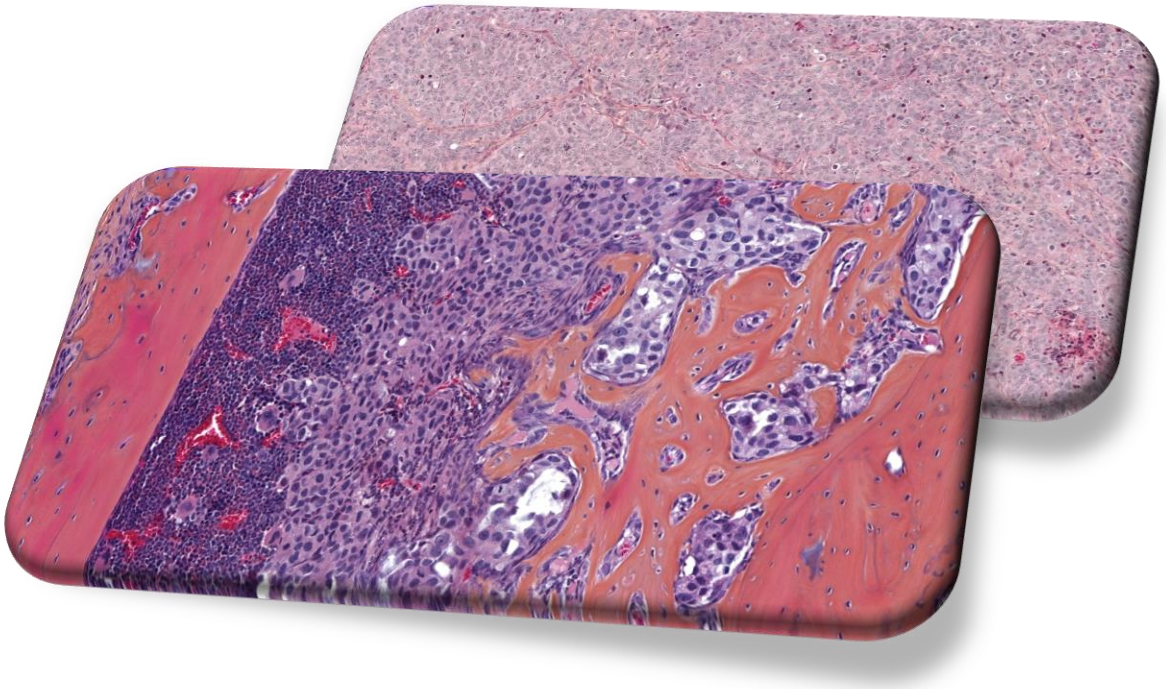
Novel bone metastasis models in humanized mice

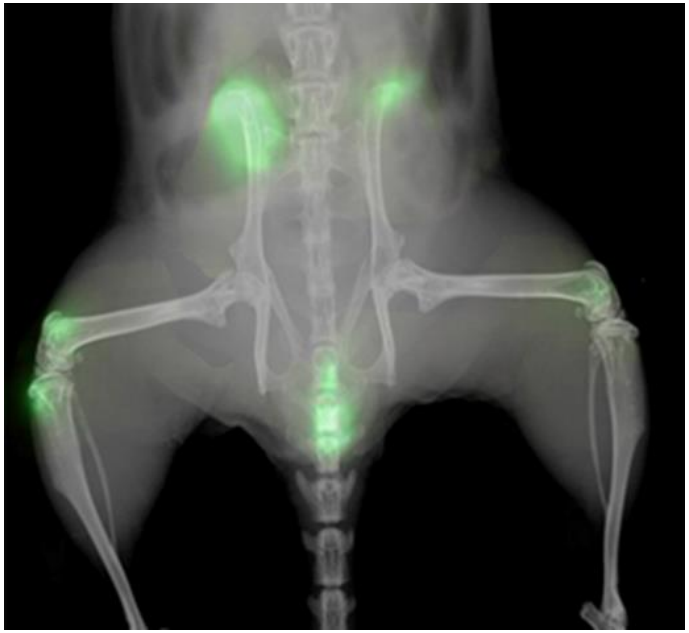
BIOCOM CRO event
San Diego 01/2018

Mari Suominen
Research Director
Pharmatest Services



Contents

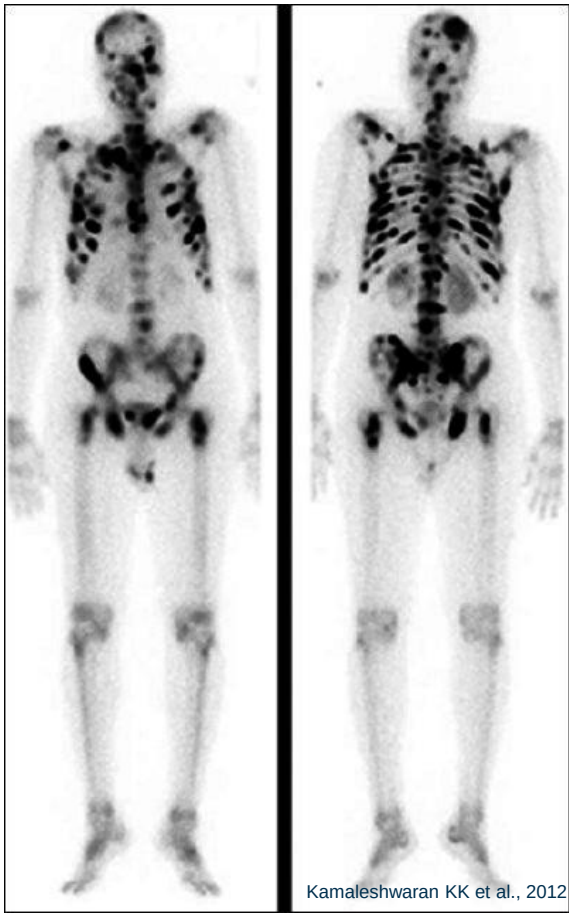
- Why study bone metastases?
- Osteoimmunology basics
- Immunotherapy and bone metastases
- Animal models



Bone metastases are a significant source of morbidity and mortality

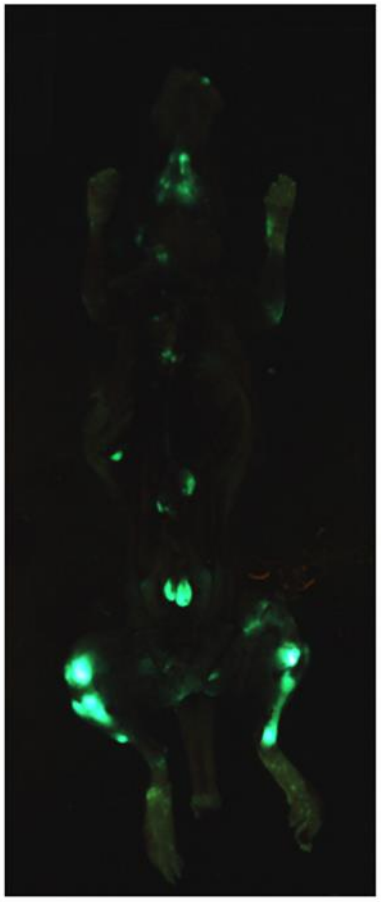
- > 300.000 patients in US
  (Hernandez et al 2015)
- Breast, prostate and lung cancer

