

Hormone Receptor and HER2/HER3 Expression in Preclinical Breast Cancer Models of Primary Tumor and Bone Metastasis

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Introduction

Breast cancers expressing estrogen receptor (ER) and progesterone receptor (PR) are classified as hormone receptor positive, and triple-positive breast cancers express also human epidermal growth factor receptors (mainly HER2).

Despite available hormonal and HER2 targeted treatments, breast cancer metastasizes to bone in high frequency and may develop resistance against the used treatment. Prevention and treatment of bone metastases is challenging, and hormones are strong regulators of both bone and immune system.

The aim of the study was to verify and compare ER, PR, HER2 and HER3 status in preclinical primary breast cancer and bone metastasis models utilizing immunodeficient and human immune system engrafted mice.

Materials and Methods

In an orthotopic tumor study, BT-474 human breast cancer cells (ATCC, USA) were inoculated into the mammary fat pad (4×10^6 cells) of female immunodeficient CIEA NOG mice (NOG, Taconic Biosciences, USA). Placebo or 17β -estradiol rods (E2, 5 μ g per day, PreclinApps Ltd., Finland) were implanted prior to orthotopic cancer cell inoculation. In a bone tumor study, BT-474 breast cancer cells were inoculated into the tibia (1×10^6 cells) of intact female CIEA NOG or CD34+ human immune system engrafted NOG mice (huNOG, Taconic Biosciences, USA). After 8 weeks of tumor growth, histopathological tumor evaluation from hematoxylin-eosin (H&E) stained sections and immunohistochemical (IHC) stainings for ER, PR, HER2 and HER3 were performed.

Orthotopic BT-474 tumor growth in NOG mice

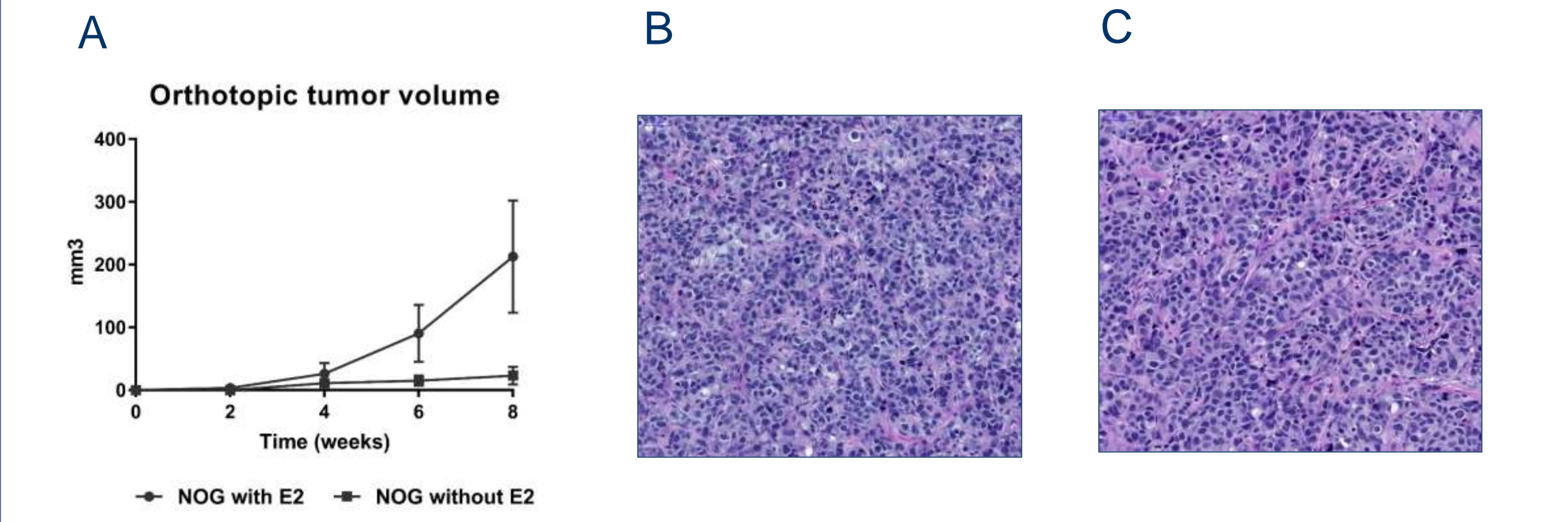


FIGURE 1. Orthotopic tumor growth and histology (H&E). (A) BT-474 tumor growth shows that E2 promotes orthotopic tumor growth. (B) Representative histological image when tumor has grown with E2 supplement. (C) Representative histological image when tumor has grown without E2 supplement.

