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A paired-agent fluorescent molecular imaging strategy for quantifying antibody drug target engagement in *in vivo* window chamber xenograft models

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ABSTRACT

A paired-agent fluorescent molecular imaging strategy is presented as a method to measure drug target engagement in whole tumor imaging. The protocol involves dynamic imaging of a pair of targeted and control imaging agents prior to and following antibody therapy. Simulations demonstrated that antibody "drug target engagement" can be estimated within a 15%-error over a wide range of tumor physiology (blood flow, vascular permeability, target density) and antibody characteristics (affinity, binding rates). Experimental results demonstrated the first in vivo detection of binding site barrier, highlighting the potential for this methodology to provide novel insights in drug distribution/binding imaging.
Keywords: paired-agent, fluorescent imaging, window chamber, binding potential.

1. INTRODUCTION

Since the advent of the first clinically approved anti-CD20 antibody rituximab, receptor-specific cancer therapy with monoclonal antibodies (mAbs) has become a major mode of treatment in modern oncology. Several different surface receptor-specific mAbs have become clinically approved for the treatment of different cancers, such as Herceptin (trastuzumab) for the treatment of HER2-positive breast cancers, as well as Erbitux (cetuximab) for the treatment of EGFR-positive head and neck cancer (1). By binding to a specific antigen or overexpressed receptor, mAbs are able to facilitate the destruction of cancer cells via mechanisms such as antibody-dependent cell-mediated cytotoxicity (ADCC) (2).

In the field of cancer imaging, labeled-mAbs have been vital to the development of imaging methods to quantify tumor dynamics such as drug extravasation into tumors and tumor receptor expression. By exploiting the tumor-to-normal tissue contrast from overexpression of specific antigens by cancer cells, numerous groups have attempted to image drug extravasation and binding dynamics in tumors (3-5). However, because of the relatively large size of antibodies (150-180 kDa), mAb-based therapeutics have shown limited tumor penetration and effectiveness, even in patients with antigen-positive tumors. This is because antibodies are often immobilized shortly after extravasation into the interstitial space, resulting in minimal penetration into solid tumors. Consequently, central portions of the tumor can go untreated resulting in the growth of treatment resistant cancer cells and increased intratumoral receptor heterogeneity (6). This phenomenon, termed by Fujimori et al. as "binding site barrier" (7), results from a multitude of factors such as antigen density, binding affinity, antibody molecular weight, abnormal tumor vasculature, and increased tumor interstitial pressure (8).

In order to improve penetration, different teams have developed "small-molecule" affibody-based treatments to act as both primary therapeutics and in conjunction with existing mAb therapeutics (9-10). Affibodies are highly specific small proteins (<12kDa) that have been engineered to target a growing library of antigens, such as HER2 and EGFR. Due to their smaller size, affibodies exhibit increased diffusivity into tumors and the effects of binding site barrier are

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negligible. Additionally, affibodies may be capable of interacting with difficult to reach antigens or bind to active sites normally inaccessible to bulky antibodies (10). Using these smaller molecules, Davis et al. developed a non-invasive method for assessing available receptor density in EGFR expressing cancer cells using a paired-agent fluorescent imaging technique (11). In this technique, two fluorescently labeled tracers are injected simultaneously. One of the tracers is targeted to a specific receptor, allowing for measurement of specific uptake of the tracer. The second tracer is left untargeted, allowing for measurement of nonspecific uptake. By accounting for both specific and nonspecific uptake of the therapeutic, a parameter called binding potential (BP) can be extracted from the tumor tissue. This study aims to track whole tumor BP throughout before and after antibody therapy, allowing for the first *in vivo* demonstration of binding site barrier.

2. METHODS AND RESULTS

2.1 Animal Experiment

In this cancer targeted imaging study , 3 months old, female athymic nude mice are used. To perform the window chamber surgery, mouse is anesthetized on a heated bed with isoflurane and oxygen (3% induction, 2% continuos). 5mm skin biopsy puncher is used to remove skin from right flank. 8mm diameter acrylic window chamber is placed underneath the skin edges then the disk is sutured to the skin. Antibiotic ointment is applied around the sutures, and the flank is covered by transparent wound film dressing (Bioclusive Plus ®). Buprenorphine hydrochloride (0.3 mg.ml -1) is diluted and 100 ml 0f 0.1 mg/kg mouse is injected subcutaneously to the mouse. One day after, the same procedure is performed for left flank on same mouse.

5 days after surgery, subcutaneous tumor implantation is done for right flank with 10^6 human glioma cell line (U251). Left flank have no cells to be control.