Prostate cancer patient, Tc99m-MDP



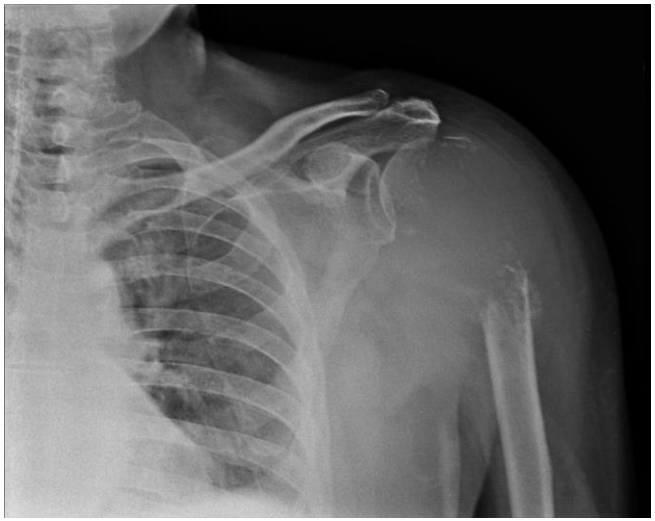
Kamaleshwaran KK et al., 2012

Mouse, GFP

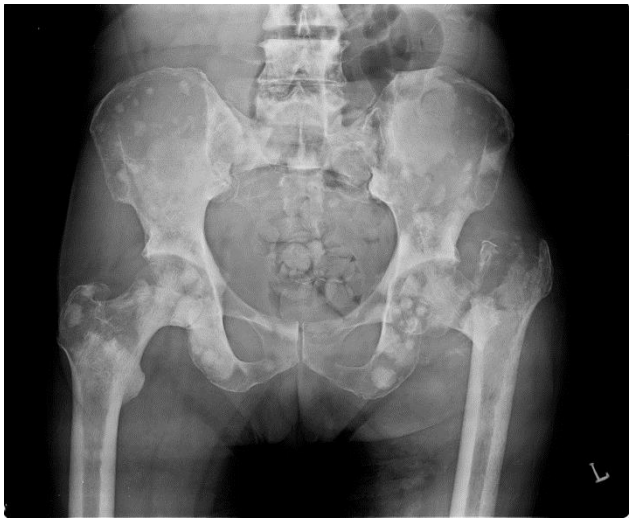


Bone metastases are a significant source of morbidity and mortality

Osteolytic



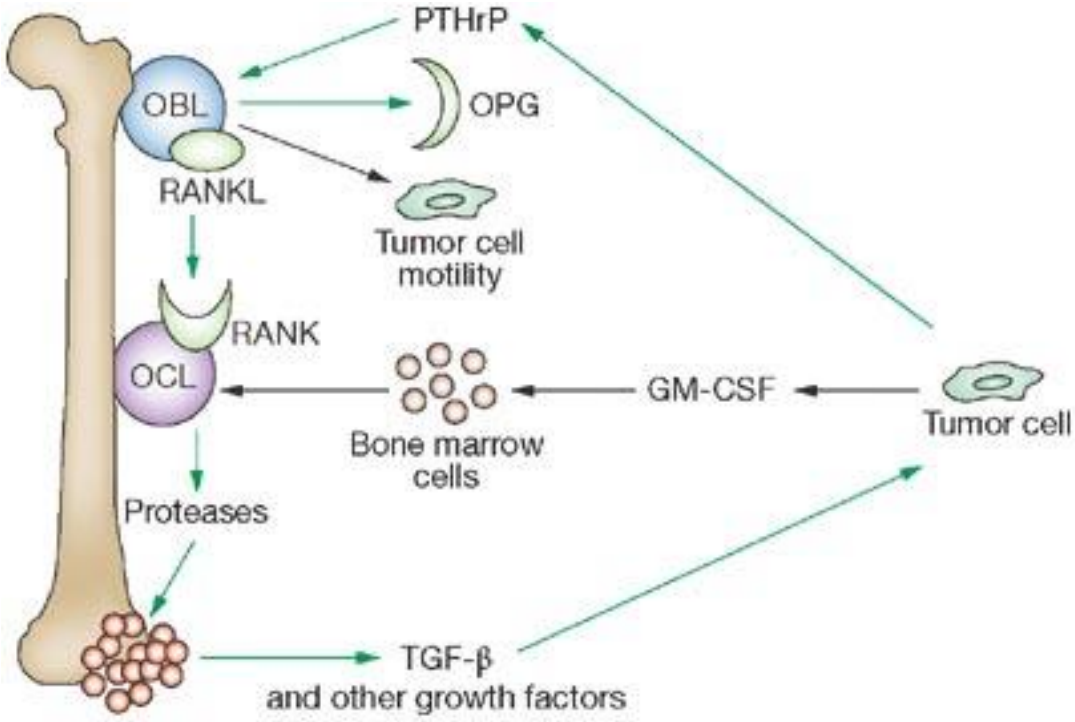
Osteoblastic



Left: Osteolytic lesion in the humerus. Case courtesy of Dr Maulik S Patel, Radiopaedia.org, rID: 19359.
Right: Osteosclerotic (osteoblastic) lesions in the pelvis. Case courtesy of Dr Nafisa Shakir Batta, Radiopaedia.org, rID: 38894.
Both: Creative Commons license CC BY-SA 3.0.

The vicious cycle

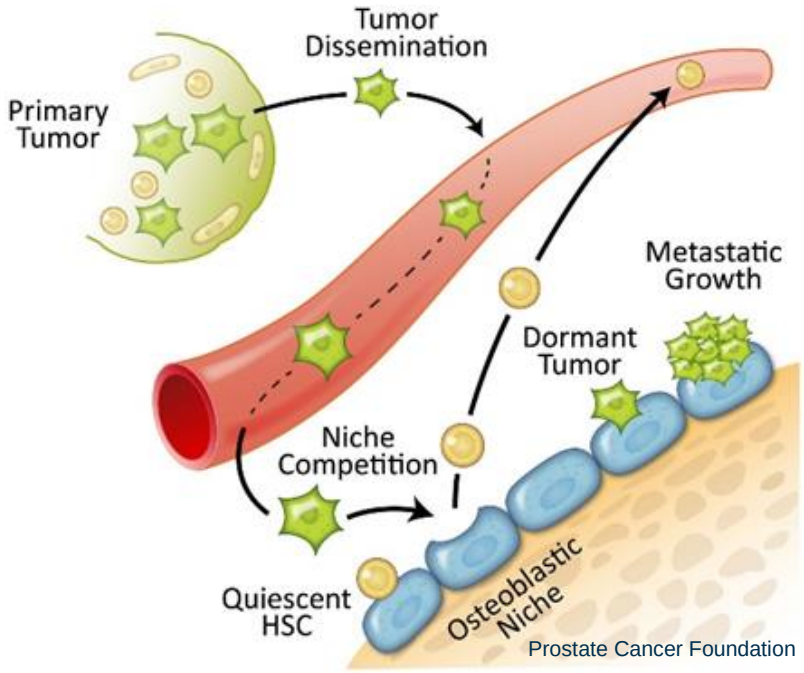
- Cancer cells induce changes in bone microenvironment that further support their growth -> “The vicious cycle”



Steeg PS and Theodorescu D (2008) Metastasis: a therapeutic target for cancer *Nat Clin Pract Oncol* doi:10.1038/ncponc1066

Dormancy

Disseminated tumor cells (DTC:s) are found in 30% and 72% of early breast and prostate cancer patients, respectively



Sänger et al 2011, Morgan et al 2009

Drug resistance

Cell

Sensitizing Protective Tumor Microenvironments to Antibody-Mediated Therapy

Christian P. Pallasch,[1,2] Ilya Leskov,[1] Christian J. Braun,[1] Daniela Vorholt,[2] Adam Drake,[1] Yadira M. Soto-Feliciano,[1] Eric H. Bent,[1] Janine Schwamb,[2] Bettina Iliopoulou,[1] Nadine Kutsch,[2] Nico van Rooijen,[3] Lukas P. Frenzel,[2] Clemens M. Wendtner,[2] Lukas Heukamp,[4] Karl Anton Kreuzer,[2] Michael Hallek,[2] Jianzhu Chen,[1,*] and Michael T. Hemann[1,*]

[1]Koch Institute for Integrative Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

[2]Department of Internal Medicine, Center of Integrated Oncology, University of Cologne, Cologne 50931, Germany

