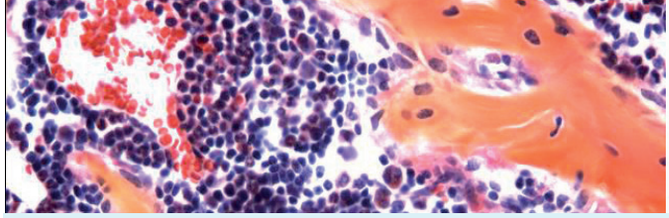
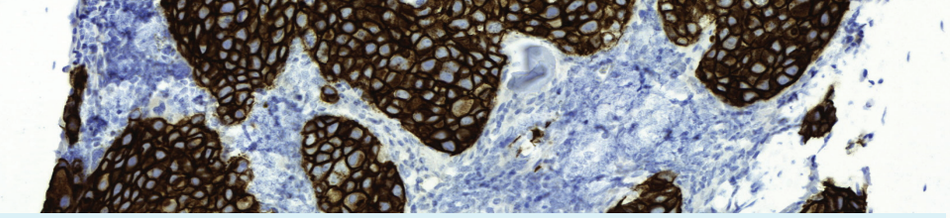




# Drug efficacy evaluation in bone metastasis models - Why and How

**WHITE PAPER | January 2017**



pharmatest

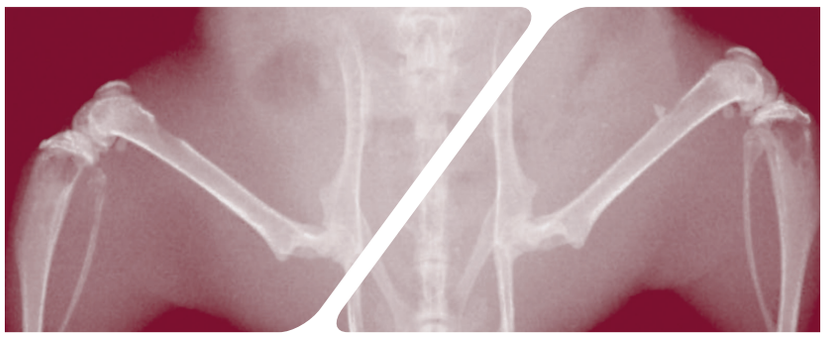
# 5 FACTS ABOUT METASTASIS MODELS

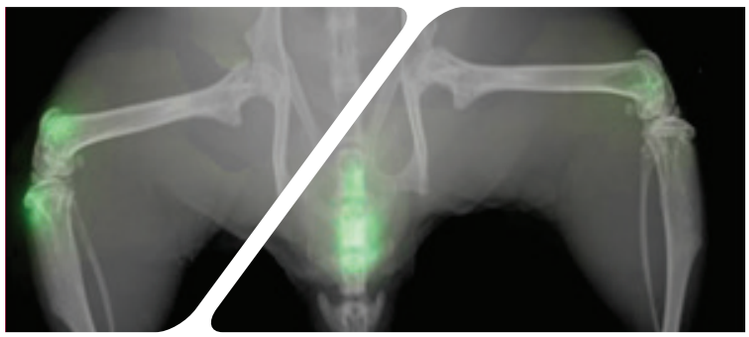
### 1. Secret is in the combination

It has become apparent that no single model can fully replicate all aspects of the human disease or accurately predict response to therapy. Consequently the investigative or predictive power of these models is typically established by utilizing multiple platforms to overcome the limitations of individual models.

### 2. Shortcut to the metastatic process

Experimental transplantation models involve the injection of tumor cells directly into the vascular system, thus bypassing the formation of a primary tumor and early stages of the metastatic cascade. This allows rapid tumor growth and homogenous treatment groups.





## WHY SHOULD YOU TEST YOUR COMPOUND IN A METASTASIS MODEL?

Metastatic and primary tumors are known to respond differently to chemotherapy.[1] Therefore, when establishing drug efficacy it is important not to rely only on experiments in primary tumor models but to also study the test compound in metastasis models. Organs can favor certain types of primary tumor cells, and therefore it is highly important to test compounds in models that provide the correct microenvironment for the metastases. Bone metastases in particular are a frequent source of pain for late-stage cancer patients and often very resistant to treatment due to the characteristics of the bone.[2] A compound effectively treating or preventing bone metastases may also increase the overall survival.[3,4]