IHC of orthotopic BT-474 tumor

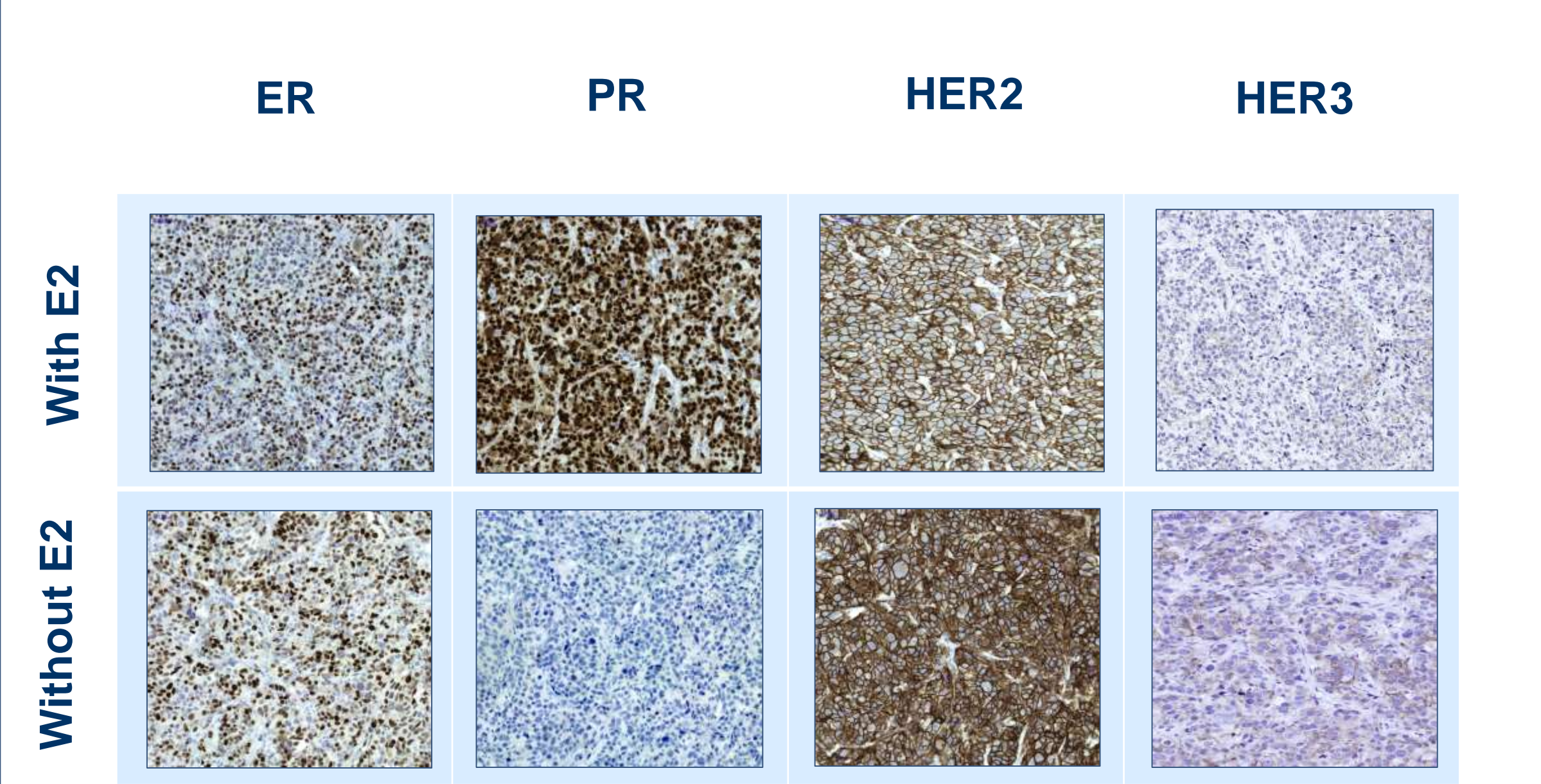


FIGURE 2. Comparison of ER, PR, HER2 and HER3 expression in orthotopic BT-474 tumor in NOG mice with or without E2 supplement. PR is not expressed in the absence of E2 supplement.

TABLE 1. Antibodies used in IHC stainings.