[3]VFM Amsterdam 1081, Netherlands

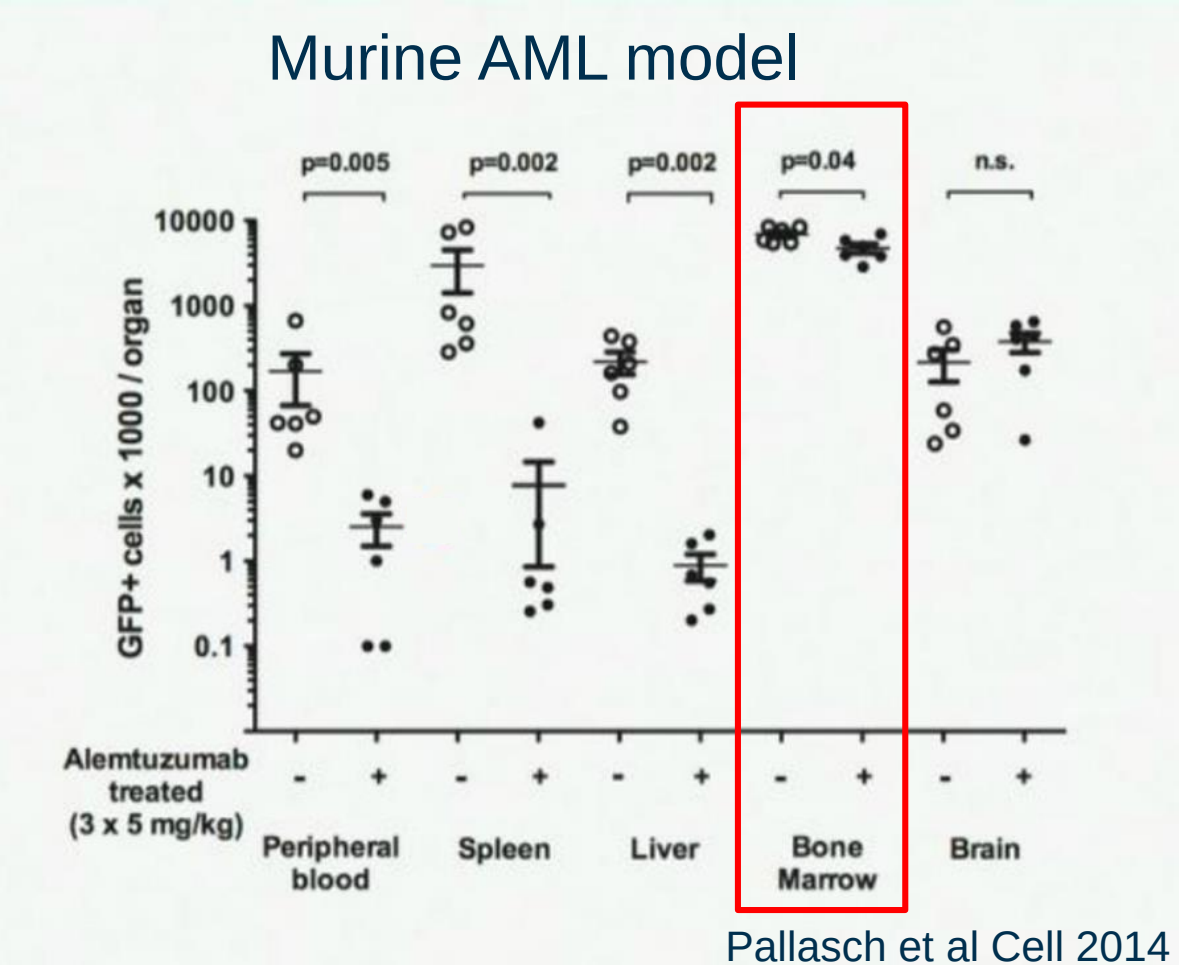
[4]Department of Pathology, University Hospital of Cologne 50937, Germany

*Correspondence: jchen@mit.edu (J.C.), hemann@mit.edu (M.T.H.)

http://dx.doi.org/10.1016/j.cell.2013.12.041

Cell *156*, 590–602, January 30, 2014

Drug resistance



Pallasch et al Cell 2014

- Macrophages
- Sc vs orthotopic tumor: Response to immuno-therapeutics (Westwood et al. 2014)

Drug resistance

- Anti-apoptotic signals, chemokines and growth factors
  - GAS6 from osteoblasts protects prostate cancer cells from docetaxel (Lee et al., 2016)
  - IL-6 from bone marrow stromal cells protects multiple myeloma cells from dexamethasone (Grigorieva et al., 1998)

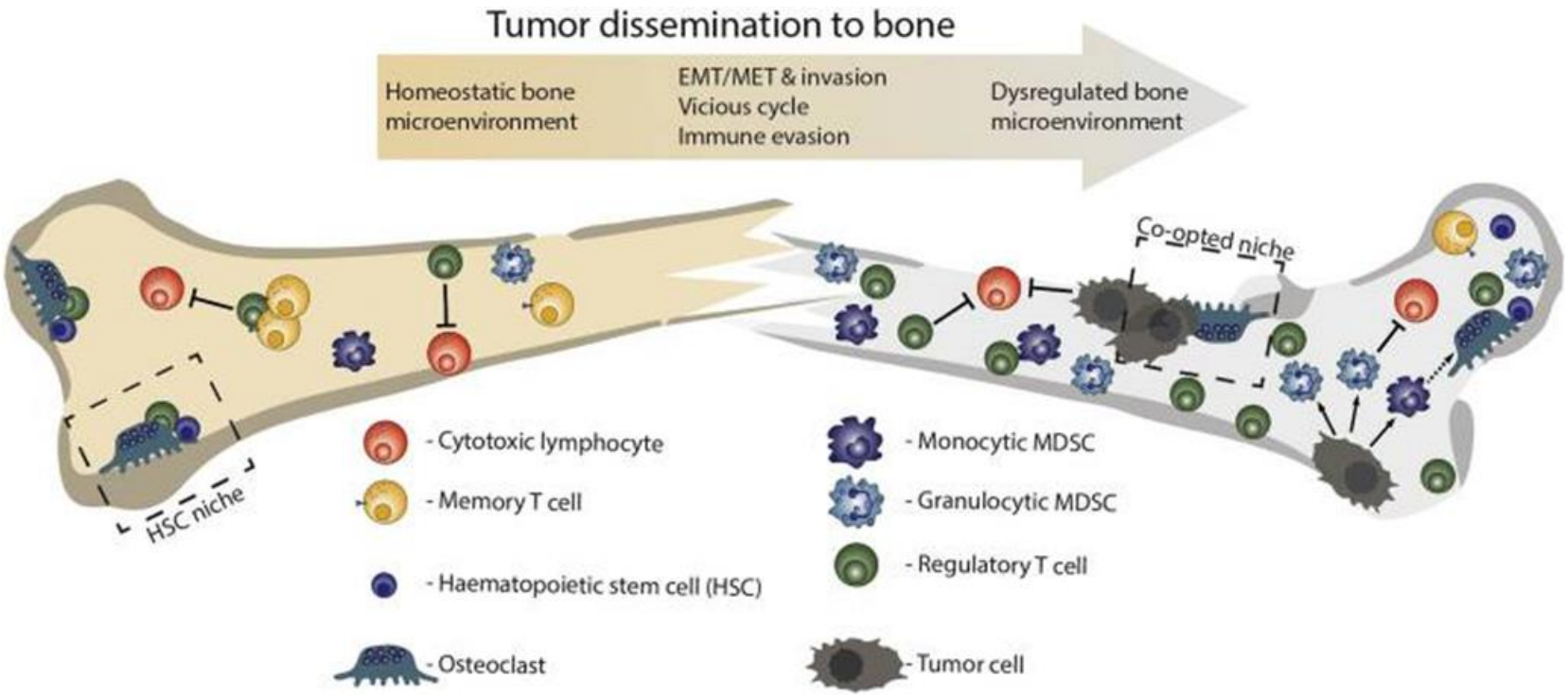
Drug resistance

- The bone microenvironment may serve as a rehab center
- Availability of other survival signals may lower the dependency on e.g. hormones in a hormone-dependent cancer
- Treatment targeting the addiction becomes less efficient

Osteoimmunology - basics

- Common origin of immune cells and osteoclasts
- Bone marrow holds only few mature T-cells, but a lot of mature B-cells
- Activated T-cells induce bone loss, local and systemic
- Bone forming cells, osteoblasts, are necessary for B-cell development and maturation

Osteoimmunology - basics



Baschuk et al. BoneKEy Reports 2015

Osteoimmunology - CPIs

- CTLA4 knock-out mice have more active osteoclasts, resulting in osteopenia
- PD-1 knock-out mice have less osteoclasts, resulting in osteopetrosis

Osteoimmunology – Current IO therapies and bone metastases

Critical Reviews in Oncology/Hematology 117 (2017) 114–127

Contents lists available at ScienceDirect

Critical Reviews in Oncology/Hematology

journal homepage: www.elsevier.com/locate/critrevonc

Overcoming immunosuppression in bone metastases

Zachary Z. Reinstein[a,b,c], Sahithi Pamarthy[a,b], Vinay Sagar[a,b], Ricardo Costa[a], Sarki A. Abdulkadir[b,d,e], Francis J. Giles[a], Benedito A. Carneiro[a,*]

[a] Developmental Therapeutics Program, Division of Hematology/Oncology, Feinberg School of Medicine, Chicago, USA

Table 1
Standard of care treatment of bone metastases.

