. PC3 lesion has been segmented at 100, 200, and 300 μ m from the bone interface (dotted lines). Bar = 100 μ m. **D**) Comparison of PC3 cell nuclear status overtime at days 4, 7, and 11 post-Rad-223 treatment. Dot, interphase (IP); green circle, mitosis; red circle, apoptosis; B = bone. PC3 lesion has been segmented at 100, 200, and 300 μ m from the bone interface (dotted lines). Bar = 100 μ m. A miniature of the original image is included. **E**) Number of mitotic and apoptotic cells (represented as percent of the total [tot] number of cells) at different distances from bone in Rad-223-treated and control mice (mean [SD], $n = 4$ lesions). **F**) Ratio between mitotic and apoptotic cells at different distances from bone in control-treated mice at the baseline (mean [SD], $n = 4$ lesions). **G**) Ratio between mitotic and apoptotic cells at different distances from bone at day 4, 7, and 11 post-Rad-223 treatment (mean [SD], $n = 3$ –4 lesions per time point). P value by one-way ANOVA followed by Tukey's HSD post-hoc test.

300, or greater than 300 μ m equidistance from the bone interface, and the number of mitotic and apoptotic H2B/eGFP-positive events in each zone was quantified at day 4, 7, and 11 posttreatment (Figure 3B–D). Four days after Rad-223 administration, the zone closest to bone (up to 100 μ m) displayed an almost quadrupled increase in apoptosis and negligible mitotic frequency compared with farther zones (>300 μ m; means [SD]:

13.1 [2.3%] vs 3.6 [0.2%] apoptotic cells, $P = .01$; 0.9 [0.5%] vs 6.1 [0.8%] mitotic cells, $P < .001$; Figure 3A–E). The amount of apoptotic and mitotic cells in the more distant core reached levels comparable to control-treated tumors, which did not exhibit any zonal distribution (means [SD], 0–100 μ m vs >300 μ m distance from bone: 2.5 [0.5%] vs 2.3 [0.9%] apoptotic cells; 5.3 [1.4%] vs 5.9 [2.2%] mitotic cells; Figure 3, E and F).

Figure 4. In silico simulation of tumor subregions during Radium-223 (Rad-223) therapy response in bone. **A,B** Schematic representation of an in vivo tumor lesion in the miniaturized tissue-engineered bone construct (**A**) compared to in silico topology created by agent-based modeling (ABM). **B** White horizontal arrows in the lower panel indicate emission of α -particles by bone treated with Rad-223 for xy (**top panels**) and xz (**bottom panels**) orthogonal views. Bar = 100 μ m. **C** Representative cell states (Apopt = apoptosis; IP = interphase; Mit = mitosis) in untreated tumor lesion created through the ABM after initialization of growth for 1 hour. Empty dot, interphase (IP) cell; red dot, apoptotic cell; green dot, mitotic cell. Bar = 100 μ m. **D** Tumor growth curves in silico ($n = 10$ simulations) and in vivo (PC3; $n = 10$ tibiae). Data represent the means (SD). **E** Functions describing Rad-223 decay over time and spatial distribution of apoptosis or mitosis probabilities in the presence of Rad-223. **F,G** Topologic representation (**F**) and quantification of therapy response with single-cell resolution (**G**). **F** Visualization of the graphic interface of treated and untreated tumors, including tumor subregions based on distance from bone and cell states defining the therapy response. Bar = 100 μ m. **G** Time-dependent cell growth as a function of distance from the bone interface, $n = 10$ simulations per group.

The ratio of mitosis to apoptosis within 0–100 μ m near the bone decreased over time to the level of control mice 11 days posttreatment (means [SD]: 1.1 [0.5] vs 2.2 [0.7] in Rad-223 vs control-treated mice, respectively; Figure 3, F and G). These data indicate that Rad-223 induces direct lethality in tumor subregions adjacent to bone, in line with the predicted short efficacy radius of alpha particles (3). Notably, however, the viability and growth of distal tumor portions remained unperturbed with ongoing mitotic activity.

In Silico Modeling of Topology and Efficacy of Rad-223 Response

To recreate the dose and space conditions of the tumor response to Rad-223 in bone and investigate their effects on tumor regression or persistence across a broad multi-parameter space, an in silico agent-based model of tumor in bone was developed (Figure 4, A and B). Through a bottom-up approach, the biological rules recorded in vivo (Figure 3E–G) were

implemented to simulate the zonal therapy effects relative to the tumor–bone interface. Therefore, the agent-based model tracked mitosis, apoptosis, and nondividing states of individual cells and their combined consequences for tumor evolution or regression (Figure 4C). As baseline, we simulated the exponential growth of a tumor lesion, achieving kinetics similar to in vivo experiments (Figure 4D; compare Figure 1B).

