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
Bone metastases are a significant source of morbidity and mortality.

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”

*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
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. Hemann1,*

1Koch Institute for Integrative Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA
2Department of Internal Medicine, Center of Integrated Oncology, University of Cöologne, Cologne 50931, Germany
3VFM Amsterdam 1081, Netherlands
4Department 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

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

Pallasch et al Cell 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

- Bone is an immuno-privileged site
- Thought to protect HSC compartment
- Small pool of effective cytotoxic cells
- Large pool of immature or suppressor immune cell types, such as MDSCs and Tregs
- Imnosuppressive cytokines TGF-β and RANKL

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
Overcoming immunosuppression in bone metastases

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

\textsuperscript{a} Developmental Therapeutics Program, Division of Hematology/Oncology, Feinberg School of Medicine, Chicago, USA

Table 1
Standard of care treatment of bone metastases.

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

www.pharmatest.com
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. Immune cells and PD-L1 and PD-1 expression in spleen and lymph 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) 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 (mm²).
Osteoblastic lesions were associated with increased bone mineral density

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/cm²) 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) Serum TRACP 5b levels indicate decreased osteoclast number in huNOG mice.

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

<table>
<thead>
<tr>
<th>Day 0</th>
<th>1 weeks</th>
<th>2 weeks</th>
<th>3 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood collection, MDA-MB-231SA intratibial or orthotopic inoculation</td>
<td>X-ray</td>
<td>X-ray</td>
<td>Blood collection, X-ray, DXA, BLI, Sacrifice, Tissue sample collection and ex vivo analysis</td>
</tr>
<tr>
<td>BLI</td>
<td>BLI</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
MDA-MB-231SA in mammary fat pad vs bone

Orthotopic tumor volume

Orthotopic model, Control Donor 1

Orthotopic model, Control Donor 2

Bone model, BLI

Donor 1, tumor in bone

Donor 2, tumor in bone
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

Endpoints in survival
Paraplegia
Weight loss
Effects of anti-PD-1 on survival in multiple myeloma model

Survival

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-privileged 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

• BioSiteHisto
• Vincit
• Tiina Kähkönen

Thank you!

Contact: mari.suominen@pharmatest.com