Orthotopic and metastasis models for preclinical efficacy testing of novel cancer drugs

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pharmatest

Introduction

Lack of efficacy is one of the major causes of attrition in the clinical development of new compounds to treat cancer. Therefore, selecting the correct and the most predictive animal models for your research is critical before entering the clinical efficacy studies. Choosing the right model can help you determine the efficacy of your compound quickly and reliably. When testing the preclinical efficacy of a new antitumor therapeutic, it is important to:

- Take into account the tumor microenvironment[1, 2]
- Test immunomodulatory drugs in intact immune systems[3]
- Test compounds also in metastasized disease[4]
- Evaluate the potential adverse effects on bone (cancer treatment-induced bone loss, CTIBL)[5]

Pharmatest Services Ltd provides a selection of cancer and skeletal disease models that take into account these crucial elements in developing novel antitumor therapies. We can provide you with models that allow you to test the efficacy of your compounds in broad experimental settings with improved clinical predictivity. We can also test your compound on osteoblast and osteoclast bone cell cultures to determine the possible beneficial or adverse effects of your compounds on bone cells[6].

"Testing your compound in orthotopic models provides you with more relevant data on the efficacy of your compound than subcutaneous models."

How can you generate more accurate preclinical efficacy data?

In vivo xenograft tumor models can be constructed in several different ways, depending on the site and route of the tumor cell inoculation. In orthotopic models, the tumor cells are inoculated directly into the relevant organ where they proliferate to form solid tumors. This allows the implanted tumor to interact with the correct tumor microenvironment, which is important because the immune cells and signaling molecules along with stromal cells and other components of the microenvironment are known to play a crucial role in the sensitivity of the tumor to antitumor compounds[1]. Therefore, testing your compound in orthotopic models provides you with more relevant data on the efficacy of your compound than subcutaneous models, and an improved clinical predictivity.

Pharmatest offers efficacy testing in orthotopic tumor models for breast cancer, prostate cancer, multiple myeloma, and pancreatic cancer. Data on body weight change and immunohistochemical analyses are always available, and depending on the model, also additional parameters such as primary tumor volume, tumor burden, cachexia, osteolytic area by x-ray, or tumor and bone histomorphometry. Orthotopic tumor growth can be measured by imaging using luminescence in solid tumors, and in multiple myeloma also by detecting paraprotein IgG2b levels in serum samples. Figures 1, 2, and 3 are examples of the parameters that can be measured to monitor the study.

