Title: Forward Translation: Determining the Accuracy, Sensitivity, and Specificity of Our Fluorescence Imaging System in Dogs with Cancer

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Background: Curative treatment of solid tumors, such as soft tissue sarcomas (STS) and canine mast cell tumors (MCT) depends upon complete excision of the tumor. However, obtaining clean margins with no residual cancer cells remaining in the tumor bed can be challenging. Furthermore, there is no way to assess the tumor bed intraoperatively for the presence of residual disease. The presence of residual tumor after excision is not determined directly, but rather inferred by histopathological examination of the margins surrounding the excised tumor. Incomplete tumor excision is associated with increased risk for local recurrence and decreased disease-specific survival.

To address this need for a technology that allows real-time examination of the entire tumor bed for residual cancer cells, we developed a wide-field-of-view handheld imaging device prototype that, when used in conjunction with fluorescent probes, can detect microscopic residual cancer intraoperatively.   We have used the imaging device to image tumor beds of genetically engineered mice undergoing marginal and wide resections of sarcomas. Microscopic quantities of residual cancer could be detected in tumor beds consisting of skeletal muscle; the presence of residual fluorescence correlated with local recurrence; and resection of fluorescent tissue improved local control. We have also reported results of a pilot clinical trial assessing the imaging system in 9 dogs with naturally occurring STS or MCT that received a fluorescent imaging agent 24 hours before surgery.

Questions/Purposes: The purpose of this clinical trial was to assess the accuracy, sensitivity, and specificity of this imaging system designed for intraoperative detection of residual cancer in tumor beds of dogs undergoing resection of soft tissue sarcomas (STS) and mast cell tumors (MCT).

Patients and Methods: This was a non-randomized prospective clinical trial in which 19 client-owned dogs with spontaneously occurring STS (12) or MCT (7) were enrolled. A proprietary fluorescent imaging agent, LUM015, which is activated by proteases *in vivo* was administered intravenously to the dogs 6 or 24 hours prior to tumor resection. During surgery, a handheld imaging device was used to measure fluorescence intensity within the cancerous portion of the excised specimen and determine an intensity threshold for subsequent identification of cancer. Selected areas within the resection specimen and tumor bed were then imaged, and biopsy samples (n=101) were obtained from areas that did or did not have a fluorescence intensity exceeding the threshold. Results of intraoperative fluorescence and histopathology were compared.

Results: The imaging system correctly distinguished neoplastic from normal tissue in 93/101 biopsy specimens (92.1%). Using histopathology as the reference, the sensitivity and specificity of the imaging system for identification of cancer were 91.9% and 92.2%, respectively.

Conclusions: A fluorescence-based imaging system designed for real-time, intraoperative tumor bed assessment distinguishes cancer from normal tissue in dogs with a high degree of accuracy.

Figures and Tables:

Table 1: Detail of histopathology results and corresponding fluorescence results for 101 biopsy samples

Histopathology results	Number	Imaging results: Number correct/number incorrect
Cancerous Tissue	33	33/0
Cancerous and Normal Tissue Intermixed	4	1/3 (false negatives)
Normal Skeletal Muscle	19	19/0
Normal Fat	15	12/3 (false positives)
Unspecified Normal Tissue	11	11/0
Normal Collagenous Tissue	6	6/0
Normal Fat and Collagenous Tissue Intermixed	7	5/2 (false positives)
Normal Skin	5	5/0
Hemorrhage	1	1/0

Table 2: Summary of correlations between imaging results and histopathology for 101 biopsy specimens

Imaging Results	Histopathology Results	
	Positive for Cancer	Negative for Cancer
Positive for Cancer	34 (true positives)	5 (false positives)
Negative for Cancer	3 (false negatives)	59 (true negatives)