**Table 1.** Xenograft metastasis models available at Pharmatest. Preventive, treatment and survival settings are available with most of the models. In addition, Pharmatest has a lot of experience from customized models using many other cell lines and study designs. ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; AR, androgen receptor; PSA, prostate-specific antigen.

| Tumor model | Can you test effects on metastases? | Tumor type | Tumor characteristics | Inoculation route | Metastatic growth in | See case examples (page) |
|---|---|---|---|---|---|---|
| MDA-MB-231(SA) | Yes | Breast, human | ER-, PR-, HER2- | Intracardiac<br>Orthotopic | Bone<br>Lung, lymph nodes | p. 3 |
| MCF-7 | Yes | Breast, human | ER+, PR+, HER2- | Intratibial | Bone | p. 5 |
| BT-474 | Yes | Breast, human | ER+, PR+, HER2+ | Intratibial | Bone | p. 4 |
| PC-3 | Yes | Prostate, human | AR- | Orthotopic<br>Intracardiac<br>Intratibial | Lymph nodes<br>Bone<br>Bone | p. 6 |
| LNCaP | Yes | Prostate, human | AR+, PSA+ | Intratibial | Bone | p. 7 |

### 3. Spontaneous metastasis for early stages

Models of spontaneous metastasis encompass early and late stages of metastasis and involve the transplantation of cells into either an ectopic or orthotopic site to form a primary tumor which may subsequently metastasize.

### 4. Site does matter

The site of tumor implantation influences metastatic distribution. While tumor cell injection into the lateral tail vein predominantly results in pulmonary metastases, intracardiac injection increases the frequency of hepatic, ovary, adrenal gland, brain and bone metastases.

### 5. Vive la Microenvironment

Inoculation of tumor cells into correct microinvironment allows the assessment of compatible tumor-stroma interactions and endocrine signaling, which has great impact on tumor growth and treatment efficacy.

## BREAST CANCER MODELS WITH MDA-MB-231 CELLS

MDA-MB-231 is a triple-negative (ER-, PR-, HER2-) human breast cancer cell line derived from metastatic pleural effusion. MDA-MB-231(SA) is a bone-seeking variant of this cell line. The cells are labeled with GFP, enabling easy detection of tumor burden using fluorescence imaging. When the MDA-MB-231(SA) cells are inoculated intracardially, metastatic growth can be observed primarily in bone. Tumor-induced changes in bone can be detected using X-ray imaging. This model is relatively aggressive and forms osteolytic tumors in approximately two weeks.[3, 9, 10] Orthotopically inoculated cells tend to metastasize to lungs and lymph nodes.

Histomorphometric analysis can be used to measure parameters such as intraosseous tumor or trabecular bone area, number of osteoclasts at tumor-bone interface, and e.g. apoptotic index in the intracardiac MDA-MB-231(SA) model.

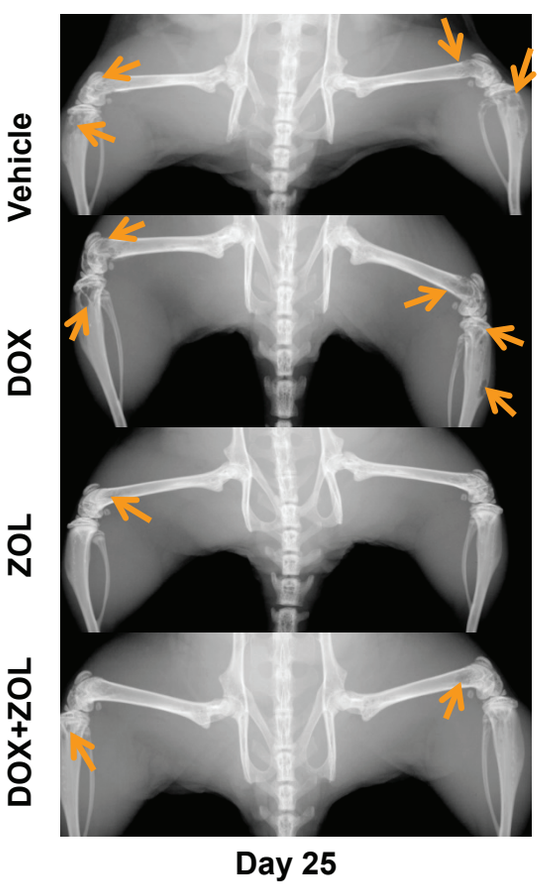



**Figure 1.** Representative X-ray images showing established osteolytic MDA-MB-231(SA) lesions at sacrifice in the intracardiac model. DOX, Doxorubicin; ZOL, Zoledronic acid.