Treatment	Mechanism	Effect on Bone metastases	Notes	Refs
SRE Treatments				
Zoledronic acid	inhibition of farnesyl pyrophosphate synthase	Inhibition of Osteolysis	Action against TAMs as well. Recommended for adjuvant use	(Vignani et al., 2016)
denosumab	mAb against RANKL	Reduces osteoclast activity		(Vignani et al., 2016)
Radium-223	Localizes to bone, releases alpha radiation	Cytotoxic to tumor cells by inducing dsDNA breaks	Offers less myelosuppression due to shorter range of alpha radiation	(Aragon-Ching and El-Amm, 2016)
Immunotherapies				
nivolumab	Anti-PD-1 mAb		Currently unstudied in bone metastases	
ipilimumab	Anti-CTLA-4 mAb		Currently unstudied in bone metastases	
pembrolizumab	Anti-PD-1 mAb		Currently unstudied in bone metastases	
atezolizumab	Anti-PD-L1 mAB		Currently unstudied in bone metastases	
Sipuleucel-T	Dendritic cells stimulated with GM-CSF and PAP	Unknown		(Vanneman and Dranoff, 2012)

Examples of Bone Metastasis models suitable for testing IO therapies

Breast cancer

- Syngeneic: 4T1, intracardiac
- Humanized: BT-474 or MDA-MB-231SA, intratibial

Multiple myeloma

- Syngeneic: 5TGM1, tail vein

Bladder cancer

- Syngeneic: MBT2, intratibial

Novel approach:
Tumor growth in bone of humanized mice

- High relevance and need to develop new treatment options against bone metastases
- Bone marrow is the original site of HSCs and an important site for immune cell development, indicating their role also in bone metastases
- A clinically predictive preclinical model that combines tumor, bone and immune system to functional entity
  - Osteo-immuno-oncology model

Schematic layout of the study

- Female huNOG mice (HSCFTL-NOG-F, Taconic Biosciences) from two donors;
  - Engraftment rate 40% (donor 1) and 60% (donor 2)
  - Age-matched CIEA NOG mice as controls

- BT-474 human breast cancer cells
  - Ductal carcinoma, 60 year old female
  - ER and PR positive and HER2 overexpressing

Day 0
Blood collection,
BT-474 intratibial
inoculation

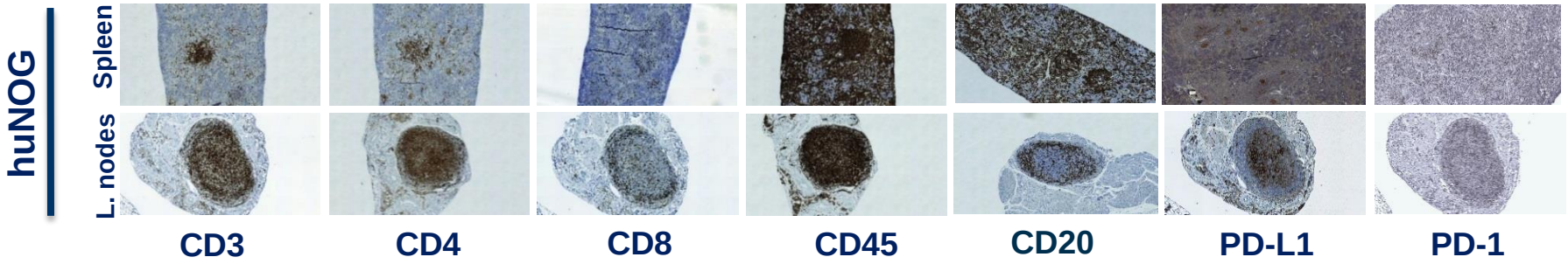
4 weeks
X-ray

6 weeks
X-ray

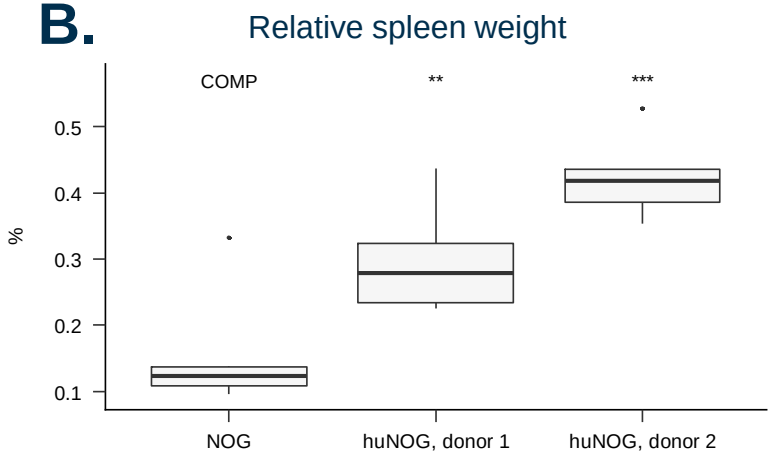
8 weeks
Blood collection,
X-ray,
DXA,
Sacrifice,
Tissue sample
collection and
ex vivo analysis

Expression of immune cell markers in huNOG mice

A.



B.

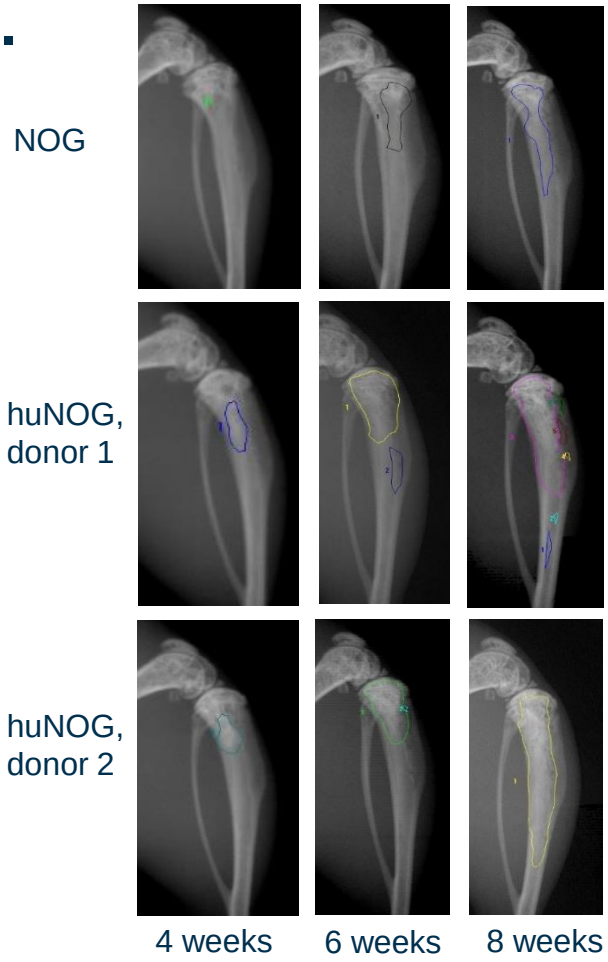


A) Immune cells and PD-L1 and PD-1 expression in spleen and lymp nodes of huNOG mice. 10x magnification.

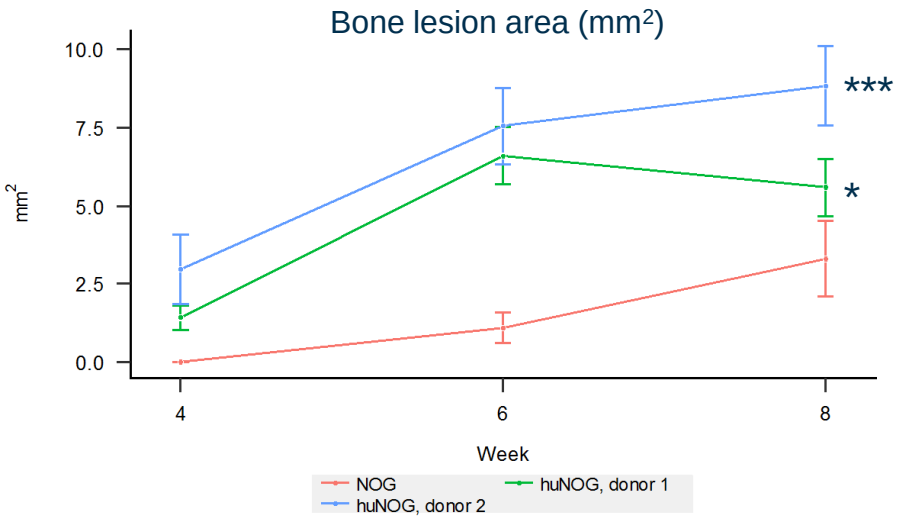
B) Relative change in spleen weight corrected to body weight (%).

Tumor-induced bone changes and bone lesion development during the study

A.



B.

