Radium-223 dichloride exhibits dual mode-of-action inhibiting both tumor and tumor-induced bone growth in two osteoblastic prostate cancer models

ABSTRACT

Radium-223 dichloride (radium-223), an alpha particle-emitting calcium-mimetic, improves overall survival in prostate cancer patients with symptomatic bone metastases. Here, we define radium-223 mode-of-action and efficacy in two clinically relevant prostate cancer xenograft models demonstrating PSA expression and osteoblastic growth upon intratibial inoculation of cancer cells. Immunocompromized male mice were inoculated with human LNCaP or patient-derived LuCaP 58 prostate cancer cells in the intratibial compartment and subsequently stratified into treatment groups based on lesion grade and/or serum PSA levels. Radium-223 (300 kBq/kg) or vehicle was administered intravenously, two times at 4-week intervals during the experiment. X-rays and serum samples were obtained biweekly and at sacrifice. Soft tissue tumors were examined macroscopically at sacrifice and tissue samples were collected and processed for γ-counter measurements, micro-CT, autoradiography and histology. Radium-223 treatment inhibited tumor-induced osteoblastic bone growth as indicated by reduced bone volume and surface in LNCaP and LuCaP 58 prostate cancer mouse models. In addition, radium-223 treatment suppressed metabolic activity in bone as evidenced by decreased number of osteoblasts and osteoclasts relative to bone surface and reduced levels of the bone formation marker PINP. Radium-223 resulted in lower PSA values as early as two weeks after the first dose, indicating constrained tumor growth following treatment. This phenomenon was further supported by reduced total tissue and tumor area in tibia in LNCaP and LuCaP 58 models and increased percentage of necrotic tumor area in the LuCaP 58 model in radium-223-treated mice as compared to vehicle-treated mice. Moreover, DNA double-strand breaks were increased in cancer cells 24 hours post radium-223 treatment in the LuCaP 58 model providing further evidence of anti-tumor effects. Radium-223-treated mice exhibited less visceral metastases in the LuCaP 58 model (not significant). Based on autoradiography, radium-223 was deposited in the intratumoral bone matrix and inhibits tumor growth in both cell line- and patient-derived osteoblastic prostate cancer metastasis models. We demonstrate that radium-223 dichloride is successfully incorporated into the intratumoral bone matrix and inhibits tumor growth and tumor-induced bone reaction, both important players in the destructive vicious cycle of osteoblastic bone metastasis in prostate cancer.

Poster presented at the Hanson Wade, Tumor Models
July 21 – July 23, 2015, Boston, USA

For additional information, please contact:
Mari Suominen
e-mail: mari.suominen@pharmatest.com
Tel: +358 2 278 4700

Arne Scholz
e-mail: arne.scholz@bayer.com
Tel: +49 30 468 16369
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INTRODUCTION

Prostate cancer is frequently associated with metastasis to bone.

Bone tumors are often osteoblastic and lead to the formation of fragile bone, increased chance of fractures, severe bone pain, significant morbidity and poor prognostic consequences.

Radium-223 dichloride (Ra-223, Xafogo®), an alpha-emitting calcium-mimetic, binds to hydroxyapatite in bone and provides targeted radiation therapy against bone metastases.

Ra-223 improves overall survival in prostate cancer patients with bone metastases and Xafogo® has been approved for the treatment of castration-resistant prostate cancer with symptomatic bone metastasis and no known visceral metastatic disease throughout the world.

Ra-223 reduces development of osteolytic lesions and improves survival in a mouse model of osteolytic breast cancer bone metastasis via a dual mode-of-action on both tumor cells and osteoclasts.

AIM OF THE STUDY

To investigate the efficacy and mode-of-action of radium-223 dichloride (Ra-223, Xafogo®) in two clinically-relevant prostate cancer xenograft models.

