Validation of a Preclinical Model for Bone Metastatic Triple Negative Breast Cancer

AACR 2019
Poster number 2007

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Introduction

Metastatic breast cancer (MBC) is the most advanced stage of breast cancer, diagnosed as stage IV. Typically, MBC metastases occur in the bones, liver, brain, and lungs. Bone metastases cause significant morbidity and mortality in late-stage breast cancer patients. At present, there is no efficient curative or preventive treatment for bone metastases. Therefore, establishment and validation of well characterized *in vivo* models of breast cancer bone metastasis is of utmost importance.

The aim of this study was to validate an intracardiac model of bone MBC utilizing GFP tagged triple negative human breast cancer cells that could be used to study efficacy of new potential treatments.

Materials and Methods

Female athymic nude mice (Hsd: Athymic Nude-Foxn1nu, Envigo) were used in this study. Bone seeking MDA-MB-231(SA)-GFP (originally received from Dr. Theresa Guise) triple negative (ER, PR and HER2 negative) human breast cancer cells (10^5 cells) were inoculated into the left cardiac ventricle of 4-6 weeks old female mice. Allocation to treatment groups (n=15) was performed by stratification procedure based on animal weight prior to the beginning of the study. To validate the model, the current standard-of-care (SOC) treatment (Paclitaxel, Fresenius Kabi Limited) was administered between study days 13-17 at the therapeutic dose (9 mg/kg, i.p., once daily). Serum bone resorption marker tartrate- resistant acid phosphatase 5b TRACP5b, IDS Ltd) was measured before cancer cell inoculation and at sacrifice. Planar X-ray of hind limbs (Faxitron) and *ex vivo* fluorescence imaging (LT 9 GFP-imaging system LT-MACIMSYSPLUSC Lightools Research) of the whole body were performed at sacrifice.

Study design

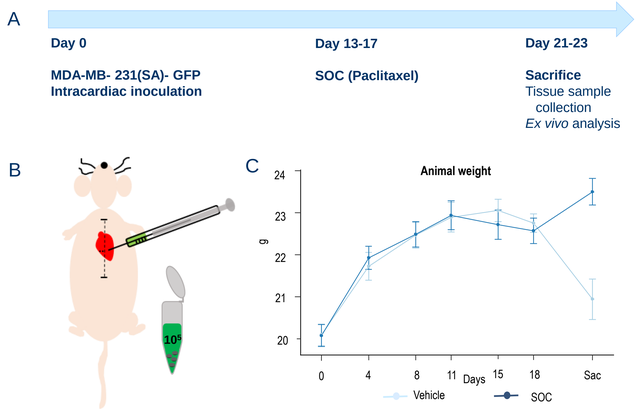

FIGURE 1. (A) Schematic layout of the intracardiac model using MDA-MB-231(SA)-GFP cancer cells. (B) A cartoon representation of intracardiac inoculation. Anatomical landmarks are shown with horizontal dashed lines on the sternum. A 30G needle was introduced via the 4th intercostal space into the left ventricle of the heart. Sternal notch and xyphoid process served as landmarks. (C) Mice were weighed twice a week following tumor cell injection (day 0). The data are presented as mean ± SEM (bars) ** p < 0.01.

Ex vivo tumor burden measurements

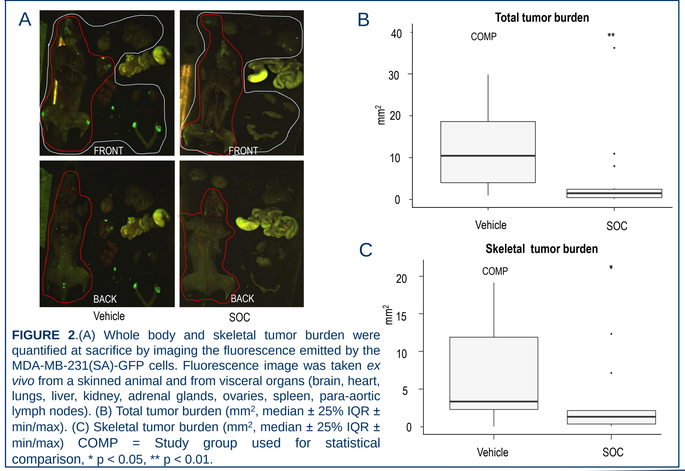

FIGURE 2.(A) Whole body and skeletal tumor burden were quantified at sacrifice by imaging the fluorescence emitted by the MDA-MB-231(SA)-GFP cells. Fluorescence image was taken *ex vivo* from a skinned animal and from visceral organs (brain, heart, lungs, liver, kidney, adrenal glands, ovaries, spleen, para-aortic lymph nodes). (B) Total tumor burden (mm^2, median ± 25% IQR ± min/max). (C) Skeletal tumor burden (mm^2, median ± 25% IQR ± min/max) COMP = Study group used for statistical comparison, * p < 0.05, ** p < 0.01.