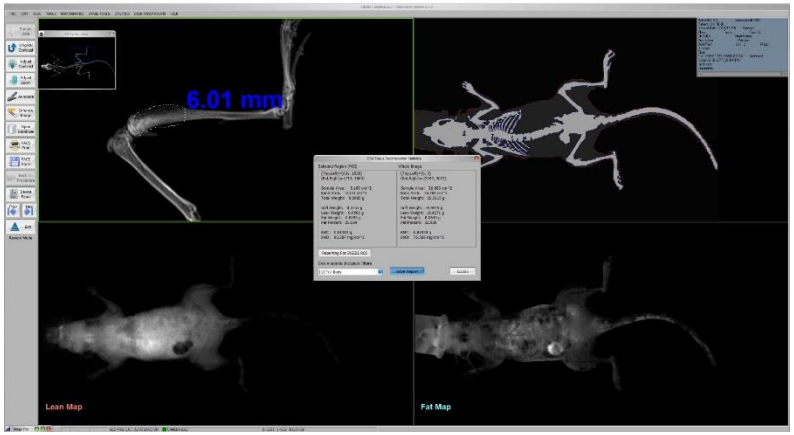


A) Examples of bone lesion development during the study in NOG and huNOG mice.

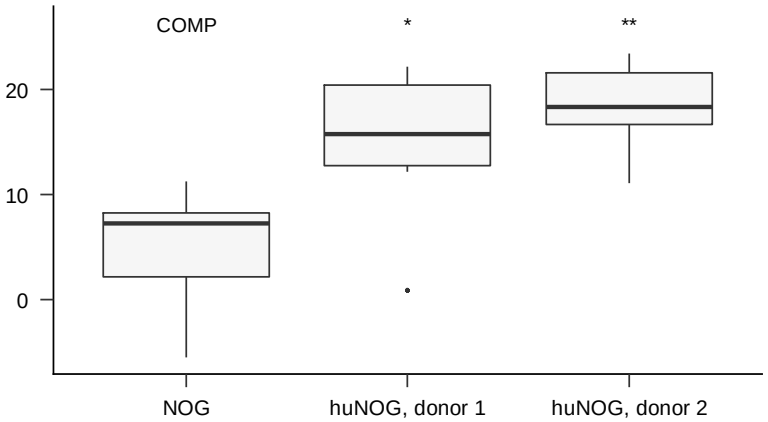
B) Monitoring of tumor-induced bone changes by X-ray imaging. Bone lesion area was quantified and presented as mean lesion area (mm^2).

Osteoblastic lesions were associated with increased bone mineral density

A.



B. Change in BMD in tumor-bearing tibia

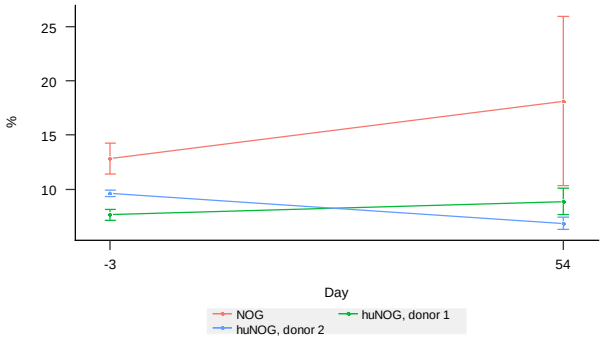


A) Dual X-ray absorptiometry (DXA) can be used to study bone changes *in vivo* during the study.

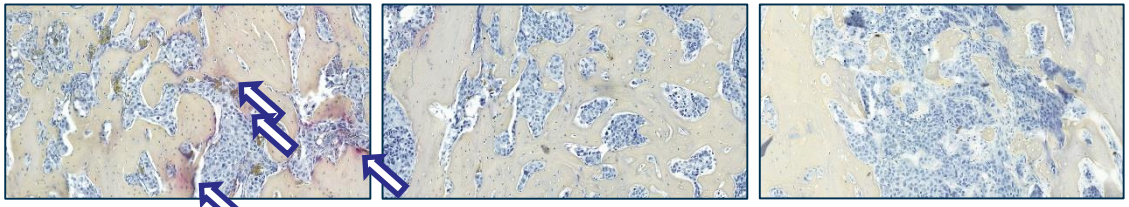
B) Quantitation of changes in bone mineral density (BMD, mg/cm^2) in tumor-bearing tibia at endpoint (8 weeks). Values of the contra-lateral tibia subtracted.

Larger bone amount in huNOG mice was partially caused by decreased number of bone resorbing osteoclasts

A. Relative TRACP 5b serum level



B.



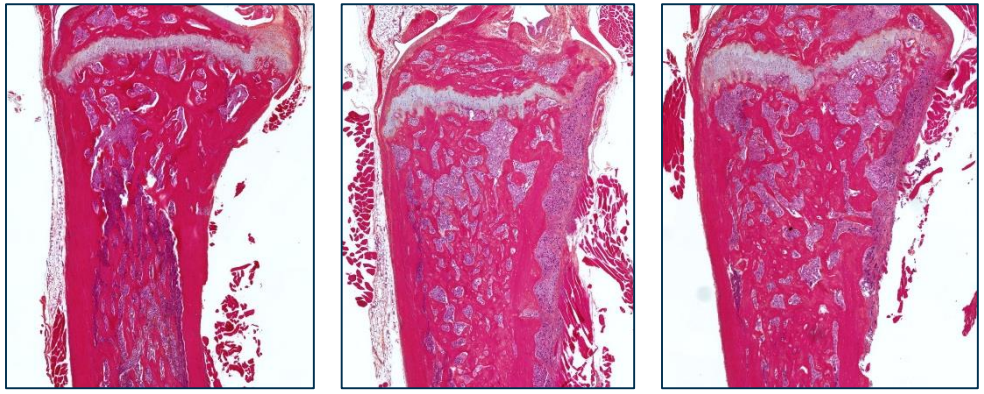
NOG | huNOG, donor 1 | huNOG, donor 2

A) Serum TRACP 5b levels indicate decreased osteoclast number in huNOG mice

B) Activated resorbing osteoclasts in the tumor-bearing tibia visualized by TRACP staining

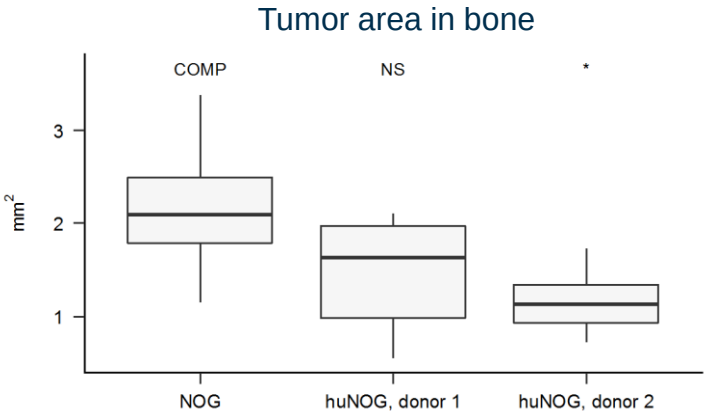
Quantitation of tumor area in bone marrow

A.



