Targeted in vivo imaging of tumor vasculature using a near infra-red labeled tomato lectin agent

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Abstract

Tumor neo-vasculature, 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 microvessel 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 imaging 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. Yields of greater than 95% were achieved, based on absorbance, 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-vasculature was performed in two models: matrigel plugs containing bFGF, VEGF and heparin injected into the flank of SKH-1 mice and nude mice bearing Lewis Lung Carcinoma tumors. 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 +/- 17.44 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 (R^2= 0.90) 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 +/- 6 nM, 13.4 vessels/field, and matrigel plugs 73.6 +/- 9 nM, 5.5 vessels/field. There was also an excellent correlation (R^2= 0.99) between CD-31 and tLectin 680 signals: Lewis Lung Carcinomas, 27.7 vessels/field with tLectin vs. 32 vessels/field with CD-31; HT-29, 13.4 vessels/field vs. 15.4 vessels/field, and matrigel plugs 5.5 vessels/field vs. 7 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.

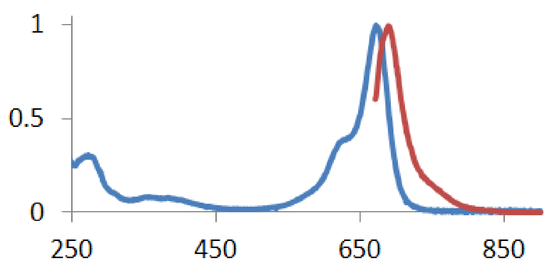
1 Description of the Agent

A. Physicochemical properties

Property	Specification
MW	~ 72,000 g mol^{-1}
Fluorescence[1]	
• Excitation	670 nm
• Emission	690 nm
Absorbance[1]	675 ±10nm
Purity[2]	>95%
Appearance	Blue solid

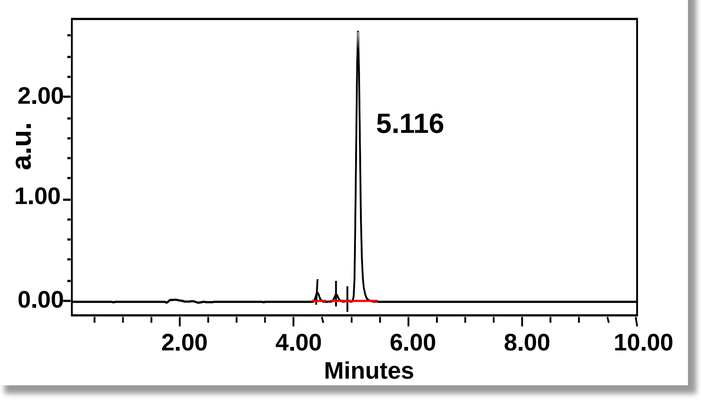
1. Absorbance, excitation, and fluorescence maxima in PBS.
2. As determined by SE-HPLC and measuring absorbance at 675 nm.

B. Absorbance / emission spectra



Abs/Em Spectra in 1X PBS

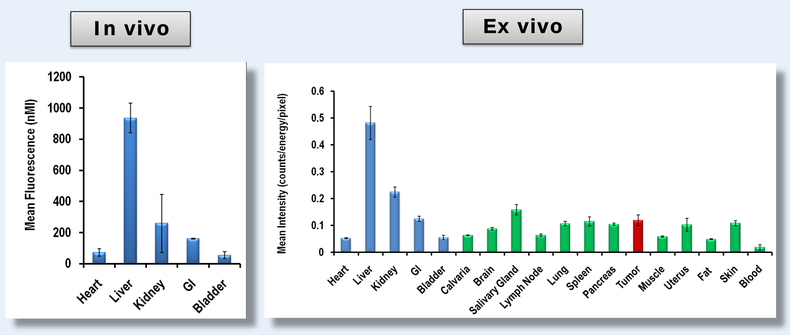
C. HPLC-UV



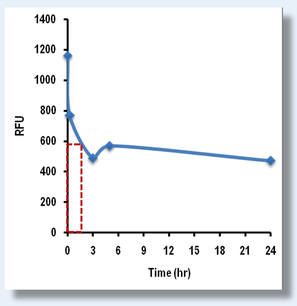
A. Tomato lectin (*Lycopersicon esculentum*) was conjugated with a near infrared fluorophore, VivoTag 680XL (ε=210,000/M/cm; abs/em max 665/688 nm). **B.** The resulting agent (TL680) has an absorption peak at 670 nm and emission 690 peak nm in 1x PBS. **C.** The purity of TL680 was determined by HPLC-UV.

