PD-1/PD-L1 Expression and Tumor-Infiltrating Immune Cells in Triple-Negative Breast Cancer: Characterization of Preclinical Primary Tumor and Bone Metastasis Models in Humanized Mice

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Introduction

Expression of programmed cell death -1 (PD-1) receptor, programmed death-ligand 1 (PD-L1) and tumor-infiltrating lymphocytes (TILs) in triple-negative breast cancer (TNBC) has been shown in many studies supporting development of immunotherapies against TNBC.

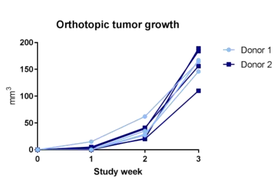
In order to develop novel immunotherapies especially against bone metastatic TNBC, better understanding of PD-1/PD-L1 axis, TILs, and tumor associated macrophages (TAMs) in the bone microenvironment is warranted.

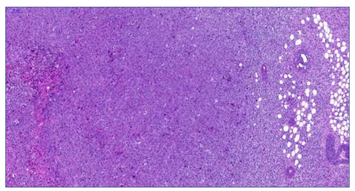
The aim of the study was to characterize immune cell infiltration to TNBC primary and bone metastatic tumors in humanized mice. This information can be used to support development of immunotherapies that affect both primary tumor and bone metastases in advanced breast cancer.

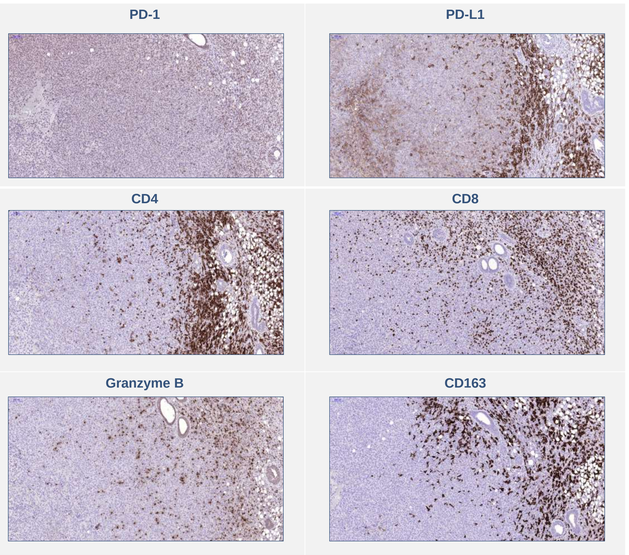
Materials and Methods

MDA-MB-231(SA) human breast carcinoma cells were inoculated into the mammary fat pad or tibia of female CIEA NOG mice (n=8 per group, Taconic Biosciences) engrafted with human pluripotent CD34+ cells from two donors. Tumor growth was followed by caliper measurements and the tumor volume was calculated using the formula π/6*Width*Lenght*Height. After 3 weeks of tumor growth, histopathological evaluation of primary tumors and intratibial tumors was performed from hematoxylin-eosin stained sections. Tumor area in bone was analyzed by color-thresholding, and immunohistochemical stainings were performed to characterize expression of PD-1, PD-L1, CD4, CD8, Granzyme B and CD163 (Table 1). TILs and TAMs were assessed by a 4-scale immunoscoring system (0-3) and PD-L1 expression was determined by Tumor Proportion Scoring (TPS, Table 2). PD-L1 positivity of the TAM's was omitted in TPS interpretation.

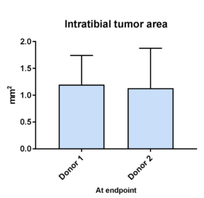
Orthotopic MDA-MB-231(SA) model in huNOG mice

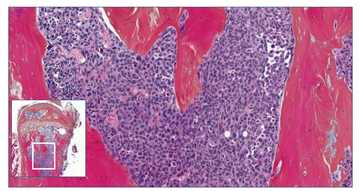






Intratibial MDA-MB-231(SA) model in huNOG mice





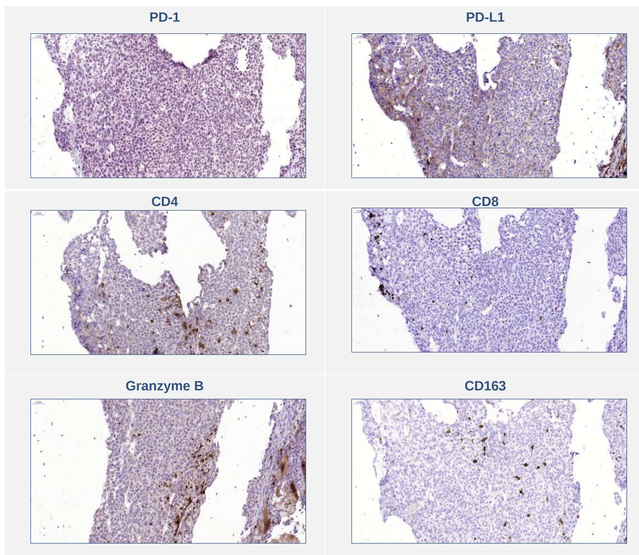


TABLE 1. Antibodies used in immunohistochemical stainings.

Antibody		Clone	Supplier
PD-1	Programmed cell death-1 receptor	BSR1	Nordic Biosite
PD-L1	Programmed death-ligand 1	ZR3	Nordic Biosite
CD4	T helper cells	BSR4	Nordic Biosite
CD8	Cytotoxic T cells	BSR5	Nordic Biosite
Granzyme B	Serine protease secreted by e.g. CD8	BSR150	Nordic Biosite
CD163	Macrophages	EDHu-1	Bio-Rad Antibodies

TABLE 2. Immunoscoring criteria for stromal and intratumoral TILs and PD-L1 expression.

Score for TILs	Description
0	No TILs or very low number
1	Low number of TILs
2	Moderate number of TILs
3	High number of TILs

TPS for PD-L1	Description
< 1 %	Negative
1–49 %	Low or moderate expression
> 50 %	High expression

Summary

- When analyzing the whole tumor section, intratumoral and peritumoral variation of the expression of PD-1 and PD-L1, and location of TILs and TAMs were observed.
- Orthotopic tumors exhibited mainly low or moderate heterogeneous expression of PD-L1 (TPS score 1-49%). Moderate or high number of human CD4+ and CD8+ TILs (scoring 2-3) and low PD-1 expression was observed. Granzyme B expression correlated with CD8 positivity.
- Intratibial tumors had similar PD-L1 expression than orthotopic tumors. Tumor-infiltrating CD4+ and granzyme B+ human immune cells were observed. However, in the bone microenvironment, less CD8+ immune cells were observed compared with the orthotopic tumor, and PD-1 expression was variable.

Conclusions

The obtained results comparing primary and bone metastatic tumors indicate intratumoral and peritumoral variation in the expression of immune cell markers.

Different expression of targets of immuno-therapies in primary and metastatic sites highlights the importance of testing compound efficacy also in relevant preclinical metastasis models.