Targeted Magnetic Nanoparticles Detectable by both SPMR and MRI

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Introduction

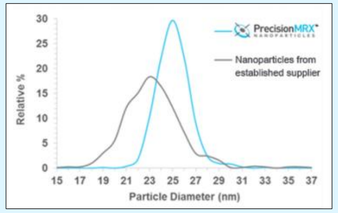
To achieve true diagnostic utility an *in vivo* functional imaging tracer, or imaging agent, must be able to provide both specificity and sensitivity. Previously we have reported that targeted superparamagnetic iron oxide nanoparticles (SPION) can be used for *in vivo* detection by SuperParaMagnetic Relaxometry (SPMR). SPMR is a highly sensitive detection technology that is able to differentiate the magnetic signature of nanoparticles bound to tumor cells from unbound nanoparticles. Nanoparticles that reach and bind to the target cells are detectable by their Néel relaxation, while unbound nanoparticles such as those freely circulating in the bloodstream are not detected due to their rapid Brownian relaxation. Here we show that the same targeted SPION accumulated in tumor which are detectable by SPMR also provide contrast for magnetic resonance imaging (MRI), offering an opportunity for MRI to achieve diagnostic utility as molecular imaging modality.

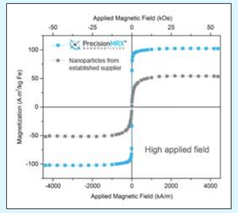
Study Objective

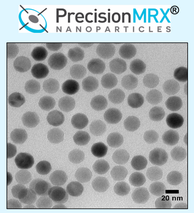
The current study aim is to demonstrate that biomarker target–specific iron oxide nanoparticles can be used for image-based diagnosis and treatment monitoring by both SPMR and MRI.

HER2 Targeting Magnetic Nanoparticles

PrecisionMRX® Nanoparticles (NP)s are precision made 25nm superparamagnetic magnetite (Fe_3O_4) cores with high magnetic relaxivity (r_2 = ~570 @ 7T). Particles are monodispersed with narrow size distribution and exhibit high magnetic saturation.

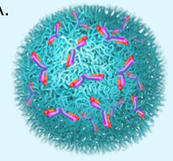






To bio-functionalize the NP, cores were encapsulated by a layer of polymer and then functionalized with carboxylate (COO^-) surface. PEG + anti-Her2 mAb were subsequently conjugated onto the polymer surface. Size of resulting NPs were measured by DLS. Bound and free mAb were determined via ELISA.

Surface	Diameter	PDI	# of Ab/NP	% of free Ab
PEG + anti-Her2	70-80 nm	<0.10	3-5	<10%



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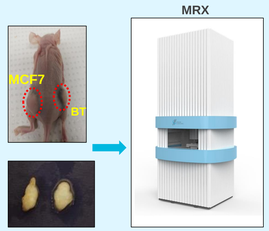
Method - SPMR

Specific binding in vitro

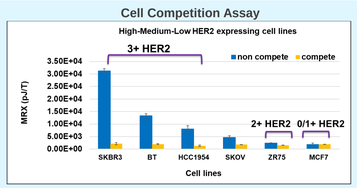
- A variety of cell lines with different levels of HER2 expression were incubated with 100ug of Anti-HER2 NP overnight.
- Cells were washed, harvested, centrifuged, and pellets were subsequently measured on the MRX instrument.
- Cell competition study was done by pre-incubate cells with free anti-HER2 antibody.

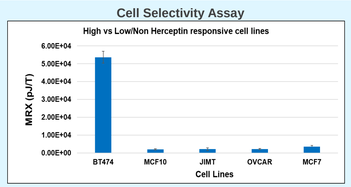
Specific binding in vivo

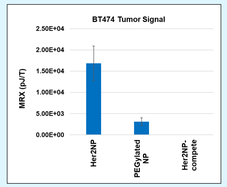
- Anti-HER2 mAb conjugated NPs or PEGylated NPs (20mg/kg) were injected into BT474 (HER2 (3+)) and MCF-7 (HER2 (1/0+)) dual implanted tumor bearing mice (Nude) by tail vein (IV), intraperitoneal (IP), or peritumoral (PT) delivery.
- For in vivo competition, free anti-HER2 mAb were injected 24hr prior to NP injection.
- After 24 hr post NP injection, mice were euthanized, and tumors and organs were excised for *ex vivo* MRX measurement.

