HER2 Functionalized Nanoparticles Are Safe and Specific for in vivo HER2+ Breast Tumor Cell Detection

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Poster 1953

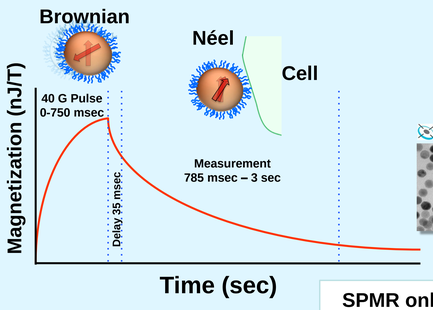
Introduction

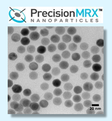
Iron oxide nanoparticles (NPs) have been used for a variety of *in vivo* and *ex vivo* applications in the biomedical sciences. Moreover, when intended for *in vivo* clinical applications, NPs need to meet rigorous requirements to ensure safety as well as bio-functionality, including blood circulation time and specificity for cellular targets. PrecisonMRX® NPs are extensively characterized superparamagnetic NPs composed of 25 nm magnetite cores that are currently used in a variety of pre-clinical *in vivo* applications including non-invasive *in vivo* diagnosis of cancer, Magnetic Particle Imaging, MRI contract, and magnetic hyperthermia.

Objective

Conduct development and pre-clinical functionality studies of anti-HER2 antibody (mAb) conjugated NPs for *in vitro* and *ex vivo* detection of HER2+ tumor cells by Magnetic Relaxometry (MRX).

Superparamagnetic Relaxometry





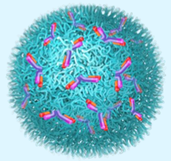
1. Inject NPs
2. Apply small magnetizing pulse
3. Turn off field
4. NPs relax to their equilibrium states.
   - **Brownian** motion of unbound NPs (fast)
   - **Néel** relaxation of NPs bound to cells (slow and measurable)

SPMR only detects NPs bound to cells/tissues

Anti-HER2 NP Functionalization and Characterization

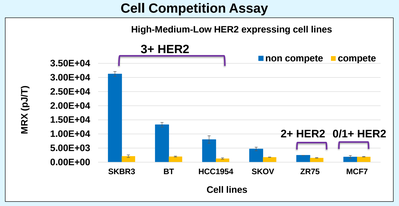
PrecisionMRX® NPs were encapsulated by a layer of polymer and then functionalized with carboxylate (COO^-) surface. PEG + anti-HER2 mAb were subsequently conjugated onto the polymer surface. Size of resulting NPs were measured by DLS. Bound and free mAb were determined via ELISA.

Surface	Diameter	PDI	# of Ab/NP	% of free Ab
PEG + anti-HER2	70-80 nm	<0.10	3-5	<10%

