Establishment of a HER2 Positive Breast Cancer Bone Metastasis Model for Validation of Novel Therapies

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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 with overexpression of human epidermal growth factor receptor 2 (HER2+) have aggressive clinical behavior, and at advanced stages are associated with increased risk for developing metastases to distant organs including bones, brain and lungs.

The aim of this study was to establish a metastasis model for HER2+ breast cancer with a special interest in bone metastasis. The effects of cell number and estrogen supplementation on metastasis formation were studied in two different mouse strains.

Materials and Methods

In the study, 5-6 weeks old athymic nude (Hsd: Athymic Nude-*Foxn1*^{nu}) and Rag2 (R2G2™: B6; 129-*Rag2*^{tm1Fwa}*Il2rg*^{tm1Rsky}/DwlHsd) mice (Envigo, n = 10-13 per group) were used. Half of the mice received estrogen supplementation (E2-releasing rods 5 µg/day, PreclinApps) one week before inoculation of the cancer cells. 1x10^5 or 5x10^5 luciferase-labelled human BT-474 breast cancer cells (ER, PR positive and HER2 overexpressing; obtained from Japanese Collection of Research Bioresources*) were inoculated to mice intracardially. The formation of metastases was followed by bioluminescence imaging (BLI, PerkinElmer Inc) at inoculation and once a week for the duration of the study. The mice were sacrificed individually if they developed severe symptoms or had extensive bone metastases, and latest after 90 days in the study. At sacrifice, X-ray imaging (Faxitron) was performed and the bones were collected for histological analysis.

Tumor burden and bone lesions

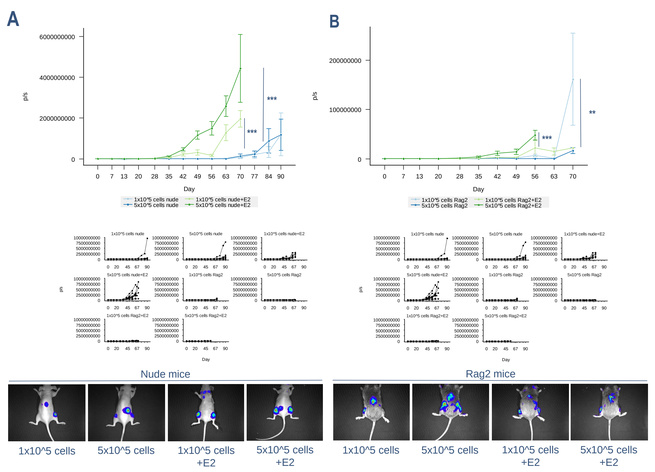


FIGURE 1. Total tumor burden was analyzed by BLI from A) nude mice and B) Rag2 mice. Total flux from the whole body (mean ± SEM) is presented for all study groups. E2 increased tumor burden in nude mice inoculated with 1x10^5 cells and 5x10^5 cells, and tumor burden in Rag2 mice inoculated with 5x10^5 cells. Increasing the cell number in Rag2 mice increased tumor burden in non-E2 supplemented mice. Representative BLI images are presented for each study group. Note: Detection of metastases in Rag2 mice was challenging due to dark fur which was hindering the signal transmittance. For this reason the values are not comparable between nude and Rag2 mice. ** p < 0.01, *** p < 0.001.

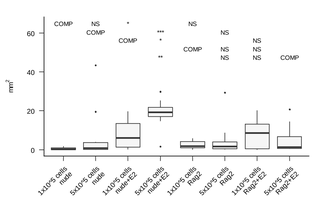
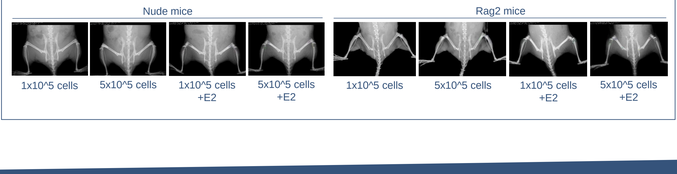


FIGURE 2. Bone lesion area was analyzed from X-ray images collected at sacrifice. Total bone lesion area (mm², median ± 25%IQR ± min/max) is presented for each study group. In nude mice, E2-supplement increased bone lesion area in mice inoculated with 1x10^5 cells and 5x10^5 cells. In Rag2 mice, no significant differences were observed in bone lesions. Representative X-ray images are presented for each group. COMP = Study group used for statistical comparison, * p < 0.05, *** p < 0.001.



