

**Title:** Non-Invasive In-Vivo Detection of HER2 Overexpression in Breast Cancer Using Dual-Labeled Trastuzumab-Based Imaging Agent

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## **Introduction**

The Human Epidermal Growth Factor Receptor (HER) family comprises of four trans-membrane receptor tyrosine kinases that are involved in intracellular signaling pathways that regulate cell growth and differentiation. The second member, HER2, is of particular importance in breast cancer because overexpression or gene amplification of HER2 is closely associated with aggressive tumor progression and poor prognosis. This affects approximately 25% of breast cancer patients. Biopsy of tissue samples continues to be the gold standard for analyzing tumor profiles of breast cancer patients. However, there is a need for non-invasive imaging agents to assess disease markers and monitor response to therapy. This will play a major role in personalized medicine.

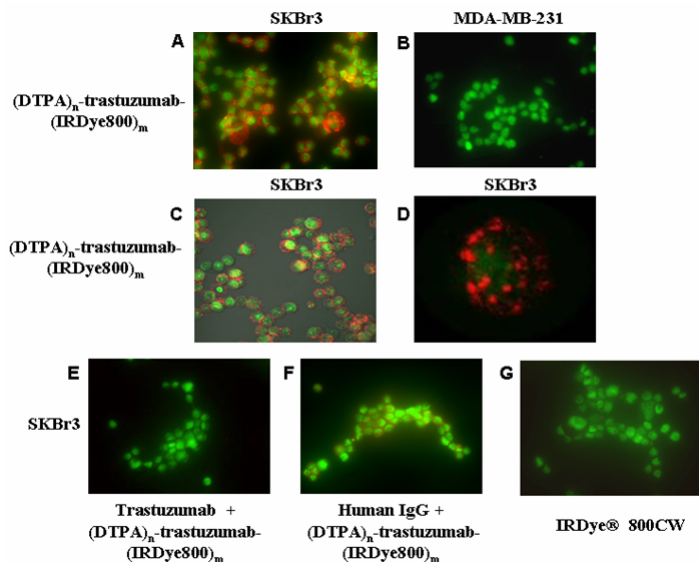
*During the last year, I have been working on the synthesis and characterization of a novel dual-labeled nuclear and optical agent to detect HER2 overexpression in-vivo. This agent is designed for clinical translation; and in this summary I will present preclinical experimental data that validate the specificity of the agent in-vitro and in-vivo in a murine model.*

Herceptin (trastuzumab) is a humanized anti-HER2 antibody which is currently involved in numerous clinical trials as a therapeutic agent. Owing to the importance of HER2 in breast cancer, several investigators have developed trastuzumab-based imaging agents as a diagnostic marker against HER2. For review, see Sampath et al. (J Nuc Med, in press) [1]. Recently, 111-Indium labeled trastuzumab has been clinically translated for scintigraphy to identify HER2 positive tumors [2].

*In this study, we have exploited the extracellular binding property of trastuzumab to synthesize (<sup>111</sup>In-DTPA)<sub>n</sub>-trastuzumab-(IRDye800)<sub>m</sub>, a dual labeled probe that employs (i) the stable emission of an NIR dye, IRDye 800CW, that has no finite half-life (ii) the gamma emission of Indium-111 for scintigraphy employed in sentinel lymph node mapping (SLNM); and (iii) the opportunity to cross validate NIR and nuclear imaging modalities. We expect that the NIR fluorescent reporter to aid in the intraoperative identification of HER2 positive lymph nodes.*

## **In-vitro Characterization of (<sup>111</sup>In-DTPA)<sub>n</sub>-trastuzumab-(IRDye800)<sub>m</sub>**

Human breast cancer cells that either (i) express high levels of HER2 (SKBr3) or (ii) do not express HER2 (MDA-MB-231) were grown on 10 cm tissue culture dish until they reached 95% confluence. Cells were then trypsinized, washed in excess PBS and resuspended in 100µl of culture medium with IRDye 800CW (1µM) or (DTPA)<sub>n</sub>-trastuzumab-(IRDye800)<sub>m</sub> (1µM equivalent IRDye 800CW). For the blocking study, SKBr3 cells were pre-treated with Herceptin (22µM) or human IgG (22µM) for 30 minutes at 37°C, followed by (DTPA)<sub>n</sub>-trastuzumab-(IRDye800)<sub>m</sub> treatment for 1 hour at 37°C. At the end of this incubation period, the cells are washed twice with PBS and incubated in a solution of Sytox Green in 95% ethanol (1 µM; Molecular Probe, Eugene, OR) for 15 min to fix and stain cell nuclei.

