Grant Mechanism: CCTSI Phase I - Novel Methods Application 2015

Title of the Proposal: Non-Invasive Lymph Node Imaging in Breast Cancer Models

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Requesting Phase II Investigator: Natalie Serkova, PhD, Associate Professor of Anesthesiology, Radiology and Pharmacology, UCCC/CCTS Imaging Core

Key Words: Tumor Associated Macrophages (TAM); Magnetic Resonance Imaging (MRI); Superparamagnetic Iron Oxide (SPIO) Nanoparticles; Stromal Microenvironment; Tumor Inflammation

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Background, Objectives, and Aims

We seek to obtain a non-invasive imaging technique to detect inflammation-induced lymphangiogenesis at the primary tumor site and to detect lymph node metastases in mouse models of breast cancer.

Background: Distant metastases are the major cause of mortality in breast cancer (BCa) patients. A large body of evidence indicates that dissemination of breast cancer (BCa) occurs via lymphogenous spread and lymph node (LN) involvement remains the best prognostic indicator for BCa. However, there is an unmet need to improve the non-invasive diagnosis of LN involvement and to image the interplay between the tumor microenvironment and formation of peri-tumor lymphatics or lymphangiogenesis. We have recently shown that inflammation triggers cyclooxygenase-2 (COX-2) to increase lymphangiogenesis, which leads to increased LN and distant metastases formation in mouse inflamed BCa models 1,2. Non-invasive imaging quantitation of BCa inflammation (tumor-associated macrophages, TAMs), simultaneously with imaging of lymphangiogenesis and LN metastases could provide a revolutionary diagnostic tool and also guide anti-inflammatory and anti-lymphangiogenic therapies. Radiologically, magnetic resonance imaging (MRI) is used to determine and monitor tumor margins and vascularity of primary BCa lesions; unfortunately, conventional MRI is not sensitive enough to characterize LN metastases3. Thus, sentinel node biopsy is presently the standard of care for LN staging in BCa patients. We seek to refine conventional MRI methods to allow for detection of LN metastasis in a non-invasive manner. Our mechanistic rationale for the proposed studies is based on iron uptake and metabolism in the reticuloendothelial system including macrophages and innate lymph cells4-6. In this Novel Methods application we aim to develop a quantitative MRI protocol, using iron-oxide based contrast, for non-invasive characterization of the inflamed microenvironment in primary BCa (TAMs) simultaneously with its effect on the LN status. Additionally, this method will be employed for quantitatively imaging the efficacy of anti-inflammatory (COX-2 inhibitors and bisphosphonates) and anti-lymphangiogenic therapies (anti-VEGFR2/3) in mouse models of inflamed and disseminated BCa.

Aims: This proposal seeks to develop a non-invasive contrast-enhanced T2-weighted magnetic resonance imaging (CE T2-MRI) method for lymph node imaging in mouse models of breast cancer. It has been recently shown that a pro-inflammatory stromal milieu, with resulting intra-tumoral macrophage infiltration, correlates with poor prognosis in breast cancer. Influx of macrophages with strong similarity to tumor-associated macrophages (TAMs) also drives lymphangiogenesis. TAM and lymphatic vessels are usually assessed in breast tissue specimens using immunohistochemistry (IHC) and other ex vivo assays, severely limiting the assessment of TAM infiltrate over time or with chemotherapeutic treatments. The primary function of macrophages is phagocytosis, therefore they have a very high uptake of iron oxide. Super-paramagnetic iron oxide (SPIO) nanoparticles have a variety of applications for cellular MR imaging, since they are so-called T2-MRI contrast agents, which shorten T2-relaxation times of surrounding tissues and produce negative contrast in T2-MRI. We hypothesize that intravenous administration of SPIO into mammary tumor-bearing mice will result in T2-MRI-detectable iron accumulation at the site of inflammation allowing for in vivo TAM imaging. Furthermore, as macrophages exit the primary tumor site they will travel to the lymph node via newly formed or existing lymph vessels. Thus, we predict that benign lymph nodes will contain macrophages and retain the iron contrast. In contrast, malignant lymph nodes will accumulate tumor cells, which will partially replace the immune cell component, and fail to retain the iron. To test this hypothesis we propose the following steps:

Step 1: Determine a threshold for T2/T2* relaxation times that changes as a function of iron accumulation in TAMs (inflamed BCa) and in negative vs. positive LNs.

Step 2: Validate inflame-invasion BCa imaging scores for inflamed BCa in correlation with positive LNs.

Step 3: Establish specificity and sensitivity of the qT2wMRI for inflamed disseminated BCa using anti-inflammatory and anti-lymphangiogenic treatment.