NOG | huNOG, donor 1 | huNOG, donor 2

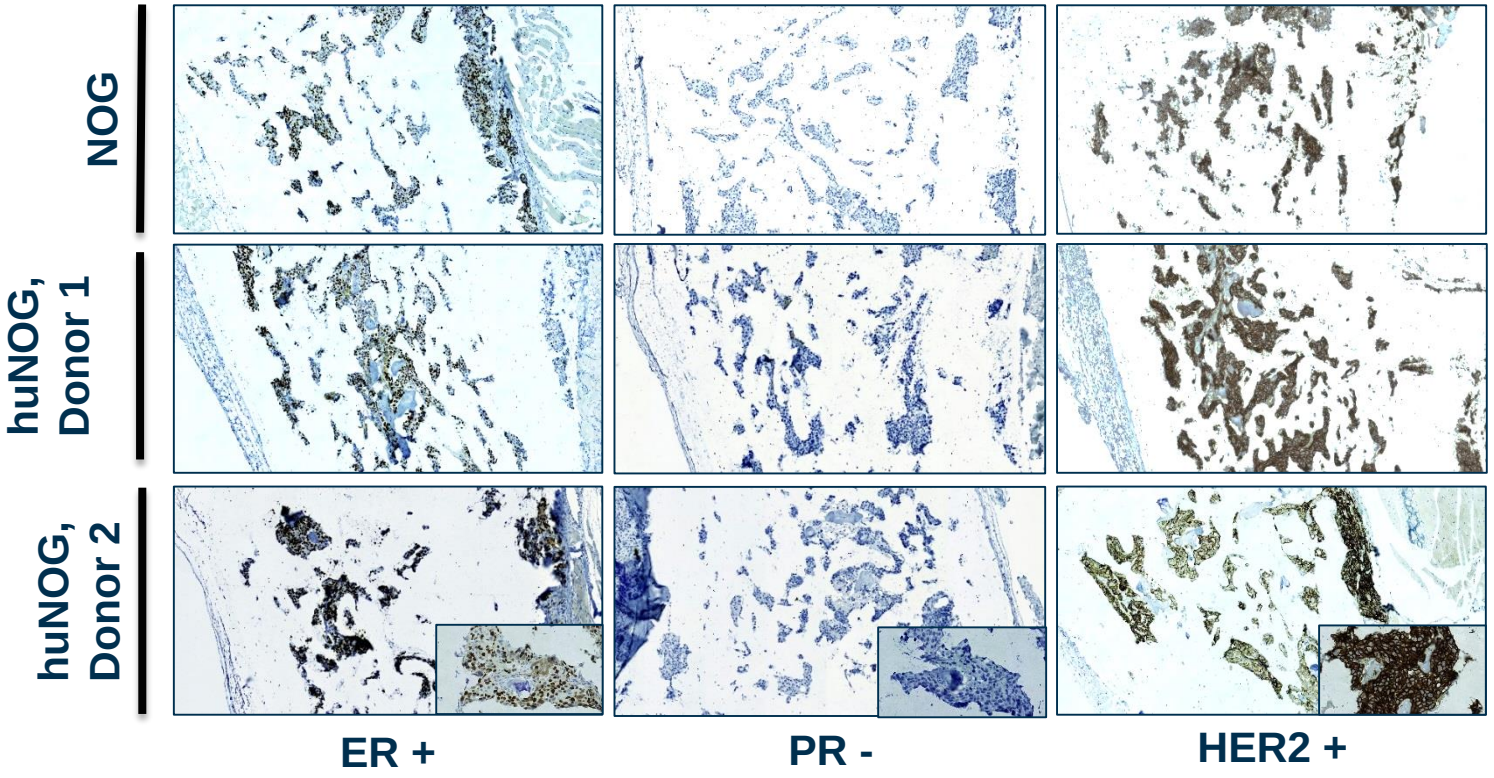
B.



A) Representative hematoxylin and eosin (HE) staining from tumor-bearing tibias

B) Quantitation of intratibial tumor area from the HE-stainings

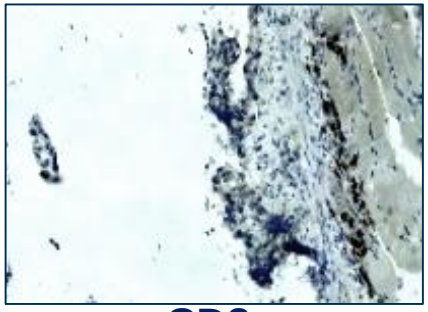
Expression of ER, PR and HER2 in tumor area

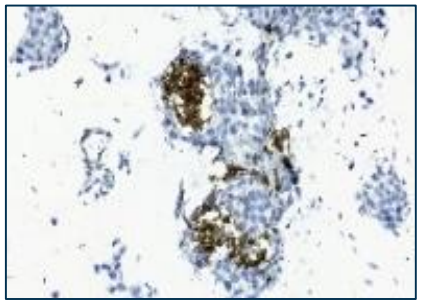


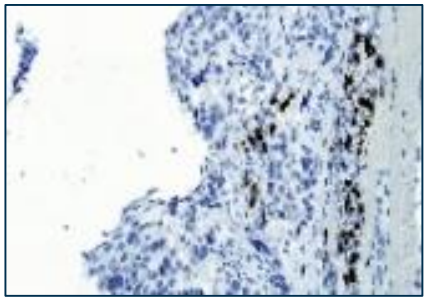
Immunohistochemical stainings for estrogen receptor alpha (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2). Magnification 4x and 40x.

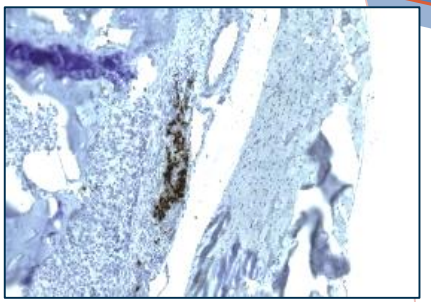
Immune cell markers in the tumor

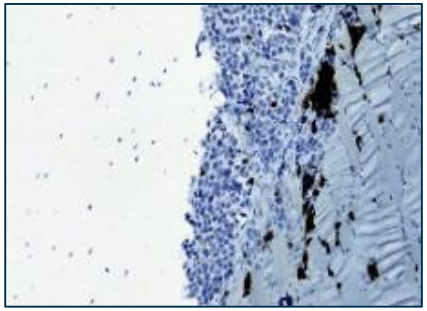
huNOG Tumor

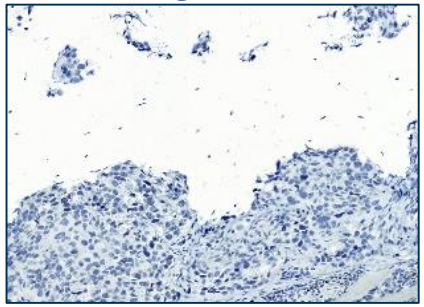

CD3

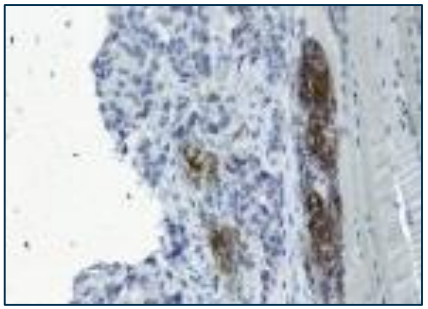

CD4

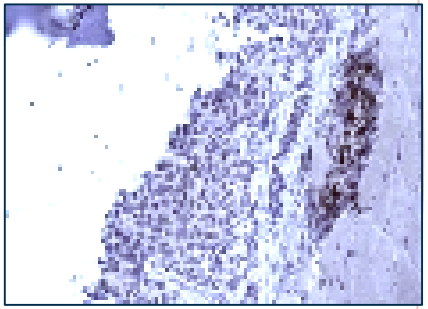

CD8


CD20


CD45


CTLA4


PD-L1


PD-1

Immune cell markers in the tumors of huNOG mice

- **CD3:** T cells
- **CD4**: Helper T cells
- **CD8**: Cytotoxic T cells
- **CD20**: B cells
- **CD45:** Leukocyte common antigen

Additional markers tested in this model

- **CTLA-4**
- **PD-L1**
- **PD-1**

Schematic layout of the study

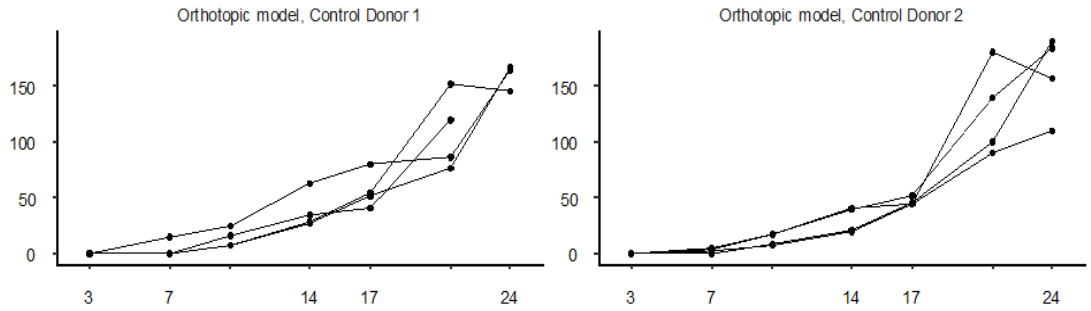
- Female huNOG mice (HSCFTL-NOG-F, Taconic Biosciences) from two donors;
  - Age-matched CIEA NOG mice as controls

- MDA-MB-231SA human breast cancer cells
  - Adenocarcinoma derived from pleural effusion of a 51 year old female
  - Triple-negative
  - Orthotopic vs bone immune milieu

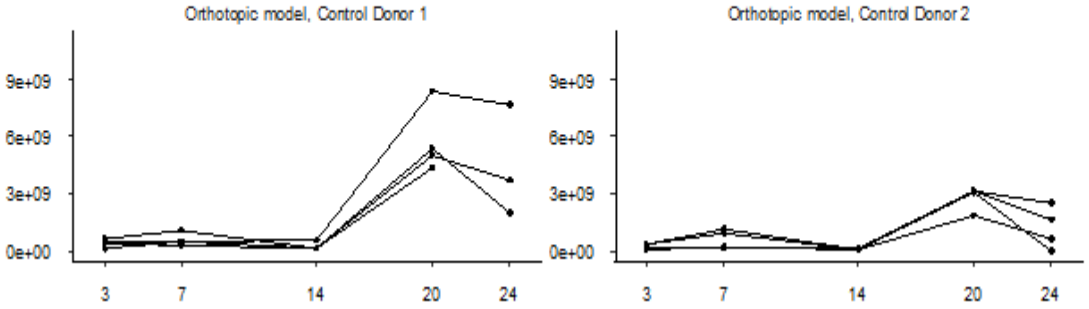
Day 0	1 weeks	2 weeks	3 weeks
Blood collection, MDA-MB-231SA intratibial or orthotopic inoculation	X-ray BLI	X-ray BLI	Blood collection, X-ray, DXA, BLI, Sacrifice, Tissue sample collection and ex vivo analysis

