# Multi-Parametric mpMRI to Characterize Brain and Bone Metastases in Disseminated Breast Cancer

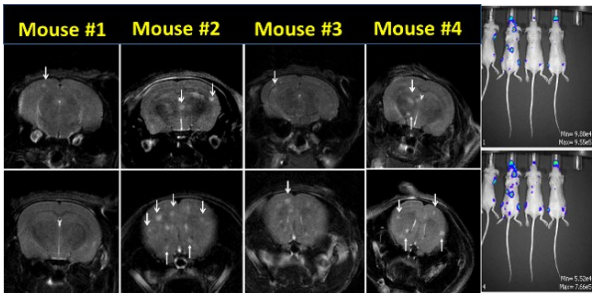
Natalie Julie Serkova[1], Diana M Cittelly[1], Kendra M Huber[1], and Carol A Sartorius[1]
[1]*University of Colorado Anschutz Medical Center, Aurora, Colorado, United States*

**Introduction**: The most prevalent site of breast cancer metastasis is bone. Bone metastases ultimately cause dire clinical complications including pain, fracture, hypercalcemia, cachexia, and bone marrow suppression. Bone lesions are often over time accompanied by other distal metastases that contribute to fatality. Approximately 16% of patients with breast cancer develop symptomatic brain metastases (BMs) and the majority (80%) will die subsequently. It has been shown that bone metastasis microenvironment differs from brain lesions and, as such, different treatment strategies might be necessary. In the past, the radiological assessment of tumor dimensions (so-called anatomical imaging) was considered the "gold standard" for clinical diagnosis. Standard clinical practice predominantly used CT, sometimes supported by T1- and T2-MRI and ultrasound, for tumor detection and therapy planning in women with disseminated breast cancers. Unfortunately, the true extent and phenotype of viable tumor is often obscured on standard imaging, meaning that edema, cysts, fibrosis, or necrosis cannot be distinguished from primary and, moreover, metastatic lesions. With the recent advances in targeted anti-cancer therapies, there is an increasing demand for oncologic imaging of tumor "microenvironment", the interplay between the host environment, the tumor, and the tumor vasculature. Currently, sophisticated multiparametric (mp) functional and molecular imaging end-points are emerging which provide more specific physiological information than just the margin of neoplasm. Using modern mpMRI protocols the tumor phenotype and microenvironment, such as cell density, apoptosis, angiogenesis, perfusion, tumor metabolism, can be non-invasively evaluated. The goal of this study was to establish mpMRI endpoints for micro-metastasis detection (by high-resolution dimensional MRI) and microenvironment characterization (ADC by DWI for cellularity and $K^{trans}$ by DCE-MRI) in a mouse model for disseminated breast cancer and to validate them among bone and brain lesions.

**Methods**: GFP-luciferase labeled MDA-MB-231 cells were injected in the left cardiac ventricle of female nude mice (n=19) and the extent of bone and brain metastases was monitored using luciferase expression once a week MRI was performed when the first optical signals were detected. All MRI scans were obtained on anesthetized mice using a 4.7 Tesla Bruker PharmaScan MRI with a 31mm-diamter Bruker volume coil and acquired using Bruker Paravision 4.0.1 software. After obtaining tri-pilots, a high-resolution anatomical and physiological mpMRI protocol was applied (Table 1).

| | MSME T1w-MRI | RARE T2w-MRI | DWI 6b-Values | GRE DCE-MRI |
|---|---|---|---|---|
| Field of view | 3.0 cm | 3.0 cm | 4.6 cm | 4.0 cm |
| Slice thickness | 1.0 mm | 1.0 mm | 2.0 mm | 1.5 mm |
| # Slices | 16 | 16 | variable | variable |
| TR | 700 ms | 4,000 ms | 3,000 ms | 18.4 ms |
| TE | 11 ms | 80 ms | 40 ms | 3.3 ms |
| # Evolutions | n/a | n/a | n/a | 128 |
| # Averages | 4 | 4 | 16 | 1 |
| b-Values | n/a | n/a | 0,150,300,600, 800, 1000 s/mm$^2$ | n/a |
| Matrix size | 256x256 | 256x256 | 64x64 | 128x128 |
| Flip angle | 180 degrees | 180 degrees | 90 degrees | 20 degrees |
| Acquisition time | 11min 56s | 8min 32s | 6min 2s | 5min 1s |