2 Pharmacokinetics and Biodistribution

A. Biodistribution

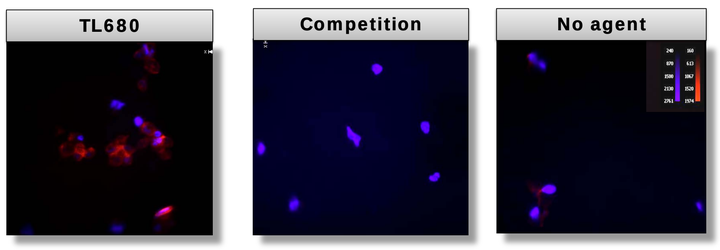


B. Pharmacokinetics



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.

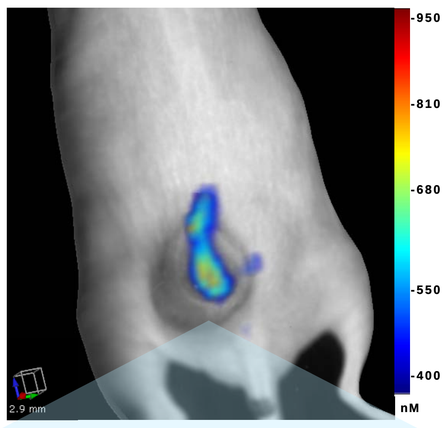
3 Cell Binding



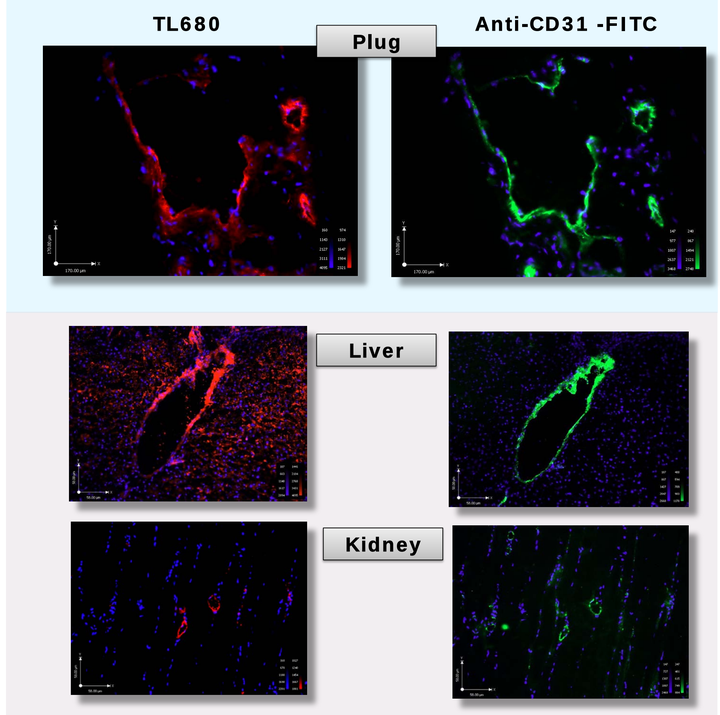
Cells were incubated with TL680 in the presence or absence of unlabeled lectin (50x concentration, competition) and visualized by fluorescence microscopy. In blue: DAPI nuclear stain, red: TL680 (200x,. VivoTag 680XL did not label cells and incubation in the presence of FBS did not affect labeling (not shown).

4 Validation in matrigel plugs

A. Fluorescence Tomography



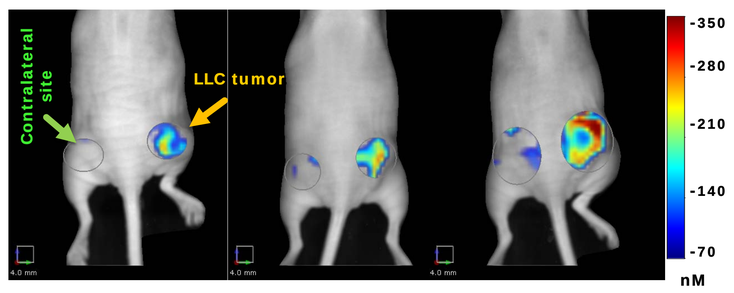
B. Fluorescence microscopy



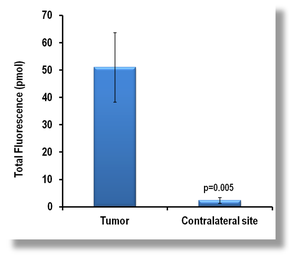
Matrigel containing bFGF, VEGF and heparin was implanted in the flank of SKH-1 mice. Eight-14 days later, mice were injected intravenously with 4 nmoles of TL680 and imaged at 6h. **A.** Tomographic image of matrigel plug-bearing mouse. **B.** Localization of the fluorescent signal in a the plug, liver and kidney (Blue: DAPI nuclear staining, Red: TL680, green anti-CD31-FITC).

