Radium-223 dichloride inhibits tumor growth and tumor-induced bone growth in osteoblastic prostate cancer models

MI Suominen1, KM Fagerlund2, D Mumberg3, K. Ziegelbauer4, SM Kälkönen5, JM Halleen5, RL Vessella4 and A Scholz2

1Pharmatest Services Ltd, Turku, Finland,
2Bayer HealthCare, Global Drug Discovery, TRG-Onc/GT, Berlin, Germany,
3University of Turku, Turku, Finland, 4University of Washington, Seattle, WA

ABSTRACT

Radium-223 dichloride (radium-223), an alpha particle-emitting calcium-mimetic, improves overall survival in prostate cancer patients with symptomatic bone metastases. We have defined 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. Immuno-compromised 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 bone lesion tissue and tumor area 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 in conjunction with osteoblasts in osteoblastic metastases. Our results demonstrate that radium-223 dichloride is successfully incorporated into the intratumoral bone matrix and inhibits tumor growth in both cell line- and patient-derived osteoblastic prostate cancer metastasis models. Given the α-particle range of 50-80 μm, potent radiation effects on the tumor microenvironment are evident whereas relevant effects on the more distant bone marrow are not expected. Taken together, radium-223 therapy exhibits a dual mode-of-action that impacts tumor growth and tumor-induced bone reaction, both important players in the destructive vicious cycle of osteoblastic bone metastasis in prostate cancer.
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1Pharmatest Services Ltd, Turku, Finland, 2Bayer HealthCare, Global Drug Discovery, TRG-Onc/GT, Berlin, Germany, 3University of Turku, Turku, Finland, 4University of Washington, Seattle, WA

INTRODUCTION

Prostate cancer is frequently associated with metastasis to bone. Bone tumors are often osteolytic and lead to the formation of fragile bone, increased chance of fractures, severe bone pain, significant morbidity and poor prognosis. 

Radium-223 dichloride (Ra-223, Xofigo®), an alpha-emitting calcium mimic, tends to hydrolyze apatite in bone and provides targeted radiation therapy against bone metastases.

Ra-223 improves overall survival in prostate cancer patients with bone metastases1 and Xofigo® has been approved for the treatment of castration-resistant prostate cancer with symptomatic bone metastases 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, Xofigo®) in two clinically relevant prostate cancer xenograft models.

MATERIALS AND METHODS

Mouse models and treatments

Radium-223 therapeutic effects were investigated in two clinically-relevant osteoblastic prostate cancer models.

1) LuCaP cell line model (ATCC, European distributor GIGA, Germany).

2) LuCaP 58 patient-derived xenograft (PDX) model (lentirium from the University of Washington, Seattle, WA, and investigated in two in vivo studies).

These tumor cells were implanted into tails of 5-7 week old nude mice (Charles River, Germany). Mice were dosed with the 223Ra as oral control as indicated in the data, design table and figure legends. Tumor burden was measured by monitoring changes in body weight, tumor volume and tumor growth rate.

Study design

A Brief overview of the study design is shown in the study design. Study parameters include the mouse type, LuCaP or LuCaP 58, and the time points used for the different analyses. The study design also includes the time points used for the different analyses. The study design also includes the time points used for the different analyses.


2. ALSPANDO M., FEHERDNEK, Tender 0.207-0.245, 2014.


Analysis of biochemical markers

Distal segments were collected from the ex vivo specimens at different time points after Radium-223 treatment. Serum PSA concentration was determined using Sandwich human PSA enzyme-linked immunosorbent assay (ELISA) placed in a 96-well microtiter plate (Biomedica, Vienna, Austria). The ELISA was performed in a 96-well microtiter plate (Biomedica, Vienna, Austria). The ELISA was performed in a 96-well microtiter plate (Biomedica, Vienna, Austria). The ELISA was performed in a 96-well microtiter plate (Biomedica, Vienna, Austria).

Analysis of bone morphology and tumor metastases

Axs were obtained at time (Radium223) or at time 223Ra (time 223Ra) as below and scanned at 5x on X-ray. The Radium223 treatment was performed in a 96-well microtiter plate (Biomedica, Vienna, Austria). The Radium223 treatment was performed in a 96-well microtiter plate (Biomedica, Vienna, Austria). The Radium223 treatment was performed in a 96-well microtiter plate (Biomedica, Vienna, Austria). The Radium223 treatment was performed in a 96-well microtiter plate (Biomedica, Vienna, Austria).

