Active human immune system induces more severe osteoblastic bone reaction in a humanized mouse model of breast cancer bone metastasis

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Disclosure

- Employee of Pharmatest Services
  - CRO specialized in preclinical efficacy services in oncology and skeletal diseases
Immuno-oncology

- Current trend in drug discovery
- 1264 clinical studies with "immunotherapy and tumor"
- 107 studies with breast cancer, many of which on advanced, recurrent or metastatic tumors
- 56 studies with bone included
Mouse models for immuno-oncology

• Syngeneic and humanized mouse models

• CIEA NOG mice
  • Irradiation
  • Engrafted with human CD34+ hematopoietic stem cells (HSCs)
  • Verification of hCD45+ cells in circulation
  → mice with functional T and B cells
Tumor growth in bone of humanized mice

- About 60% of advanced breast cancer patients, 70% of advanced prostate cancer patients, and 25% of advanced lung cancer patients develop bone metastases.

- Despite the recent progress in cancer drug development, bone metastases are incurable and dramatically increase mortality.

- High relevance and need to develop new treatment options against bone metastases.

- Novel precision medicines, including immunotherapies, hold the potential to treat metastatic tumors.
Bone is the reservoir for hematopoietic stem cells (HSCs)


Late-breaking poster:
Bone Phenotype of Human Immune System Engrafted Mice
#LB-SU0365
9/10/2017 12:30-2:30 PM
Aim of the study

To establish a clinically predictive preclinical model that combines tumor, bone and immune system
Schematic layout of the study

- **huNOG** mice (HSCFTL-NOG-F, Taconic Biosciences) from two donors, super immunodeficient CIEA NOG mice as control

- BT-474 human breast cancer cells (ER, PR and HER2+)

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<th>Day 0</th>
<th>4 weeks</th>
<th>6 weeks</th>
<th>8 weeks</th>
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<tr>
<td>- Blood collection</td>
<td>- X-ray</td>
<td>- X-ray</td>
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<tr>
<td>- Breast cancer cell inoculation</td>
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<td>- <em>Ex vivo</em> analysis</td>
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BT-474 breast cancer cells induced osteoblastic lesions

A.) Osteoblastic lesions in NOG and huNOG mice at endpoint

B.) Tumor-induced bone changes were monitored throughout the study 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 the bone changes \textit{in vivo} during the study

B) Quantification of changes in bone mineral density (BMD, mg/cm$^2$) in tumor-bearing tibia compared to healthy tibia
Formation of osteoblastic bone lesions induced trabecular bone formation

A) µCT reconstruction of NOG and huNOG tibia
B) Trabecular bone volume in tumor-bearing tibia
C) Trabecular thickness in tumor-bearing tibia
The number of bone resorbing osteoclasts was decreased on tumor site

A. Relative TRACP 5b serum level

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

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
Quantification of tumor area in bone marrow

A) Representative hematoxylin and eosin (HE) staining from BT-474 tumor-bearing tibias

B) Quantification of intratibial tumor area from the HE-stainings
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**: Cytotoxic T-lymphocyte-associated protein 4
- **PD-L1**: Programmed death ligand 1
- **PD-1**: Programmed cell death 1
Summary

• Immune-related organs of female huNOG mice have high prevalence of mature human T and B cells that also infiltrate into the tumors

• Functional immune system induced more severe osteoblastic bone lesion formation in the experimental breast cancer bone metastasis model
  – Larger bone metastatic lesions
  – Increased trabecular bone volume and BMD
  – Decreased number of osteoclasts

• A novel model was established for studying efficacy of immuno-oncology compounds in bone microenvironment
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