

# Understanding the Heterogeneity of Brain Metastases from Breast Cancer: Lessons from New Models and Experimental Magnetic Resonance Imaging

Donna Murrell<sup>1,2</sup>, Robbert van Gorkum<sup>1</sup>, Amanda Hamilton<sup>1</sup>, Christiane Mallet<sup>1</sup>, Brunilde Gri<sup>3</sup>, Ann Chambers<sup>2,4</sup>, Patricia Steeg<sup>3</sup>, and Paula Foster<sup>1,2</sup>

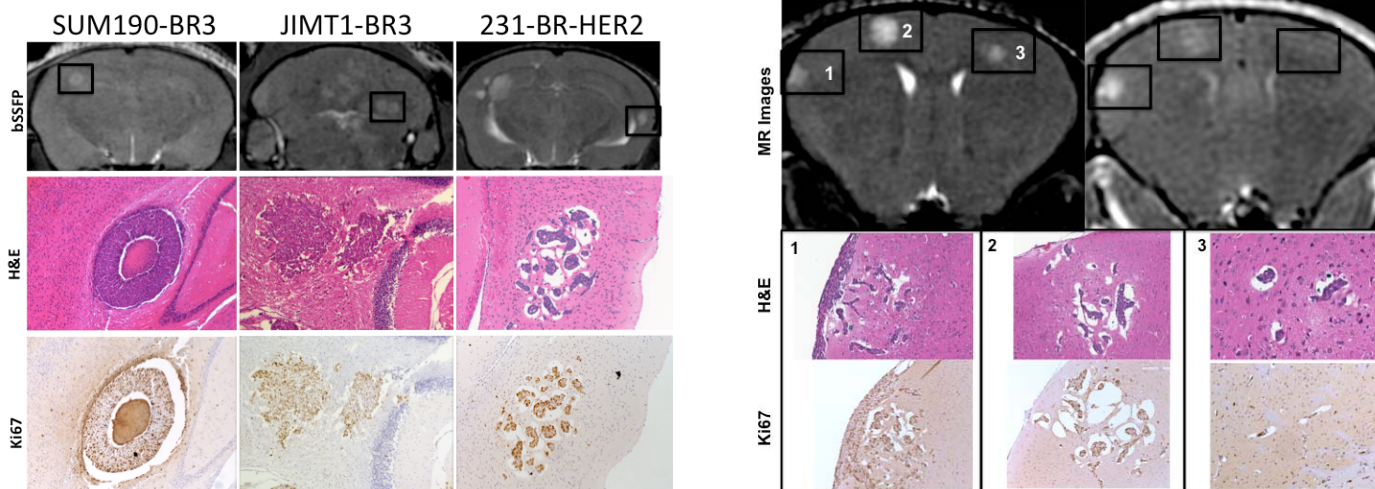
<sup>1</sup>Imaging Research Laboratories, Robarts Research Institute, London, Ontario, Canada, <sup>2</sup>Medical Biophysics, Western University, London, Ontario, Canada, <sup>3</sup>National Cancer Institute, Maryland, United States, <sup>4</sup>London Regional Cancer Program, London, Ontario, Canada

**INTRODUCTION:** Metastasis to the brain occurs in up to 50% of breast cancer patients who over express the human epidermal growth factor receptor 2 (HER2)<sup>1</sup>. The median survival time for these patients is 4-6 months and only 20% of patients can expect to live to one year<sup>2</sup>. Preclinical studies are crucial to understanding the development of brain metastases and for investigating new treatments. However, animal models to study brain metastatic breast cancer are extremely limited<sup>3-6</sup>. Furthermore, these models are often studied only by traditional histology *ex vivo*; this limits analysis to specific regions of interest (rather than whole brain) and it cannot account for the dynamic nature of metastatic growth and changes in permeability previously seen over time<sup>7</sup>. Here, we characterize three mouse models of HER2+ brain metastatic breast cancer developed in the Steeg lab using high resolution MRI and correlative histology. We present 3D anatomical MRI of the mouse brain that reveals the incidence, distribution and size of brain metastases and contrast-enhanced MRI that provides information about the integrity of the blood-tumour barrier (BTB). Our findings reflect the substantial heterogeneity of this disease.