Step 4: Correlate the inflame-invasion BCa imaging score with standard IHC/flow cytometry scores for inflammation, lymphangiogenesis and LN staging.
Traci R Lyons, the applicant of the Phase I proposal, is an expert in breast cancer and lymphangiogenesis. Her group is investigating the role of the tumor microenvironment in pre-clinical models of breast cancer with a goal of translation into clinical breast cancer research\textsuperscript{2,7}. A validated non-invasive technique, which allows for the longitudinal assessment of lymph node status in mouse models of inflamed breast cancer is crucial for the existing grants of Dr. Lyons’ group (NCI:R21CA185226-01; NIH:5KL2TR001080-02). By accomplishing the goals outlined in this proposal we will establish a solid background for applying for large R01-type of grants. Dr. Lyons is requesting Natalie Serkova’s expertise (the Phase II investigator) for developing a non-invasive CE T2-MRI method for lymphatic and lymph node imaging. Dr. Serkova is director of the UCCC/CCTSI Animal Imaging Core; her recent studies on C3- complement imaging in mouse models of renal inflammation using targeted SPION (4, 5) will provide a sound ground for the Phase II Method Development.

**Significance:** Clinically, LN positivity is a first step for detecting tumor dissemination and LN positivity remains a main prognostic indicator for breast cancer patients. Tumor-associated LNs (anatomically known as “axillary” LNs) and lymphatic drainage (known as lymphatics) are a major pathway leading to metastases of breast carcinomas\textsuperscript{8}. In addition, primary BCa often exhibit an inflamed phenotype, which is characterized by the presence of tumor-associated macrophages TAMs in tumor microenvironment\textsuperscript{1,9,10} and correlates with poor prognosis\textsuperscript{11-14}. This inflamed milieu induces increased lymphangiogenesis, which are used by cancer cells to travel to the axillary LN, to distant LNs, the bloodstream and other vital organs\textsuperscript{1,15}. To determine if a LN is positive for malignancy, axillary LNs are removed during surgeries or needle biopsies are taken for pathologists’ evaluations. However, sequential biopsies to monitor the LN status come at great cost to patients and often are prohibitive. In addition, it is almost impossible to reliably biopsy all axillary LNs for suspicious BCa spread. MRI using gadolinium contrast is routinely employed clinically to determine margins and vascularity of the primary BCa, but it is not sensitive enough to be used routinely for evaluation of LNs as benign or malignant. As of today, non-invasive means to determine the malignancy status of axillary LNs in BCa are not clinically available. Based on our preliminary data, and supported by existing clinical reports, we hypothesize that utilization of an alternative MRI contrast agent, will allow physicians to simultaneously characterize the inflamed and lymphogenic status in BCa patients based on the appearance of primary lesions and axillary LNs on post-contrast MRI scans. Quantitative physiological imaging is the future in oncologic radiology. The proposed inflamed-invasion BCa imaging score, once established will provide a quantitative platform for diagnostic and prognostic BCa imaging.

MRI is a non-invasive imaging procedure, which is routinely used in BCa patients. Herein, we propose to use FDA-approved SPION (Ferumoxytol) as our T2-MRI contrast for inflammation and LN staging, which can be readily translated into human trials\textsuperscript{16}. Ferumoxytol is safe for use in humans and routinely used as intravenous iron supplement in patients with renal failure; we have experience using Ferumoxytol off-label as an MRI contrast in clinical trials (with all precautions after issuing the FDA black box-warning). As such, the proposed animal MRI studies will provide impetus for novel imaging modalities to be directly translated into clinical imaging protocols on BCa patients in order to stage inflammatory microenvironment and to monitor lymphatic spread and LN metastasis. The ultimate long-term goal of this proposal is to simultaneously measure LN involvement (non-enhancing LNs) and tumor inflammation (enhancing primary BCa lesions) non-invasively in patients with breast cancers undergoing novel anti-inflammatory and anti-lymphangiogenic treatment (in collaboration with Virginia Borges, MD).

**Innovation:** Metastatic spread in breast cancer patients represents one of the major challenges in both medical oncology as well as oncologic imaging. MRI is one of the major imaging techniques for patients with breast cancer. Most recently utilized protocols are gadolinium-based (T1-contrast for imaging of cancer margins and vascularity) which have low sensitivity in detecting LN involvement. This projects aims to establish a unique non-invasive quantitative qT2mMRI approach for real-time cross-talk between inflammation and LN staging in mouse inflamed BCa models based on iron-oxide induced decrease in T2/T2* relaxation times. As of today, only ex vivo protocols are widely available on collected specimens. While very first reports on TAMs imaging with SPION have recently appeared\textsuperscript{17}, to the best of our knowledge, there is not a single animal MRI study on interplay between TAMs and LN metastases using SPION-enhanced T2-MRI in any kind of cancer. The innovation of establishing of an inflame-invasion BCa score from a quantitative MRI approach is remarkable. In parallel, we are also working on synthetizing molecular SPION tracers to target specific surface markers for M1- and M2-macrophages for molecular imaging by qT2wMRI.