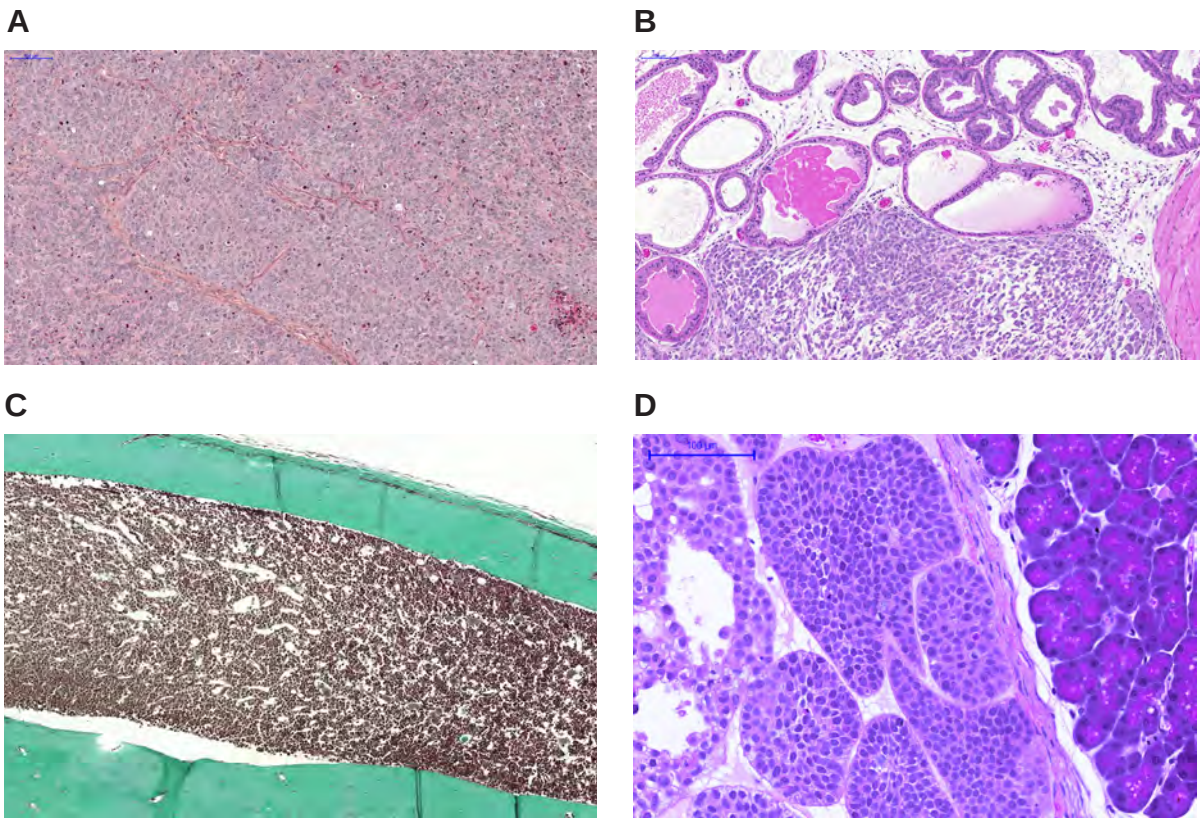

Figure 1. Orthotopic tumor tissue in MCF-7, PC-3, 5TGM1, and BxPC3 cancer models *in vivo*.
A. MCF-7 breast cancer xenograft tumor in breast tissue (Hematoxylin and eosin (H&E) staining)
B. PC-3 prostate cancer xenograft tumor in prostate tissue (H&E staining)
C. 5TGM1 multiple myeloma xenograft tumor in bone marrow (Masson-Goldner Trichrome staining)
D. BxPC3 pancreatic cancer xenograft tumor in pancreatic tissue (H&E staining)

