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Molecular Imaging of Breast Cancer Lymph Node Metastasis

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Axillary lymph node (ALN) status is a key determinant of prognosis and adjuvant therapy in breast cancer. Pre-operative lymph node mapping for sentinel lymph node (SLN) identification and surgical biopsy for standard histologic analysis remains the current gold standard for the evaluation of ALNs in breast cancer.^{1, 2} Numerous un-targeted agents have been used to map or identify SLNs including sulfur colloids, blue dyes and nanomaterials. However, these untargeted probes collect non-selectively into the draining lymph nodes with only transient and non-specific visualization of the lymphatic system.^{3, 4} Surgical biopsy of SLNs is invasive, morbid⁵ and expensive. Further, SLNs in most (74%) clinically node-negative breast cancer patients are also pathologically negative for metastatic disease and removing them provides no therapeutic value to breast cancer patients.⁶ Recent data even suggests that axillary clearance in select node-positive patients has no impact on survival or local recurrence,⁷ and current surgical trends are to minimize axillary surgery for breast cancer due to the potential long-term morbidity.^{8, 9} The accuracy of nodal staging has been challenged by the observed heterogeneity of outcomes for node-negative breast cancer, and high rate of false negatives.^{10, 11}

MRI is a noninvasive imaging modality that can generate high-resolution, multiplanar, three-dimensional (3D) images of any organ of the human body with excellent soft tissue contrast (see¹² for review). Development of an MRI contrast agent that specifically targets only ALNs with metastasis would represent a major advance in the care of breast cancer patients. Such an advance would allow surgeons to transcend the use of sentinel lymph nodes for identification of metastasis, to the point where only those patients with metastasis would be considered for selective ALN surgery to remove metastatic nodes.

By gene-expression profiling of patient tissue samples, we have identified 5 unique cell-surface biomarkers that are highly and broadly expressed among breast cancer lymph node metastases, but are not expressed in normal/unaffected lymph nodes or surrounding tissue (CA9, CA12, CEACAM6, SCGB2A2 and MMP9).^{13, 14} Over-expression, based on normalized DNA microarray intensity values, has shown that as few as three of these

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markers can cover nearly all ALN metastases, where at least one of each is expressed in >99% of axillary metastases of breast cancer.^{13, 14} We have confirmed protein expression for 4 of these markers by immunohistochemistry (IHC) of patient ALN metastasis samples in the Moffitt breast cancer tissue microarray (TMA): carbonic anhydrases 9 & 12 (CA9 & CA12), mammaglobin-A (SCGB2A2) and matrix metalloproteinase-9 (MMP9) (Table 1). As a proof of concept, we have used monoclonal antibodies that are highly specific for mammaglobin-A, and carbonic anhydrases 9 & 12 conjugated to near infrared fluorescent dye and in vivo fluorescence imaging to detect orthotopic breast cancer ALN metastases in nude mice. Following intravenous injection, we have shown that these imaging probes have high selectivity for retention in marker expression tumors compared to non-expressing tumors (Figure 1A), and following peri-tumoral injection into the mammary fat pad, we have shown that these antibody probes are retained in lymph node metastases that express the target protein (Figure 1B) and that as few as 1000 cells are detectable (Figure 1C).

In conclusion, this method may be translated for use in the clinic by combining it with other more established imaging modalities such as MRI by developing gadolinium-based T1 contrast agents such as Gd-fullerenes or -nanotubes, or biocompatible super-paramagnetic iron oxide nanoparticles as T2-weighted contrast agents conjugated to these targeting antibodies¹⁵. Such agents could be used for the pre-operative non-invasive detection of ALN metastasis with high sensitivity and specificity, sparing the majority of patients from surgery; or in the case of surgery, to generate a map of positive nodes prior to surgery. Further labeling of these probes with a radionuclide or NIR fluorescent dye could guide the surgeon to the node in real-time using a gamma-counting wand or intra-operative real-time fluorescence imaging.