Antibody	Clone	Manufacturer
ER	Estrogen receptor α	SP1 Spring Bio
PR	Progesterone receptor	SP2 Spring Bio
HER2	Human epidermal growth factor receptor 2	SP3 Spring Bio
HER3	Human epidermal growth factor receptor 3	DAK-H3-IC Dako/Agilent

Intratibial BT-474 tumor in NOG and huNOG mice

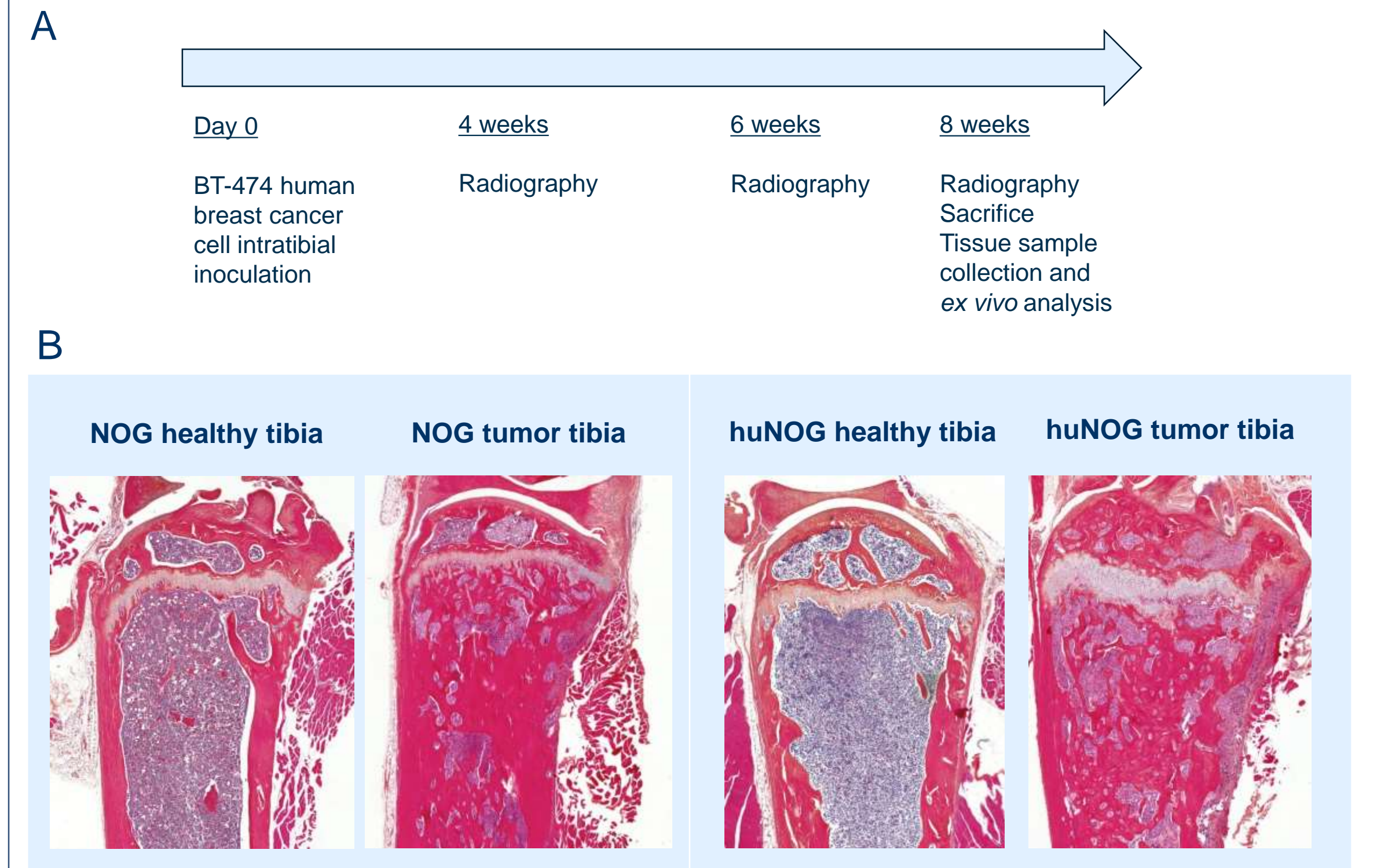


FIGURE 3. Schematic layout of the intratibial breast cancer model and histology (H&E). (A) Cancer cells were inoculated intratibially into intact mice and tumor growth was assessed by histology after 8 weeks of intratibial tumor growth. (B) Representative histological images showing healthy tibia compared to tumor-bearing tibia in NOG and huNOG mice. Intratibial tumors grew without E2 supplement.

