HER2 Positive Breast Cancer Bone Metastasis Model for Studying Efficacy of Novel Therapies

ASBMR 2019
SAT-137

Tiina E. Kähkönen[1], Mari I. Suominen[1], Jenni H.E. Mäki-Jouppila[1], Jussi M. Halleen[1], Jenni Bernoulli[1], Derek Grant[2]
[1] Pharmatest Services, Turku, Finland; [2] Bayer AS, Oslo, Norway

E-mail correspondence to Tiina Kähkönen (tiina.kahkonen@pharmatest.com)

Introduction

Breast cancers overexpressing human epidermal growth factor receptor 2 (HER2+) are associated with an increased risk for developing metastases to distant organs including bones, brain and lungs. Despite recent progress in drug development, bone metastases remain incurable.

The aim of this study was to establish a systemic metastasis model for HER2+ breast cancer with a special interest in bone metastasis.

Materials and Methods

Five to six weeks old athymic nude mice (Envigo, n = 10-13 per group) were used. About half of the mice received estrogen (E2) supplementation (E2-releasing rods, 5 µg/day, Preclinapps) one week before cancer cell inoculation. The mice were inoculated intracardially with 1 or 5 x 10^5 luciferase-labelled triple-positive (ER, PR positive and HER2 overexpressing) human BT-474 breast cancer cells †. Formation of metastases was monitored by bioluminescence imaging (BLI, PerkinElmer) at inoculation and once a week for the duration of the study. X-ray imaging (Faxitron) and histology were performed at sacrifice to visualize cancer-induced changes in bone.

Acknowledgements

† BT-474/CMV-luc cells were obtained from Japanese Collection of Research Bioresources (ID: JCRB1450, established by Murakami, T.). We thank all Pharmatest personnel who contributed to the study, and Esa Alhoniemi (Vincit, Finland) for assistance in statistical analysis.

Tumor burden and histology

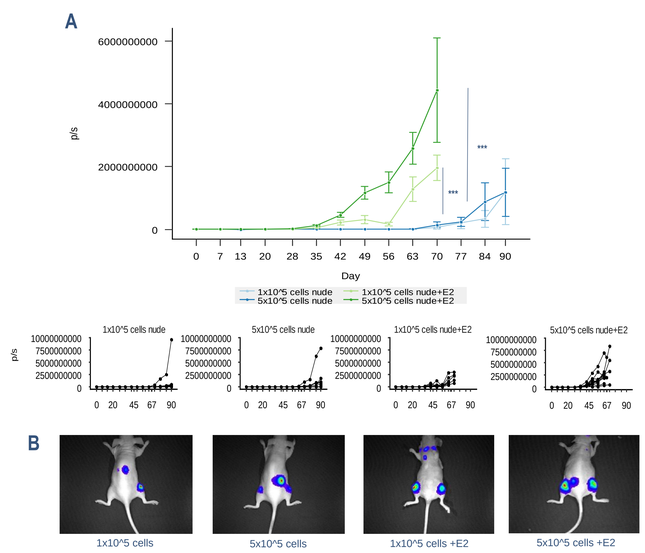

FIGURE 1. A) Total tumor burden was analyzed by BLI from nude mice. Total flux from the whole body (mean ± SEM) is presented for all study groups. E2 increased tumor burden in nude mice inoculated with 1x10^5 cells (*** p < 0.001) and 5x10^5 cells (*** p < 0.001). B) Representative BLI images for each study group.

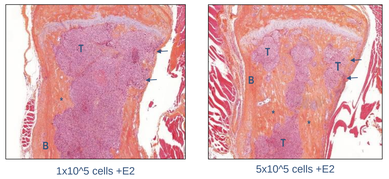

FIGURE 2. Representative images of hematoxylin and eosin (HE) stainings, magnification 5x. The images show E2-induced bone growth. In non-E2 supplemented mice, tumors did not induce bone effects as evaluated by X-ray imaging (see Figure 3), and the samples were not processed to histological analysis. Abbreviations: 'T' = Tumor, 'B' = normal bone, '*' = E2-induced bone growth, 'arrows' = tumor-induced bone resorption.

Bone lesions

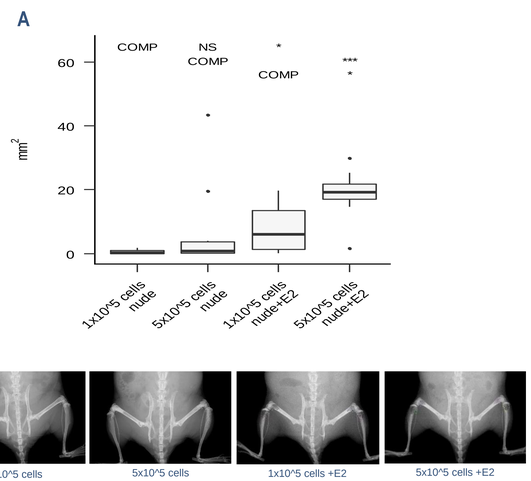

FIGURE 3. Bone lesion area was analyzed from X-ray images collected at sacrifice. Osteolytic bone lesion area (mm², median ± 25%IQR ± min/max) is presented for each study group. E2-supplement increased tumor-induced bone lesion area in mice inoculated with 1x10^5 cells (* p < 0.05) and 5x10^5 cells (*** p < 0.001). Also, E2-induced new bone growth can be observed. NS = not significant compared to the comparison (COMP) group. B) Representative X-ray images for each group.

TABLE 1. Summary of the number and type of bone metastases observed in each study group based on *in vivo* BLI and X-ray imaging on sacrifice day, and the range of sacrifice days in the study group. During the study, the mice were sacrificed individually when they met the predefined sacrifice criteria, including more than 20% loss on body weight from the maximum weight obtained during the study, appearance of cachexia, severe E2-induced adverse effects, development of bone-effects (i.e. extensive growth of bone metastases or fractures), or at latest at study day 90.

Study group	N of bone metastases based on BLI	Bone lesion characterization based on X-ray	Sacrifice days
1x10^5 cells, nude mice	2/10, 20%	No lesions	D70 - D90
5x10^5 cells, nude mice	3/10, 30%	No lesions	D90
1x10^5 cells, nude mice, +E2	12/12, 100%	Osteolytic	D25 - D70
5x10^5 cells, nude mice, +E2	13/13, 100%	Osteolytic	D50 – D70

Summary

- Bone metastases were dominant in the model, and metastases in soft tissues including brain and ovaries were occasionally observed.
- Bone metastases were detected between days 28 to 35 in E2 supplemented mice and around day 63 in non-E2 supplemented mice.
- Increasing cell number in E2 supplemented mice accelerated the growth of bone metastases and tumor burden was highest in the mice inoculated with 5 x 10^5 BT-474-luc cells.
- All E2 supplemented mice and 20-30% of non-E2 supplemented mice had bone metastases based on BLI imaging at sacrifice
- X-ray imaging showed large tumor-induced osteolytic lesions in E2 supplemented mice, while no lesions were observed in non-E2 supplemented mice.
- E2 and tumor-induced effects on bone led to fragile bone and bone fractures were occasionally observed.
- E2 induced adverse effects such as skin lesions and urinary distress and obstruction in some mice. Due to severity of these effects, E2 supplemented mice were sacrificed earliest at day 25, and the maximum length of a study should be 50 days.

Conclusions

A high rate of bone metastasis was achieved in athymic nude mice supplemented with E2. This model can be used to study the efficacy of anti-cancer therapies, such as HER2-targeted compounds, on the prevention of bone metastases. However, special attention should be paid to E2 caused adverse effects and humane endpoints should be carefully followed.