Ex vivo MR Imaging; A High Throughput Approach for Characterization of Melanoma Brain Metastases Mouse Models

Amr Morsi[1,2], Avital Gaziel[3], Hana Baig[1], John G. Golfinos[4], Eva Hernando-Monge[3], and Youssef Zaim Wadghiri[5]

[1]*Radiology, NYU Langone Medical Center, New York, NY, United States,* [2]*Neurosurgery, NYU Langone Medical Center, NY, NY, United States,* [3]*Pathology, NYU Langone Medical Center, New York, NY, United States,* [4]*Neurosurgery, NYU Langone Medical Center, New York, NY, United States,* [5]*Radiology, NYU School of Medicine, New York, NY, United States*

Introduction: Melanoma has a high predilection for neuronal tissue, with approximately 75% of patients with metastatic melanoma developing brain metastases (B-Mets) during the course of their disease. Once metastasized to the brain, prognosis is dismal with a median survival of less than 6 months (1,2). Unfortunately, the scarcity of physiologically relevant *in vivo* models and an accurate non-invasive modality to monitor tumorigenesis has significantly hindered the investigation of melanoma brain tropism, adaptation and response to novel therapies. We previously reported our ability to implement a clinically relevant *in vivo* Magnetic Resonance Imaging (MRI) protocol on a B16F10 (B-Mets) mouse model following intracarotid surgery to provide a non invasive three-dimensional evaluation of the extent of the tumor (3). However, the use of *in-vivo* MRI proved to be a slow screening tool requiring 1- to 2-hours to characterize each individual subject, resulting in severe hampering of our ability to analyze large cohorts of mice. In this study we used ultrasound guided intracardiac injections to reduce the morbidity and improve the safety of the surgical procedure that enables tumor induction via hematogeneous spread. In order to develop a more clinically relevant model, we compared the effect of the well established murine cell line B16F10 to the 5B1 human cell line. In addition, we capitalized on the paramagnetic nature of melanin that makes tumor tissue visible in *contrast-free* T1 datasets to examine whether *ex-vivo* scanning could help circumvent the limited availability of the MRI instrument while increasing the throughput by using an MRI probe that can accommodate the simultaneous examination of multiple whole head samples. The loss of image quality resulting from this larger sample set can be compensated by the signal accumulation of repetitive unattended scans over long imaging hours acquired overnight.

Methods: *Animals & surgery:* A 100-µl Ultrasound intracardiac injection of 10^5 B16-F10 and 5B1 melanoma cells was injected in C57black6 mice (N=40) and Nude mice (N=40) respectively. Mice were monitored daily, perfused when they showed neurological deficits or weight loss and the heads were prepared for *ex vivo* imaging. *Imaging:* All µMRI experiments were performed with a 7T Bruker Avance II console (Bruker Biospin, Ettlingen Germany). The protocol consisted of a T1- weighted (non-contrast) (110 µ m)^3, 54 mins TR = 50 ms, TE =5.9 ms, 1-echo, FA = 29 °, BW= 50 Khz, Matrix size = 256^3, FOV = (282mm)3, Nav =2. T2/T2*-Multi-echo (110 µ m)^3, 1hrs27min TR = 80 ms, TE =4 ms, 4-echoes, Echo spacing 6.7 ms, FA = 12°, BW = 50 Khz, Matrix size= 256^3, FOV =(282mm)3, Nav = 2

Results: *Ex vivo* MRI experiments conducted on both the B16F10 and 5B1 models recapitulates the characteristic clinical radiological findings of melanoma B-Met (4): T1 brightening from melanin without contrast (1a,b) and melanin susceptibility effect on T2* (1c,d). Using this high throughput imaging modality, we were able to accurately characterize the metastatic lesions in both models. *Ex vivo* data acquired from B16F10 model (n=40) revealed exclusive ventricular and leptomeningeal spread (2a,b) while preliminary data from the 5B1 model (n=16) shows parenchymal lesions (2c). In addition, ex vivo imaging allowed for the exploration of the multicentric nature of the two melanoma cell lines used, where 90% of the 5B1 mice with brain tumors showed multiple lesions (2d) (maximum of 16 lesions), in comparison with 18% in the B16F10 model (maximum of 3 lesions). Finally, preliminary three dimensional volume studies data conducted on both models revealed a higher brain penetrance 69%, delayed onset of tumor detection day 41 post injection and a slow growth rate of the 5B1 cell line compared to a lower brain penetrance 27.5%, early onset of tumor detection on day 15 and an exponential growth rate of tumors in the B16F10 mouse model (3).

