Optimization of Second Window Indocyanine Green for Intraoperative Near-Infrared Imaging of Thoracic Malignancy

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BACKGROUND: Near-infrared (NIR) imaging using the second time window of indocyanine green (ICG) allows localization of pulmonary, pleural, and mediastinal malignancies during surgery. Based on empirical evidence, we hypothesized that different histologic tumor types fluoresce optimally at different ICG doses.

STUDY DESIGN: Patients with thoracic tumors biopsy-proven or suspicious for malignancy were enrolled in an NIR imaging clinical trial. Patients received a range of ICG doses 1 day before surgery: 1 mg/kg (n = 8), 2 mg/kg (n = 8), 3 mg/kg (n = 13), 4 mg/kg (n = 8), and 5 mg/kg (n = 8). Intraoperatively, NIR imaging was performed. The endpoint was to identify the highest tumor-to-background fluorescence ratio (TBR) for each tumor type at each dose. Final pathology confirmed tumor histology.

RESULTS: Of 45 patients, 41 had malignancies (18 non-small cell lung cancers [NSCLC], 3 pulmonary neuroendocrine tumors, 13 thoracic metastases, 4 thymomas, 3 mesotheliomas). At doses of 4 to 5 mg/kg, the TBR from primary NSCLC vs other malignancies was no different (2.70 vs 3.21, p = 1.00). At doses of 1 to 3 mg/kg, the TBR was greater for the NSCLCs (3.19 vs 1.49, p = 0.0006). Background fluorescence from the heart or ribs was observed in 1 of 16 cases at 1 to 2 mg/kg, 5 of 13 cases at 3 mg/kg, and 14 of 16 cases at 4 to 5 mg/kg; this was a major determinant of dose optimization.

CONCLUSIONS: This is the first study to demonstrate that the optimal NIR contrast agent dose varies by tumor histology. Lower dose ICG (2 to 3 mg/kg) is superior for nonprimary lung cancers, and high dose ICG (4 to 5 mg/kg) is superior for lung cancers. This will have major implications as more intraoperative imaging trials surface in other specialties, will significantly reduce costs and may facilitate wider application. (J Am Coll Surg 2019;228:188−197. © 2018 by the American College of Surgeons. Published by Elsevier Inc. All rights reserved.)

Complete surgical resection provides the best chance for cure or prolonged survival in most thoracic malignancies including non-small cell lung cancer (NSCLC), limited pulmonary metastases, mediastinal masses, and mesothelioma.[1-4] Thoracic cancer resections present several challenges to the surgeon including identification of small pulmonary nodules; delineation of mediastinal mass margins and discrimination of tumor from surrounding critical structures such as the phrenic nerve; and complete debulking in mesothelioma. Intraoperative near-infrared (NIR) imaging is a new technology that can meet many of these challenges.[5-7]

Indocyanine green (ICG) is the only US FDA-approved NIR contrast agent. It is a water-soluble small molecule

Abbreviations and Acronyms

DAPI = 4′6-diamidino-2-phenylindole
ICG = indocyanine green
MFI = mean fluorescence intensity
NIR = near-infrared
NSCLC = non-small cell lung cancer
OM = other malignancies
TBR = tumor-to-background ratio

(775 Da) which facilitates low toxicity, rapid clearance, and favorable pharmacokinetics. However, ICG can act as a macromolecule in circulation, where it is 98% albumin and lipoprotein bound.[8] Indocyanine green has traditionally been used for vascular perfusion imaging; common applications include assessment of blood flow to bowel anastomoses and tissue flaps.[9,10] When used for these indications, a low dose (5 to 10 mg) is given immediately before imaging; this is the first time window. It was recently demonstrated that there is a second window for NIR imaging with ICG that can be used for tumor imaging.[11] Specifically, ICG has tumor imaging properties when given at significantly higher doses (5 mg/kg) with imaging at longer time intervals (24 hours) than those recommended on the product label.[12,13] Near-infrared imaging with this dosing regimen can identify NSCLC, pulmonary metastases, mediastinal masses, and mesothelioma.[13-16]

In preclinical animal studies, the ICG dose was optimized in NSCLC at 5 mg/kg.[11,12] When it was translated to human studies, the dose was maintained at 5 mg/kg. Now, with several years of experience, we have learned through empirical evidence that NIR imaging in humans is different than in canine and murine models. Using NIR imaging with second window ICG, we have also observed that different tumor histologies have a wide range of tumor-to-background ratios (TBR). Finally, background fluorescence from the heart and ribs can be distracting and difficult to interpret for surgeons new to the technology. Therefore, we chose to optimize the dose in humans because animal models are inadequate to do this investigation. In this study, we hypothesized that the optimal second window ICG dose varies by tumor histology. Our goals were to determine if the optimal ICG dose varies by histology, and if so, to define an initial nomogram for the optimal ICG dose based on tumor histology.