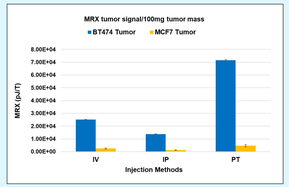


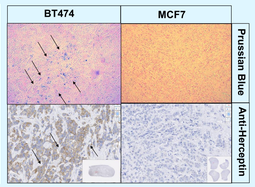
Results - SPMR











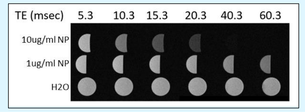
- Anti-HER2 NPs can clearly distinguish high, medium and low HER2 expressing cell lines.
- NP targeting can be competed out by free anti-HER2 mAb, indicating specificity.
- Anti-HER2 NPs generate higher binding signal than PEG-only NP, indicating targeting is a function of the antibody not EPR or other non-specific mechanisms.
- Dual flank tumor study demonstrates that BT474 tumor generated much higher binding signals compared to MCF7 tumor. These results were confirmed by the presence/absence of anti-HER2 mAb and iron (Prussian Blue) in BT474 and MCF7 tumor respectively in IHC study.

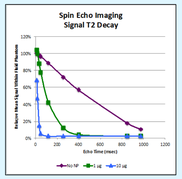
Method - MRI

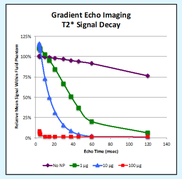
- All MRI measurements were conducted using a 1.5T GE HDx TwinSpeed clinical MRI with research capabilities using a wrist coil (Univ. California San Diego).
- Different concentrations of nanoparticles were prepared and imaged to establish MRI measurement parameters.
- Different concentrations of BT474 cells were pre-labeled with anti-HER2 nanoparticles and mixed with agarose gel to be evenly distributed in a solid medium for MRI measurement.
- Different concentrations of BT474 cells were pre-labeled with anti-HER2 nanoparticles and implanted at flank region of mice for MRI measurement.

Results - MRI

Nanoparticle in solution contrast

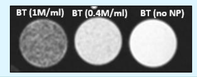


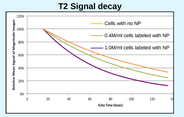


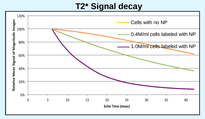


- NP produce negative T2 and T2* contrast
- Decay increases (shorter T2 and T2*) with an increase in NP concentration

BT474 cell bound nanoparticle contrast

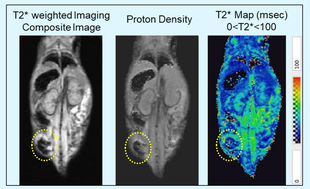


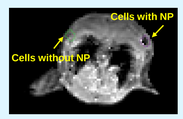


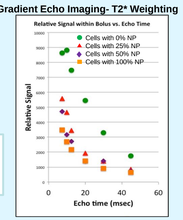


- T2 and T2* signal decay increase with amount of NP labeled cells

BT474 cell bound nanoparticle implant in mice contrast







- NP-labeled cells are observable at implanted site with a negative contrast signal

Conclusions – Future Work

Together, these results suggest that our HER2 nanoparticles can provide targeted and specific delivery to cancerous tissue and generate measurable signal by SPMR. Further study using these HER2 nanoparticles demonstrated its utility in providing tumor specific magnetic contrast agent in MRI to improve its detection sensitivity and specificity. These studies lay out groundwork for early and metastatic breast cancer tumor detection as well as monitoring therapy response.