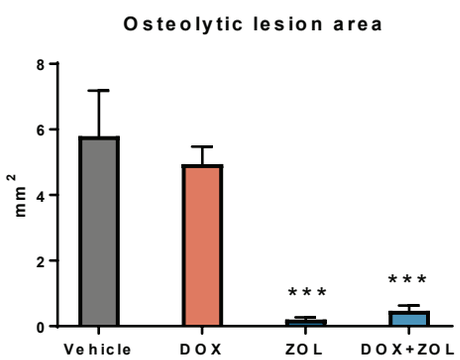



**Figure 2.** Zoledronic acid (ZOL) decreases osteolytic lesion area in the intracardiac MDA-MB-231(SA) model (mean+SEM). ***, p<0.001 compared with vehicle. DOX, Doxorubicin.

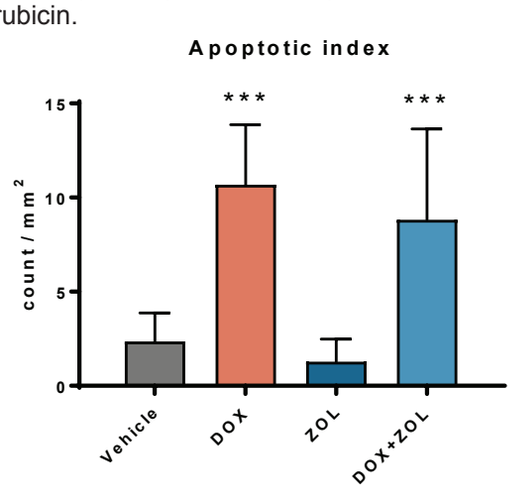



**Figure 3.** Doxorubicin (DOX) increases the number of apoptotic cells in the intracardiac MDA-MB-231(SA) model (mean+SEM). ***, p<0.001 compared with vehicle. ZOL, Zoledronic acid.

MCF-7 human breast cancer cells derived from metastatic pleural effusion are ER+, PR+ and HER2-. When inoculated orthotopically into mammary fat pad, MCF-7 cells need estradiol (E2) supplementation for tumor growth. However, when inoculated intratibially MCF-7 breast cancer cells grow also without E2 supplement. In the absence of E2 supplement MCF-7 cells induce mainly osteoblastic bone lesions after intratibial cancer cell inoculation.

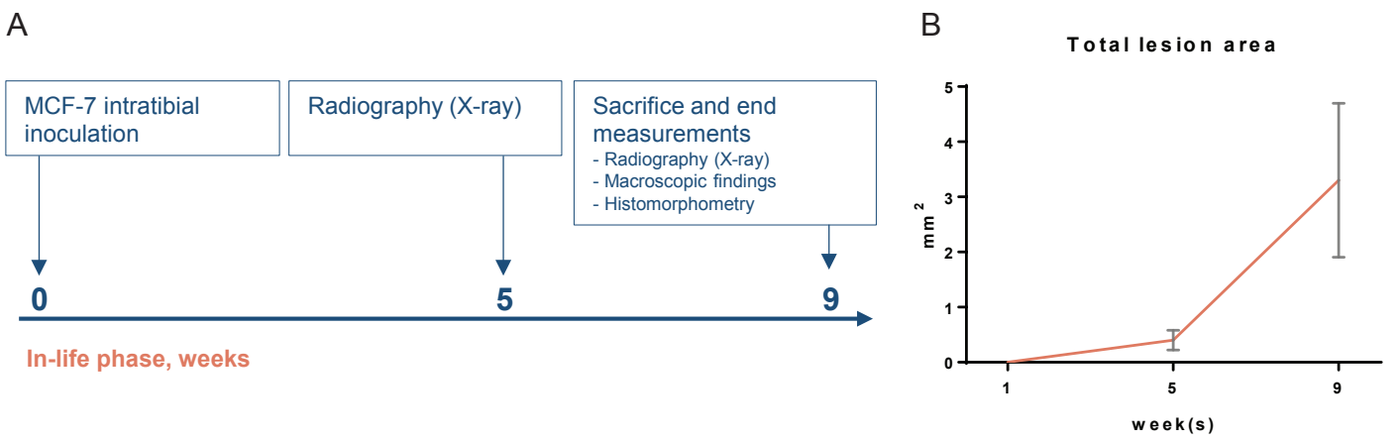



**Figure 4.** (A) Timelines of intratibial MCF-7 human breast cancer model. Five weeks after intratibial MCF-7 inoculation tumor growth induces bone lesions. (B) Development of bone lesion area in MCF-7 human breast cancer model (mean+SEM).