MDA-MB-231SA in mammary fat pad vs bone

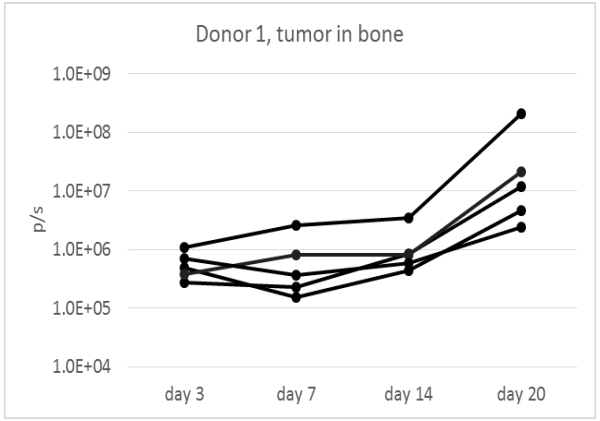
Orthotopic tumor volume

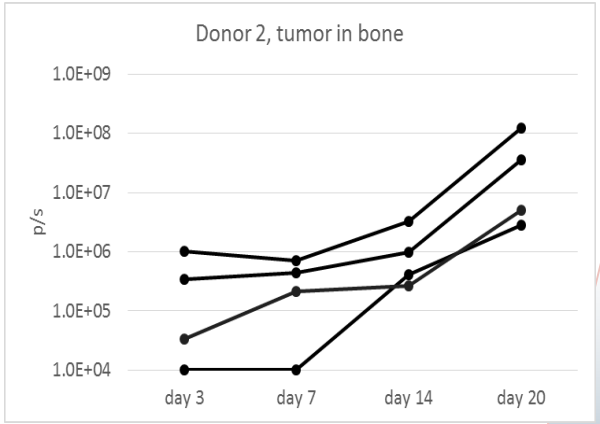


Orthotopic BLI



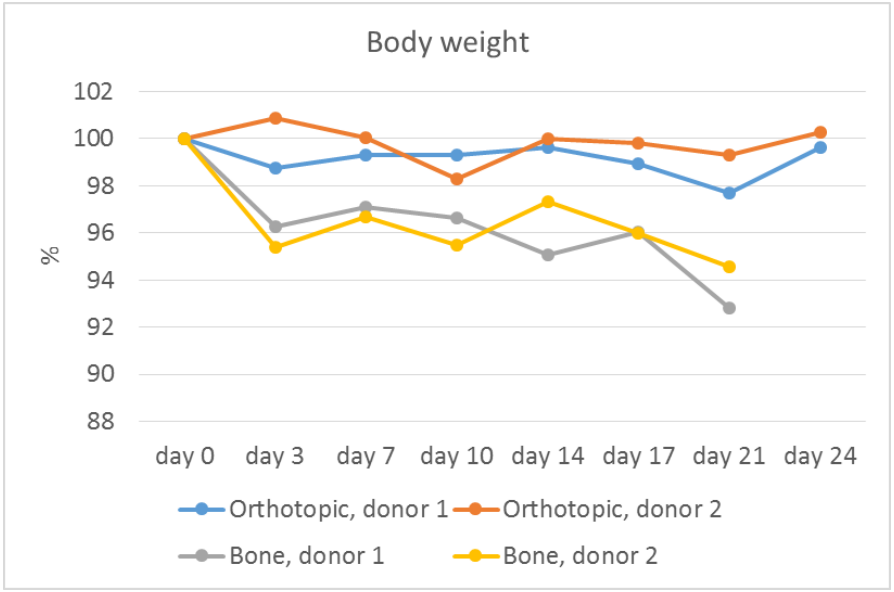
Bone model, BLI



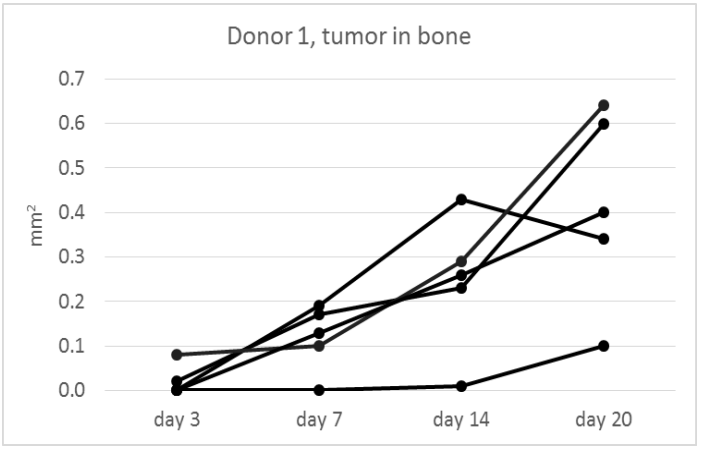


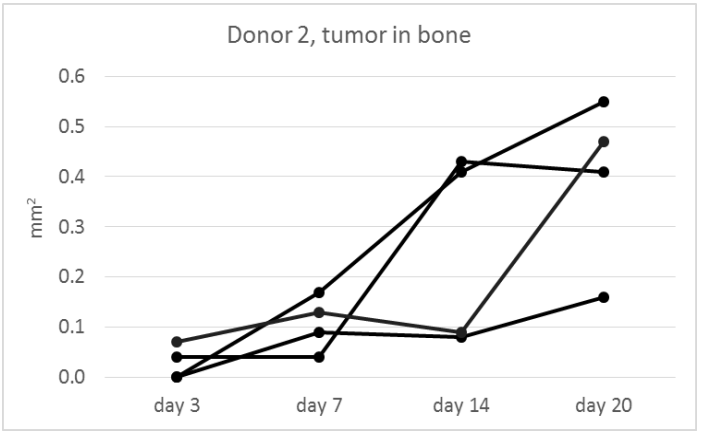
MDA-MB-231SA in mammary fat pad vs bone

A. Body weight

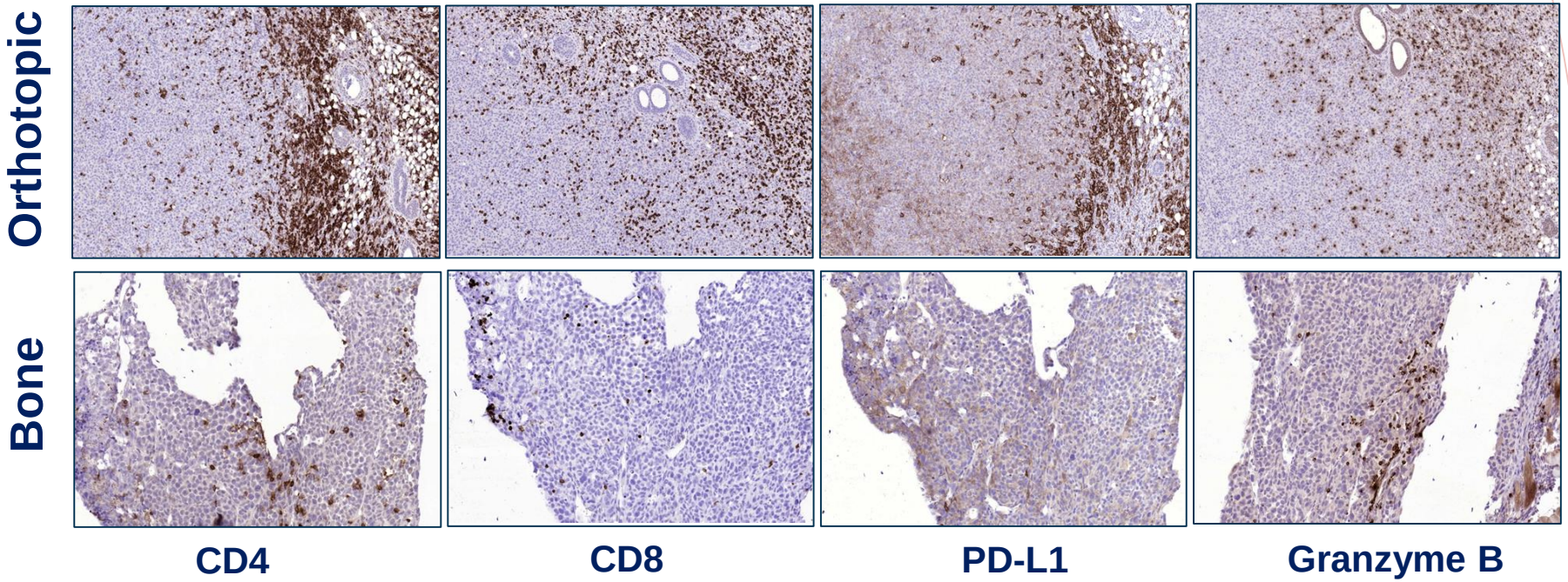


B. Osteolytic bone lesion area



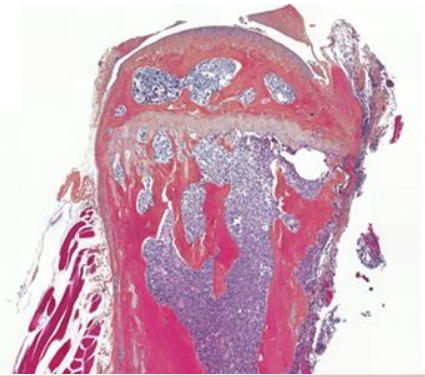


MDA-MB-231SA in mammary fat pad vs bone



Immune markers in the tumors of huNOG mice

- **CD4**: Helper T cells
- **CD8**: Cytotoxic T cells
- **PD-L1**: Expressed in tumor cells and APCs
- **Granzyme B:** Activated cytotoxic T-cells and NK cells



New results:

Differential efficacy of PD-1 targeted immunomodulation in preclinical models of primary and bone metastatic triple-negative breast cancer

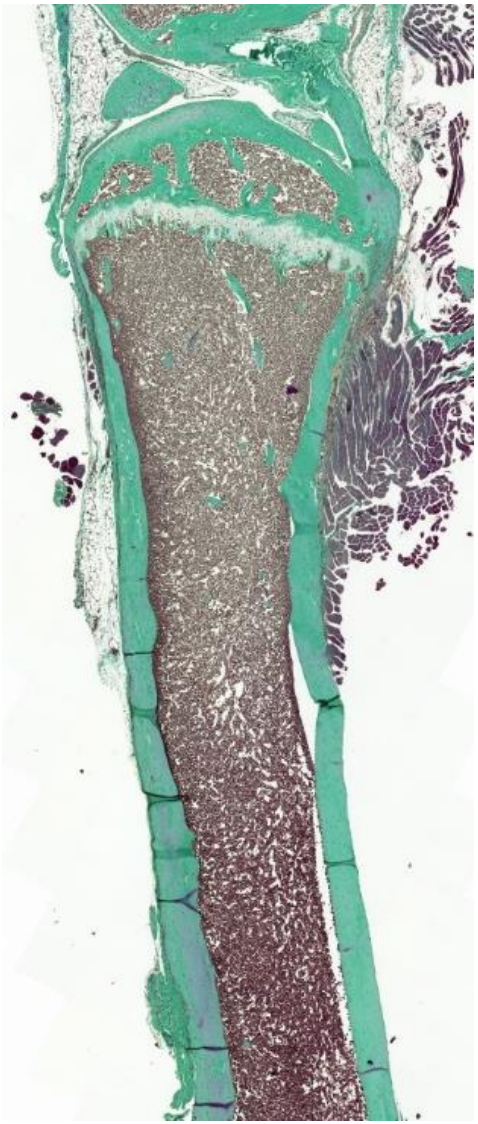
Abstract submitted to AACR annual meeting

Syngeneic MM model: 5TGM1 tail vein

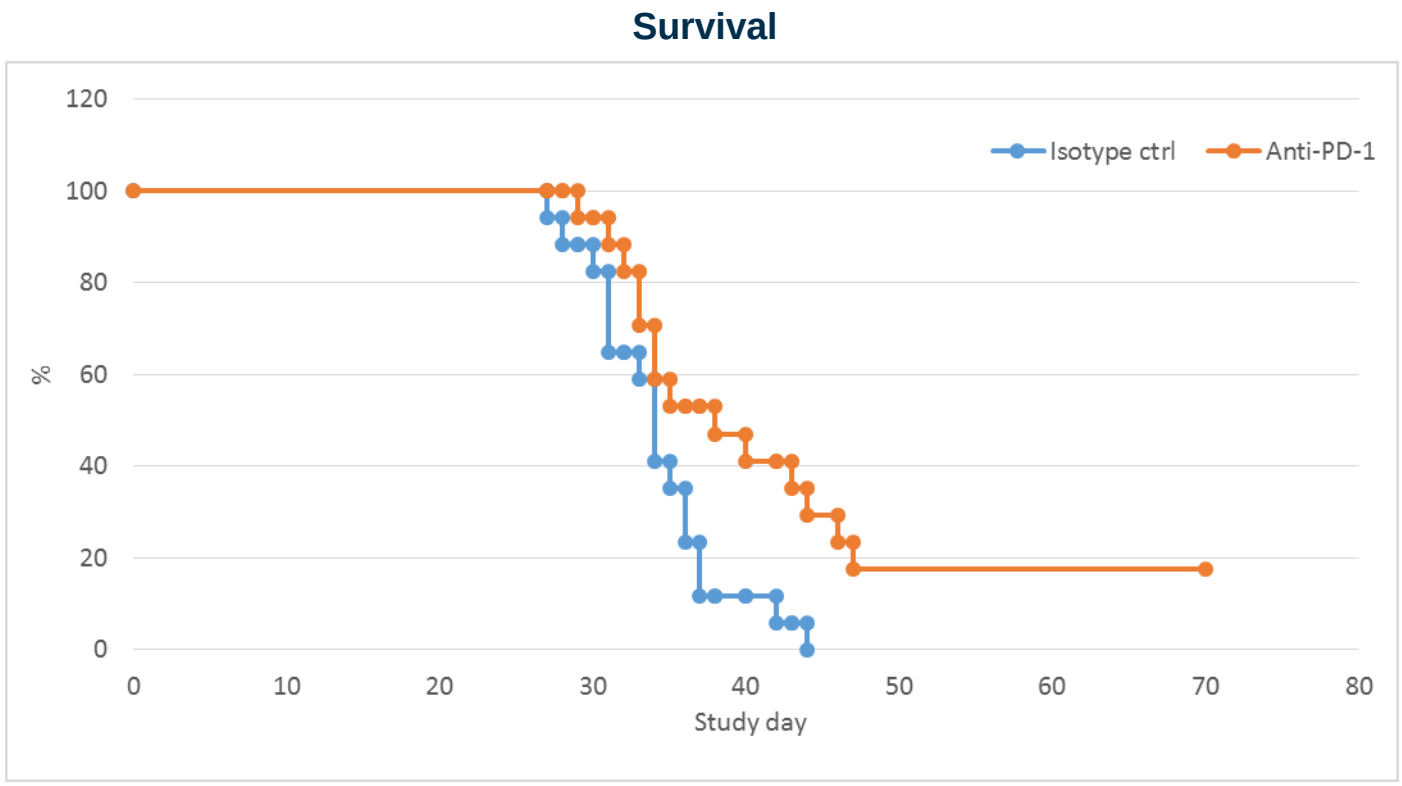
5TGM1 murine
multiple myeloma cells

Efficacy: 32 days
Survival: 70 days

<u>Endpoints in survival</u>
Paraplegia
Weight loss



Effects of anti-PD-1 on survival in multiple myeloma model



n =17 in both groups

New model and results:

Anti-PD-1 therapy reduces bone lesion growth in a novel syngeneic bladder cancer bone metastasis model

Abstract submitted to AACR annual meeting

Summary: Why study bones in oncology

- Bone is a common site for metastasis and significant cause of morbidity and mortality
- Bone microenvironment confers dormancy and drug resistance
- Cancer treatment induced bone loss is a clinical problem
- Lack of negative bone effects is an advantage for a cancer drug candidate

Summary: Bone metastasis and immunotherapy

- Bone is immune-priviledged site
- Special means in overcoming the local immunosuppression are needed
- Even though part of the patients in IO therapy trials have bone metastases, information on IO therapy efficacy on bone metastases is scarce

Acknowledgements

- TACONIC Models For Life.
- BioSiteHisto
- Vincit
- Tiina Kähkönen

Thank you!

Contact: mari.suominen@pharmatest.com