To describe the niche-dependent therapeutic response to Rad-223, we attributed probabilities of mitosis, apoptosis, or G₁/S/G₂ (interphase) nuclei to tumor cells based on their distance from bone and the temporal decay of Rad-223 (Figure 4E) to reflect frequencies retrieved from Rad-223-treated mice (Figure 3E–G). For Rad-223-treated conditions, the growth rate of tumor cells was inverse to their distance from the bone interface, with growth arrest in cells directly adjacent to bone and growth rates similar to control conditions cells for distances greater than 300 μ m (Figure 4, F and G). As overall response to treatment with Rad-223, macroscopic tumor growth transiently stalled followed by growth, whereas untreated tumors grew exponentially (Figure 4G). These data suggest that lesions that are close enough to bone in their

Figure 5. In silico simulation of Radium-223 (Rad-223) response in tumors of different sizes in bone. **A)** Comparison of the growth pattern in lesions with different initial cell number (i #); **Red**, Rad-223-mediated reduction (%). Data show the means from 10 simulations. @ = sum of the layer analysis for the conditions represented in **Figure 4G**. **B)** Probability of cell distribution relative to the bone interface for tumors of different sizes. **C)** Occurrence of apoptotic and mitotic cells as percentage of total cells for different time points and initial tumor size in untreated or Rad-223-treated condition. Triangles indicate increasing lesion size (4192, 597, 2439, 9800, 27 306 initial cell number; 10 simulations/lesion; initial cell number, i #). Data represent the means (SD). **D,E)** Topologic representation with single-cell resolution of therapeutic response in Rad-223 simulations in two representative lesions (192 9800 initial cell number, i #). The overall size of the lesions is shown in **D**; bar = 240 μm . Dotted box, magnification. **E)** Magnification, visualization of the graphic interface (Apopt = apoptosis; IP = interphase; Mit = mitosis) over time. Bar = 100 μm .

entirety, such as micro-tumors, should be fully eradicated by Rad-223, whereas tumors with high mass proportions extending beyond the 300 μm from bone should persist.

To verify this prediction, we simulated tumors ranging from one cell to macro-lesions (up to 27 306 cells) and tested their sensitivity to Rad-223 as a function of tumor size and proximity to bone (**Supplementary Figure 2**, available online; **Figure 5A**). Whereas most cells in small lesions were located within a 100- μm distance to the bone, in larger tumors more than 90.5% of the cells were located beyond 300 μm in the core (**Figure 5B**). In this setting, Rad-223 was efficient in

halting the growth of single cells, with eradication rates up to 88.0% and only 1.0% of the surviving cells reaching nine-cell volumes 15 days post Rad-223 treatment (**Supplementary Figure 2**, available online). In small to mid-sized lesions (from 4 to 597 cells), Rad-223 decreased cell growth by 97.5% and 78.1%, respectively, with a 42.0% eradication probability for four-cell lesions, whereas macro-tumors consisting of 27 306 initial cells retained growth rates similar to untreated tumors (with 16.2% growth reduction; **Figure 5A**; **Supplementary Movies 1–3**, available online). Notably, the percentage of apoptotic cells decreased in lesions of

increasing dimensions, and the opposite occurred for mitosis (Figure 5, D and E; [Supplementary Movie 1–3](#), available online). Thus, *in silico* analysis predicts that cytotoxicity induced by Rad-223 targets micro-tumors located near the bone interface with particular efficacy.

In Vivo Validation of In Silico Predictions

We then aimed to confirm the *in silico* prediction of the ability of Rad-223 to control or even eradicate small, but not large, bone tumors. Increasing numbers of PC3 cells expressing luciferase were administered into the tibia of mice belonging to different groups (0.1 , 0.25 , 1 , 1.5×10^6 cells per tibia; $n = 10$ – 14 tibiae per group), treated with Rad-223, and the therapeutic response was followed over time (Figure 6, A and B). Rad-223 statistically significantly reduced the growth of lesions generated with an initial load of 0.1×10^6 (means [SD]: 1.2×10^6 [1.7×10^6] in control-treated mice vs 4.9×10^4 [8.8×10^4] in Rad-223-treated mice, $P = .048$; Figure 6, A and B) and 0.25×10^6 cells (means [SD]: 5.7×10^6 [4.3×10^6] in control-treated mice vs 2.1×10^6 [2.0×10^6] in Rad-223-treated mice, $P = .01$; Figure 6A), including a subset of mice remaining below the detection limit of the luciferase signal (Figure 6C, dashed circle). As size threshold, larger tumors beyond 1×10^6 implanted cells retained growth competence despite Rad-223 therapy (means [SD]: 8.5×10^6 [7.9×10^6] in control-treated mice vs 6.1×10^6 [5.2×10^6] in Rad-223-treated mice; Figure 6A–C). As histological outcome, Rad-223 therapy resulted in small nodular lesions near the bone interface, whereas both control tumors and treated macro-tumors reached near-complete replacement of the bone marrow (Figure 6D). The size-dependent response to Rad-223 therapy identified in PC3 osteolytic tumors was confirmed using C4–2B osteoblastic tumors (0.5×10^6 or 1×10^6 cells per tibiae, $n = 10$ tibiae per group; means [SD] for the dose of 0.5×10^6 : 3.9×10^6 [1.9×10^6] in control-treated mice vs 2.0×10^5 [2.7×10^5] in Rad-223-treated mice, $P < .001$; means [SD] for the dose of 1×10^6 : 5.9×10^6 [4.3×10^6] in control-treated mice vs 1.2×10^6 [9.5×10^5] in Rad-223-treated mice, $P = .003$) that proved overall more sensitive to Rad-223 treatment (Figure 6E). In summary, for osteolytic and osteoblastic PCa tumors, these results show an up to eightfold gain of Rad 223 efficacy for targeting micro-lesions, whereas macroscopic tumors were increasingly nonresponsive.

