Sensitive, specific detection of Her-2 positive tumors in mice using superparamagnetic relaxometry (SPMR)

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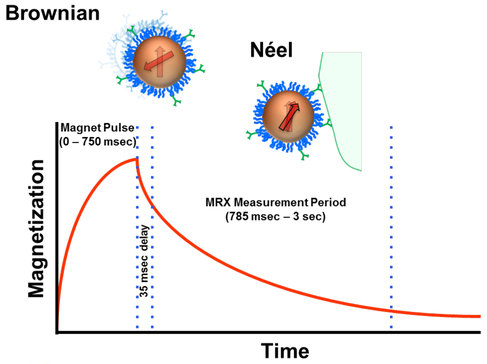
Introduction

Superparamagnetic Relaxometry (SPMR) utilizes superconducting quantum interference device (SQUID) detectors to localize and quantify superparamagnetic iron oxide (Fe_3O_4) nanoparticles (NPs) specifically bound to cancerous tumors. In an SPMR measurement, polyethylene glycol (PEG) coated NPs are functionalized with a tumor-targeting monoclonal antibody (mAb) and injected intravenously. NPs that reach and bind to the target tissue are measured by the MRX™ instrument, while unbound nanoparticles, such as those freely circulating in the bloodstream, are not detected.

Objectives

- Develop long-circulating, anti-HER2 mAb conjugated PrecisionMRX® NPs
- Specific detection of HER2 positive breast cancer cells in vitro and in vivo detected by SPMR

Background



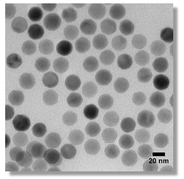
1. NPs injected into subject
2. Small magnetizing pulse is applied
3. Field turned off
4. NPs relax to their equilibrium states.
   - **Brownian** motion of unbound NPs (fast)
   - **Néel** relaxation of NPs bound to cells (slow and measurable)

SPMR detects only nanoparticles bound to cells/tissues

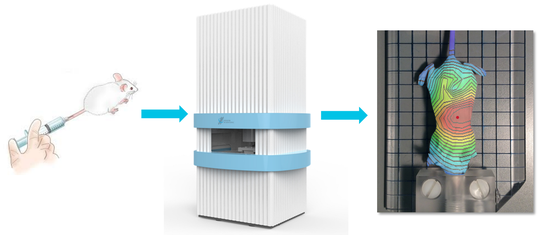
Methods

Characterization of anti-HER2 PrecisionMRX® NPs

PrecisionMRX™ NANOPARTICLES



Analytical Test	Method
Particle Size	Small angle X-ray scattering (SAXS)
Hydrodynamic size	Dynamic light scattering (DLS)
Anti-HER2 Content	Direct ELISA
Specificity	Competition ELISA
Specificity	SPMR, digital photography
Stealth	Zeta potential, agarose gel electrophoresis



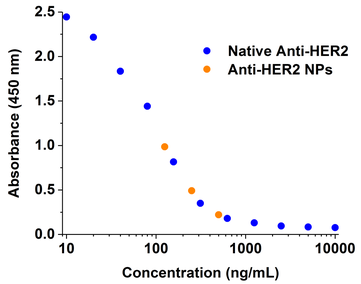
- Injection of NPs into NSG mice (20 mg/kg)
- MRX measurements over 4 hour time course
- Magnetic dipole map generated for signal localization
- Organs, tumors excised for ex-vivo SPMR measurements

Results

DLS

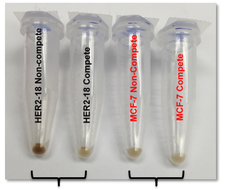
Surface	Diameter (nm)	PDI	Zeta Potential (mV)
COO^-	46	0.04	-40
PEG	70	0.10	-10
PEG + anti-HER2	85	0.10	0

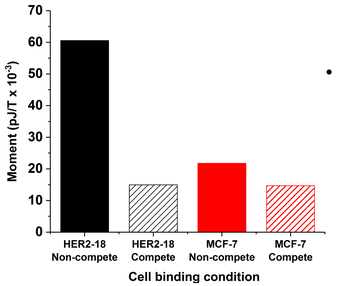
- Neutral charge of anti-HER2 NPs indicative of stealth in vivo
- Anti-HER2 NPs compete with biotinylated Anti-HER2 for antigen binding with equal potency to native anti-HER2 mAb



Results

Specific binding in vitro

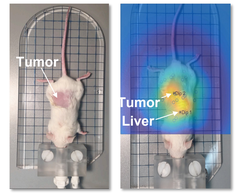




- Competitive binding of anti-HER2 NPs can be correlated to the expression of HER2 on cells

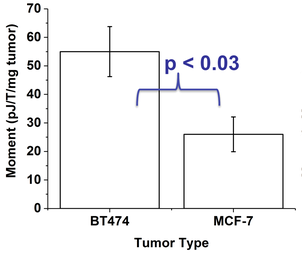
Specific binding in vivo

In vivo dipole map

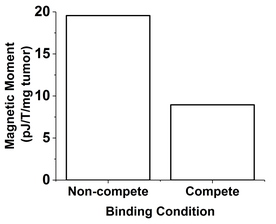


- 4% NP delivery to tumor

Ex vivo measurements



- SPMR signal dependent on HER2 expression



- ~50% competition of anti-HER2 NPs by native anti-HER2

Conclusions and Future Work

- Developed an anti-HER2 conjugated, PEG-coated PrecisionMRX® NPs that specifically bind to HER2 expressing breast cancer cells in vitro and in vivo
- Future work is focused on a NP formulation with optimal targeting and stealth for clinical detection of sentinel lymph node metastases

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