Title: Indocyanine Green Fluorescence for Intraoperative Margin Assessment in Musculoskeletal Tumor Surgery: A Single-Institution Experience

Authors: Joseph O. Werenski BS[1,2*], Shreya R. Halur BS[1*], Emma R. Kerimo BA[1], Juliet S. Moncho BS[1], Joseph J. Connolly BS[1], Erik T. Newman MD[1], Kevin A. Raskin MD[1], Yin P. Hung MD PhD[3], Anand T. Kumar PhD[4], Santiago A. Lozano-Calderón MD PhD[1]

1. Orthopaedic Oncology Service, Department of Orthopaedic Surgery, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114
2. University of Massachusetts Chan Medical School, Worcester, MA 01655
3. Department of Pathology, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114
4. Department of Radiology, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA 02129

[*]These authors contributed equally to this work.

jwerenski@mgh.harvard.edu
shalur@mgh.harvard.edu
ekerimo@mgh.harvard.edu
jmoncho@mgh.harvard.edu
jconnolly28@mgh.harvard.edu
etnewman@mgb.org
kraskin@mgh.harvard.edu
yphung@mgh.harvard.edu
atkumar@mgh.harvard.edu
slozanocalderon@mgh.harvard.edu

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Significance and hypothesis/techniques: Achieving negative margins is critical for local tumor control. Indocyanine green (ICG) is an emerging intraoperative imaging agent that may augment margin assessment in bone and soft tissue tumor resection by providing real-time fluorescence to identify residual disease. Although the mechanism of tumor uptake is not fully understood, ICG accumulation likely reflects differential clearance and enhanced permeability and retention within tumor tissue.

Prior studies in breast and sarcoma populations have used a normalized fluorescence approach, calibrating the brightest region of the excised tumor to 100% and comparing to the tumor bed. A relative fluorescence intensity (RFI) threshold of ≥77% has been proposed to identify positive margins, demonstrating >50% accuracy and improved specificity compared to surgeon assessment. However, these findings are limited by small cohorts and require validation across varying ICG doses and infusion-to-imaging intervals.

We hypothesized that this threshold-based approach could be validated and improve intraoperative margin detection.

Innovation: This study evaluates variable ICG dosing in a larger cohort with exploratory analysis of infusion-to-imaging timing to refine its clinical utility and optimize intraoperative margin assessment.

Approach: A prospective, non-randomized study of adult patients (≥18 years) undergoing resection of confirmed or suspected bone or soft tissue tumors was conducted at a tertiary academic center (11/2023-11/2026). Patients with ICG contraindications were excluded, and all procedures were performed by fellowship-trained musculoskeletal oncologists. An escalated ICG dosing protocol was used (0.5, 1.0, 1.5, and 2.0 mg/kg administered intravenously the morning before surgery). Following resection, margins were assessed by the operating surgeon, followed by near-infrared fluorescence imaging of the excised specimen and tumor bed. Fluorescence images were analyzed using Fiji (ImageJ, NIH) with a normalized approach. The primary outcome was concordance with final pathology, with diagnostic performance evaluated overall and stratified by ICG dose and infusion-to-imaging interval.

Results: Of 61 enrolled patients, 47 were included in the final cohort (median age 55 years), consisting predominantly of malignant (97.9%) and soft tissue (78.7%) tumors, most commonly in the lower extremity, with positive margins identified in 27.7% of cases (Table 1). Surgeon-perceived margin assessment demonstrated high specificity (97.1%) but limited sensitivity (46.2%), indicating frequent under-recognition of positive margins. RFI-based assessment showed threshold-dependent performance, with improved specificity at higher thresholds and optimal diagnostic accuracy at 2.0 mg/kg, achieving 100% sensitivity and 81.8% accuracy (Table 2). A combined surgeon and ≥77% RFI approach increased sensitivity to 92.3%, reducing false negatives, though specificity decreased to 44.1%. Imaging timing was not associated with performance continuously, but exploratory analyses suggested an optimal window of 100-160 minutes post-infusion (Table 3).

Discussion and impact: ICG near-infrared fluorescence may serve as an adjunct for intraoperative margin assessment, improving detection of residual disease when combined with surgeon judgment, particularly at higher doses and lower RFI thresholds, but is limited by false positives, technical challenges, and workflow constraints. More accurate modalities, such as fluorescence lifetime imaging, warrant further investigation for intraoperative deployment. However, small sample size and exploratory timing analyses preclude definitive conclusions on optimal dosing and infusion-to-imaging intervals, necessitating larger studies before standardization.