**Figure 5.** (A) X-ray, (B) µCT and (C) histology images of intratibial MCF-7 human breast cancer model demonstrating tumor-induced osteoblastic bone lesions.

BT-474 human breast cancer cells from ductal mammary carcinoma overexpress ER, PR, and HER2. When BT-474 cells are inoculated orthotopically into mammary fat pad, E2 supplementation is needed to support tumor growth. However, when inoculated intratibially BT-474 cells grow also without E2 supplement, and ER and PR expressions change upon E2 supplementation (Figure 7).

ER, PR, and HER2 expression makes this model an important tool for preclinical studies when developing new therapeutics against HER2 overexpressing breast cancer.

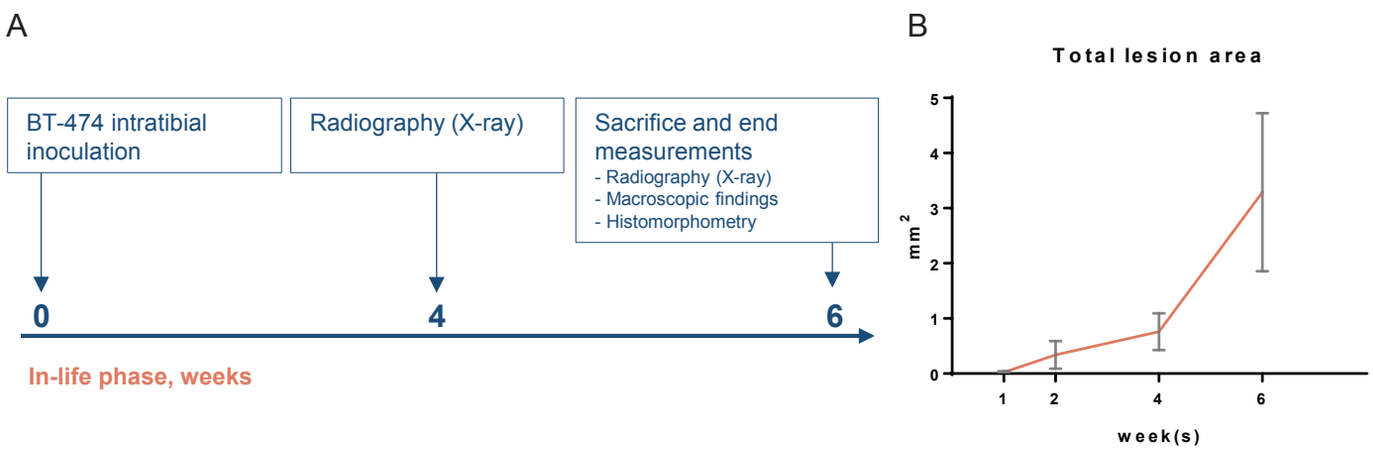



**Figure 6.** (A) Timeline of intratibial BT-474 human breast cancer model. Tumor induced bone lesions are allowed to form for 4 weeks, after which the animals are stratified to groups and test compound dosing is started. (B) Bone lesion area in intratibial BT-474 human breast cancer model (mean+SEM).