METHODS
Study design
A prospective intraoperative NIR imaging clinical trial was approved by the University of Pennsylvania Institutional Review Board. All patients gave written informed consent before trial participation. The primary objective of this trial was to determine the optimal second window ICG dose for thoracic tumors. The "optimal" dose was defined as the lowest possible dose that would achieve the highest TBR in order to maximize fluorescence and minimize patient risk. Background fluorescence in the heart or ribs was also considered an additional determinant of the optimal dose. Forty-five patients provided written informed consent and were recruited between August 2017 and May 2018. Included subjects were scheduled for resection of a thoracic tumor based on preoperative radiographs. Eight patients each received 1, 2, 3, 4, or 5 mg/kg ICG 1 day before resection of a thoracic solid tumor. An additional 5 patients received 3 mg/kg ICG 1 day before surgery to provide additional data points to confirm key findings.

Study drug
Indocyanine green (ICG) (Akorn) is an NIR fluorophore with a peak excitation wavelength of 805 nm, peak emission wavelength of 830 nm, and a molecular weight of 775 Da.

Near-infrared imaging
Macroscopic surgical fluorescent imaging in situ and ex vivo was performed using the Iridium system (Visionsense Corps). Lesions that were not fluorescent ex vivo were bisected and examined for fluorescence after bisection. Additional macroscopic specimen mapping for margin status and additional lesions was performed using the Elvis (LiCor), a prototype 3-dimensional rotating NIR imaging device.

Microscopic specimen mapping
Cross sections of excised specimens were next analyzed using the Odyssey (LiCor), an NIR digital scanning device. Mean fluorescence intensity (MFI) in the tumor was compared with that in adjacent uninvolved tissue. Serial 5-μm sections of tumor were also cut for pathology slides. Slides were stained with 4′6-diamidino-2-phenylindole (DAPI), and fluorescence microscopy for ICG and DAPI was performed. Indocyanine green was pseudo-colored green, and DAPI was pseudo-colored blue. Slide fluorescence was captured using a Leica DM-6 microscope (Leica), and images were recorded using a Leica DMC4500 camera system. Areas of fluorescence were compared with the corresponding areas imaged by standard hematoxylin and eosin (H&E) staining. Positive and negative controls were used for all images. Final histopathologic diagnosis was determined by a thoracic pathologist after specimens were formalin fixed and paraffin embedded.

Statistical analysis

Post hoc image analysis was performed using region-of-interest software and HeatMap plugin within ImageJ (National Institutes of Health; http://rsb.info.nih.gov/ij/). The fluorescence in the tumor and in adjacent uninvolved tissue was quantified with this technology, and a TBR was calculated for each case. A TBR $\geq$ 2.0 was considered positive for fluorescence based on previous studies using this as a cutoff for fluorescence with this imaging system.[16-18] Background fluorescence from the heart or ribs in situ was defined as any perceptible fluorescent signal from the heart or ribs at the gain that produced optimal contrast between tumor and adjacent normal tissue or 100% gain if there was no tumor fluorescence. The percentages of cases with tumor and background heart or rib fluorescence stratified by histology and ICG dose were compared by the Fisher exact test. Tumor-to-background ratios between doses and microscopic fluorescence differences between tumor and uninvolved tissue were compared by the Wilcoxon rank sum test. The associations of clinicopathologic characteristics with tumor fluorescence were compared using the Pearson's correlation coefficient.

RESULTS

Study population

Between August 2017 and May 2018, 45 patients with a radiographic thoracic tumor were enrolled in this study. There were 18 men and 27 women. Mean age at surgery was 61.5 $\pm$ 12.8 years. The mean time from ICG infusion to imaging was 22.8 $\pm$ 3.1 hours. Full patient characteristics are provided in Table 1. Infusion of ICG was safe, with no serious drug-related adverse events.

