International Journal on Magnetic Particle Imaging
Vol 12, No 1, Suppl 1, Article ID 2603028, 3 Pages

Infinite Science Publishing

Proceedings Article

In vivo MPI of HER2-targeted tumor SPIO uptake is hindered by its current dynamic signal range

Asif Itoo[a,†] · Ali Shakeri-Zadeh[a,b,†] · Janani Gurumurthy[a,b] · Preethi Korangath[c] · Sudath Hapuarachchige[a,d] · Dmitri Artemov[a,d] · Robert Ivkov[c] · Jeff W.M. Bulte[a,b,*]

[a]The Russell H. Morgan Department of Radiology and Radiological Science, Division of MR Research, The Johns Hopkins University School of Medicine, Baltimore, MD, USA
[b]Cellular Imaging Section and Vascular Biology Program, Institute for Cell Engineering, The Johns Hopkins University School of Medicine, Baltimore, MD, USA
[c]Department of Radiation Oncology and Molecular Radiation Sciences, The Johns Hopkins University School of Medicine, Baltimore, MD, USA
[d]Department of Oncology, The Sidney Kimmel Comprehensive Cancer Center, The Johns Hopkins University School of Medicine, Baltimore, MD, USA
[†]These authors contributed equally.
[*]Corresponding author, email: jwmbulte@mri.jhu.edu

Abstract

Trastuzumab (Tz, herceptin) is a clinically approved monoclonal antibody targeting HER2 on tumor cells. We investigated if Tz-conjugated superparamagnetic iron oxide (SPIO) could be used for MPI of HER2$^+$ breast tumors. Successful conjugation and preservation of specific HER2-binding of Tz after SPIO conjugation was confirmed in vitro by immunostaining and MPI. Following intravenous injection of Tz-SPIO in a transgenic (spontaneously orthotopic) HER2$^+$ breast cancer mouse model, in vivo MPI was unable to show discernable tumor signal due to the close anatomical proximity of high-signal organs (i.e., liver, spleen, and lungs) to the thoracic tumor. The pulmonary signal disappeared by day 3, indicating SPIO redistribution and/or clearance. However, ex vivo MPI confirmed that tumor accumulation of Tz-SPIO was approximately 1.7- to 3.7-fold higher than that for IgG_1-SPIO and unconjugated-SPIO controls, respectively. Hence, the limited dynamic range of current MPI technology prevents successful in vivo tumor visualization at low tumor to non-tumor tissue ratios, which was 1:8 to 1:15 in our studies. While antibody-mediated SPIO tumor targeting works in principle, there is an urgent need to develop methods for increasing the dynamic range of MPI.

I. Introduction

In breast cancer, 15–20% of cases overexpress human epidermal growth factor receptor 2 (HER2) [1], a biomarker associated with aggressive disease and poor prognosis. Trastuzumab (Tz, herceptin), a clinically approved monoclonal antibody against HER2, provides a promising targeting moiety for enhancing tumor accumulation of imaging probes and therapeutics [2]. Magnetic particle imaging (MPI) has emerged as a powerful modality for imaging SPIOs with high sensitivity, zero tissue background, and linear signal quantification. These at-

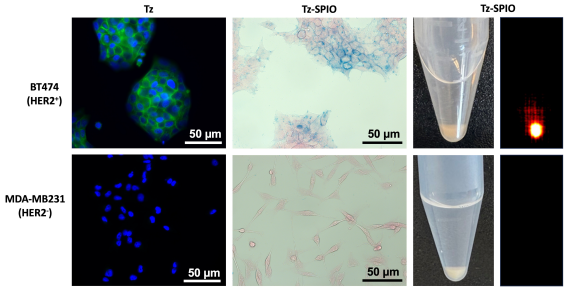

Figure 1: Immunostaining, Prussian Blue staining, and *in vitro phantom* MPI of human HER2$^+$ (top) and HER2$^-$ (bottom) breast cancer cells.

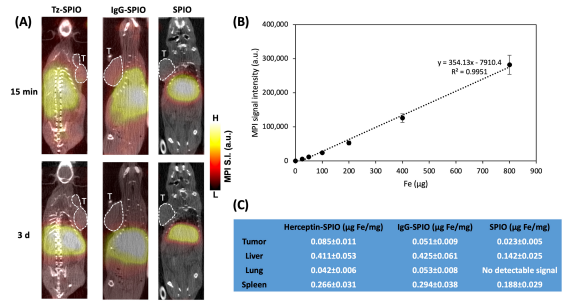

Figure 2: **(A)** Representative MPI/CT images at 15 min and 3 d post-injection for Tz-SPIO, IgG$_1$-SPIO, and unconjugated-SPIO. T=tumor. **(B)** Calibration curve showing linear relationship between MPI signal and iron content, enabling quantitative iron measurements. **(C)** *Ex vivo* MPI quantification of average iron content (µg Fe/mg wet tissue) at 3 d post-injection for Tz-SPIO (n=8), IgG$_1$-SPIO (n=3), and SPIO (n=5).

tributes make MPI attractive for monitoring SPIO biodistribution and tumor targeting *in vivo* [3]. Despite these advantages, MPI is constrained by its limited dynamic range [4]. Strong SPIO accumulation in organs such as the liver, spleen and lungs can significantly "overshadow" tumor signal. This problem is worsened when tumors are located near these organs, leading to poor *in vivo* tumor visibility despite significant uptake. Targeted SPIO delivery and detectability in spontaneous (transgenic) tumor models remain uninvestigated. We evaluated Tz-SPIO tumor targeting in a transgenic HER2$^+$ breast cancer mouse model and show here the impact of the limited dynamic range of MPI on *in vivo* tumor visualization.