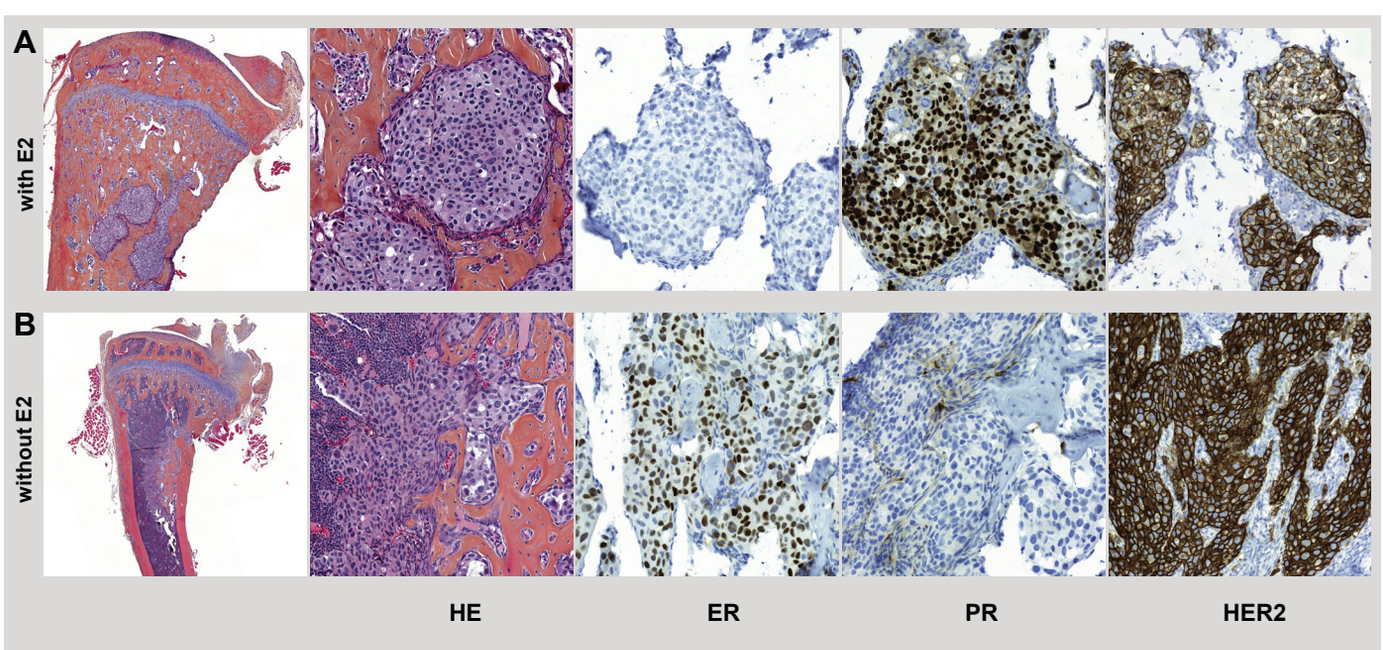



**Figure 7.** (A) E2 supplemented BT-474 cells in bone express PR and HER2 but less ER. (B) In contrast, without E2 supplement intratibial BT-474 cells are PR negative but ER and HER2 positive.

PC-3 human prostate cancer cells are androgen receptor negative cells derived from prostate cancer bone metastatic site. The cells are inoculated into proximal tibia of male athymic nude mice, and the mice are sacrificed within 5 weeks of the inoculation. PC-3 cells induce large osteolytic lesions clearly visible in X-rays. However, histologically also osteoblastic components can be observed. The model can be used in evaluating the effects of anti-resorptive compounds or compounds targeting AR negative prostate cancer.

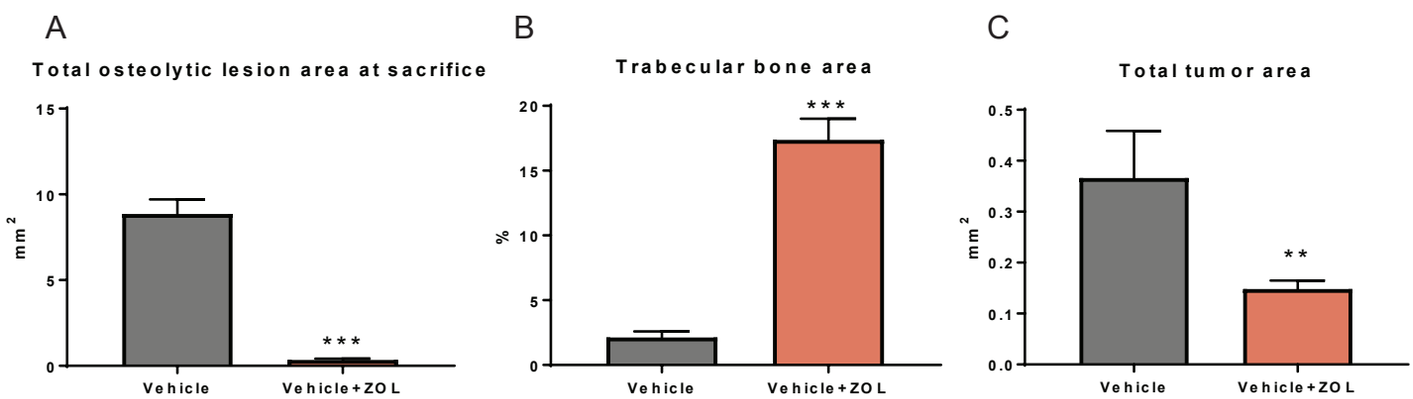



**Figure 8.** Zoledronic acid (ZOL) radically decreased osteolytic lesion area (A), increased trabecular bone area (B), and decreased total tumor area (C) in intratibial PC-3 model (mean+SEM). **, $p<0.01$; ***, $p<0.001$ compared with vehicle.