Tumor histology in bone

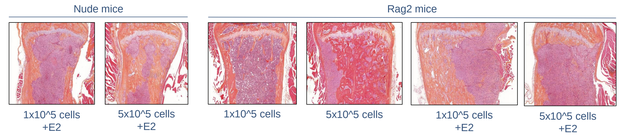


FIGURE 3. Representative images of hematoxylin and eosin (HE) stainings, magnification 5x. The images show estrogen-induced bone growth in both mouse strains, and cancer cell induced bone growth in Rag2 mice not supplemented with E2. In non-E2 supplemented nude mice, tumors did not induce bone effects as evaluated by X-ray imaging, and these samples were not processed to histological analysis.

Survival and summary of bone metastasis

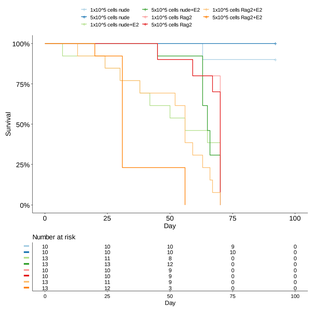


FIGURE 4. Survival (% and number at risk) is presented for each study group. During the study, the mice were sacrificed individually when they met the predefined sacrifice criteria which were more than 20% loss on body weight from the maximum weight obtained during the study, appearance of cachexia, severe E2-induced adverse effects or development of bone-effects (i.e. extensive growth of bone metastases or fractures). Sacrifice due to any cause is included in the survival analysis.

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.

Study group	N of bone metastasis based on BLI	Bone lesion characterization based on X-ray
1x10^5 cells, nude mice	2/10, 20%	No lesions
5x10^5 cells, nude mice	3/10, 30%	No lesions
1x10^5 cells, nude mice, +E2	12/12, 100%	Osteolytic
5x10^5 cells, nude mice, +E2	13/13, 100%	Osteolytic
1x10^5 cells, Rag2 mice	5/10, 50%	Osteoblastic
5x10^5 cells, Rag2 mice	4/10, 40%	Osteoblastic
1x10^5 cells, Rag2 mice, +E2	6/13, 46%	Osteolytic
5x10^5 cells, Rag2 mice, +E2	6/13, 46%	Osteolytic

Summary

- Bone metastases were dominant in the model, and metastases in soft tissues including brain and ovaries were occasionally observed.
- Bone metastases were observed in both strains, and they were largest in nude mice supplemented with E2 and inoculated with 5x10^5 BT-474 cells.
- In nude mice, E2 supplement increased tumor burden. In Rag2 mice, E2 supplement and increase in cell number increased tumor burden.
- Bone metastasis take rate was 100% in nude mice with E2 supplement compared to 20-30% in nude mice without E2 supplement. In Rag2 mice, the rate of bone metastasis was 40-50%.
- X-ray imaging showed E2-induced bone growth and large tumor-induced osteolytic lesions in hind limbs. Without E2 supplement, the mice developed osteoblastic lesions.
- Survival of mice was different between the study groups, and mice were lost earlier in the E2 supplemented groups than in the non-E2 supplemented groups. Attention should be drawn to E2-induced adverse effects.

Conclusions

A high rate of bone metastasis was achieved in athymic nude mice supplemented with estrogen. This model can be used to study the efficacy of anti-cancer therapies such as HER2-targeted compounds on tumor growth at metastatic locations, or on prevention of metastasis formation.

Acknowledgements

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