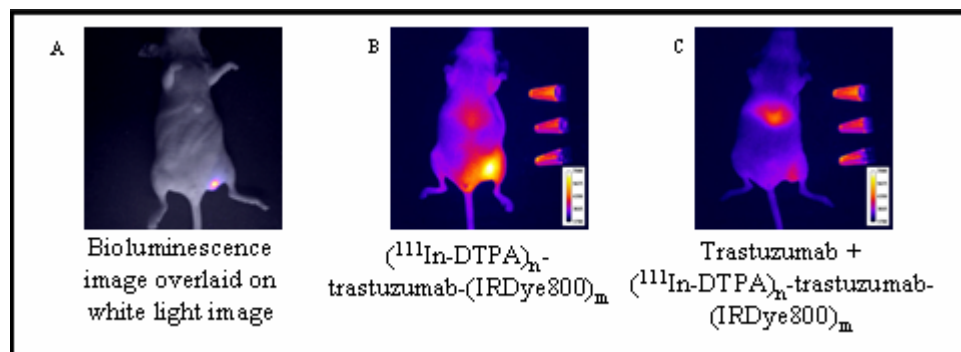


**Figure 1** compares the fluorescence images of SKBr3 cells and MDA-MB-231 incubated with  $(\text{DTPA})_n\text{-trastuzumab-(IRDye800)}_m$ . The imaging agent binds with high specificity to SKBr3 cells but not to MDA-MB-231 cells (Fig. 1, A and B). Confocal microscopy imaging of SKBr3 cells confirm that the agent bound to the extra-cellular domain of the cell (Fig. 1C) and appears as spots or clusters on the surface membrane as seen in higher magnification (Fig.1D). Furthermore, binding of  $(\text{DTPA})_n\text{-trastuzumab-(IRDye800)}_m$  was abolished when

SKBr3 cells were pre-treated with 200x molar excess of trastuzumab (Fig. 1E), but not when pre-treated with the same amount of non-specific human IgG (Fig. 1F). As a measure of control, SKBr3 cells incubated with an equivalent dose of free IRDye 800CW showed negligible binding (Fig. 1G).

### **In-vivo Characterization of $(^{111}\text{In-DTPA})_n\text{-trastuzumab-(IRDye800)}_m$**

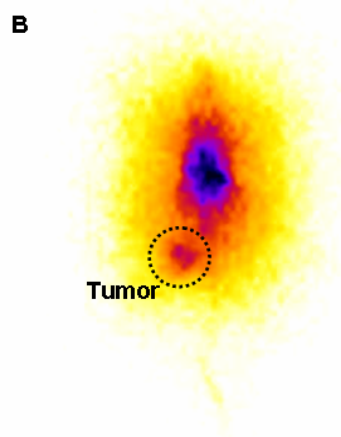
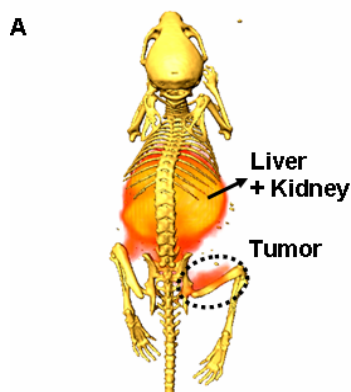
Three- to four-week-old female athymic nude mice were inoculated subcutaneously in the left flank with SKBr3-luc cells ( $2\text{--}3 \times 10^6/\text{animal}$ ). Initial tumor growth was monitored using bioluminescence imaging. When the tumors reached a size of  $5\text{--}8\text{mm}^3$ , whole body fluorescence images were taken at 24 and 48 hours after i.v. injection of  $(^{111}\text{In-DTPA})_n\text{-trastuzumab-(IRDye800)}_m$  (0.43 nmole,  $80\mu\text{g}$ ). A detailed description of the *in vivo* optical fluorescence imaging system used in this study has been previously described [3]. For the blocking study, animals were pre-injected with 200x molar excess of trastuzumab (86nmole, 16mg) 24 hours prior to administering  $(^{111}\text{In-DTPA})_n\text{-trastuzumab-(IRDye800)}_m$ . At 48 hours, mice were sacrificed and fluorescence imaging was performed on the excised organs. For quantitative comparison, ratios of fluorescence signal intensities in region-of-interest (ROIs) corresponding to the tumor and normal tissue regions were determined.