Intraoperative near-infrared imaging

Among the first 40 patients, 8 patients each received 5, 4, 3, 2, or 1 mg/kg ICG. The tumors imaged at each dose are summarized in Table 2. At ICG doses of 4 to 5 mg/kg, 8 of 9 NSCLC tumors were fluorescent (mean TBR 2.70 $\pm$ 0.91), and 9 of 9 other malignancies (OM) (thoracic metastases, thymoma, or mesothelioma) were fluorescent (mean TBR 3.21 $\pm$ 1.14) (p = 1.00). At ICG doses of 1 to 3 mg/kg, 1 of 9 NSCLCs (mean TBR 1.49 $\pm$ 0.91) and 13 of 14 OMs were fluorescent (mean TBR 2.96 $\pm$ 1.12) (p < 0.001) (Fig. 1A). Three tumors in 2 patients (1 sarcoma metastasis at 4 mg/kg and 2 colorectal adenocarcinoma metastases at 3 mg/kg) could not be seen with standard white light and were detected only with NIR imaging. In situ and ex vivo fluorescence correlated for all malignant tumors. The only nonfluorescent OM was a 0.7 cm small bowel

Table 1. Characteristics of the Study Subjects

Variable	Dose de-escalation cohort (n = 40)	Validation cohort (n = 5)
Age, y, mean $\pm$ SD	60.8 $\pm$ 13.1	67.6 $\pm$ 9.2
Sex, n		
Male	15	3
Female	25	2
Final pathology, n		
NSCLC		
Adenocarcinoma	14	
Squamous cell carcinoma	4	
Pulmonary neuroendocrine tumor		
Large cell neuroendocrine tumor	1	
Typical carcinoid	2	
Thoracic metastases		
Adrenal cortical carcinoma	1	
Colorectal adenocarcinoma	2	
Small bowel adenocarcinoma	1	
Germ cell tumor	1	
Sarcoma	4	2
Endometrial adenocarcinoma	1	
Renal cell carcinoma		1
Other		
Thymoma	3	1
Mesothelioma	2	1
Benign		
Granuloma	2	
Alveolar adenoma	1	
Sclerosing pneumocytoma	1	
Time, h, mean $\pm$ SD	22.8 $\pm$ 3.2	22.2 $\pm$ 2.0

NSCLC, non-small cell lung cancer.

adenocarcinoma that was 0.1 cm from the pleural surface. This patient received an ICG dose of 1 mg/kg, with imaging 28.7 hours after ICG infusion. Representative cases with TBR at each ICG dose are shown in Figure 1B.

There were 5 benign nodules in 4 patients at doses of 1 to 2 mg/kg: 3 granulomas, 1 alveolar adenoma, and 1 sclerosing pneumocytoma. Two of these lesions had false positive fluorescence. One of the granulomas was fluorescent both in situ and ex vivo (TBR = 2.18), and the sclerosing pneumocytoma was not fluorescent in situ but was fluorescent after tumor bisection

Table 2. Tumor Types in Each Dosing Group

Histology	Dose, n				
	1 mg/kg	2 mg/kg	3 mg/kg	4 mg/kg	5 mg/kg
NSCLC	3	2	4	4	5
Neuroendocrine tumor	—	—	2	1	—
Metastases	3	3	2	2	—
Thymoma	1	—	—	1	1
Mesothelioma	—	—	—	—	2
Benign	1	3	—	—	—

NSCLC, non-small cell lung cancer.

ex vivo (TBR = 3.84). The sclerosing pneumocytoma was 1.5 cm in diameter and 2.6 cm from the pleural surface.

Three-dimensional fluorescence analysis and fluorescence microscopy

In the initial dose de-escalation cohort, there were no close margins or additional nodules identified in the ex vivo specimen with the Elvis. A representative case in Figure 2 demonstrates in situ and ex vivo fluorescence, no close margins or additional lesions with the Elvis (supplemental video 1), and more microscopic dye accumulation within the tumor compared with adjacent normal lung. For all tumors that were fluorescent in situ and ex vivo, microscopic tumor fluorescence was observed. A representative case with microscopic fluorescence at the lowest ICG dose (1 mg/kg) is shown in Figure 3.

