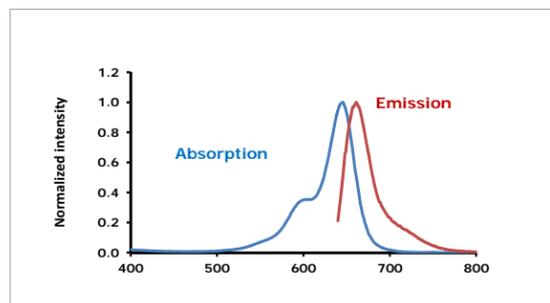


Abstract

Upregulation of tumor HER2 occurs in approximately 25% women with breast cancer and is often associated with poor prognosis. We developed a red fluorescent imaging agent to non-invasively image and quantify tumor-associated HER2 expression *in vivo*. A red fluorescent dye (VivoTag 645; $\epsilon=210,000$ M/cm; abs/em max 643/660 nm) was used to label trastuzumab, which is currently used to treat breast and stomach cancer. The red-labeled trastuzumab (VM4003) preferentially labeled HER2⁺ SKOV-3 human ovarian adenocarcinoma cells over HER2⁻ human colorectal adenocarcinoma Colo-205 cells (10 fold), and the specificity of binding was confirmed by control experiments using free dye, labeled non-specific IgG, and competitive blockade with unlabeled excess trastuzumab. Fluorescence microscopy confirmed the expected membrane localization of fluorescence. *In vivo* and *ex vivo* imaging by fluorescence molecular tomography (FMT), showed significantly higher signal within the tumors, peaking at 6-72 hours following intravenous injection of 2 mg/kg VM4003, decreasing thereafter with a tissue half-life of 3 days. *In vivo* quantification of tumor signal in nude mice showed significantly higher tumor signal in HER2⁺ than in HER2⁻ tumors (14.36 ± 4 versus 2.39 ± 0.74 pmol, at 6h imaging time, $p=0.007$; 18.77 ± 4.45 versus 3.50 ± 0.98 pmol, at 24h, $p=0.001$). Specificity of targeting was confirmed by competition with excess intravenous unlabeled trastuzumab, which achieved 70% signal inhibition in the tumors (tumor signal 16.12 ± 3.03 versus 4.72 ± 1.96 pmol, $p=0.022$ at 24h). Tumor volumes, as determined by direct measurements of tumor size, were comparable between both groups of mice ($p=0.193$). Fluorescence microscopy of *ex vivo* frozen tissue sections confirmed tumor fluorescence and signal localization associated to cell membranes and cytoplasm. In summary, red fluorescent-labeled trastuzumab selectively targets HER2, allowing both imaging *in vitro* and the non-invasive real-time tomographic imaging and quantification *in vivo* of HER-2 expression.

1 Description of the Agent

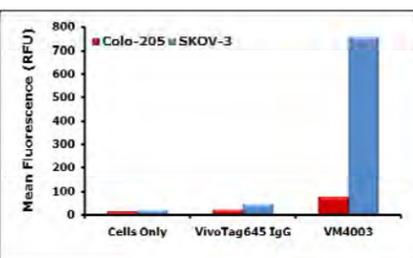
Absorption and Emission Spectra



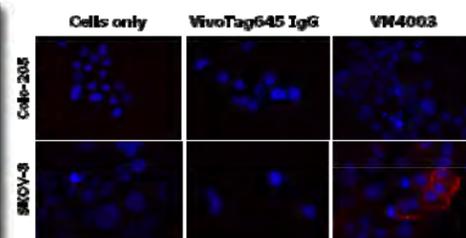
The agent has an absorption peak at 645 nm and emission peak at 661 nm in 1x PBS.