MATERIALS AND METHODS

Mouse models and treatments

Ra-223 therapeutic effects were investigated in two clinically-relevant prostate cancer xenograft models:

1) LuCaP cell-line model (ATCC, European distributor LGG Standards)
2) LuCaP SB patient-derived xenograft (PDX) model (licensed by Bayer from the University of Washington; abiraterone resistance demonstrated in an in-house in vivo study)

These tumor cells were injected into tibia of 6-7 weeks old male NOD SCID mice (Charles River). Mice were dosed with Ra-223 or vehicle as described in the study design below and drug tolerability was assessed by monitoring animal body weight twice weekly.

Study design

Analysis of tumor growth and metastasis to bone

Tumor cell inoculation into mice

Tumor cell inoculation into mice

Stratification based on PSA levels & tumor size

Stratification based on PSA levels

Two i.p. dosings (1 day and 4 days after stratification)

Single i.c. dosing: Vehicle or Ra-223 (300 MBq/kg)

Vehicle or Ra-223 (100 µg/kg)

Sacrifice 6 days after the first dose

Sacrifice 24, 48 or 72 hrs after the dose

Analysis of biochemical markers

Blood samples were collected from the saphenous vein at different timepoints following LuCaP SB and LuNPc tumor cell inoculation. LNCaP prostate cancer xenograft was analyzed using Quantikine Human Kallikrein 1/PSA ELSA kit (R&D Systems) and PINP (N-terminal propeptide of type I procollagen) concentration was determined using rat/mouse PINP EIA (IDS, Germany).

Analysis of bone morphology and tumor metastasis

X-rays were obtained at three (LuCaP SB) or six (LNCaP) weeks and bleomycin-theraderived (Faxitron Specimen Radiography System MX-20 D12, Faxitron Corp., Illinois, USA). Micro-computed tomography (micro-CT) was performed (SkyScan 1022 ex vivo micro-CT, Bruker micro-CT, Kontich, Belgium) on unprocessed tibia. Masson-Goldner trichrome (MGT) and immunohistochemical stainings were performed on decalcified paraffin sections to determine osteoblast and osteoclast cell numbers. DNA double-strand breaks were detected using γH2AX antibody and the Leica DM8000 B Research Microscope (Leica Microsystems, Wetzlar, Germany). Autoradiography was performed on histological sections of undecalcified tibia (embedded in methyl methacrylate) and used to assess localization and extent range of 14C-labeled Ra-223 in 1-31 ATP-bound particles. Soft tissue tumors were analyzed macroscopically at sacrifice.

Statistical methods

Statistical analysis included (version 3.1.1, packages rsm and multcomp) was used for encounters. Unless otherwise indicated, p-values were calculated using the Kruskall-Wallis test followed by pairwise comparison using Mann-Whitney U test or t-test. Survival analysis and Cox proportional hazards model (SPSS) were used for hazard mixed-effects model and comparisons were made using model coefficients. LuCaP xenograft model was used for total tissue area and bone volume of LuCaP SB model, respectively, prior to statistical analysis.

RESULTS

Figure 1. Ra-223 inhibits tumor-induced osteoblastic reaction. A-D) Ra-223 (100 MBq/kg, i.v.) i) dramatically reduces bone volume in tumor-bearing tibia in both the LNCaP and the LuCaP SB models. Representative micro-CT reconstructions of mice tibia (measurement area = 5 mm starting from below the growth plate, medial side view on the left and sagittal sections on the right) are shown. A-B) Plot representing bone volume (mm3) in tumor-bearing tibia in both the LNCaP and the LuCaP SB models in different duration of dosing and groups, represents the average value of 5 mice. C) PINP levels (ng/ml) reflects new bone formation rate in mice bearing LuCaP SB (n = 13) or LuCaP SB (n = 11) tumors, respectively. *p < 0.05, **p < 0.01.

Figure 2. Ra-223 reduces total bone area, relative trabecular bone area and osteoblast and osteoclast numbers in tumor-bearing mice. A) Representative H&E staining of bone architecture in LNCaP and LuCaP SB tumor-bearing mice treated with vehicle or Ra-223 (100 MBq/kg, i.v.). B) Box plots representing total bone area (mm2) (n = 11-13) and osteoclast (n = 8.11) cell numbers relative to tumor-bearing bone (TIB) in mice bearing LNCaP or LuCaP SB tumors and treated with vehicle or Ra-223. The horizontal lines represent 5th, 25th, 50th, 75th and 95th percentiles and the crosses indicate mean values. *p < 0.05, **p < 0.01, ***p < 0.001, a > p = 0.1, ns = not significant.