Determination of optimal indocyanine green dose

In order to determine the optimal ICG dose, the TBR and the background fluorescence from the heart and ribs at each dose were compared. The mean fluorescence intensity (MFI) for tumor and background and the mean TBR at each dose are shown in Figures 4A and 4B. For NSCLC, the TBR increased with increasing doses. For OM, the TBR was highest at a 3 mg/kg dose. Fewer cases had background fluorescence from the heart or ribs at 3 mg/kg compared with 4 to 5 mg/kg (p = 0.02) and at 1 to 2 mg/kg compared with 3 mg/kg (p = 0.05) (Fig. 4C). Near-infrared imaging detected 1 additional OM at a dose of 4 mg/kg, 2 additional OMs at a dose of 3 mg/kg, and no additional lesions at doses of 1 to 2 mg/kg. It was determined that 4 to 5 mg/kg was the optimal dose for NSCLC because only 1 of 9 tumors was fluorescent at doses lower than 4 mg/kg. It was determined that 3 mg/kg was the optimal dose for OMs because all OMs were fluorescent, the TBR was highest, there was decreased background

fluorescence from the heart and ribs compared with 4 to 5 mg/kg, and 2 additional nodules were detected at this dose.

Confirmation of optimal indocyanine green dose

Finally, NIR imaging was performed prospectively in 5 patients at 3 mg/kg with expected tumor histology other than NSCLC. Tumor fluorescence was observed in each case; there were 7 total tumors in 5 patients detected with the Iridium (mean TBR 3.65 ± 2.08). Three representative cases are presented in Figure 5. In 1 case, there was some suggestion of fluorescence outside the primary tumor, but no obvious second nodule was seen with the Iridium system. However, a second nodule within the specimen was more apparent with the Elvis (supplemental video 2) and was discovered on final pathology analysis. Background fluorescence from the heart and/or ribs was observed in 2 of 5 cases. On microscopic analysis, MFI in fluorescent tumors was higher than that in adjacent uninvolved tissue (1,598 vs 939 arbitrary units [AU], p = 0.04). In all patients who received 3 mg/kg (dose de-escalation and validation cohorts), there was no association between time from infusion to imaging and TBR (r = -0.007, p = 0.96).

DISCUSSION

In a prospective clinical trial, we demonstrated for the first time that different tumor types are optimally fluorescent at varying dosing parameters during intraoperative near-infrared (NIR) imaging of thoracic malignancies. With NIR imaging 1 day after indocyanine green (ICG) infusion, nearly all thoracic metastases, mediastinal masses, and mesothelioma (other malignancies [OMs]) were fluorescent at ICG doses of 1 to 3 mg/kg; this dose was insufficient for non-small cell lung cancer (NSCLC). The lower dose also improved fluorescent detection of OMs by decreasing background fluorescence from the heart and ribs. With prospective NIR imaging on 5 patients with tumor histologies that were expected to accumulate
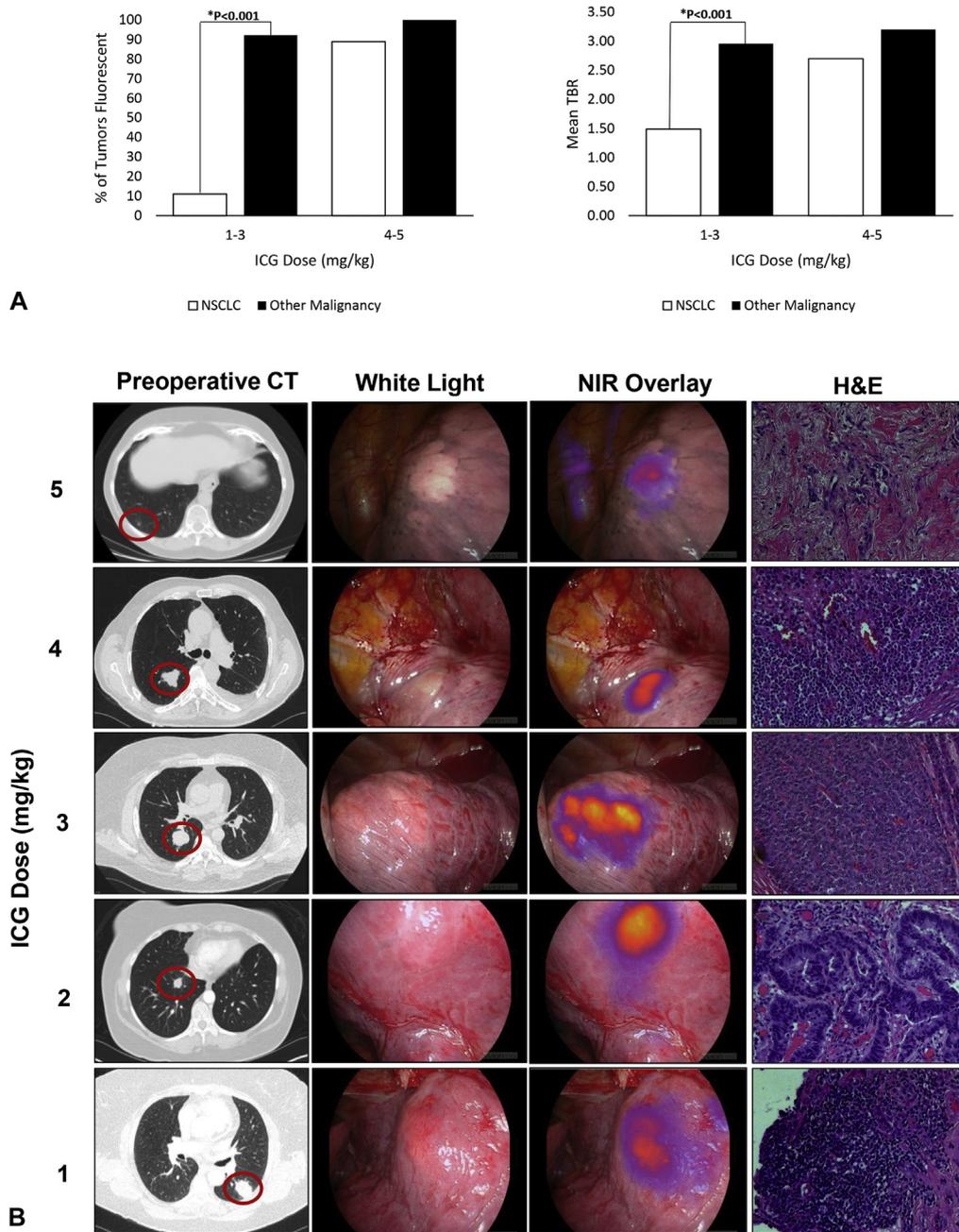
Figure 1. (A) Percentage of fluorescent tumors and mean tumor-to-background ratio (TBR) of non-small cell lung cancer (NSCLC) and other malignant tumors at low (1 to 3 mg/kg) and high (4 to 5 mg/kg) indocyanine green (ICG) doses. (B) Representative preoperative CT, intraoperative white light, intraoperative near-infrared (NIR) overlay, and hematoxylin and eosin (H&E) (20×) images of a pulmonary nodule at each ICG dose in the dose de-escalation trial. Final pathology for each case was: 5 mg/kg, pulmonary adenocarcinoma; 4 mg/kg, adreno-cortical carcinoma; 3 mg/kg, typical carcinoid; 2 mg/kg, colorectal adenocarcinoma; and 1 mg/kg, endometrial carcinoma.