Cell labeling

a. Flow Cytometry



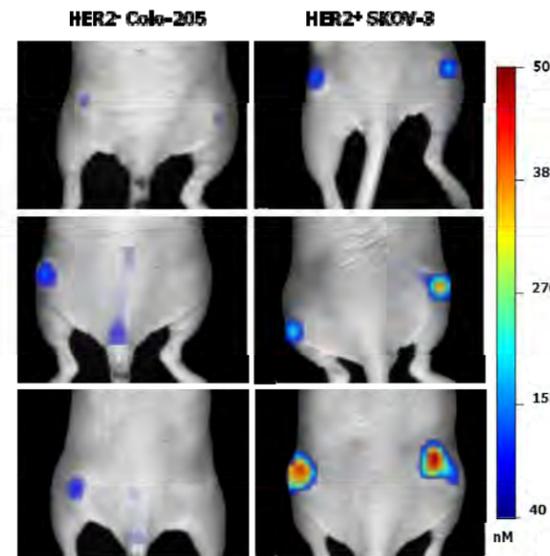
b. Fluorescence Microscopy



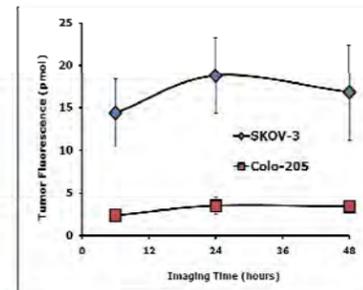
SKOV-3 (HER2⁺) or Colo-205 (HER2⁻) were incubated with VM4003 (0.25 μ M), VivoTag 645-labeled IgG or VivoTag 645 (not shown) for 1h. Cells were analyzed by a. flow cytometry and b. fluorescence microscopy using appropriate lasers and filters appropriate for detection of 645 nm wavelength. VM4003 only labels SKOV-3 cells (shown in red). Cell nuclei are stained with DAPI (blue).

2 FMT and Quantification

a. Imaging SKOV-3 and Colo-205



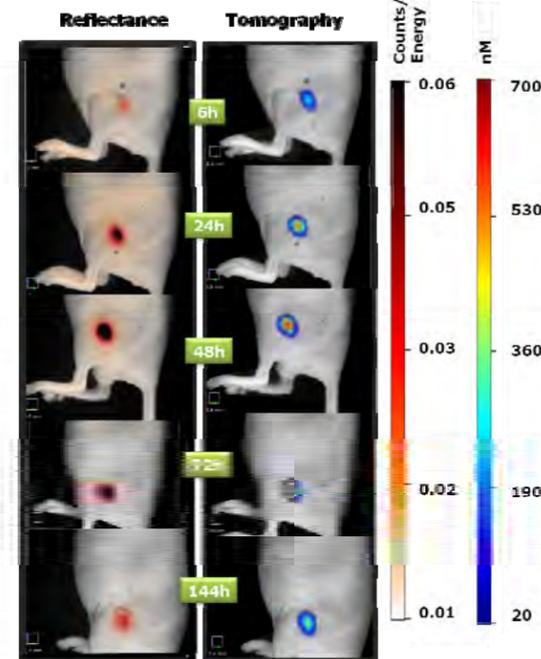
b. Quantification



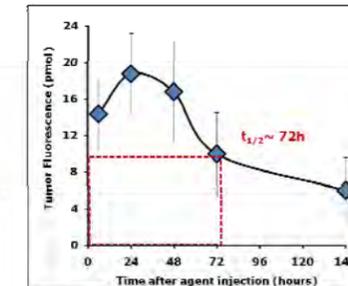
Nu/Nu mice were implanted with human ovarian SKOV-3 or colorectal Colo-205 tumors. When tumors reached desired volume, mice were injected intravenously with 2 mg/kg VM4003 and imaged tomographically (FMT 2500LX) at 6, 24 and 48h. a. Shown are 3 representative mice per group at 24h. b. Tomographic imaging datasets were used to quantify tumor region fluorescence associated with HER2 expression.