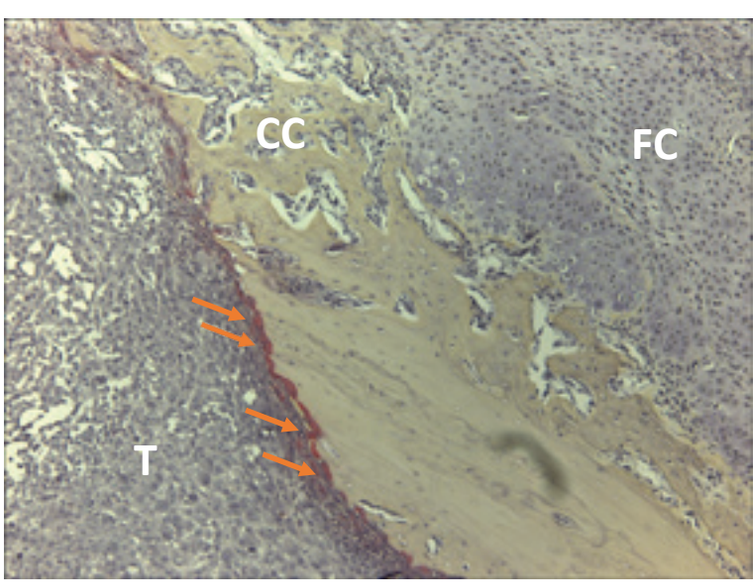



**Figure 9.** Induction of osteoclasts and bone destruction are prominent features in the intratibial PC-3 model. This results in fractures, followed by formation of callus tissue in an attempt of fracture healing. Arrows indicate osteoclasts. T, tumor; FC, fibrocartilage; CC, calcified callus.

LNCaP human prostate cancer cells derived from prostate cancer lymph node metastatic site express PSA and AR.[8] Intratibially inoculated LNCaP cells induce osteoblastic and osteolytic changes in bone. Overall more bone is formed than destroyed, resulting in net increase of bone volume. The model can be used to test the effects of compounds on bone metastases in the correct microenvironment.

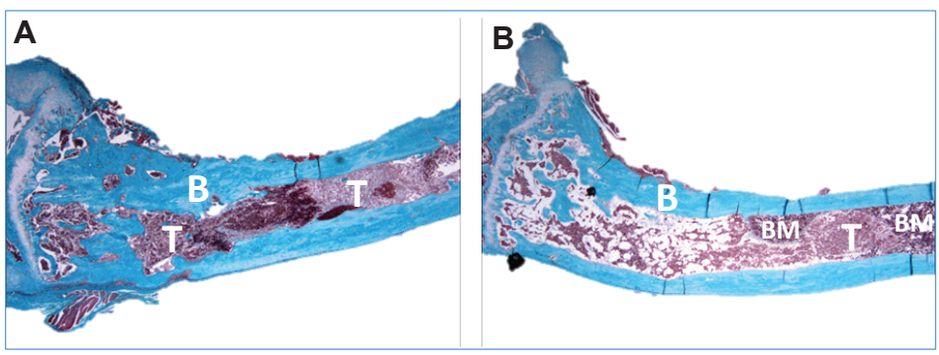



**Figure 10.** Radium-223 dichloride (Ra-223) reduces total bone area in tumor-bearing mice. Representative images of bone histology architecture in LNCaP tumor-bearing mice treated with vehicle (A) or Ra-223 (300 kBq/kg, i.v.) (B). Staining was performed with Masson-Goldner trichrome on decalcified paraffin sections. B, bone; T, tumor; BM, bone marrow.