ICG at a dose of 3 mg/kg, the tumors were fluorescent in each case.

Intraoperative NIR imaging is an attractive new technology for localization and margin assessment during resection of thoracic tumors. Advantages over other preoperative or intraoperative localization techniques include low risk of serious complications, no exposure to ionizing radiation, the ability to visualize large surfaces in real
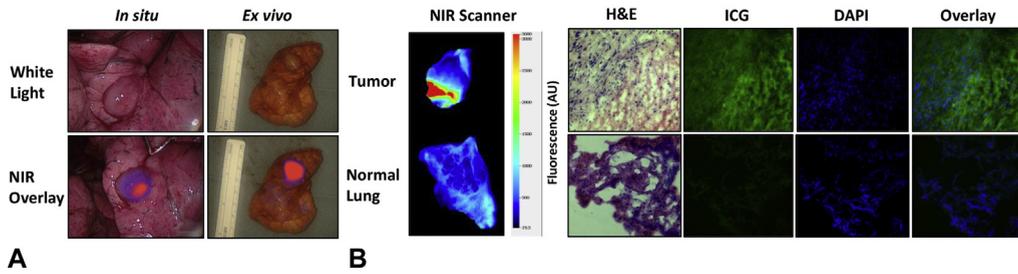
Figure 2. Representative case of specimen mapping for a pulmonary leiomyosarcoma metastasis. (A) Gross imaging. The tumor displayed in situ and ex vivo fluorescence. (B) Microscopic imaging. Near-infrared scanner and fluorescence microscopy (20×) images demonstrate more fluorescence in the tumor than in adjacent uninvolved lung. DAPI, 4′6-diamidino-2-phenylindole; H&E, hematoxylin and eosin; ICG, indocyanine green; NIR, near-infrared.

time, and the ability to integrate it into the normal flow of an operation. Indocyanine green is currently the only clinically approved intraoperative NIR imaging agent. Near-infrared imaging with ICG was traditionally used to assess perfusion.[9,10] It was recently demonstrated that a high