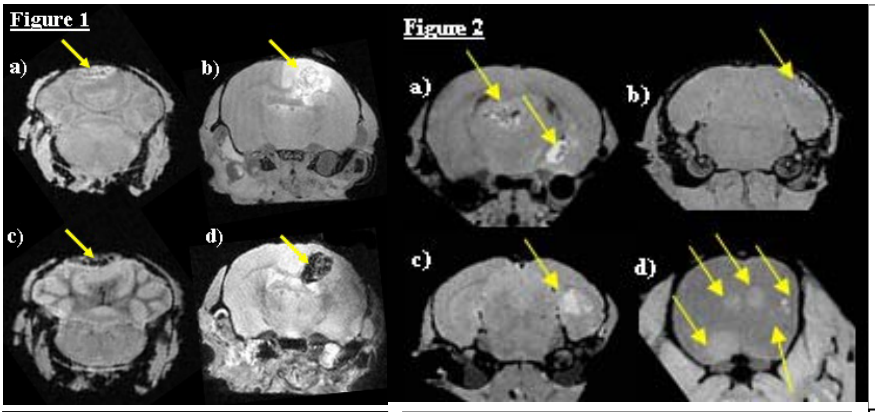

Figure 1: **Imaging features of melanoma brain metastasis:** Endogenous T1-brightening of melanin in B16F10(**a**) and 5B1(**b**) Melanin T2* darkening in B16F10 **(c)** and 5B1(**d**)

Figure 2: **Site specific growth of melanoma brain metastasis:** **(a)**B16F10 ventricular growth **(b)**B16F10 meningeal growth **(c)**5B1 parenchymal growth **(d)**5B1 shows multiple metastasis.

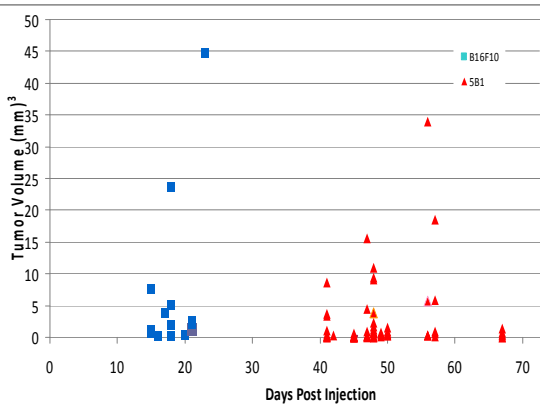

Figure 3: ***Ex vivo*** **MRI and 3D volumetric studies:** Data presented on a scatter plot reveals early detection and exponential growth rate of metastatic lesions in B16-F10 melanoma mouse model (blue squares) while lesions in the 5B1 model (red triangles) exhibit a delayed onset of tumor detection and a slower growth rate.

Conclusion: The *ex vivo* MRI protocol suggested in this study successfully reflected the paramagnetic nature of melanin through T1 brightening and susceptibility effect on T2* which was crucial to our contrast free *ex vivo* imaging studies. The large MRI probe used in our study allowed for simultaneous examination of four mouse whole heads to screen large cohorts of mice, providing us with a high throughput imaging modality to characterize tumor growth in two different melanoma (B-Met) models. *Ex vivo* Imaging and preliminary three dimensional volume analyses revealed that the 5B1 mouse model exhibits parenchymal tumor growth, high brain penetrance, multicentric tumor spread within the brain and a slow and predictable growth rate of the metastatic lesions. In contrast, the B16F10 model showed mostly focal involvement, leptomeningeal growth, low brain penetrance, and an exponential growth rate of melanoma lesions. Results of our *ex vivo* study suggest that the 5B1 parenchymal infiltration is a more clinically relevant model of melanoma brain metastasis for preclinical studies. The slower tumor growth observed so far may offer a more amenable timeframe to test therapeutic strategies being developed.

References: 1)Kehrli *et al.* Epidemiology of brain metastases Neurochirurgie. Dec;45(5):357-63. 1999 2)Sawaya *et al.,* Considerations in the diagnosis and management of brain metastases. Oncology (Williston Park). 2001 Sep;15(9):1144-54(2001)3) Morsi *et al.,* ISMRM Stockholm (2010) 4)Gaviani *et al.,* Improved Detection of Metastatic Melanoma by T2*-Weighted Imaging. American Journal Neuroradiology 27:605– 08(2006)