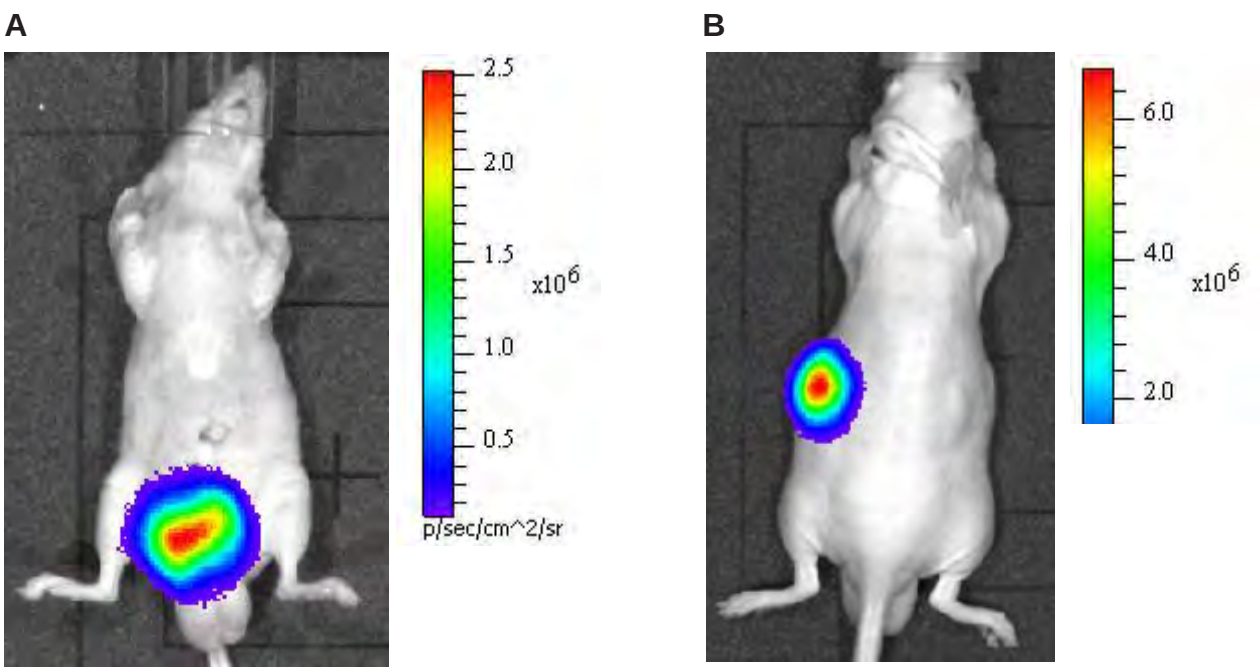

Figure 2. In order to follow orthotopic tumor growth, luciferase-expressing cell lines have been generated. (A) PC-3-luciferase orthotopic prostate tumor (B) BxPC-3-luciferase orthotopic pancreatic tumor.

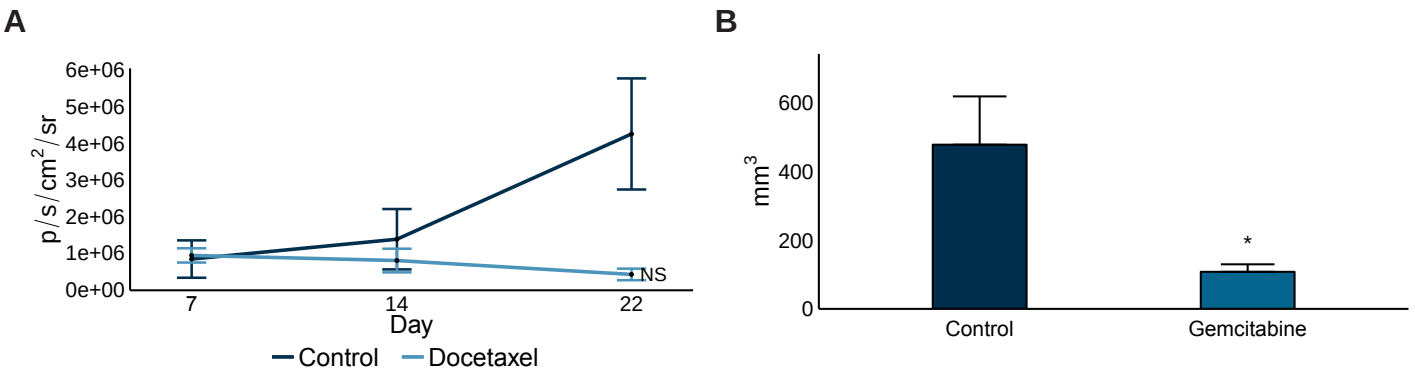

Figure 3. Treatment response of docetaxel and gemcitabine on PC-3 and BxPC3 orthotopic xenograft models in mice.

A. Orthotopic PC-3 prostate tumor growth is reduced with docetaxel as measured by bioluminescent imaging
B. Orthotopic BxPC3 pancreatic tumor growth is reduced with gemcitabine as measured by a caliper, *ex vivo* measurement

Orthotopic models available:

Tumor model	Tissue type	Tumor characteristics	Metastatic growth in
MDA-MB-231(SA)	Breast	ER-, PR-, HER2-	Lung, lymph nodes
4T1	Breast	ER-, PR-, HER2-	Lung, liver, lymph nodes
MCF-7	Breast	ER+, PR+, HER2+	None
PC-3	Prostate	AR-	Lymph nodes
LNCaP	Prostate	AR+, PSA+	None
5TGM1	Multiple myeloma	Paraprotein (IgG2b)	Bone
BxPC3	Pancreatic cancer		None

Syngeneic models for testing anticancer efficacy of immunomodulators

Drugs targeting the immune system have been shown to be very promising as the next generation of cancer therapeutics. Molecules known as immune checkpoints regulate T cell functions and proliferation in normal cells, but in cancer, there can be an imbalance between the stimulatory and inhibitory signals. This can allow tumor cells to escape the control of the immune system. However, compounds such as immune checkpoint inhibitors can restore the immune functions in the tumor microenvironment[3]. Syngeneic models, where mouse tumors are expressed in mice with intact immune systems, are very useful tools for studying the efficacy of your immunomodulatory compound.

Data on body weight change and immunohistochemical analyses are always available for syngeneic models. Depending on the model, additional parameters such as primary tumor volume, tumor burden, radiography, tumor and bone histomorphometry are also available. In multiple myeloma, tumor growth can be measured by detecting paraprotein IgG2b levels in serum samples (Figure 4).