Bone metastasis

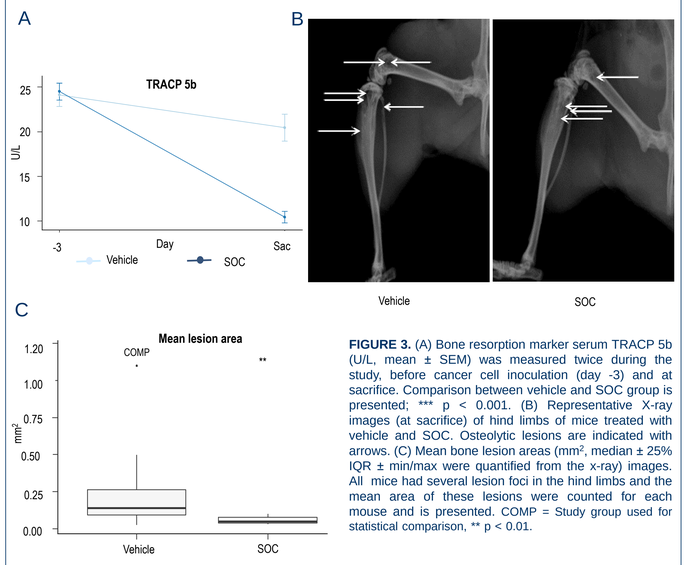

FIGURE 3. (A) Bone resorption marker serum TRACP 5b (U/L, mean ± SEM) was measured twice during the study, before cancer cell inoculation (day -3) and at sacrifice. Comparison between vehicle and SOC group is presented; *** p < 0.001. (B) Representative X-ray images (at sacrifice) of hind limbs of mice treated with vehicle and SOC. Osteolytic lesions are indicated with arrows. (C) Mean bone lesion areas (mm^2, median ± 25% IQR ± min/max were quantified from the x-ray) images. All mice had several lesion foci in the hind limbs and the mean area of these lesions were counted for each mouse and is presented. COMP = Study group used for statistical comparison, ** p < 0.01.

Animal condition

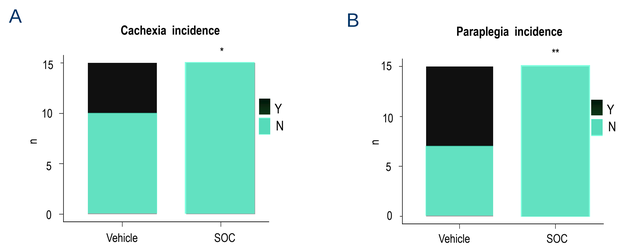

FIGURE 4. (A) The onset of cachexia and paraplegia in the animals was followed. The animals were considered cachectic when two out of three of the following conditions were met: curved spine, dehydration and/or 20% or more reduction in weight. Comparison between vehicle group and SOC group is presented. (B) Animals were considered paraplegic when visible paralysis of hind limbs was observed. * p < 0.05, ** p < 0.01.

Summary

- The observed tumor take rate was 100%.
- Tumors were observed mainly in skeletal sites but also in soft tissues.
- The SOC treatment decreased both total and skeletal tumor burden.
- Based on X-ray images, tumor induced osteolytic bone lesions were smaller in the SOC treated group compared to the vehicle group, but no differences were observed in total bone lesion area.
- Serum TRACP 5b was increased in the vehicle group as a result of osteolytic lesions, and decreased with the SOC treatment.
- 33% of mice in the vehicle group were cachectic, but mice treated with SOC exhibited only mild and temporary weight loss.
- 53% of mice in the vehicle group developed paraplegia, which was not observed in the SOC group.

Conclusions

An intracardiac model of metastatic breast cancer was successfully validated with the SOC treatment paclitaxel that efficiently inhibited disease progression. This model provides a promising tool for testing new treatments against bone and soft tissue metastatic breast cancer *in vivo*.

Acknowledgements

We thank all Pharmatest personnel who contributed to the study, and Esa Alhoniemi (Vincit, Finland) for assistance in statistical analysis.