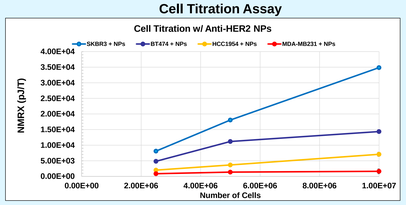


Results – In Vitro Specificity, Sensitivity

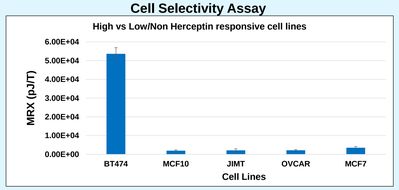
Cell Competition Assay



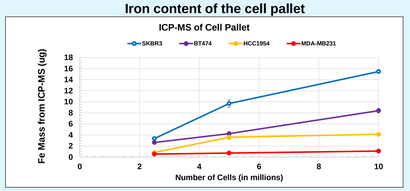
Cell Titration Assay



Cell Selectivity Assay

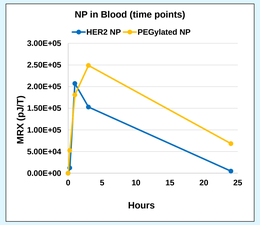


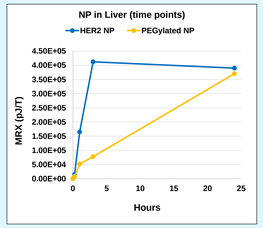
Iron content of the cell pallet

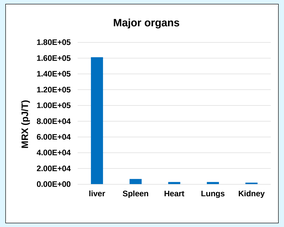


- **Nanoparticles can clearly distinguish high, medium and low HER2 expressing cell lines, demonstrating specificity and sensitivity.**

Results – In vivo Distribution, Specificity

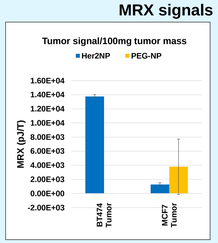


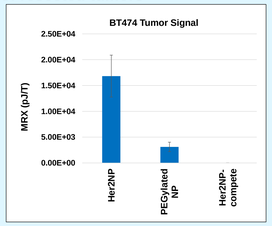




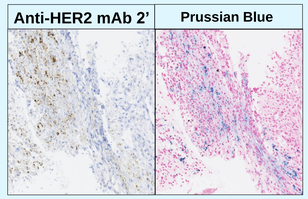
- **All major organs except liver (clearance) have no significant NP accumulation indicating anti-HER2 mAb NP is safe.**

MRX signals of resected tumors





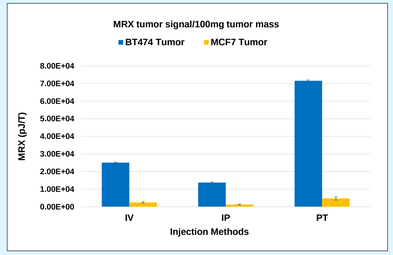
Prussian Blue Staining



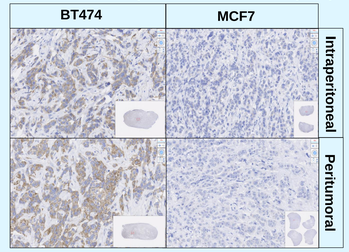
- **MRX tumor binding signal can be competed out by free anti-HER2 mAb indicating specificity.**
- **Anti-HER2 mAb conjugated NP generated higher binding signal than PEGylated NP indicating specificity**

Dual Flank Tumors of BT474 (HER2 3+) and MCF7 (HER2 1/0+)

MRX signals of resected tumors



IHC of Tumor (Anti-HER2 Secondary Ab)



- **Dual flank tumor study demonstrated that BT474 tumor generated much higher binding signals compared to MCF7 tumor. These results were confirmed by the presence/absence of anti-HER2 mAb in BT474 and MCF7 tumor respectively in IHC study.**

Methods

Specific Binding *in vitro*

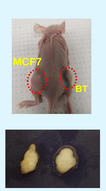
- A variety of cell lines with different levels of HER2 expression were incubated with 100ug of anti-HER2 mAb NPs overnight.
- Cells were washed, harvested, centrifuged, and pellets were subsequently measured on the MRX instrument.
- Cell competition study was done by pre-incubating cells with free anti-HER2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

Nanoparticle Distribution and Clearance *in vivo*

- Anti-HER2 mAb conjugated NPs or PEGylated NPs (20mg/kg) were injected into Balb/C mice via intraperitoneal (IP) delivery.
- Major organs as well as blood samples were taken post injection at different time points after euthanization for MRX measurement.

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.



Conclusions and Future Work

Together, these results suggest that our anti-HER2 antibody conjugated nanoparticles are safe; can provide targeted and specific delivery to cancerous tissue in *vivo* and generate measurable signal on our MRX detection instrument. These studies lay out ground work for our future human clinical study for *in vivo* breast cancer detection.