Targeted Detection/Imaging of Prostate Cancer by SPMR and MRI

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Introduction

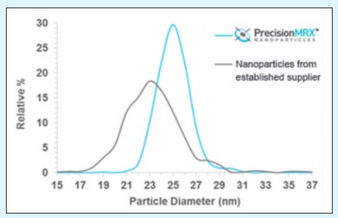
To achieve true diagnostic utility an *in vivo* molecular imaging tracer, or imaging agent, must be able to provide both specificity and sensitivity. Iron oxide nanoparticles (NPs) have been used in a variety of preclinical and clinical cancer detection/imaging applications. Here we show that targeted superparamagnetic iron oxide nanoparticles (SPION) can be used for *in vivo* detection by two different magnetic imaging methods. Superparamagnetic Relaxometry (SPMR) is a highly sensitive *in vivo* detection technology that can differentiate the magnetic signature of nanoparticles by their Néel relaxation when bound to tumor cells. Unbound nanoparticles such as those freely circulating in the bloodstream are not detected due to their rapid Brownian relaxation. The same targeted SPIONs accumulated in tumor provide molecular magnetic resonance imaging (MRI) contrast as well. Recently, prostate-specific membrane antigen (PSMA) PET tracers have been reported to be effective in detecting prostate cancer as well as its metastases. Here we show a non-radioactive SPION-based approach to achieving molecular imaging by two methods using the same targeting capability as PSMA-PET while obtaining better anatomical image resolution by MRI.

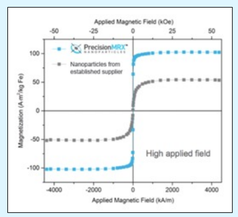
Study Objective

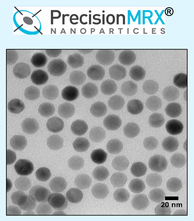
Previously, we have reported the success of developing an *in vivo* anti-HER2 targeted SPION-based imaging agent for SPMR and MRI detection. This program is currently in clinical testing for the detection of nodal metastases in HER2-positive breast cancer. Leveraging the same targeted SPION platform, the purpose of this current study is to demonstrate a PSMA target–specific imaging agent can be used for magnetic imaging in prostate cancer.

PSMA Targeting Magnetic Nanoparticles

Nanoparticle (NP) cores of 25nm superparamagnetic magnetite (Fe_3O_4) are made with high magnetic relaxivity (r_2 = 180 mM^{-1} s^{-1} at 3 T and 590 mM^{-1} s^{-1} at 7 T) providing excellent Néel relaxation and T2 contrast. Particles are monodisperse with narrow size distribution and exhibit high magnetic saturation.

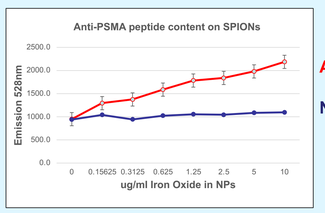






To bio-functionalize the NP, cores were encapsulated by a layer of polymer and then functionalized with carboxylate (COO^-) surface. PEG + an anti-PSMA peptide were subsequently conjugated onto the polymer surface. Size of resulting NPs were measured by DLS. Anti-PSMA peptide content on the nanoparticles were measured using a plate-based ELISA. PEG-only NPs served as a control.

Surface	DLS (size)	PDI
PEG + anti-PSMA peptide	67.2nm	0.08
PEG	59.2nm	0.07



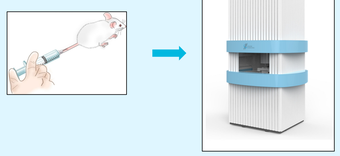
Method - SPMR

Specific binding *in vitro*

- A variety of prostate cancer cell lines with different levels of PSMA expression were incubated with 200 µg of Anti-PSMA NP per million of cells in 6-well plate as well as the non-targeted PEG NP control overnight.
- Cells were washed, harvested, centrifuged, and pellets were subsequently measured for SPMR signal on a Magnetic Relaxometry (MRX) instrument.
- PSMA expression levels were confirmed by flow cytometry for all the cell lines used in the study.

Specific binding *in vivo*

- Anti-PSMA peptide NPs or PEGylated NPs (20mg/kg) were injected into LNCaP (PSMA+ve, high), 22RV1 (PSMA+ve, medium) and PC3 (PSMA-ve) tumor bearing mice by tail vein (IV) delivery.
- After 24 hr post NP injection, mice were euthanized, and tumors and organs were excised for *ex vivo* SPMR measurement.

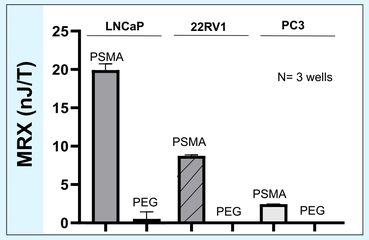


NP distribution and PK study *in vivo*

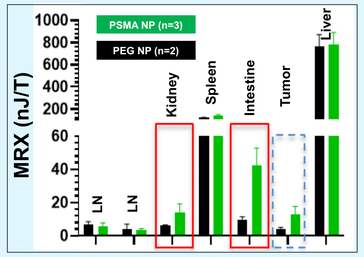
- Anti-PSMA peptide NPs (20mg/kg) were injected into naïve Balb/c mice by tail vein (IV) delivery. Mice were euthanized at different time points post-injection. Organs and blood were harvest to access NP contents and measured for SPMR signal.