5 Imaging in Lewis Lung Carcinoma Xenografts

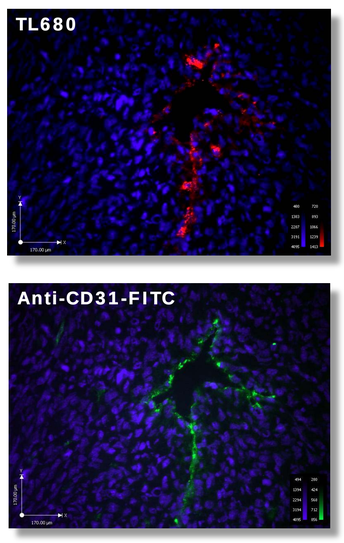
A. Imaging



B. Quantification



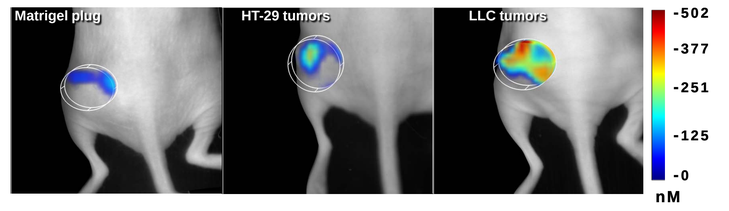
C. Localization



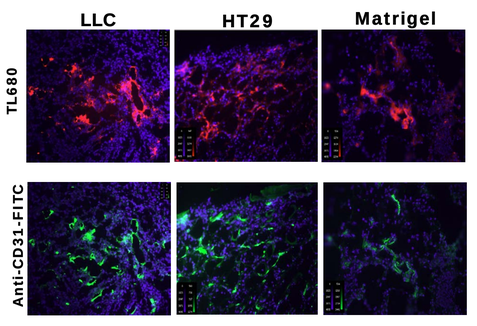
A. Lewis Lung Carcinoma cells were injected into the right flanks of Nu/Nu female mice and grown for 10 days. Mice were imaged tomographically (FMT 2500LX) 6h after intravenous TL680 injection (2 nmoles; three representative mice are shown. **B.** Quantification of the tumor and contralateral site signal (pmoles) and **C.** localization of the fluorescent signal in a frozen tumor section (Blue: DAPI nuclear staining, Red: TL680, Green: anti-CD31).

6 Correlation to anti-CD31

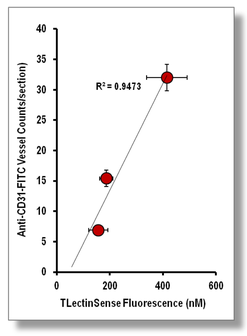
A. Imaging



B. Fluorescence microscopy



C. Correlation



LLC, human colorectal HT-29 tumors and matrigel plugs were implanted in mice. **A.** Once tumors reached the desired size, mice were injected with TL680 and imaged tomographically 6h later. Un-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.

Summary

There is considerable interest in oncology research and drug development in the potential of therapeutic agents to disrupt and/or inhibit angiogenesis. Standard techniques of assessing drug efficacy in relevant animal models include tumor volume measurement or non-specific dye uptake. However, these techniques cannot accurately monitor or quantify the underlying biology, as they rely on macroscopic changes in tumor volume or require sacrifice of the subjects. There is a need for accurate quantification of the disease biology and therapeutic response profiles non-invasively, in real time and *in vivo*. The results presented here demonstrate the utility of fluorescence-based quantitative tomography with a vascular imaging agent, TLectinSense 680, in providing *in vivo* data on disease-related vascularity in oncologic models, suggesting further opportunities for the use of these technologies in a range of other ischemic, inflammatory, infectious, and immune disorders.

7 References

1. Folkman J. Tumor angiogenesis: therapeutic implications. N Engl J Med. 1971; 285: 1182-1186
2. Pathak AP *et al.* A new lectin-targeted contrast agent for MR and optical molecular imaging of vascular endothelium. Proc. Intl. Soc. Mag. Reson. Med. 2007; 15

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