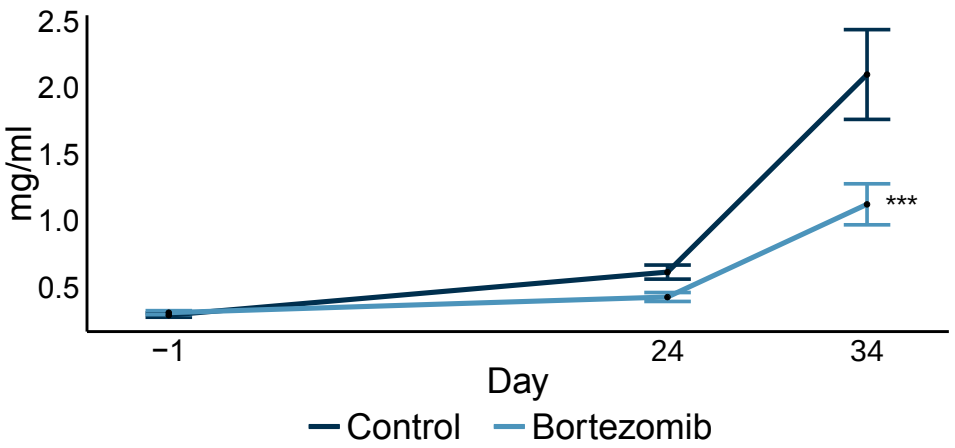

Figure 4. Bortezomib treatment lowers the IgG2b paraprotein levels in serum in 5TGM1 syngeneic multiple myeloma model.

Syngeneic cancer models available:

Tumor model	Tissue type	Tumor characteristics	Inoculation route	Tumor growth in
4T1	Breast	ER-, PR-, HER2-	Intracardiac Orthotopic	Bone Lung, liver, lymph nodes
5TGM1	Multiple myeloma	Secretes IgG2b	Tail vein	Bone, ovary, calvaria, lymph nodes

Why should you test your compound in metastasis models?

Metastatic and primary tumors are known to respond differently to chemotherapy. Secondary tumors can, for example, be resistant to the same drug that efficiently prevented the growth of the corresponding primary tumor[7]. 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.

Cancers can metastasize to several different organs such as lung, liver, and bone, but particular preferences for target organs have been discovered for certain tumor types, leading to the seed and soil -theory[8]. The microenvironment of each organ can favor certain types of tumor cells, thereby aiding homing of the circulating primary tumor cells and providing a fertile environment for the formation of metastases.

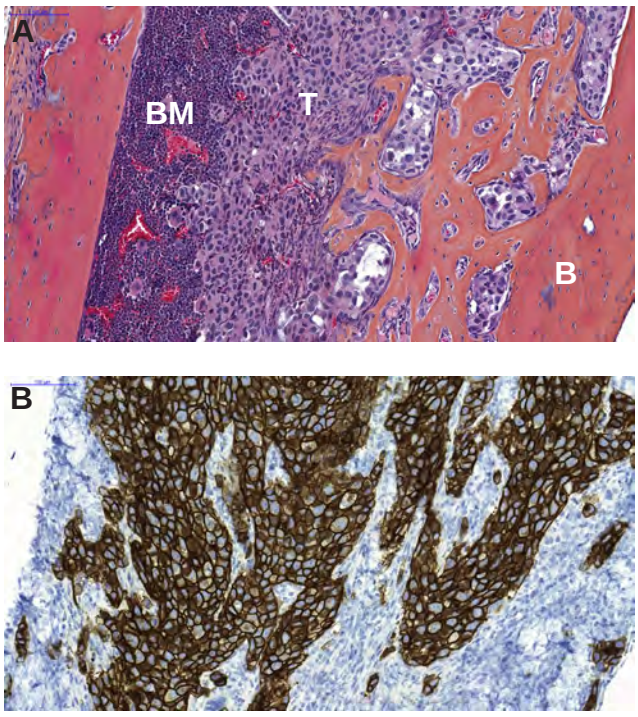

Figure 5. Histology images of BT-474 xenograft tumors of triple-positive (ER+, PR+, HER+) breast cancer growing in bone after intratibial inoculation.