Table 1. Patient, tumor, and intraoperative fluorescence characteristics

Variable		n (%)
Age at surgery (years)[1]		55 (44.5-69.0)
Tumor size (cm^3)[1]		322.9 (103.5-520.4)
ICG dose (mg/kg)	0.5	14 (29.8)
	1.0	9 (19.1)
	1.5	13 (27.7)
	2.0	11 (23.4)
Time from infusion to imaging (min)[1]		131 (87.8-173.8)
TBR[1]		2.30 (1.77-3.02)
RFI[1]		0.80 (0.70-0.94)
Sex	Female	19 (40.4)
	Male	28 (59.6)
Race	Asian	1 (2.1)
	Black	1 (2.1)
	White	44 (93.6)
	>1 race	1 (2.1)
Tumor type	Bone	10 (21.3)
	Soft tissue	37 (78.7)
Tumor behavior	Benign	1 (2.1)
	Malignant	46 (97.9)
Anatomical location	Lower extremity	29 (61.7)
	Upper extremity	8 (17.0)
	Pelvis/sacrum	6 (12.8)
	Trunk	4 (8.5)
Tumor histology	Liposarcoma	8 (17.0)
	Myxofibrosarcoma	8 (17.0)
	Chordoma	5 (10.6)
	Solitary fibrous tumor	4 (8.5)
	Rhabdomyosarcoma	3 (6.4)
	Synovial sarcoma	3 (6.4)
	Other sarcomas	16 (34.0)
Surgeon-perceived margins	Negative	40 (85.1)
	Positive	7 (14.9)
Pathology margins	Negative	34 (72.3)
	Positive	13 (27.7)

[1]Continuous variables are reported as medians (IQR).

cm: Centimeter; *ICG*: Indocyanine green; *IQR*: Interquartile range; *min*: Minute; *mg/kg*: Milligram/kilogram; *RFI*: Relative fluorescence intensity (maximum fluorescence intensity of tumor bed/excised tumor); *TBR*: Tumor-to-background ratio (mean fluorescence intensity of tumor/normal adjacent tissue).

Table 2A. Diagnostic performance of relative fluorescence intensity thresholds for detecting positive margins

RFI threshold	Rationale	Accuracy (%)	Sensitivity (%)	Specificity (%)
0.77	Literature-based Favors sensitivity	51.1	69.2	44.1
0.94	Youden's index Optimal/balanced performance	69.8	46.2	80.0

RFI: Relative fluorescence intensity (maximum fluorescence intensity of tumor bed/excised tumor)
Accuracy was defined as the proportion of correctly classified cases relative to pathology. Sensitivity represents the ability to detect positive margins, and specificity represents the ability to correctly identify negative margins. The 0.77 threshold was selected based on prior literature. The 0.94 threshold was derived using Youden's index from receiver operating characteristic analysis.

Table 2B. Relative fluorescence intensity performance stratified by dye dose and threshold

ICG dose (mg/kg)	n	RFI threshold	Accuracy (%)	Sensitivity (%)	Specificity (%)
0.5	14	0.77	71.4	80.0	66.7
		0.94	71.4	20.0	100
1.0	9	0.77	33.3	50.0	28.6
		0.94	66.7	0	85.7
1.5	9	0.77	11.1	33.3	0
		0.94	44.4	33.3	50.0
2.0	11	0.77	54.5	100	37.5
		0.94	81.8	100	75.0

ICG: Indocyanine green; *IQR*: Interquartile range; *mg/kg*: Milligram/kilogram; *RFI*: Relative fluorescence intensity (maximum fluorescence intensity of tumor bed/excised tumor)
Accuracy was defined as the proportion of correctly classified cases relative to pathology. Sensitivity represents the ability to detect positive margins, and specificity represents the ability to correctly identify negative margins.

Table 3A. Performance of surgeon, dye, and combined assessment for detecting positive margins

Model	Accuracy (%)	Sensitivity (%)	Specificity (%)
Surgeon alone	83.0	46.2	97.1
RFI threshold ≥0.77	51.1	69.2	44.1
Combined (positive If either surgeon-perceived or RFI ≥0.77)	57.4	92.3	44.1

RFI: Relative fluorescence intensity (maximum fluorescence intensity of tumor bed/excised tumor)
Accuracy was defined as the proportion of correctly classified cases relative to pathology. Sensitivity represents the ability to detect positive margins, and specificity represents the ability to correctly identify negative margins.

Table 3B. Time from dye infusion to imaging and its association with concordance of device-based margin assessment

Time from infusion to imaging (min)	Concordant margins	Discordant margins	p
Continuous	127.0 (103.5-165.3)	136.0 (80.8-194.0)	0.73
<100	4 (28.6)	10 (71.4)	0.01
100-160	13 (81.3)	3 (18.8)	
>160	7 (43.8)	9 (56.3)	

ICG: Indocyanine green; *IQR*: Interquartile range; *RFI*: Relative fluorescence intensity (maximum fluorescence intensity of tumor bed/excised tumor)
Values are presented as median (IQR) for continuous variables and n (%) for categorical variables. Concordant margins were defined as agreement between device-based (RFI) margin classification and final pathology, whereas discordant margins were defined as disagreement. Continuous variables were compared using the Mann-Whitney U test. Categorical variables were compared using the chi-square test, with Fisher's exact test used when expected cell counts were <5. A p-value <0.05 was considered statistically significant. Time intervals (<100, 100-160, and >160 min) were selected based on the distribution of time from infusion to imaging within the cohort.