II. Methods and materials

Tz-SPIO was prepared by conjugating thiol-functionalized Tz to aminated SPIO nanoparticles (size=20 nm, SHA-20, Ocean NanoTech, San Diego, CA) using sulfo-SMCC conjugation chemistry. Control formulations included IgG$_1$-SPIO (non-specific isotype control) and unconjugated-SPIO. HER2 immunospecificity was assessed on BT474 (HER2$^+$) and MDA-MB-231 (HER2$^-$) breast cancer cells by anti-HER2 and Prussian Blue staining. Female transgenic human (Hu)HER2 mice on an FVB/NJ background (n=8), which spontaneously develop HER2$^+$ multiple mammary tumors and lung metastases, were obtained from Genentech through an MTA and further maintained as a breeding colony. Tumors typically appeared at approximately 9 months of age. Mice received Tz-SPIO or control IgG$_1$-SPIO/unconjugated-SPIO at a dose of 35 mg Fe/kg bw via intravenous injection. In vivo imaging was performed at 15 min, 2 h, 1 d, and 3 d post-injection using 2D and 3D MPI in "standard mode" (Momentum scanner), followed by micro-CT imaging for anatomical reference (IVIS Spectrum/CT). After the last imaging time point, tumors and major organs (liver, spleen, and lungs) were excised and scanned ex vivo with MPI for further analysis. Quantification of iron content was performed using a fixed-concentration calibration curve and background annulus subtraction to minimize blooming artifacts.

III. Results and discussion

Tz was verified to retain its immunospecificity after SPIO conjugation (Figure 1). In vivo MPI revealed strong signals in the liver and lungs at 15 min post-injection for Tz-SPIO and IgG-SPIO, but only in the liver for unconjugated-SPIO, while tumor signal remained indistinguishable in all animals due to the overwhelming signal from these organs (Figure 2A). Pulmonary signal from Tz-SPIO and IgG-SPIO largely disappeared by day 3, likely reflecting transient pulmonary vascular sequestration following NP injection, followed by systemic redistribution and uptake by the reticuloendothelial system. Using a calibration curve, the MPI signals were quantified (Figure 2B). Ex vivo MPI quantification showed higher tumor iron accumulation with Tz-SPIO compared to IgG$_1$-SPIO and unconjugated-SPIO (Figure 2C), confirming that targeted delivery did indeed occur. Antibody conjugation may further influence early biodistribution and clearance kinetics through altered hydrodynamic size, Fc-mediated interactions, and opsonization, contributing to differences in organ-level signal dominance despite enhanced tumor accumulation. However, the limited dynamic range of the current MPI technology prevented in vivo tumor visualization at the low tumor to non-tumor tissue ratios (1:8–1:15). Recent reconstruction-based approaches have been proposed to address dynamic range limitations of MPI that enable simultaneous visualization of widely differing NP concentrations [4]. In the context of thoracic tumor imaging, such approaches could suppress dominant liver and lung signals while preserving sensitivity to lower-amplitude tumor-associated signal, thereby improving detectability without altering NP design.

10.18416/ijmpi.2026.2603028

IV. Conclusion

While our findings underscore the principal feasibility of antibody-mediated targeting, there is an urgent need for improved reconstruction algorithms, adaptive windowing, and/or spectral separation strategies to enhance the current dynamic range of MPI. This limitation is particularly important for thoracic tumors located near high-signal lung and liver regions. Unless the MPI hardware is advanced and reconstruction algorithms are in place to extend the dynamic range, tumor-targeted MPI will remain challenging.

Acknowledgments

This study was funded by NIH grants R01 CA257557 and S10 OD026740. Conflict of interest: J.W.M.B is a shareholder of SuperBranche. This arrangement has been approved by JHU according to its policies.

References

[1] Exman P, Tolaney SM. HER2-positive metastatic breast cancer: a comprehensive review. Clin Adv Hematol Oncol. 2021;19(1):40-50.

[2] Sitia L, Sevieri M, Signati L, Bonizzi A, Chesi A, Mainini F, et al. HER-2-targeted nanoparticles for breast cancer diagnosis and treatment. Cancers. 2022;14(10):2424.

[3] Salimi M, Kuddannaya S, Bulte JWM. Pharmacokinetic Profiling of Unlabeled Magnetic Nanoparticles Using Magnetic Particle Imaging as a Novel Cold Tracer Assay. Nano Lett. 2024;24(49):15557-64.

[4] Boberg M, Gdaniec N, Szwargulski P, Werner F, Möddel M, Knopp T. Simultaneous imaging of widely differing particle concentrations in MPI: problem statement and algorithmic proposal for improvement. Physics in Medicine & Biology. 2021;66(9):095004..

