Purpose: Metastatic breast cancer (MBC) is incurable. The clinical gold standard for assessing tumor microvessel density (MVD), an independent prognostic marker in MBC, is CD105 staining. The goal of this study is to develop a positron emission tomography (PET)/near-infrared fluorescent (NIRF) probe for imaging of CD105 expression in MBC (i.e. non-invasive measurement of MVD), as well as other applications such as early detection of metastasis, intraoperative guidance, etc.

Methods: TRC105, a chimeric anti-CD105 mAb, was dual-labeled with a NIRF dye and $^{89}$Zr to yield $^{89}$Zr-Df-TRC105-800CW. Luciferase-transfected 4T1 murine breast cancer cells were injected intravenously into female BALB/c mice to establish a lung MBC model. Bioluminescence imaging (BLI) was carried out to non-invasively monitor the lung tumor burden. Comprehensive in vivo/ex vivo studies were performed to investigate $^{89}$Zr-Df-TRC105-800CW in this MBC model. Cetuximab was used as an isotype-matched control.

Results: Radiolabeled TRC105 has high tumor uptake in many tumor types in addition to MBC (e.g. pancreatic/prostate cancer and brain tumor), revealing broad clinical potential for TRC105-based agents. FACS analysis of HUVECs showed no difference in CD105 binding between TRC105 and Df-TRC105-800CW. PET imaging revealed that 4T1 lung tumor uptake of $^{89}$Zr-Df-TRC105-800CW was 8.7±1.4, 10.9±0.5, and 9.7±1.1 %ID/g at 4, 24, and 48 h post-injection (n = 4), with excellent tumor contrast. Biodistribution studies, blocking, control studies with $^{89}$Zr-Df-cetuximab-800CW, ex vivo BLI/PET/NIRF imaging, and histology all confirmed CD105 specificity of the tracer. NIRF imaging-guided removal of 4T1 tumors with Df-TRC105-800CW in a subcutaneous model was also straightforward.

Conclusions: We report the first PET/NIRF imaging of CD105 expression in a MBC model. Broad clinical potential of TRC105-based agents was shown in many tumor types, which also enabled early detection of small metastases and provided intraoperative guidance for tumor removal.