Noninvasive Detection of Lymph Node Involvement in Subjects with Human Epidermal Growth Factor Receptor 2 Positive (HER2+) Breast Cancer
A First-In-Human Phase 1 Study Using the MagSense® HER2 Imaging Agent

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Introduction

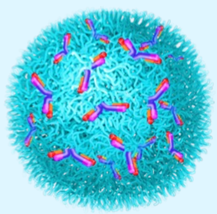
The standard of care for axillary staging in breast cancer requires lymph nodes be surgically removed for histopathological examination. Superparamagnetic iron oxide nanoparticles (SPIONs) have been used in preclinical and clinical research as imaging agents for decades because of their magnetic properties and their known safety profile, including for evaluation of tumor status of lymph nodes. However, the SPIONs used to-date have been non-targeted, typically dextran coated particles, that result in image contrast associated with non-specific uptake by macrophages. The MagSense® HER2 Imaging Agent has been developed as a molecular imaging agent specific for patients with Human Epidermal Growth Factor Receptor 2 (HER2) - positive breast cancer as an aid in detecting nodal disease. The imaging agent incorporates an anti-HER2 antibody covalently conjugated to a SPION to provide targeted specific binding of the imaging agent when HER2 expressing tumor cells are present. Here we present the clinical results from the first six patients dosed with MagSense® HER2 in the ongoing phase 1 study (ACTRN12621000126819).

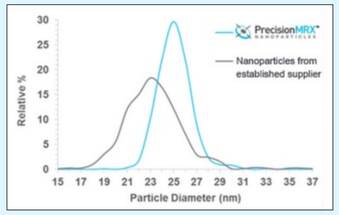
Study Objective

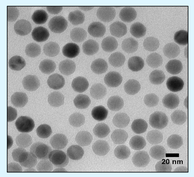
This study is designed as a preliminary proof-of-principle for the HER2 targeted imaging agent. The primary objective of this first-in-human study is an initial assessment of the safety and tolerability of the injectable imaging agent. A secondary objective of the study is the confirmation that the route of administration is effective in allowing the imaging agent to reach the patient's lymph nodes. The exploratory objectives of the study include a comparison of two imaging modalities: magnetic resonance imaging (MRI) and a novel technology known as superparamagnetic relaxometry (SPMR). Results of the imaging methods are compared to standard clinical tissue histopathology to achieve a preliminary assessment as to whether the MagSense® HER2 imaging agent, when used with one or both imaging modalities, might provide improved axillary nodal assessment for clinical decision making.

HER2 Targeted Magnetic Nanoparticles

The MagSense® HER2 imaging agent is designed for use with the magnetic relaxometry detection instrument and as an MRI contrast agent.

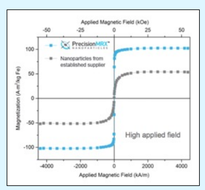




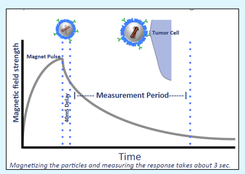


Surface	Diameter	PDI	# of Ab/NP	% of free Ab
PEG + anti-Her2	70-80 nm	<0.10	3-5	<10%

Superparamagnetic magnetite (Fe_3O_4) cores 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 monodispersed with narrow size distribution and exhibit high magnetic saturation. To make a molecular imaging agent, cores are encapsulated with a polymer and then functionalized with carboxylate (COO^-) surface. Polyethylene Glycol (PEG) and an anti-HER2 antibody are conjugated onto the polymer surface.



Superparamagnetic Relaxometry (SPMR) can differentiate the magnetic signature of nanoparticles by their Néel relaxation when bound to tumor cells. Unbound nanoparticles are not detected due to their rapid Brownian relaxation.



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Study Design

Patient Eligibility

- Newly diagnosed HER2-positive breast cancer patients prior to treatment
- Suspicion of nodal disease by clinical evaluation, *e.g.,* ultrasound or biopsy

Study Protocol

- Breast MRI on Day 1 prior to MagSense® HER2 administration (pre-dose)
- Subcutaneous injection (peri-tumoral or areolar) of 30mg dose of MagSense® HER2
- Breast MRI on Day 2 (~ 24 hours post-dose)
- Breast MRI on Day 4 (~ 72 hours post dose) for patients 1-6 only
- Following last MRI, either dissected nodes if surgery planned before systemic therapy or biopsy (core needle) of a clinically "suspicious" lymph node obtained
- Dissected nodes or biopsied tissue(s) analyzed *ex vivo* for magnetic relaxometry and histology
- Day 7 safety follow up and Day 28 study completion

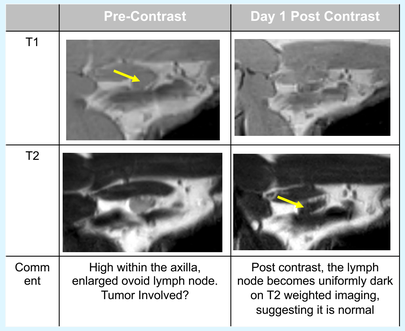
Safety & Tolerability

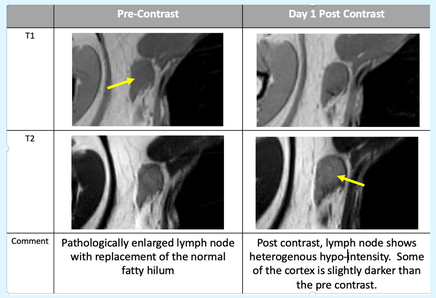
- A Safety Review Committee (SRC) reviewed safety data following the first cohort of patients (N=6).
  - No dose limiting toxicities reported.
  - Injection Site Reactions (ISR) – majority reported as mild or moderate, mostly discoloration at the injection site.
  - No imaging agent or procedure related adverse events (AEs) reported.
- Subjects enrolled after the SRC review show similar safety and tolerability.