Results - SPMR

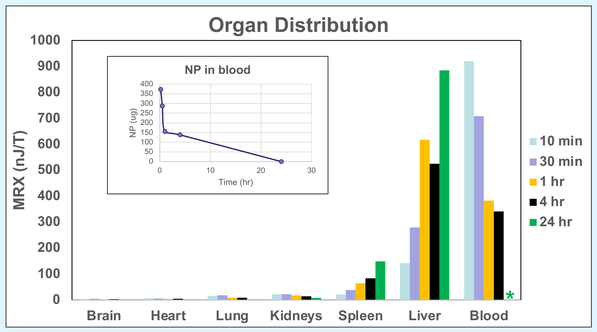
In vitro cell binding study



In vivo tumor mice study



- The SPMR signal is proportionate to the PSMA expression level of the cell line showing anti-PSMA NPs can clearly distinguish high, medium and low PSMA expressing cells *in vitro* while non-targeted NPs (PEG NP) do not produce an SPMR signal indicating PSMA NPs function with targeting specificity *in vitro*.
- *In vivo* models show anti-PSMA NPs generate higher binding signal than PEG NPs in PSMA+ve expressing tumors as well as in organs such as kidney/intestine where there is a high level of endogenous PSMA expression indicating targeting is predominately a function of the targeting peptide and not EPR or other non-specific mechanisms *in vivo*.



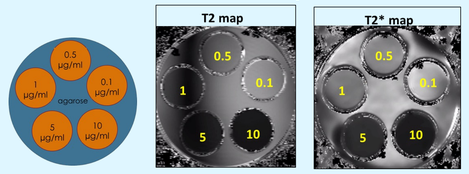
- Anti-PSMA NPs still maintained ~30% of injected amount in the blood at 4-hours post injection, but was completely cleared from blood at the 24-hour time point.
- NPs accumulated in the liver and spleen, but not in other organs such as brain, heart and lung.

Method - MRI

- All MRI measurements were conducted using a 11.7 T horizontal Bruker Biospec MRI at the Molecular Imaging Center at Sanford Stem Cell Consortium, San Diego, CA.
- Different concentrations of NP Phantoms samples were prepared and imaged to establish MRI measurement parameters.
- Pre-labeled LNCaP cells with anti-PSMA NPs and non-labeled cells were implanted at flank region of mice for MRI measurement to optimize MRI measurement parameters.
- Anti-PSMA NPs (20mg/kg) were injected into LNCaP tumor bearing mice by tail vein (IV) delivery.
- MRI images of the tumor mice were taken pre- and 24-hour post dosing of the animal and analyzed.

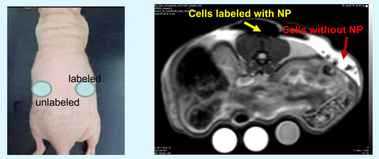
Results - MRI

Nanoparticle Phantom Study



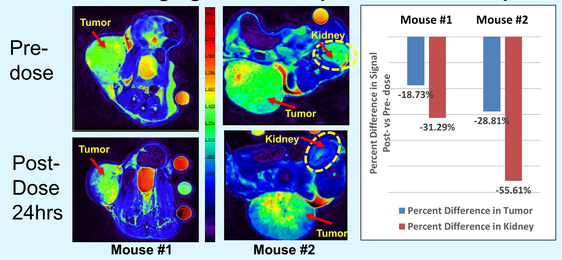
- NP produce negative T2 and T2* contrast
- Decay increases (shorter T2 and T2*) with an increase in NP concentration
- $r2$ = 323 $s^{-1}mM^{-1}$ $r2^*$ = 460 $s^{-1}mM^{-1}$

LNCaP cell bound nanoparticle implants in mice



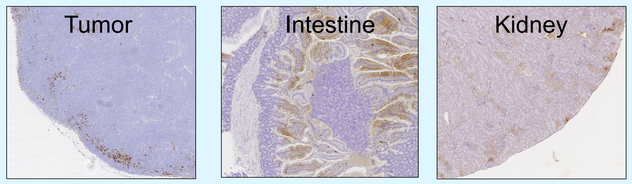
- NP-labeled cells are observable at implanted site with a negative contrast signal

In vivo MRI imaging PSMA NP uptake in LNCaP implanted tumor in mice (T2)



- Normal kidney proximal tubule cells express endogenous PSMA
- Pseudo colored images showed hypo-intensity in implanted PSMA+ve LNCaP tumor as well as kidney post-dose vs pre-dose, demonstrated that PSMA NP accumulated in these tissue specifically and were observable by MRI

Tissue Fe Staining Study



- PSMA NP presence in the tumor, intestine and kidney were confirmed by enhanced Fe Prussian Blue staining

Conclusions – Future Work

Together, these results suggest that our anti-PSMA nanoparticles can provide targeted and specific delivery to cancerous tissue and generate measurable signal by SPMR. The same anti-PSMA nanoparticles demonstrated utility as a tumor specific molecular contrast agent in MRI, showing that SPIONs can provide non-radioactive detection of prostate cancer. These studies lay out groundwork for potential future clinical prostate cancer tumor detection as well as monitoring therapy response.