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

Justyna Zdrojewska, Mari I. Suominen, Katja M. Fagerlund, Jussi M. Halleen, Jenni Bernoulli, Johann Zimmermann
1 Pharmatest Services, Turku, Finland; 2 Polyphor Ltd, Allschwil, Switzerland

E-mail correspondence to Justyna Zdrojewska (justyna.zdrojewska@pharmatest.com)

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 (10^6 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 (Pacitaxel, 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 GFT-imaging system LT-MACIMYSPLUS Lighttools Research) of the whole body were performed at sacrifice.

Study design

Bone metastasis

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 TRACP5b 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 paradigm 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 Eva Alhennroth (Vant, Finland) for assistance in statistical analysis.