ICG dose will also accumulate in tumors over 24 hours by the enhanced permeability and retention (EPR) effect.[11,12] The enhanced permeability and retention effect is based on the concept that tumor angiogenesis creates an environment with excess but defective, leaky
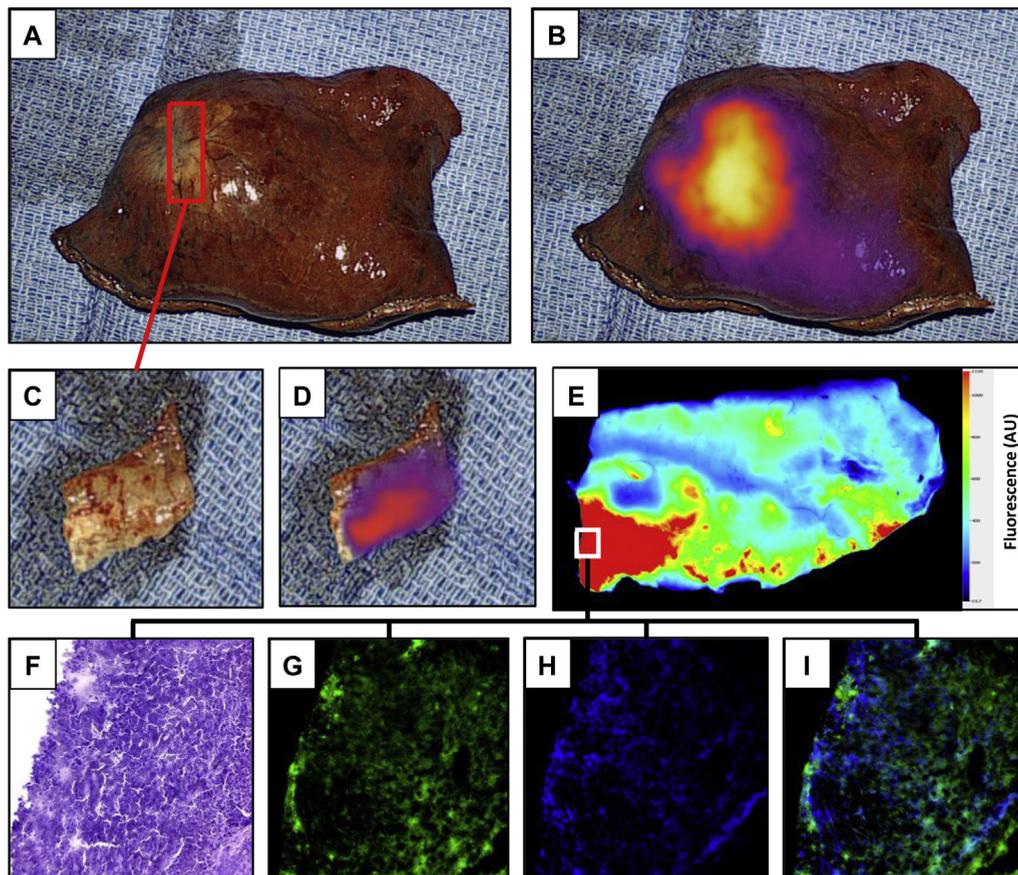