A. BT-474 breast cancer cells growing in bone (H&E staining). B, bone; T, tumor; BM, bone marrow.

B. Expression of human epidermal growth factor receptor 2 (HER2) in BT-474 cells growing in the bone.

Bone metastases, in particular, are frequently source of pain for late-stage cancer patients and they are often very resistant to treatment due to the characteristics of the bone[9].

Tumor cells can also lie dormant in bone for years or even for decades, as for example is the case in breast cancer[9]. Furthermore, bone metastases have been shown to promote secondary metastases to other organs such as lung, brain, liver, or adrenal gland[10]. Therefore, compounds that effectively treat or prevent bone metastases may also increase the overall survival[11, 12].

While any cancer can metastasize to bone, bone marrow tends to provide an especially attractive soil for occult cells that originate from breast and prostate tumors. Pharmatest offers *in vivo* bone metastasis models for both of these primary cancers. Figures 5 and 6 show examples.

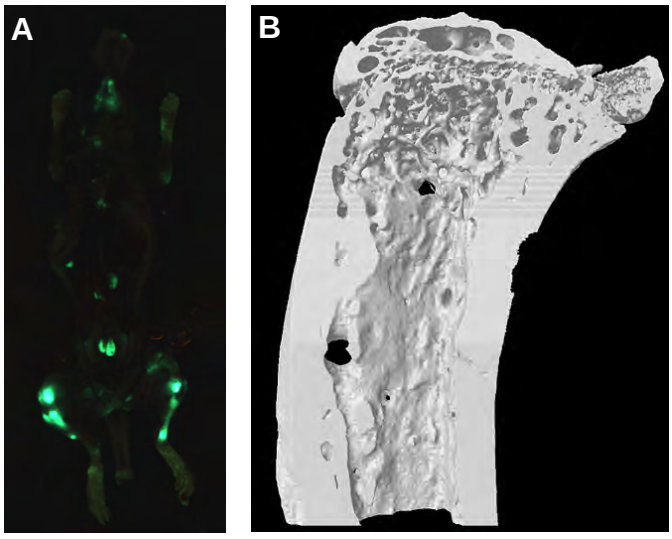

Figure 6. GFP and micro-CT imaging can be used to quantitatively measure tumor growth and tumor induced effects on bone, respectively.

A. MDA-MB-231(SA) breast cancer metastases *in vivo*. Cells are inoculated intracardially and detected by bioluminescent imaging of green fluorescent protein (GFP) expression

B. Intratibially inoculated LNCaP prostate cancer tumors induce osteoblastic-mixed bone lesions, detected by micro-computed tomography (µCT)

Metastasis models available:

Tumor model	Tumor type	Tumor characteristics	Inoculation route	Tumor growth in
MDA-MB-231(SA)	Breast	ER-, PR-, HER2-	Intracardiac Orthotopic	Bone, adrenal glands Lung, lymph nodes
4T1	Breast	ER-, PR-, HER2-	Intracardiac Orthotopic	Bone, several other tissues Lung, liver, lymph nodes
BT-474	Breast	ER+, PR+, HER2+	Intratibial	Bone
PC-3	Prostate	AR-	Intratibial Orthotopic	Bone Lymph nodes
LNCaP	Prostate	AR+, PSA+	Intratibial	Bone
VCaP	Prostate	AR+, PSA+	Intratibial	Bone

Is your compound bone-protective or rendering bone susceptible to metastases?

Cancer treatments such as hormone-deprivation or chemotherapy can affect the bone, inducing increase in bone metabolism. For instance several hormone-ablative breast and prostate cancer treatments can have adverse effects on bone. This can subsequently lead to bone loss, which is often referred to as cancer treatment-induced bone loss (CTIBL)[5].

Along with osteoporosis, which the aging cancer patient population is already at risk of, CTIBL can predispose bone to formation of metastases due to the accelerated metabolism that has been shown to increase bone metastases by providing a more fertile microenvironment than unaffected bone[13]. The bone matrix can release factors that stimulate the growth of tumor cells, while the tumor cells themselves can also further increase the bone resorption by secreting factors that stimulate osteoclasts.

Pharmatest offers several *in vivo* models of skeletal diseases that you can utilize to find out if your particular test compound has potential adverse effects on bone.

