MRI Evaluation of a Peptide-Coated Nanoparticle as a Potential Therapy Against Preclinical Brain Metastatic Breast Cancer

Amanda M Hamilton[1], Sallouha Aidoudi-Ahmed[2], Venkata R Kotamraju[2], Shweta Sharma[2], Paula J Foster[1], Kazuki N Sugahara[2], Erkki Ruoslahti[2], and Brian K Rutt[3]
[1]*Robarts Research Institute, London, Ontario, Canada,* [2]*Sanford-Burnham Medical Research Institute, California, United States,* [3]*Stanford University, California, United States*

Introduction: As systemic therapies that control extracranial cancers improve, the incidence of metastasis to the brain is on the rise (1). Consequently, there is an increasing interest in the evaluation of novel systemic approaches with the potential to both cross the blood brain barrier (BBB) and confer a therapeutic effect in intracranial metastases. In this study, we used our validated cellular MRI methods, exploiting single cell sensitivity and longitudinal capabilities, to explore the treatment efficacy of a single dose of nanoparticles coated with a tumor-penetrating peptide, iRGD (iRGD-NWs) in a preclinical experimental model of brain metastasis of triple-negative breast cancer.

Methods: *Cell labeling and animal preparation***:** MDA-MB-231BR (231BR) cells were labeled with 25 μg Fe/mL MPIO beads (0.9 μm, Bangs Laboratory) in complete DMEM media for 24h. $1.5x10^5$ MPIO-labeled cells were injected into the left ventricle of anesthetized female nu/nu Foxn1 mice (6-8 weeks old; Charles River Laboratories). Mice were monitored daily until endpoint. *Treatment:* 231BR-injected mice were randomized to three intravenous treatment groups: 100μL saline (treatment day (td) 6/12 n=5), 5mg/kg iRGD-NWs (td 6 n=6, td 12 n=5) or 5mg/kg CRGDC-NWs (td 6 n=6, td 12 n=5) on either 6 or 12 days post-cell injection. *MRI:* All MRI was performed on a 3T GE Discovery MR750 whole-body clinical MR scanner using a custom-built high performance gradient coil with solenoidal radio-frequency mouse head coil and a 3D balanced steady-state free precession (bSSFP) sequence. Mice were imaged for proof of cell delivery at day 1 using the following parameters: res: 150x150x200 μm^3, TR/TE=8/4ms, FA=35°, BW=± 42kHz, 4 phase cycles, 14 min. Mice were imaged for tumor assessment on days 23 and 30 with the same parameters as above except: TR/TE=10/5ms, BW=±12.5kHz, 8 phase cycles, NEX=2, 36 min. *MRI analysis:* All images were analyzed using OsiriX image software and assessed for number of tumors, total tumor volume and signal void retention. *Histological analysis:* Animals were sacrificed 31 days post-cell injection by pentobarbital overdose. Excised brains were fixed in 10% formalin, paraffin-embedded and sectioned at 5 μm. Select sections were stained with hematoxylin and eosin (H&E) to validate MR imaging. *Statistical analysis:* All statistical analysis was performed using GraphPad Prism software by repeated measures 2-way ANOVA and Tukey post hoc test.

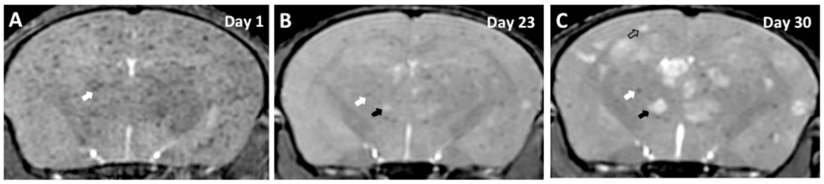

Figure 1: bSSFP MRI can be used to visualize MPIO-labeled cell delivery to mouse brain and the development of brain metastases over time.