MR Imaging Results

- MRI measurements were conducted using a 1.5T or 3T clinical scanner with a standardized 20-minute breast imaging protocol of the ipsilateral axillary region.*
- A central radiology group was used to evaluate all patient images and compare pre-dose images to post-dose images. Nodes were assessed by both conventional radiological measures such as size and morphology as well as for changes in contrast intensity. A 30% change in contrast intensity (as observed by the radiologist) between pre- and post-dose images was considered sufficient to have observable presence of nanoparticles.
- Nodes were scored as "suspicious", or "normal" or "indeterminate" both pre-dose and post-dose.

- Central Radiologists reported interpretable contrast change in post dose images for both normal and enlarged nodes vs. pre-dose images in four (4) of six (6) subjects.
- Post-dose normal nodes displayed a uniformly dark contrast (right panel) whereas post-dose enlarged nodes (below panel) showed a central heterogeneous hypointensity.
- There was no intensity change from post-dose Day 2 to Day 4





- In three (3) subjects, radiologists utilized pre-dose vs. post-dose contrast as an aid in resolving nodal status of "indeterminate" nodes (ex.: above panel)
- In 2 subjects, post-dose images were not interpretable - excess susceptibility in one and potentially lack of particle drainage in another (see pathology section)

* MR Imaging protocols were established with collaboration from Siemens Healthineers Australia

Tissue Specimens

- Central Pathology laboratory collected formalin fixed specimens from all sites.
- Whenever possible, SPMR measurements were performed prior to processing the tissue for pathology.

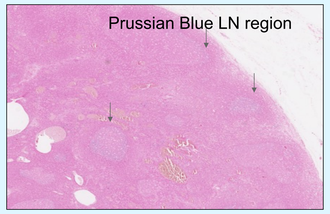
Subject	Specimens collected for each subject
1	No specimen collected due to small node size
2	3 nodes each cut into 3 slices (9 specimens)
3	2 Core biopsies from one node
4	2 Core biopsies from one node
5	2 Core biopsies from one node
6	2 Core biopsies from one node

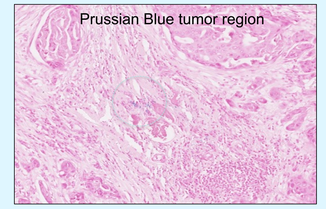
SPMR Results

- SPMR measurements were conducted *ex vivo* at Central SPMR laboratory using preclinical instrument to determine if SPMR signal was detectable in subject nodes and inform future clinical instrument parameters.
- Subject 2 samples (3 nodes sliced as 9 specimens) measured significant SPMR signal (3-10x of LOQ) in 8 of 9 specimens. LOQ~ 2.5 μg of iron.
- Core biopsy specimens did not result in measurable SPMR signals. Core biopsy represents 2-5% of a full node and are insufficient size to inform SPMR sensitivity for the clinical *in-vivo* use case.
- These data suggest feasibility for SPMR measurement in subject nodes when sufficient sample is available, More samples are needed for evaluating concordance with pathology and for future instrument optimization.

Histopathology

- Histopathology was evaluated using hematoxylin & Eosin (H&E), HER2 and Prussian Blue (iron) stains. 5 subjects had specimens available for pathology staining (see Specimen Table above).
- 4 subjects showed Prussian Blue stain in the lymph nodes confirming presence of iron particles.
- 1 subject's specimens had no iron stain. Same subject did not show any evidence of imaging agent in post MR images. Either issues with lymphatic drainage or technical issues with injection is suspected.
- 4 subjects showed HER2 - positive nodal metastasis and 1 subject was negative for tumor.





Histology slides from Subject 2 showing Prussian blue stains in the lymph region (left panel marked by arrows) and in the tumor region (right panel marked by a blue circle).

Clinical Concordance

- Four (4) of six (6) subjects were evaluable for MRI vs. pathology concordance at patient level.
- In 3 subjects, post dose MRI assessments by central radiologists were in concordance with pathological confirmation of nodal metastasis.
- Radiologists reported a suspicious node in subject 6 (pre- and post-dose) who was pathology negative**.
- ***Note that in first cohort, the biopsied node and the MR-suspicious node are not confirmed to be same (no clips or localization). Therefore, even though the biopsied node was negative, we cannot rule out the possibility of a positive pathology from the MR suspicious node. To address this issue, protocol was amended for 2nd cohort, to include an MRI compatible clip in a clinically suspicious node to allow MR imaging of the same node for evaluating concordance at node level.*

Conclusions – Future Work

These preliminary results indicate that an anti-HER2 targeted imaging agent can be safely administered and used as an aid in assessing nodal disease for HER2 - positive breast cancer. Histopathological examination of excised lymph nodal tissue confirms the presence of tumor cells and the MagSense® HER2 nanoparticles in the nodes. Comparison of pre-dose vs. post-dose MR images appear to discriminate suspicious nodes from the normal nodes by the molecular signature of the HER2 targeted nanoparticles. These data suggest that combining standard morphological assessments (size and shape) with observable changes in MR contrast has the potential to improve radiological evaluation thereby improving the standard of care clinical assessments. Evaluation of the second imaging modality (SPMR) is on-going with specimens from patients undergoing nodal dissection. The study remains open for enrollment in Australia.