**STUDY DESIGN:** Brain metastatic human breast cancer cells (231-BR-HER2, JIMT1-BR3, or SUM190-BR3) were injected intracardially in nude mice for systemic transport to the brain. Imaging was performed *in vivo* at experimental endpoint, which was approximately one month post cell injection for the 231-BR-HER2 and JIMT1-BR3 cell lines and two months for SUM190-BR3. Balanced steady state free precession (bSSFP) images were acquired at 3T to quantify the number, size, and distribution of all brain metastases. Post-gadolinium (Gad) T1-weighted spin echo (T1w SE) images were acquired to assess permeability of the BTB for each tumour. Mice were sacrificed at endpoint and brains were excised for histological analysis, including hematoxylin and eosin (H&E) and Ki67 immunostaining to assess proliferation.

**RESULTS:** The MRI appearance of JIMT1-BR3 and 231-BR-HER2 tumours was similar, appearing hyperintense compared to the normal brain in bSSFP images. SUM190-BR3 metastases appeared with a region of signal hypointensity in their centre (top panel Fig 1). Mice injected with SUM190-BR3 cells each developed only 1 or 2 metastases, compared to an average of 15.17 tumours for the JIMT1-BR3 and 20.40 for the 231-BR-HER2 groups. In the 231-BR-HER2 model, most tumours grew in the forebrain (44%); 33% were in the midbrain and 23% in the hindbrain. For the JIMT1-BR3 group, the highest incidence of metastasis was in the hindbrain (45%), with 34% in the forebrain and 21% in the midbrain. The SUM190-BR3 group did not develop spatial growth patterns; of the 4 metastases detected, 2 were found in the midbrain and 1 each in the forebrain and hindbrain. The 231-BR-HER2 metastases grew the largest; the mean tumour volume was 0.5507 mm<sup>3</sup>. The JIMT1-BR3 and SUM190-BR3 had average volumes of 0.2039 and 0.1944 mm<sup>3</sup> respectively. All of the SUM190-BR3 tumours were permeable to Gad. There was considerable heterogeneity in the permeability of metastasis for JIMT1-BR3 and 231BR-HER2 metastases; 92% of JIMT1-BR3 tumours enhanced after Gad, and, 64% of 231BR-HER2 tumours enhanced after Gad (Figure 2). Histological analysis of the stained brain sections correlated well with our imaging observations.

**SIGNIFICANCE:** 3D *in vivo* MRI is a valuable tool for providing a comprehensive accounting of the number, size, and permeability status of experimental brain metastases in the whole mouse brain. This information is practically impossible to obtain with traditional histopathological methods and is important for understanding the natural progression of murine HER2+ brain metastasis models. Moreover, understanding the disease heterogeneity presented here is vital to advancing therapeutic treatment options and improving patient survival.



**Figure 1 (left):** Representative images of mouse brains at endpoint showing metastases resulting from SUM190-BR3, JIMT1-BR3, or 231-BR-HER2 cancer cells. bSSFP images show metastasis burden (hyperintensities); correlative H&E and Ki67 stained sections show morphology and proliferation. The region of signal void in the centre of the SUM190-BR3 tumour relates to a necrotic core seen by histology (left column). JIMT1-BR3 metastases present with considerable morphologic heterogeneity; some are very dense and proliferative (as seen in middle column), while others present similar to 231-BR-HER2 tumours. Characteristic traits of 231-BR-HER2 tumours are proliferative cancer cell clusters surrounded by regions of edema (right column), which contributes to their very bright MRI appearance.

**Figure 2 (right):** Mouse brain with 231-BR-HER2 metastases visible in a bSSFP image compared to a post-Gad SE. Tumours 1 and 2 are large, proliferative and permeable to Gad; tumour 3 is small and nonpermeable, although still proliferative.

## REFERENCES:

1. Sperduto PW *et al* (2012) *Int J Radiat Oncol Biol Phys*
2. Ohno S *et al* (2004) *Breast Cancer*
3. Rye PD *et al* (1996) *Int J Cancer*
4. Yoneda T *et al* (2001) *J Bone Miner Res*
5. Palmieri D *et al* (2007) *Cancer Res*
6. Tanner M *et al* (2004) *Mol Cancer Ther*
7. Percy DB *et al* (2011) *Invest Radiol*