Figure 3. Specimen mapping from near-infrared (NIR) imaging of an endometrial carcinoma pulmonary metastasis. (A) Ex vivo white light; (B) ex vivo NIR; (C) white light bread loaf section of tumor; (D) NIR bread loaf section of tumor; (E) tumor section on NIR scanner; (F) hematoxylin and eosin (H&E) (20×) images; (G) Indocyanine green (ICG); (H) 4′6-diamidino-2-phenylindole (DAPI) and (I) ICG and DAPI overlay fluorescence microscopy (20×).
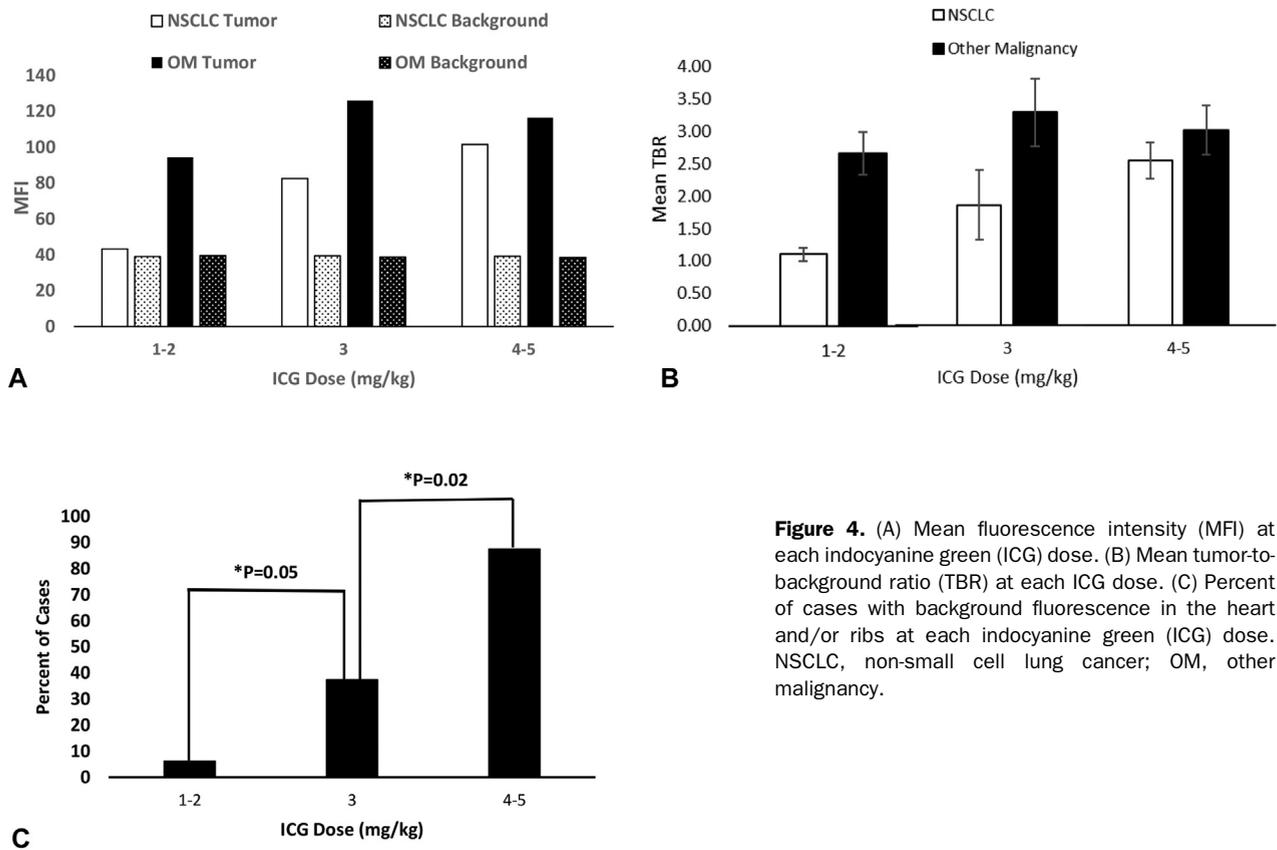
Figure 4. (A) Mean fluorescence intensity (MFI) at each indocyanine green (ICG) dose. (B) Mean tumor-to-background ratio (TBR) at each ICG dose. (C) Percent of cases with background fluorescence in the heart and/or ribs at each indocyanine green (ICG) dose. NSCLC, non-small cell lung cancer; OM, other malignancy.

capillaries.[19,20] Macromolecules (>10,000 Da) leak out and become trapped in tumors due to properties including size, shape, charge, and polarity.[21] Indocyanine green is only 775 Da, but acts as a macromolecule in circulation, where it is primarily protein bound.[8]

In this study, most thoracic malignancies were fluorescent at an ICG dose of 4 to 5 mg/kg with NIR imaging 1 day later. This is consistent with findings from NIR imaging with second window ICG in other solid tumors including breast cancer and glioma.[22,23] Interestingly, NSCLC tumors were generally not fluorescent at doses less than 4 to 5 mg/kg; all other malignancies were fluorescent at doses of 2 to 3 mg/kg. At 1 mg/kg, a sclerosing pneumocytoma could not be seen in situ but was highly fluorescent after bisection, and a peripheral small bowel adenocarcinoma pulmonary metastasis had no tumor fluorescence in situ or ex vivo. The lack of in situ fluorescence in the sclerosing pneumocytoma was most likely related to its depth at 2.6 cm from the pleural surface, as depth of penetration is a known limitation of NIR imaging.[24] We hypothesize that the small bowel adenocarcinoma metastasis was not fluorescent because imaging occurred more than 28 hours after ICG infusion. Although there was no difference in tumor fluorescence

with slight variations in time from infusion to imaging at a dose of 3 mg/kg, we believe imaging timing is more critical at the very low dose of 1 mg/kg. It is possible that consistent tumor imaging would be possible with an ICG dose of 1 mg/kg at a time less than 24 hours, but this would be impractical in clinical practice.