Results: *Detection of labeled cells and metastasis growth:* Imaging mice 1 day after MPIO-labeled cell injection using our validated single-cell protocol permitted the quantification of cell delivery to the brain (Figure 1A) and also enabled the assessment of signal void retention over time (Figs. 1B&C, example of a retained void indicated by the white arrow). At later time points, metastases appeared as high signal intensity regions in bSSFP images (Figs. 1B&C). Tumor burden increased over time in all mice imaged in this study and our imaging protocol permitted the longitudinal evaluation of tumor growth (Figs. 1B&C, example of growing tumor indicated by black arrow) as well as the identification of new tumors (Fig. 1C, example of new tumor indicated by grey arrow). *Treatment day comparison:* We compared brain metastasis formation at two time points after treatment administration. At td 6 a significant treatment effect was observed such that iRGD-NW administered mice displayed significantly fewer tumors and had significantly lower total tumor volume than those observed in saline or CRGDC-NW injected mice (Figs. 2A&C). Administration of saline, CRGDC- or iRGD-NW at 12 days post-cell injection resulted in similar tumor numbers and volumes over time and no significant treatment effect was observed (Fig. 2B&D). All experimental mice showed a significant decrease in the number of signal voids from image day 1 to 23 as well as from 23 to 30 (Figs. 2E&F). Mice treated at day 6 with either iRGD- or CRGDC-NW exhibited significantly fewer signal voids than saline control mice at image day 23 (Fig. 2E). No significant differences in signal void retention was observed between groups treated at td 12 (Figure 2F). *Histological analysis:* H&E stained sections showed typical 231BR tumor morphology with areas of edema evident as large white spaces. Identified tumors in histological sections correlated well with those observed in bSSFP images.

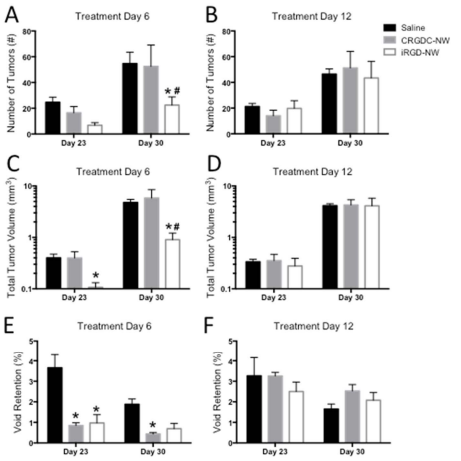

Figure 2: Quantification of brain tumor number, total tumor volume and signal void retention in saline, CRGDC- and iRGD-NW injected mice. *=sig diff than saline #=sig diff than CRGDC-NW

Discussion: Ligand-modified binding can be used to target specific overexpressed receptors on tumor surfaces, thereby increasing the payload concentration at tumor sites and significantly improving therapeutic efficacy. The tumor-homing peptide iRGD has been reported to increase tissue penetration in a tumor-specific manner (2). In the work presented here, iRGD-NW were tested in a preclinical model of breast cancer brain metastasis with a known intact BBB in early stages of disease progression (3). A single dose of iRGD-NW applied early in the time course of tumor development had a significant effect on tumor burden. This alteration in tumor growth was not observed in animals treated at td 12 indicating there was a time window of efficacy for this targeted therapeutic agent. This time dependency has previously been observed in another therapeutic trial in the 231BR brain metastasis model as reported by Fitzgerald et al., in which another therapeutic agent, TPI-287, when applied early, significantly reduced the number of large brain metastases by 55% (4). Similar to iRGD-NWs, late administration of TPI-287 did not alter metastatic tumor growth. Another observation in our current study was that both iRGD-NWs and CRGDC-NWs reduced the retention of MR signal voids, which are believed to represent single iron-labeled cancer cells that successfully arrest in the mouse brain but do not immediately proliferate. Importantly, key results in this study showed that only iRGD-NWs caused significant inhibition of metastasis development, even when administered several days after tumor cell dissemination, suggesting a potential use for this agent in metastasis prevention.

References: 1. Lin et al. Ecancermedicalscience. 2013; 7:307. 2. Teesalu et al. Front Oncol. 2013; 3:216. 3. Percy et al. Invest Radiol. 2011; 46(1):718-25. 4. Fitzgerald et al. Mol Cancer Ther. 2012; 11(9):1959-67.