3 Imaging kinetics in SKOV-3

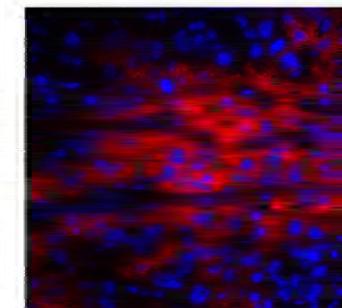
a. Imaging



b. Quantification



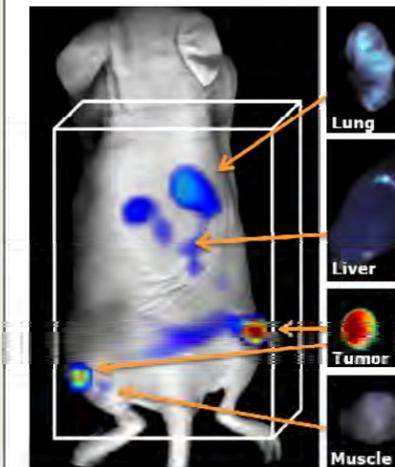
c. Localization



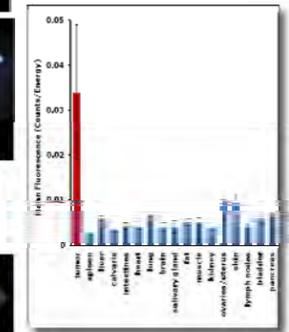
a. Reflectance and tomographic images of the same SKOV-3 tumor-bearing mouse taken at different times after VM4003 injection. b. Quantification of the tumor signal (pmoles) and c. localization of the fluorescent signal in a tumor section taken at 24h (Blue: DAPI nuclear staining, Red: VM4003).

4 Pharmacokinetics and Biodistribution

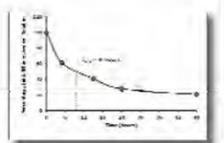
a. Biodistribution



SKOV-3 tumor-bearing mice were injected with VM4003 and imaged 24h later. Shown on the left is a full-body tomographic scan of a representative mouse and the corresponding planar images of highlighted tissues. Organs were then collected and fluorescence assessed by planar imaging (below). Mean counts/energy for each tissue were determined as a measure of tissue brightness.



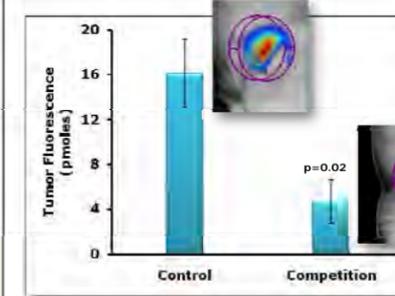
a. Pharmacokinetics



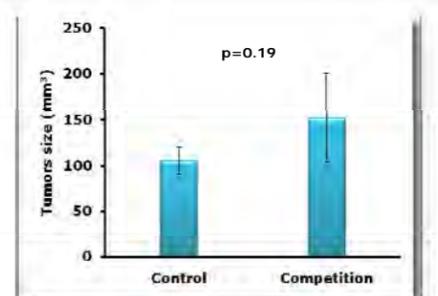
Pharmacokinetic profile was assessed by injecting CD1 mice (n=3 per time point), with VM4003, collecting blood at multiple times post-injection, and measuring the plasma fluorescence.

5 Competition

a. FMT



b. Tumor volume



a. Tomographic imaging datasets were used to quantify tumor fluorescence in SKOV-3 tumor-bearing mice injected with VM4003 (2 mg/kg) in the absence (controls) or presence (competition) of unlabeled trastuzumab (100 mg/kg) injected 5 min before VM4003. Tumor volumes were determined using caliper measurements and the formula volume = length x width²/2.

Summary

The rising field of optical imaging has the capability to provide significantly more information about different physiological and pathological states. It offers the possibility to image disease at its earliest onset compared to traditional anatomical imaging approaches. Targeted imaging agents use specific targeting moieties to detect biological markers in tissue, providing a source of image contrast with more detailed and earlier information compared to morphologic images. In these studies, we have explored a HER2 imaging agent (VM4003) as a useful biomarker of breast cancer in a relevant experimental model. The capacity to spatiotemporally visualize and quantify tissue HER2 levels *in vivo* using this targeted antibody-based fluorescent agent and quantitative imaging approach will greatly improve the ability to assess tumor development and metastasis, to develop novel therapies, and to monitor treatment efficacy longitudinally in breast cancer.

6 References

- Harari D, Yarden Y. Molecular mechanisms underlying ErbB2/HER2 action in breast cancer. *Oncogene* 2000; 19: 6102-6114.
- Valabrega G, et al. Mechanism of action, resistance and future perspectives in HER2-overexpressing breast cancer. *Ann. Onc.*, Jun 2007; 18: 977 - 984.