Discussion

By combining *in vivo* and *in silico* analyses, we here identify cytotoxicity in tumor cells along the bone interface as a contributing effector mechanism of Rad-223 therapy. This zonal response sufficiently explains the therapeutic efficacy of Rad-223 as a function of tumor load and cell fraction located in proximity to the bone. These findings complement the concept that Rad-223 acts by interrupting the “vicious cycle” of tumor growth mediated by the stroma and tumor cell cross-talk, for example, by inhibiting osteoclast- and osteoblast-derived growth factor release (1,8).

Besides proliferating cells, nondividing cancer cells including disseminated dormant cells that usually withstand therapies may be susceptible to the high energy transferred by Rad-223 ionizing particles (3). When colonizing bone, PCa cells share mechanisms of homing employed by hematopoietic stem cells, nestling within hematopoietic stem cell niches that are composed of endothelial and osteoblastic cells, through interactions

mediated by annexin II/CXCL12 and stem cell factors (15). These niches overlap with the osteoblastic environments near the bone-calcified matrix where Rad-223 accumulates and causes maximum density of alpha-particles (3).

The zonal cytotoxicity towards tumor cells may thus provide a general mechanism determining Rad-223 efficacy and a rationale to increase the eradicated tumor fraction by treating PCa bone disease at earlier stages. In support of this concept, preventive Rad-223 administration decreases the tumor burden in a preclinical model of disseminated breast cancer after intracardiac injection (9). In addition, retrospective analysis showed that men with metastatic castration-resistant PCa with a low bone scan index (0–5) tend to respond more favourably to Rad-223, with improved overall survival compared with patients with high bone scan index (> 5) (16). In aggregate, these data suggest that men at risk for metastatic progression and patients with early-stage tumors in bone may particularly benefit from Rad-223 treatment.

The locally strong but overall yet limited effectiveness of Rad-223 provides a basis for refined therapy schemes to improve its activity. Clinical efforts to extend Rad-223 efficacy by increasing its dose did not improve patients’ outcome, but resulted in a higher incidence of adverse effects (17). Similarly, we observed comparable antitumor effects using doses of 192 385 or 770 kBq/kg with no further tumor reduction at higher doses. These results suggest that an improvement of local effectiveness will require complementary targeted therapies. Broadening the zone of efficacy towards the bulky tumor core may potentially be achieved by radio sensitizers such as poly ADP ribose polymerase inhibitors, which compromise the repair of DNA single-strand breaks and induce consequent cell death (18). Furthermore, complementary efficacy may be induced by targeting the tumor core using cytokine receptor antagonists, which dampen survival-enhancing signaling patterns (19), or multi-kinase inhibitors that target vascular networks maintaining intra-tumoral viability (20).

This study design includes several limitations. We addressed the Rad-223 response in particularly aggressive neuroendocrine-like (PC3) and castrate-resistance (C4–2B) PCa models; however, we did not examine whether cancer cell susceptibility to Rad-223-mediated cytotoxicity differs in other tumor types. Varying outcomes, with increased or decreased response, may indeed arise in castrate-resistant vs sensitive PCa subsets or as a consequence of homologous repair gene defects, which may augment the DNA damage and growth delay induced by Rad-223 (21). Furthermore, survival modulation may additionally derive from stromal reprogramming in the bone, yet the importance of Rad-223-induced direct cytotoxicity vs stromal modulation remains to be further explored. Besides the contribution of bone cells, the microanatomy of bone, including the density and space between trabecular lamellae in different (eg long vs axial) bones, may also affect the local dose distribution of Rad-223. Accordingly, the more spacious anatomy of the trabecular bone, as in vertebrae (22), may support the formation of larger lesions and/or allow bone marrow to be positioned between the bone and growing tumors, hence limiting Rad-223 local efficacy. Thus, the contribution of different bone geometries to Rad-223 response should be further determined.

In conclusion, our results reveal a direct induction of tumor cell lethality in bone by Rad-223 that reaches maximum efficacy in micro-lesions and may be further enhanced by molecular-targeted sensitization.

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