Introduction

Bone metastases cause high mortality in metastatic castration-resistant prostate cancer (mCRPC) patients. Even though newly approved therapies have prolonged survival of patients with mCRPC, bone metastases still remain incurable. Several targeted radionuclide treatments for prostate cancer are under development after approval of radium-223 dichloride (Ra-223, Xofigo®), which is effective against tumors growing in bone.

The aims of this study were to evaluate the growth of LNCaP human prostate cancer cells in bone of NMRI nude mice and to validate Ra-223 as a reference compound in the model.

Materials and Methods

Male NMRI nude mice (Janvier) aged 6-8 weeks were inoculated with 2 x 10^6 LNCaP cells (ATCC) into the tibia bone marrow, modeling growth of bone metastases. The mice were randomized to treatment groups based on serum prostate-specific antigen levels (PSA, R&D Systems), and cancer-induced changes in bone (bone lesions) were evaluated by X-ray imaging (Faxitron) at 6 weeks after inoculation of the cancer cells. The mice were treated with 300 kBq/kg of Ra-223 (Oak Ridge National Laboratory ™) or vehicle at study weeks 6 and 10. PSA and the bone formation marker serum procollagen I N-terminal propeptide (PINP, IDS Inc) measurements, and X-ray imaging were performed at weeks 6, 8, 10 and 12. The study was terminated at 12 weeks after inoculation of the cancer cells. Bone, tumor and fibrotic areas were evaluated by histology.

Efficacy of Ra-223 dichloride

- FIGURE 1. A) Serum PSA levels (ng/ml, mean ± SEM) in vehicle and Ra-223 dichloride treated mice. Individual values are presented on the right panel. Ra-223 dichloride decreased serum PSA levels (**p < 0.01). B) Serum PINP levels (ng/ml, mean ± SEM) in vehicle and Ra-223 dichloride treated mice. Individual values are presented on the right panel. Ra-223 dichloride decreased serum PINP levels (**p < 0.01). C) Bone lesion area (mm², mean ± SEM) in vehicle and Ra-223 dichloride treated mice. Individual values are presented on the right panel. Ra-223 dichloride decreased the bone lesion area (**p < 0.05). D) Representative X-ray images from vehicle and Ra-223 dichloride treated mice at endpoint.

Histology

- FIGURE 2. A) Representative HE stainings from tumor-bearing tibias in vehicle and Ra-223 dichloride treated mice. Magnification 5x. B) Tumor area in bone marrow (mm², median with IQR). Ra-223 dichloride decreased tumor area in bone marrow (**p < 0.05). C) Bone area in bone marrow (mm², median with IQR). No statistically significant differences were observed (NS, p > 0.05). D) Fibrotic area in bone marrow (mm², median with IQR). Ra-223 dichloride treatment induced fibrotic changes in bone marrow (**p < 0.001). Example image of fibrotic area in bone marrow on the right, magnification 20X. Abbreviations: T = tumor; F = fibrotic area.

Summary

- LNCaP tumors induced osteoblastic-mixed bone lesions
- A tumor take rate of 50% was observed at 6 weeks after inoculation of cancer cells based on evaluation of PSA values and bone lesions
- Ra-223 was well tolerated in NMRI nude mice
- Ra-223 decreased serum PSA levels
- Ra-223 decreased serum PINP values and the progression of bone lesions
- About 50% of Ra-223 treated mice were tumor free at the end of the study based on histological evaluation
- Histological evaluation showed decreased number of bone marrow cells, increase in fibrotic area, and changes in tumor cell morphology in Ra-223 treated mice

Conclusions

- NMRI nude mice can be used in the LNCaP prostate cancer bone metastasis model. Ra-223 dichloride was successfully validated as a standard-of-care reference compound. This model is of high importance when assessing efficacy of radiopharmaceuticals as mono- or combination treatments against bone metastases.

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