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References

- [1]. Borgstein PJ, Meijer S, Pijpers R. Intradermal blue dye to identify sentinel lymph-node in breast cancer. *Lancet*. 1997; 349(9066):1668–9. [PubMed: 9186389]
- [2]. Maza S, Valencia R, Geworski L, et al. Peritumoural versus subareolar administration of technetium-99m nanocolloid for sentinel lymph node detection in breast cancer: preliminary results of a prospective intra-individual comparative study. *Eur J Nucl Med Mol Imaging*. 2003; 30(5):651–6. [PubMed: 12612811]
- [3]. Galanzha EI, Kokoska MS, Shashkov EV, Kim JW, Tuchin VV, Zharov VP. In vivo fiber-based multicolor photoacoustic detection and photothermal purging of metastasis in sentinel lymph nodes targeted by nanoparticles. *J Biophotonics*. 2009; 2(8-9):528–39. [PubMed: 19743443]
- [4]. McElroy M, Hayashi K, Garmy-Susini B, et al. Fluorescent LYVE-1 antibody to image dynamically lymphatic trafficking of cancer cells in vivo. *J Surg Res*. 2009; 151(1):68–73. [PubMed: 18599080]
- [5]. Purushotham AD, Upponi S, Klevesath MB, et al. Morbidity after sentinel lymph node biopsy in primary breast cancer: results from a randomized controlled trial. *J Clin Oncol*. 2005; 23(19): 4312–21. [PubMed: 15994144]
- [6]. Krag DN, Anderson SJ, Julian TB, et al. Technical outcomes of sentinel-lymph-node resection and conventional axillary-lymph-node dissection in patients with clinically node-negative breast

- cancer: results from the NSABP B-32 randomised phase III trial. *Lancet Oncol.* 2007; 8(10):881–8. [PubMed: 17851130]
- [7]. Giuliano AE, Hunt KK, Ballman KV, et al. Axillary dissection vs no axillary dissection in women with invasive breast cancer and sentinel node metastasis: a randomized clinical trial. *JAMA.* 2011; 305(6):569–75. [PubMed: 21304082]
- [8]. Langer I, Guller U, Berclaz G, et al. Morbidity of sentinel lymph node biopsy (SLN) alone versus SLN and completion axillary lymph node dissection after breast cancer surgery: a prospective Swiss multicenter study on 659 patients. *Ann Surg.* 2007; 245(3):452–61. [PubMed: 17435553]
- [9]. Wasif N, Maggard MA, Ko CY, Giuliano AE. Underuse of axillary dissection for the management of sentinel node micrometastases in breast cancer. *Arch Surg.* 2010; 145(2):161–6. [PubMed: 20157084]
- [10]. McMasters KM, Tuttle TM, Carlson DJ, et al. Sentinel lymph node biopsy for breast cancer: a suitable alternative to routine axillary dissection in multi-institutional practice when optimal technique is used. *J Clin Oncol.* 2000; 18(13):2560–6. [PubMed: 10893287]
- [11]. Ung OA. Australasian experience and trials in sentinel lymph node biopsy: the RACS SNAC trial. *Asian J Surg.* 2004; 27(4):284–90. [PubMed: 15564180]
- [12]. Tafreshi NK, Kumar V, Morse DL, Gatenby RA. Molecular and functional imaging of breast cancer. *Cancer Control.* 2010; 17(3):143–55. [PubMed: 20664511]
- [13]. Tafreshi NK, Bui MM, Bishop K, et al. Noninvasive detection of breast cancer lymph node metastasis using carbonic anhydrases IX and XII targeted imaging probes. *Clin Cancer Res.* 2012; 18(1):207–19. [PubMed: 22016510]
- [14]. Tafreshi NK, Enkemann SA, Bui MM, et al. A mammaglobin-A targeting agent for noninvasive detection of breast cancer metastasis in lymph nodes. *Cancer Res.* 2011; 71(3):1050–9. [PubMed: 21169406]
- [15]. Matson ML, Wilson LJ. Nanotechnology and MRI contrast enhancement. *Future Med Chem.* 2010; 2(3):491–502. [PubMed: 21426177]

Figure 1.

Representative images demonstrating *in vivo* positive tumor selectivity of the probe following intravenous injection (in this case a monoclonal mammaglobin-A specific antibody was conjugated to Vivo-Tag-S 680 from Perkin-Elmer, termed MamAb-680) and detection of ALN metastases following injection into the mammary fat pad (MFP) and clearance through the lymphatics. (A) *In vivo* fluorescence image of mouse bearing a mammaglobin-A positive MFP xenograft tumor (ZR-75.1 cells), and negative tumor (MDA-mb-231), 24 hours post-injection of the probe. Note the bright signal in the positive tumor and that the probe has cleared from the entire mouse, including the negative tumor at this time point. (B) Fluorescence image of a mouse bearing a primary MFP tumor and a spontaneous ALN metastasis, 24 h after injection of MamAb-680 probe into the MFP. (C) Fluorescence image following ultrasound-guided injection of a known number of cells into the ALN and 24 h after MFP injection of probe.

Table 1

Percentage of ALN metastases with robust expression for each marker. Inset shows representative staining for CAXII.

Marker	IHC score 4
CAIX	27%	
CAXII	39%	
MMP9	40%	
Mammaglobin-A	22%	