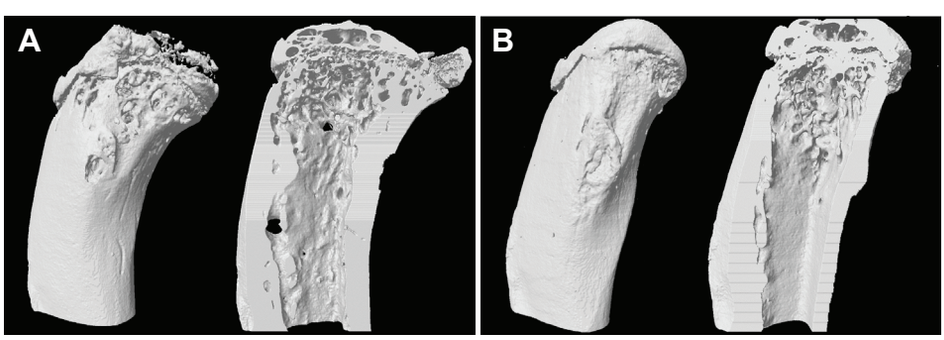



**Figure 11.** Ra-223 inhibits tumor-induced bone reaction as evidenced by the preserved bone architecture in tumor-bearing tibias. Representative µCT reconstructions of tumor bearing tibia are shown for mice treated with vehicle (A) or Ra-223 (300 kBq/kg, i.v.) (B). Medial side views are shown on the left and sagittal sections on the right in both (A) and (B).

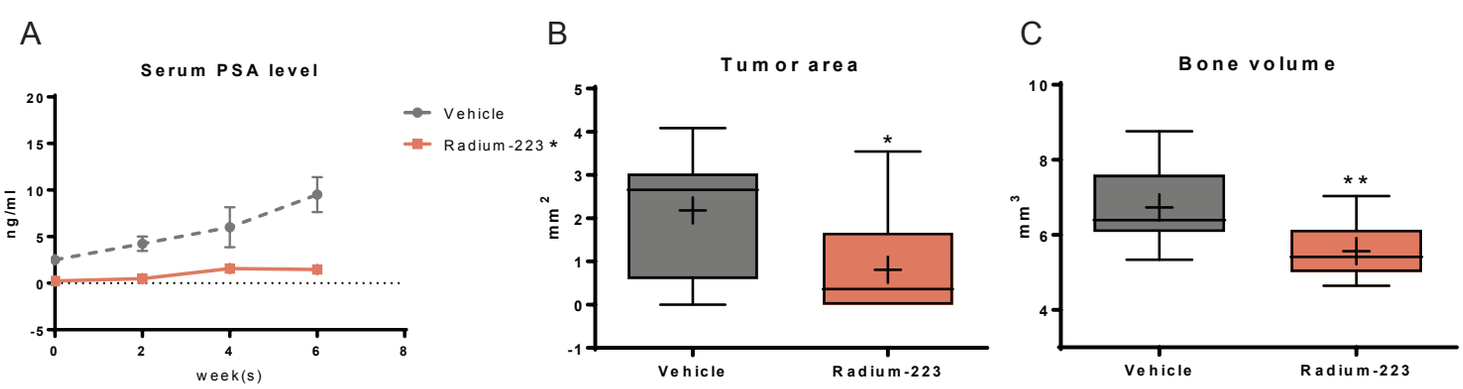



**Figure 12.** (A) Ra-223 reduced tumor burden as measured by serum PSA levels (mean+SEM). (B) Ra-223 reduced tumor area in bone quantified from histological sections. (C) Ra-223 reduced bone volume analyzed by µCT. The animals were treated with vehicle or Ra-223 (300 kBq/kg, i.v.) In (B) and (C), horizontal lines represent 5th, 25th, 50th, 75th, and 95th percentiles and the crosses indicate mean values. *, $p<0.05$; **, $p<0.01$ compared with vehicle.

## DATA DELIVERABLES IN METASTASIS MODELS

Depending on the model, parameters such as primary tumor volume, tumor burden, X-ray, DXA, serum tumor biomarkers and bone turnover markers, tumor and bone histomorphometry, and immunohistochemical analyses are available. Additionally body weight and clinical condition are always reported.