**Figure 2** shows a representative whole body fluorescence image on mice inoculated with SKBr3-luc tumors taken 48 hours after administration of

$(^{111}\text{In-DTPA})_n\text{-trastuzumab-(IRDye800)}_m$  (Fig. 2B). Bioluminescence images overlaid on a white light image enables easy identification of tumor location (Fig. 2A). The fluorescent signal intensities observed in the tumor region of these mice is significantly higher than the rest of the

body. In contrast, there is a significant decrease in fluorescent intensity in the tumor bearing mice which were pre-injected with trastuzumab (Fig. 2C) 24 hours prior to administration of  $(^{111}\text{In-DTPA})_n\text{-trastuzumab-(IRDye800)}_m$ .



SPECT/CT images were acquired consecutively using MicroCAT II (Siemens, Knoxville, TN) at 48 hr post injection of  $(^{111}\text{In-DTPA})_n\text{-trastuzumab-(IRDye800)}_m$  (1nmole, 200 $\mu\text{g}$ , 200 $\mu\text{Ci}$ ). SPECT scan was acquired for 20 projections over 360 degrees for a scan time of 1 minute per frame. SPECT/CT tomographic images were coregistered by geometric transformation and rendered to make the fused images using Amira (version

3.1, Konrad-Zuse-Zentrum fur Informationstechnik Berlin, Germany). 2-D scintigraphy images were acquired 24 hr after injecting  $(^{111}\text{In-DTPA})_n\text{-trastuzumab-(IRDye800)}_m$  (0.22nmole, 40 $\mu\text{g}$ , 50 $\mu\text{Ci}$ ). Images were acquired after removing the pin-hole collimator and integrating for 10 minutes. **Figure 3** shows accumulation of  $(^{111}\text{In-DTPA})_n\text{-trastuzumab-(IRDye800)}_m$  in the SKBr3-luc xenograft as illustrated by SPECT/CT (Fig. 3A) and 2-D scintigraphy (Fig. 3B)

## Conclusion

Preclinical studies show that dual labeled  $(^{111}\text{In-DTPA})_n\text{-trastuzumab-(IRDye800)}_m$  may be an effective diagnostic biomarker capable of tracking HER2 overexpression in breast cancer patients.

## Acknowledgement

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Lakshmi Sampath, Sunkuk Kwon, Shi Ke, Wei Wang, Rachel Schiff, Michel E. Mawad, and Eva M. Sevick-Muraca. "Dual-Labeled Trastuzumab-Based Imaging Agent for the Detection of Human Epidermal Growth Factor Receptor 2 Overexpression in Breast Cancer". *J Nucl Med.* 2007 48(9): 1501-1510. Figures 1-3

## References:

1. Sampath, L., et al., *Dual-labeled trastuzumab-based imaging agent for detection of HER-2 overexpression in breast cancer.* *J Nuc Med*, 2007 **48**(9): p. 1501-10.
2. Perik, P.J., et al., *Indium-111-labeled trastuzumab scintigraphy in patients with human epidermal growth factor receptor 2-positive metastatic breast cancer.* *J Clin Oncol*, 2006. **24**(15): p. 2276-82.
3. Ke, S., et al., *Near-infrared optical imaging of epidermal growth factor receptor in breast cancer xenografts.* *Cancer Res*, 2003. **63**(22): p. 7870-5.