Statistical methods

Graphpad software (version 1.1.2, 2.01 packages and macintosh) was used for analysis. Unless otherwise indicated, p-values were calculated using the Graphpad-StatMate test followed by pairwise comparison (Mann-Whitney or T test) for independent samples. PSA and NIH levels were analyzed in a linear regression model and the median was used to assess the difference between the two groups. The Mann-Whitney or T test was performed using Graphpad-StatMate test followed by pairwise comparison (Mann-Whitney or T test) for independent samples. PSA and NIH levels were analyzed in a linear regression model and the median was used to assess the difference between the two groups. The Mann-Whitney or T test was performed using Graphpad-StatMate test followed by pairwise comparison (Mann-Whitney or T test) for independent samples. PSA and NIH levels were analyzed in a linear regression model and the median was used to assess the difference between the two groups. The Mann-Whitney or T test was performed using Graphpad-StatMate test followed by pairwise comparison (Mann-Whitney or T test) for independent samples. PSA and NIH levels were analyzed in a linear regression model and the median was used to assess the difference between the two groups. The Mann-Whitney or T test was performed using Graphpad-StatMate test followed by pairwise comparison (Mann-Whitney or T test) for independent samples. PSA and NIH levels were anal...

RESULTS

Figure 1. Ra-223 inhibits tumor-induced osteoclast formation. A: Radium-223 (Ra-223) in vitro reduces bone volume in rats with LuCaP 58 tumors. At day 14, increased bone volume (BV) in the LuCaP 58 (Vehicle) group compared to the LuCaP 58 (Lawmen) group (mean ± SD; n = 10-11). The difference in BV was statistically significant at all time points (*** p < 0.001). B: Box plots representing bone volume of mice bearing LuCaP 58 or Lawmen tumors. The box plots represent medians, 25th and 75th percentiles, and range. Error bars represent the minimum and maximum values. The box plot represents medians, 25th and 75th percentiles, and range. Error bars represent the minimum and maximum values. The box plot represents medians, 25th and 75th percentiles, and range. Error bars represent the minimum and maximum values. The box plot represents medians, 25th and 75th percentiles, and range. Error bars represent the minimum and maximum values. The box plot represents medians, 25th and 75th percentiles, and range. Error bars represent the minimum and maximum values. The box plot represents medians, 25th and 75th percentiles, and range. Error bars represent the minimum and maximum values. The box plot represents medians, 25th and 75th percentiles, and range. Error bars represent the minimum and maximum values. The box plot represents medians, 25th and 75th percentiles, and range. Error bars represent the minimum and maximum values. The box plot represents medians, 25th and 75th percentiles, and range. Error bars represent the minimum and maximum values. The box plot represents medians, 25th and 75th percentiles, and range. Error bars represent the minimum and maximum values. The box plot represents medians, 25th and 75th percentiles, and range. Error bars represent the minimum and maximum values. The box plot represents medians, 25th and 75th percentiles, and range. Error bars represent the minimum and maximum values. The box plot represents medians, 25th and 75th percentiles, and range. Error bars represent the minimum and maximum values. The box plot represents medians, 25th and 75th percentiles, and range. Error bars represent the minimum and maximum values. The box plot represents medians, 25th and 75th percentiles, and range. Error bars represent the minimum and maximum values. The box plot represents medians, 25th and 75th percentiles, and range. Error bars represent the minimum and maximum values. The box plot represents medians, 25th and 75th percentiles, and range. Error bars represent the minimum and maximum values. The box plot represents medians, 25th and 75th percentiles, and range. Error bars represent the minimum and maximum values. The box plot represents medians, 25th and 75th percentiles, and range. Error bars represent the minimum and maximum values. The box plot represents medians, 25th and 75th percentiles, and range. Error bars represent the minimum and maximum values. The box plot represents medians, 25th and 75th percentiles, and range. Error bars represent the minimum and maximum values. The box plot re...