**Table 2.** Data deliverables in metastasis models. BLI, bioluminescence imaging; GFP, green fluorescent protein; IHC, immunohistochemistry; DXA, dual-energy X-ray absorptiometry; pQCT, peripheral quantitative computed tomography; µCT, micro computed tomography; PINP, procollagen type I N-terminal propeptide; TRACP5b, tartrate-resistant acid phosphatase isoform 5b; CTX, C-terminal cross-linked telopeptides of type I collagen.

| **Assessment on Tumor** | |
|---|---|
| Imaging | BLI<br>GFP |
| Serum Markers | Tumor related e.g. PSA |
| Histomorphometry | Tumor area and IHC from decalcified bone tumor samples |

| **Assessment on Bone** | |
|---|---|
| Imaging | Radiography (X-ray)<br>Densitometry (DXA, pQCT, µCT) |
| Bone Turnover Markers | PINP, TRACP5b, CTX |
| Histomorphometry | Bone histomorphometry from decalcified or undecalcified samples |

### Testing the mode-of-action of a compound on a cellular level *in vivo*

Several immunohistochemical and other analyses can be performed to study the effects of the compound on a cellular level.

- Morphology can be determined by H&E staining
- Proliferation can be detected by Ki-67 or phosphohistone 3 staining
- Double-strand breaks in DNA can be detected by gamma-H2AX staining
- Late apoptosis and related DNA fragmentation can be determined using TUNEL assay (terminal deoxynucleotidyl transferase dUTP nick end labeling)
- Angiogenesis can be determined via detecting microvessel density using CD34 staining

## REFERENCES


1. Day CP, Carter J, Bonomi C, Hollingshead M, Merlino G. Preclinical therapeutic response of residual metastatic disease is distinct from its primary tumor of origin. Int J Cancer. 2012;130:190-9.

2. Ren G, Esposito M, Kang Y. Bone metastasis and the metastatic niche. J Mol Med (Berl). 2015;93:1203-12.

3. Suominen MI, Rissanen JP, Kakonen R, Fagerlund KM, Alhoniemi E, Mumberg D, et al. Survival benefit with radium-223 dichloride in a mouse model of breast cancer bone metastasis. J Natl Cancer Inst. 2013;105:908-16.

4. Wissing MD, van Leeuwen FW, van der Pluijm G, Gelderblom H. Radium-223 chloride: Extending life in prostate cancer patients by treating bone metastases. Clin Cancer Res. 2013;19:5822-7.

5. Tuomela J, Valta M, Vaananen K, Harkonen P. Alendronate decreases orthotopic PC-3 prostate tumor growth and metastasis to prostate-draining lymph nodes in nude mice. BMC Cancer. 2008;8:81.

6. Morris G, Suominen M, Fagerlund K, Rissanen J, Halleen J, Vainikka S. Inhibiting androgen receptor-associated Src signaling by VAL201 inhibits prostate cancer metastasis in an orthotopic mouse model. Cancer Res. 2013;73(8 Suppl):Abstract nr 2080.

7. Stephenson RA, Dinney CP, Gohji K, Ordóñez NG, Killion JJ, Fidler IJ. Metastatic model for human prostate cancer using orthotopic implantation in nude mice. J Natl Cancer Inst. 1992;84(12):951-7.

8. Suominen M, Fagerlund K, Rissanen J, Konkol Y, Morko J, Peng Z, et al. Radium-223 dichloride exhibits dual mode-of-action inhibiting both tumor and tumor- induced bone growth in two osteoblastic prostate cancer models. Cancer Res. 2015;75(15 Suppl):Abstract nr 3447.

9. Guise TA, Yin JJ, Taylor SD, Kumagai Y, Dallas M, Boyce BF, et al. Evidence for a causal role of parathyroid hormone-related protein in the pathogenesis of human breast cancer-mediated osteolysis. J Clin Invest. 1996;98(7):1544-9.

10. Suominen M, Käkönen R, Rissanen J, Käkönen S-M, Halleen J. Sequential treatment with zoledronic acid and doxorubicin has no additive effects in a mouse model of established bone metastases. Cancer Res. 2010;70(8 Suppl):Abstract nr 5394.

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Pharmatest is a preclinical CRO that offers efficacy studies in oncology and skeletal diseases. In oncology our services include cell culture assays, orthotopic animal models and disseminated cancer models, with special expertise in bone metastasis models. We also offer customized studies and model development services for our customers.

### Reduce the risk of attrition in clinical trials

Failure to reproduce preclinical efficacy results in clinical trials is one of the major causes of the high costs of drug discovery. Improving the preclinical predictivity by using more sophisticated models will significantly decrease the cost of bringing a new drug to market and help identify the promising compounds at earlier stage, avoiding unnecessary and costly clinical trials with less than optimal compounds.

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