New preclinical model for immuno-oncology: combination of tumor, bone microenvironment and immune system

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Introduction

Breast and other solid tumors commonly metastasize to bone. Bone marrow microenvironment regulates hematopoietic stem cells and thereby immune cell differentiation. Therefore, a system with functional interaction between bone, immune system and cancer is a prerequisite in development and preclinical testing of novel immunotherapies against osseous tumors.

Aim of the Study

To establish a model where tumor, bone microenvironment and immune system would be combined to a functional entity, to characterize the tumor-induced bone changes, and to establish a validated platform for preclinical testing of immunotherapies.

Materials and Methods

An intratibial injection of 1x10^6 of BT-474 (ER+, PR+, HER2+; ATCC) human breast cancer cells was given to female NOG and huNOG mice (HSCFTL-NOG-F, provided by Taconic Biosciences). These humanized mice were produced through engrafting hCD34+ hematopoietic stem cells (HSC) into CIEA NOG mouse® (NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Sug}/JicTac; Taconic Biosciences). Tumor-induced bone changes were followed by radiography at 4, 6 and 8 weeks. Bone mineral density (BMD) was quantified by dual-energy x-ray absorptiometry (DXA), and bone volume and three-dimensional architecture was studied by micro computed tomography (µCT). Spleen, thymus, lymph nodes and hind limbs were collected and tumor and bone areas, as well as the expression of human CD3 (BSR10, Nordic Biosite), CD4 (BSR4, Nordic Biosite), CD8 (SP16, Spring Bioscience), CD16/56 (123C3, Nordic Biosite), CD20 (BS6, Nordic Biosite), CD45 (2B11+PD7/26/16, Nordic Biosite), CTLA-4 (BSB-88, BioSB) and PD-L1 (ZR3, Zeta Corporation) in immune cells was analyzed.

huNOG mice support mainly the differentiation of T- and B-cells. If differentiation towards other lineages is desired, transgenic NOG mice can be used for engraftment: hIL6-NOG: monocytes, hIL2-NOG, hIL15-NOG: NK-cells and huNOG-EXL: all lineages.

Results

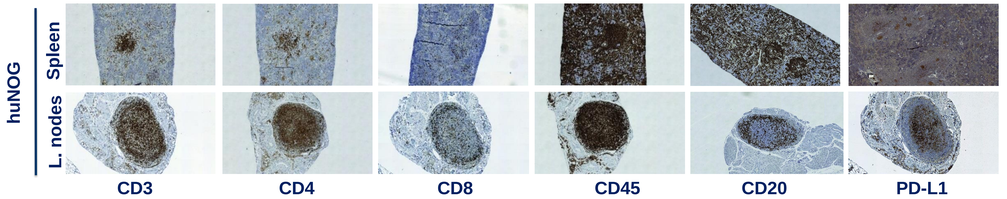

Figure 1. Maturation of immune cell in huNOGs was confirmed from spleen, lymph nodes and thymus at endpoint by immunohistochemistry. CD3, CD4, CD8, CD20, CD45 and PD-L1 expression was confirmed. Representative images are presented, magnification 10x.

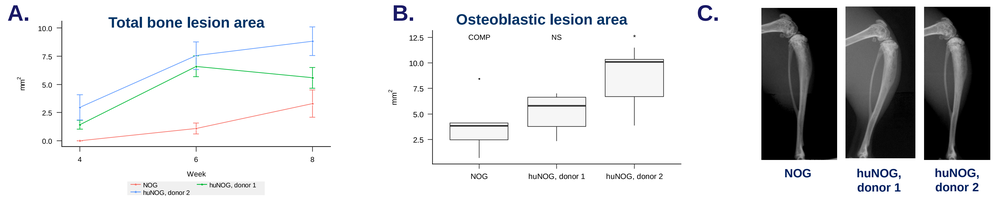

Figure 2. A) Tumor induced bone changes were monitored throughout the study by radiography (at 4, 6 and 8 weeks after inoculation of the cancer cells). The line presents mean bone lesion area (mm^2) ± SD. B) The tumors were mainly bone-forming, osteoblastic. Osteoblastic lesion area (mm^2) was quantified at endpoint from the radiography images. Box plots present 50% confidence interval with mean. COMP=comparison group, NS=non-significant, p<0,05*. C) Representative radiography images from bone lesions at endpoint are presented.

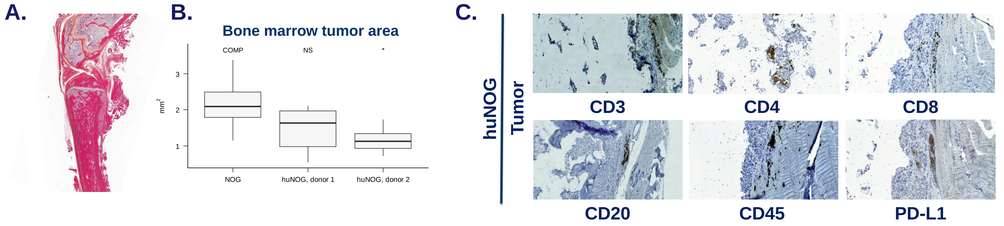

Figure 3. A) Example image of histology (Hematoxylin&Eosin staining) of tumor bearing tibia in huNOG mice. B) Quantitation of tumor area (including only cancer cells) in tibia from the histological sections. Box plots present 50% confidence interval with mean. COMP=comparison group, NS=non-significant, *p<0,05. C) Immunostainings of immune cell markers show immune filtration of CD3, CD4, CD8, CD20, CD45 in the tumor area. PD-L1 expression was observed in a subset of tumors.

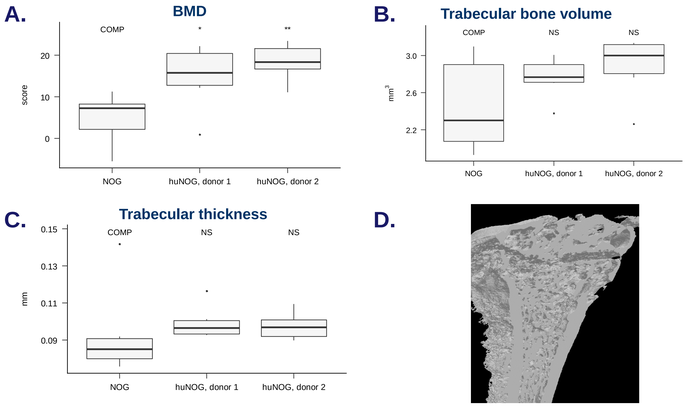

Figure 4. A) Changes in bone mineral density (BMD, mg/cm^2) were studied by dual-energy x-ray absorptiometry (DXA) at endpoint. Box plots present 50% confidence interval with mean. COMP=comparison group, *p<0,05, **p<0,01. B) Changes in trabecular bone were studied by micro computed tomography (µCT). A trend towards increased trabecular bone volume (mm^3) was observed in huNOGs correlating with C) the trend of increased trabecular thickness. Box plots present 50% confidence interval with mean. COMP=comparison group, NS=non-significant. D) A representative µCT reconstruction of tibial cross section showing tumor-induced trabecular bone in huNOG mice.

Conclusions

To our knowledge, this study describes establishment of the first humanized mouse model of tumor growth in bone. The model is characterized by tumor growth and extensive tumor-induced osteoblastic changes and tumor-infiltrating human immune cells in bone, and it mimics the late stage of breast cancer metastasized to bone. This humanized mouse model provides a completely new platform for preclinical testing of cancer immunotherapies, particularly therapies targeting cancers metastasizing to or growing in bone.

Acknowledgements

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