Figure 3. Ra-223 suppresses tumor growth. A) Line graphs representing serum PSA levels in LNCaP and LuCaP SB tumor-bearing mice treated with vehicle or Ra-223 (100 MBq/kg, i.v.) (mean ± SD, n = 10-11). B) Box plots representing tumor area (mm2) (n = 11-13), percentage of necrotic tumor area (A: n = 5), and length of tumor-bearing bone interface (D: n = 6-11). In box plots, the horizontal lines represent 5th, 25th, 50th, 75th and 95th percentiles and the crosses indicate mean values. *p < 0.05, **p < 0.01, ***p < 0.001, a > p = 0.1, ns = not significant.

Figure 4. Ra-223 suppresses the development of visceral metastases and induces DNA double-strand breaks in tumor cell populations. A) Box plots representing percentage of LNCaP tumor-bearing mice treated with vehicle or Ra-223 (100 MBq/kg, i.v.) (n = 8-11), demonstrating visceral metastases. B) Box plots representing DNA double-strand breaks in tumor cells. The light, medium and dark green and blue bars represent total number of mice, number of mice with PSA ≥ 5 ng/ml, and number of mice with PSA ≥ 5 ng/ml after dosing start, in each treatment group respectively. The numbers above the bars represent the number of mice with visceral metastases. C) Box plots representing γH2AX-positive tumor cells. In the horizontal line represents 5th, 25th, 50th, 75th and 95th percentiles and the crosses indicate mean values. *p < 0.05, **p < 0.01, ***p < 0.001, a > p = 0.1, ns = not significant.

Figure 5. Ra-223 is deposited in the intratumoral bone matrix. Representative autoradiograph is shown. Measurement was done 24 hours after single intravenous administration of Ra-223 (300 MBq/kg). Some Ra-223 deposits (black) were observed in prostate cancer cells (blue arrows) in connection with osteoblasts (green arrows).

CONCLUSIONS

Radium-223 dichloride (Xafogo®) inhibits disease progression in both the cell-line based LNCaP and the abiraterone-resistant PDX LuCaP SB prostate cancer models.

Radium-223 therapy exhibits a dual mode-of-action that impacts on tumor growth and on tumor-induced bone reaction, both important players in the destructive vicious cycle of osteoblastic bone metastasis in prostate cancer.

Our findings confirm the previously reported beneficial effects of Radium-223 and strongly support further development of Radium-223 for the treatment of patients with prostate cancer.

SUMMARY

Ra-223 inhibits disease progression (micro-CT; Fig. 1A-D), inhibits tumor-induced osteoblastic bone growth and protects normal bone architecture leading to reduced bone volume (Fig. 1E) and area (Fig. 2A-C) in two prostate cancer models.

Ra-223 suppresses bone metastases as evidenced by decreased number of osteoblasts and osteoclasts (Fig. 2D-F) and reduced level of bone formation marker PINP (Fig. 1F).

Ra-223 treatment results in lower PSA levels (Fig. 3A), suppressed tumor areas (Fig. 3B) and a trend for increased necrotic tumor area (Fig. 3C), indicating constrained tumor growth in metastatic prostate cancer.

In the LuCaP SB model, DNA double-strand breaks are enhanced in cancer cells as early as 24 hours after Ra-223 administration (Fig. 4B) and percentage of necrotic tumor area is slightly increased 48 hours after Ra-223 administration (Fig. 4C) providing further evidence of Ra-223 anti-tumor effects.

The deposition of Ra-223 in the intra-tumoral bone matrix and an α-particle range of 2-10 cell diameters (< 100 µm) suggests potential radiation effects on the tumor microenvironment.

REFERENCES

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Acknowledgements

We thank Johanna Orling, Rikka Kystamiä, and Jani Seppänen for their skilful technical assistance. Aarevet Ltd. (www.aarevet.com) is acknowledged for the editorial support funded by Bayer Pharma AG.