**Results**: After one month animals had bone and some brain metastases detectable by bioluminescence. The smallest skeletal metastases detectable by Gd-enhanced T1w-MRI were 0.5mm (Figure 1 right). Bioluminescence detected brain metastases in one out of four animals in average. High-resolution T2w-MRI detects brain metastases as small as 0.37mm not detectable by bioluminescence (Figure 1 left). Interestingly, the addition of gadolinium contrast (0.4 mmol/kg MultiHance i.v.) was beneficial only in detecting bone metastases but not brain. We found a straightforward explanation for this by establishing quantitative DCE-MRI. At the early stages, brain micrometastases exhibit a low vascularity phenotype with low-rate contrast kinetics (Figure 2) when compared to bone metastases. The median $K^{trans}$ values by DCE-MRI for brain lesions were 0.21min$^{-1}$ (n=15) as compared to 0.42min$^{-1}$ for spinal metastases (n=19, p<0.0001). The lesion cellularity also differed significantly between two sites. The median ADCs for brain micrometastases were 1.45mm$^2$/s while the bone metastases revealed a higher cellularity phenotype revealed with lower ADCs of 0.98 (p<0.002).



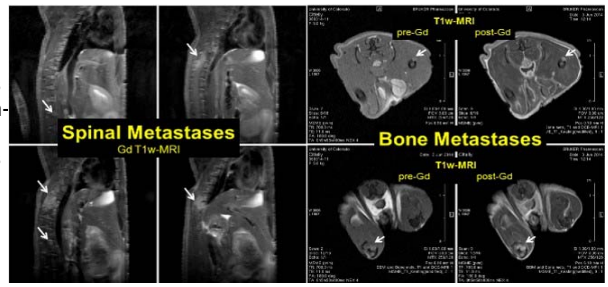




**Figure 1**. High-resolution T2w-MRI on brain metastases (left) and gadolinium-enhanced T1w-MRI on bone metastases (right) in mouse models of disseminated breast cancer. Correlation with

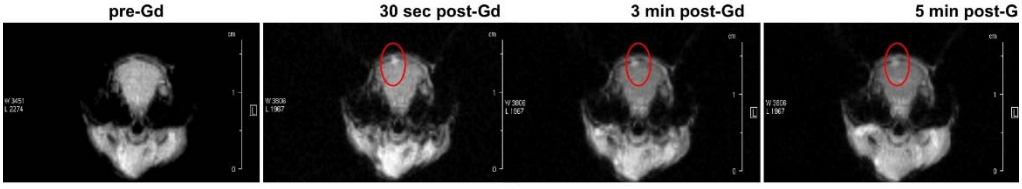



**Figure 2**. Dynamic contrast enhanced (DCE)-MRI series on a brain micrometastasis (diameter=0.57 mm) after injection of 0.4 mmol/kg MultiHance (calculated $K^{trans}$=0.19min$^{-1}$)

**Conclusions:** This pre-clinical mpMRI study reveals (i) MRI superiority in detecting brain micrometastases vs. optical imaging; (ii) low vascularity/ low cellularity brain lesion microenvironment vs. bone lesions. Non-invasive mpMRI methods to characterize the tumor microenvironment in multifocal metastases could stratify patients into those that will benefit from chemo- vs. anti-angiogenic therapies. The very same imaging protocols could be used clinically to serially monitor treatment efficacy without the need for invasive and precarious biopsies.