Targeted in vivo imaging of tumor vasculature using a near infra-red labeled tomato lectin agent
Jeffrey Morin, Jeannine Delaney, Guojie Ho, Garry Cuneo, Milind Rajopadhye, Wael Yared, Jeffrey D. Peterson, Sylvie C. Kossodo
Life Sciences and Technology, PerkinElmer, Boston, MA

Abstract
Tumor neo-vascularization, characterized by the development of abnormal, leaky, and tortuous blood vessels, represents a key target for cancer imaging and therapy. Among the various recognized tools for measuring microvascular density is tomato (Lycopersicon esculentum) lectin, a single polypeptide glycoprotein that binds to sugar-containing proteins present on the endothelium. The aim of this study was to develop a near infra-red tomato lectin agent to non-invasively assess tumor vasculature in vivo. Conjugation of the near infra-red fluorophore VivoTag 680XL (epsilon=210,000/M/cm; abs/em max 665/688 nm) to tomato lectin was carried out by addition of the fluorophore in a DMSO solution to lectin in aqueous sodium bicarbonate. Fields of greater than 95% were achieved, based on absorption, with a typical loading of 2 dyes per lectin. The resulting agent, TLectinSense™ 680 (TL680), preferentially labeled primary human umbilical vein endothelial cells. Specificity of the binding was validated by control experiments using free dye and competitive blockade with excess unlabeled tomato lectin. In vivo, non-invasive, real-time imaging and quantification of tumor neo-vascularization was performed in two models: matrigel tumors containing bFGF, VEGF and heparin injected into the flank of SKH-1 mice and nude mice bearing Lewis Lung Carcinomas. Using Fluorescence Molecular Tomography (FMT®) 6 hours after TL680 (4 nmoles) intravenous injection, tumor endothelium-associated fluorescence was detected in matrigel plugs and this signal corresponded to labeling of neo-vessels present in the plugs as assessed by fluorescence microscopy. In vivo quantification of tumor fluorescence showed significantly higher signal in flank tumors versus control (non-tumor) contra-lateral flanks (total fluorescence: 50.96 +/-12 versus 2.32 +/- 1. pmol, p=0.005; mean fluorescence concentration: 126.74 +/-7.44 versus 44.22 +/- 3.58 nm, p=0.002). Fluorescence microscopy of frozen tumor sections showed the specific localization of the agent to tumor vessels and this observation was further validated by co-labeling with FITC-labeled CD31. Vessels in other organs such as liver and kidney were also readily detectable. Importantly, tumor fluorescent signal, as quantified by FMT, correlated (R2= 0.99) with vascularization, as assessed by vessel counts using fluorescence microscopy: Lewis Lung Carcinomas 177.6 +/- 15 nm; 27.7 average vessels/field, HT-29 118.1 +/- 16 nm; 13.4 vessels/field, and matrigel plugs 73.6 +/- 9 nm, 5.5 vessels/field. There was also an excellent correlation (R2= 0.99) between CD31 and Lectin 680 signals: Lewis Lung Carcinomas, 27.7 vessels/field with lectin vs. 15.4 vessels/field, and matrigel 7.5 vessels/field vs. 5.5 vessels/field. These results highlight the value of TL680 combined with FMT imaging in assessing vascularity in vivo and in real-time, without termination of mice, excision and processing of the tissue, thus improving the efficacy, early detection and monitoring of anti-angiogenic therapies.

Pharmacokinetics and Biobdistribution
A. Biodistribution

1. Pharmacokinetics
   A. LLC tumor-bearing mice were injected with TL680, imaged tomographically at 6h (whole body, in vivo) and fluorescence quantified in 5 organs. Tissues were then collected and fluorescence assessed by planar imaging (ex vivo). Mean counts/energy for each tissue were determined as a measure of tissue brightness. B. The plasma pharmacokinetic profile was assessed by injecting CD1 mice (3/time point), collecting plasma at multiple times post-injection and measuring plasma fluorescence.

2. Pharmacokinetcs
   A. TL680 was determined by HPLC-UV.

3. Cell Binding
   Cells were incubated with TL680 in the presence or absence of unlabeled lectin (50x concentration, competition) and visualized by fluorescence microscopy. In blue: DAPI nuclear stain, red: TL680, green anti-CD31-FITC.

4. Validation in matrigel plugs
   A. Fluorescence Tomography
      - TL680 anti-CD31-FITC
      - Anti-CD31 -FITC
      - Anti-Cd31- FITC

5. Imaging in Lewis Lung Carcinoma Xenografts
   A. Imaging
      - Anti-CD31-FITC
      - TL680

6. Correlation to anti-CD31
   A. LLc HT29 tumor and matrigel plugs were implanted in mice. A. Once tumors reached the desired size, mice were injected with TL680 and imaged tomographically 6h later, im-implanted mice served as controls. B. Microvessels were counted using TL680 or anti-Cd31-FITC. C. In vivo signal was correlated with microvessel counts performed on frozen sections taken from each sample.

7. References