IHC of intratibial BT-474 tumor in NOG and huNOG mice

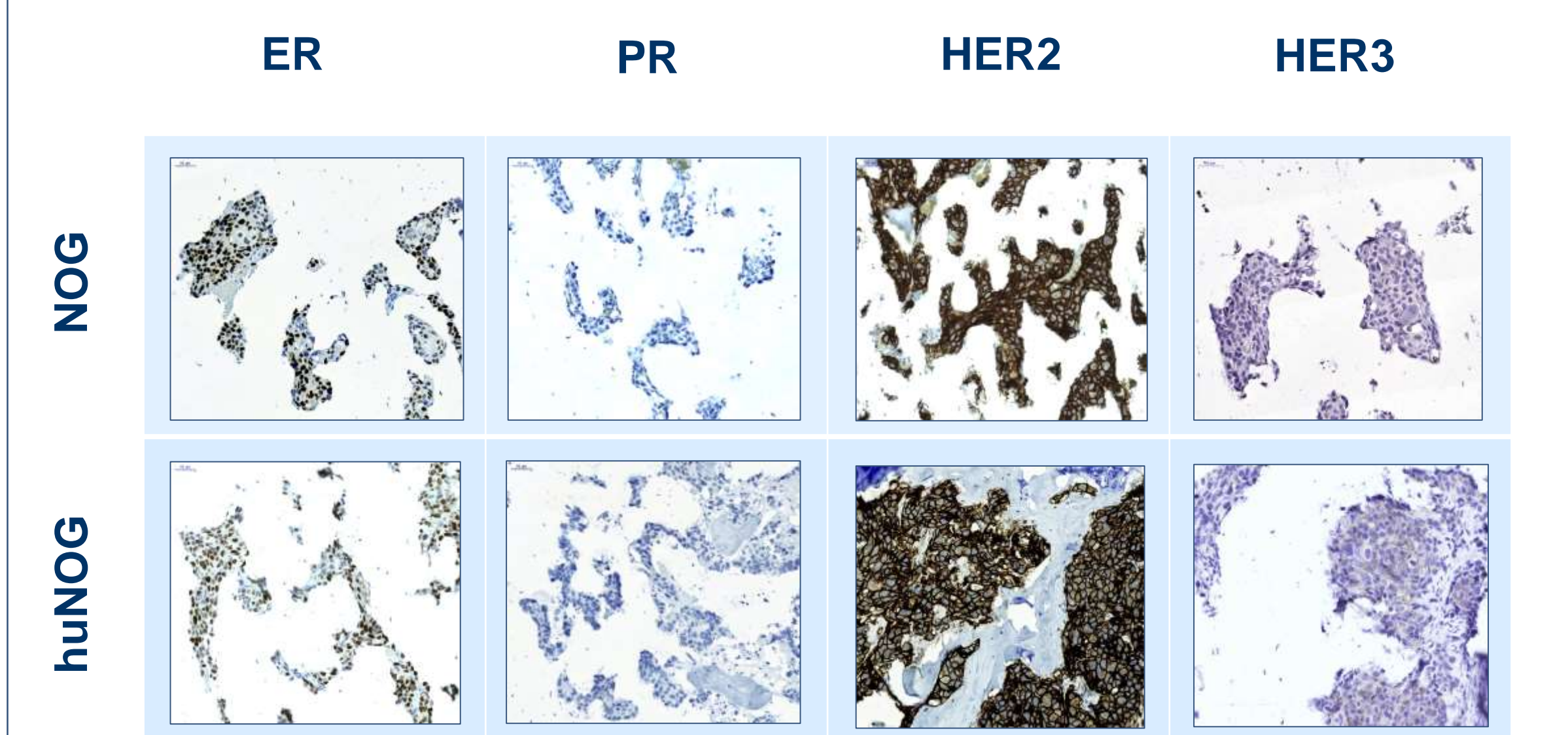


FIGURE 4. ER, PR, HER2 and HER3 staining in tumor-bearing tibia in NOG mice compared to tumor-bearing tibia in huNOG mice. No major differences in any expression was observed between NOG and huNOG mice. PR was not expressed as E2 supplement was not used in the intratibial model.

Summary

- Estrogen supplementation is needed to support breast cancer BT-474 tumor growth when cancer cells are inoculated orthotopically into mammary fat pad.
- On the contrary, BT-474 breast cancer growth was observed in bone even in the absence of supplied estrogen, highlighting importance of tumor microenvironment.
- ER and HER2/HER3 expression was observed in primary and bone tumors, but PR expression was significantly reduced if no estrogen supplement was used.
- No significant differences were observed between immunodeficient and humanized mice regarding intratibial tumor growth and ER, PR and HER2/HER3 expression.

Conclusions

When developing new therapies against breast cancer, treatment targets in preclinical models should be carefully verified.

Focus should be addressed not only on primary tumor but also on bone metastasis where cancer cells are under influence of bone microenvironment and may express differently hormone receptors and HER2/HER3.

While hormones influence breast cancer progression, they also regulate bone turnover and immune system, and therefore humanized mouse models provide an essential platform for novel therapy development.