Skeletal models also offer the unique opportunity to study effects related to osteoimmunology, the molecular communication between bone and the immune system[14]. Disorders in the crosstalk can lead to diseases such as osteoporosis.

If you are interested in studying effects of your compounds on bone cells, we can offer you our *in vitro* models of osteoclast and osteoblast diffentation and activity. Such studies can be performed quickly prior to *in vivo* testing. Figure 7 shows examples on some of the measurable parameters *in vitro*.

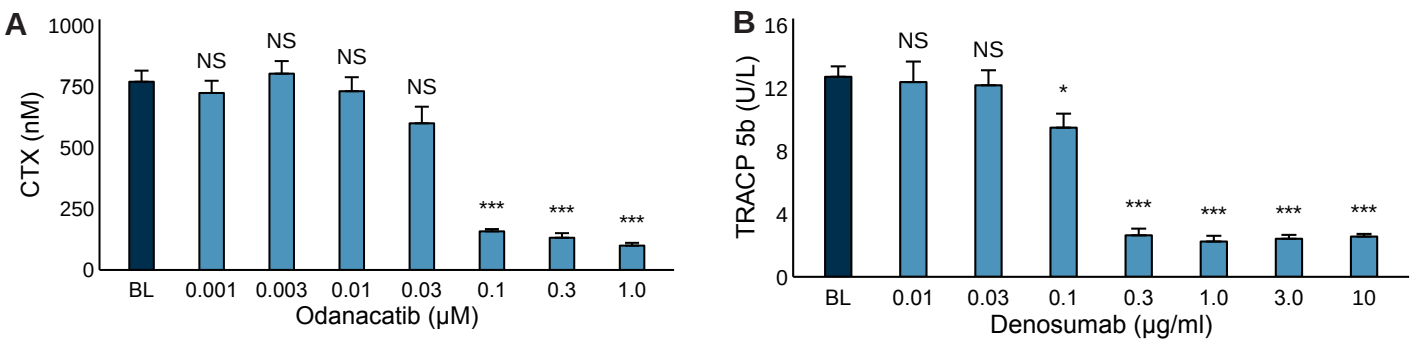

Figure 7. Reference compounds in human *in vitro* osteoclast cultures. A) The cathepsin K inhibitor odanacatib inhibits bone resorption as determined by CTX-I measurements; B) The RANKL inhibitor denosumab inhibits osteoclast differentiation as determined by TRACP 5b measurements.

Skeletal disease models:

In vitro models

Model	Species	Characteristics
Osteoclast differentation	Human	• Bone marrow-derived osteoclast precursor cells • TRACP 5b used as a marker of osteoclast formation
Osteoclast activation	Human	• Bone marrow-derived osteoclast precursor cells • CTX-I used as a marker of bone resorption
Osteoblast differentation	Mouse	• KS483 cells • Intracellular ALP used as a marker of osteoblast formation
Osteoblast activation	Mouse	• KS483 cells • Calsium and PINP used as markers of bone formation

In vivo models

Model	Species	Characteristics
Postmenopausal osteoporosis	Mouse, rat	Ovariectomy (OVX) model of female primary osteoporosis
Male osteoporosis	Rat	Orchidectomy (ORX) model of primary osteoporosis
Glucocorticoid-induced osteoporosis	Mouse	Model of secondary osteoporosis
Bone safety	Mouse, rat	Intact animals

Pharmatest provides full-service efficacy testing according to your needs

Collaboration with Pharmatest is easy. We will design a Study Proposal for your approval followed by a detailed Study Protocol; provide you with regular updates on the study progress; and at the end of the study, deliver a complete Final Report on the results.

CLINICALLY PREDICTIVE PRECLINICAL EFFICACY MODELS TO REDUCE YOUR COSTS AND ACCELERATE YOUR DRUG DEVELOPMENT

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.

Bone metastases are common and increase mortality in many cancers such as breast, prostate and lung cancers, and multiple myeloma. Bone microenvironment changes tumor properties and induces drug resistance, highlighting the importance of confirming cancer drug efficacy in models of tumor growth in bone before entering clinical trials. This should substantially decrease the currently very high failure rates of cancer drug candidates failing in clinical trials due to poor efficacy.

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