2.2 Paired-agent fluorescent molecular imaging

When the tumor size is reached to 1 cm diameter (10 day after implantation), paired-agent fluorescent molecular imaging is done to measure of imaging agent target engagement in whole tumor imaging. Dynamic imaging of trace levels of a pair of targeted and control small imaging agents for tumor and control sites are demonstrated in this study.

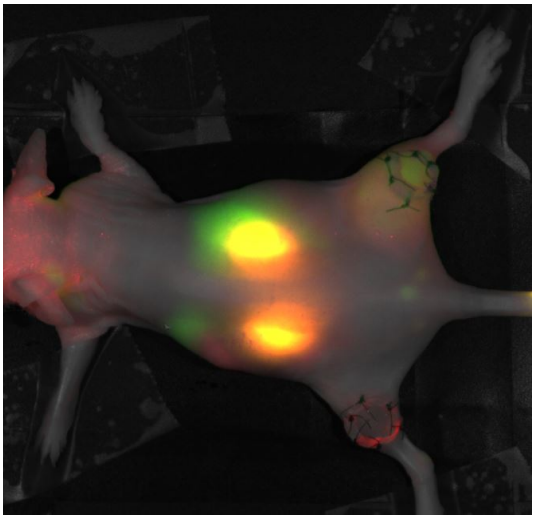
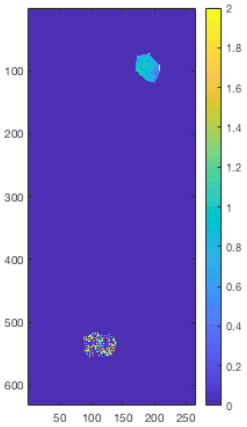
a  b 

Figure 1. a. Mouse image was taken at 100th min in PEARL ®. The color of right flank window chamber, which has tumor, is bright green shows uptake of targeted imaging agents are high here. The color of left flank window chamber, which is control, does not have any green color shows there is no targeted imaging agents uptake here. (Kidneys are bright due to accumulation of imaging agents) b. BP map for both site.

Imaging agents are administrated intravenously after the mouse is anesthetized. IRdye-700 and Negative control-affibody (100 ul of 0.2 nmoll) conjugation is used as untargeted imaging agent, IRDye-800 and ABY029 conjugation (100 ul of 0.2 nmoll) is used as targeted imaging agent. Both of them are injected together to the tail vain.

After the injection of imaging agents, images are taken during 100 min with 2 min intervals between two images. PEARL ® near infrared imaging system is used to take images.

2.3 Uptake of imaging agents

The fluorescent signal intensities in tumor and control sites are reconstructed. The untargeted imaging agent clears more quickly on tumor site than control site because it does not target any specific biomarker in the tumor. When we compare tumor and control site's uptakes, targeted imaging agent binds to specific markers on tumor site while it becomes clear by time on the control site.

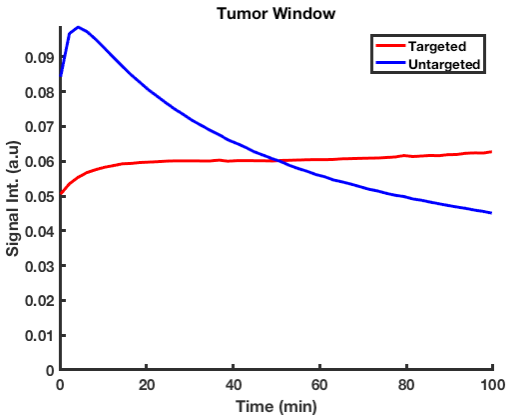


Figure 2.a. Uptake of imaging agents in tumor site

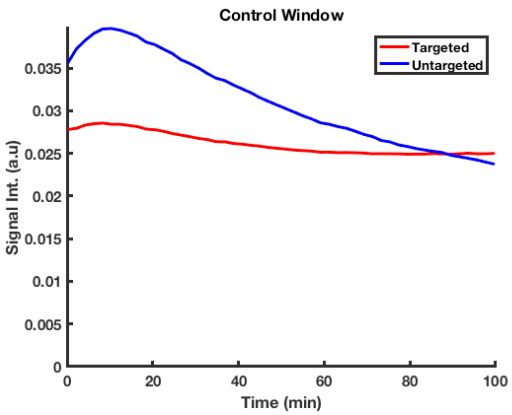


Figure 2.b. Uptake of imaging agent in control site

3. CONCLUSION

This results are very promising to answer "how much antibody-target is enough?", and "how much antibody must be engaged/bound to target?" questions. We presented a paired-agent fluorescent molecular imaging strategy to measure of drug/imaging agent engagement in whole tumor imaging.

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