To our knowledge, this is the first study to demonstrate that the optimal NIR contrast agent dose varies by histology. This suggests that previous studies of NIR imaging have oversimplified dosing parameters. It also suggests that the second window ICG dose may be able to provide a real-time prediction or "optical biopsy" based on tumor fluorescence. For example, if a 2 to 3 mg/kg second window ICG dose is given and the tumor is fluorescent, the surgeon could proceed with a wedge resection with confidence that the tumor is unlikely to be a primary lung cancer. Although we would still recommend getting a frozen section diagnosis, the knowledge gained with NIR imaging could decrease the operative time and improve the overall accuracy of frozen section diagnosis. Addition of a second fluorescent dye targeted to receptors expressed only in certain malignancies could further improve discrimination of the most likely histology based on tumor fluorescence
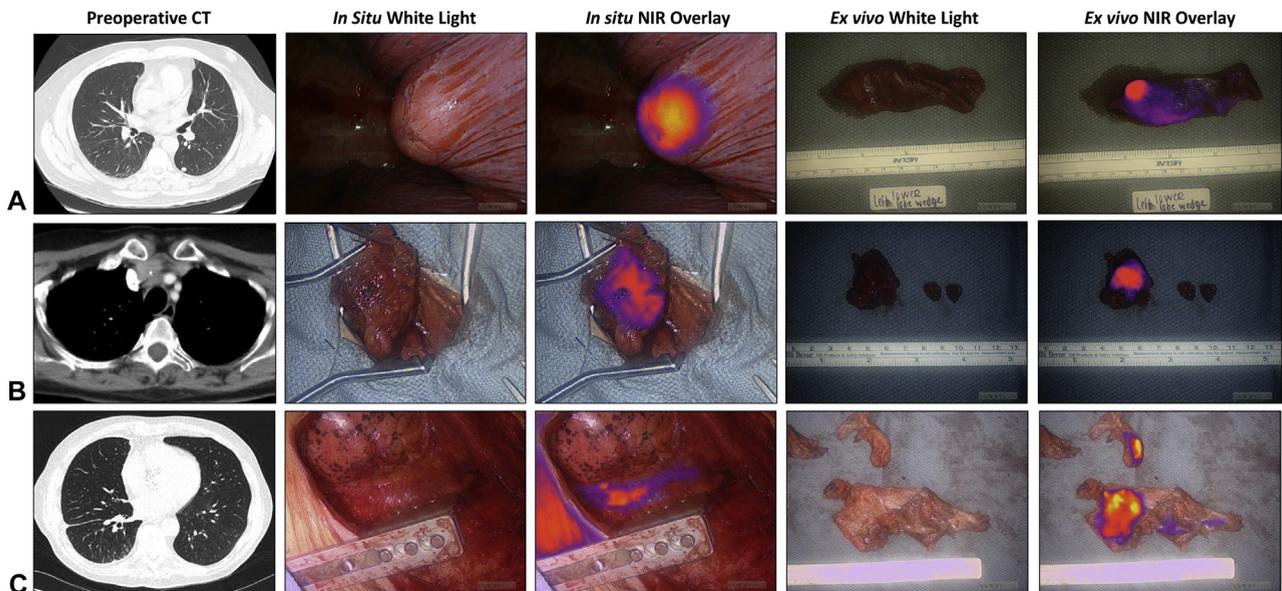
Figure 5. Representative images from near-infrared (NIR) imaging of (A) pulmonary myxofibrosarcoma metastasis, (B) thymoma, and (C) mesothelioma 1 day after infusion of 3 mg/kg indocyanine green (ICG) in the validation cohort.

while maintaining high sensitivity for detecting many different types of tumors.

The finding that NSCLC tumors were not fluorescent at lower ICG doses contrasts with another recent study of 11 pulmonary resections that used second window ICG.[25] Twenty-four hours after infusion of 1 mg/kg ICG, the authors reported fluorescence in 7 of 7 NSCLCs. Although they performed only ex vivo imaging and did not evaluate in situ fluorescence, the findings are still surprising and disparate with ours, especially considering a more sensitive imaging system was used in this study.[26]

Excellent results were achieved with NIR imaging of OM at second window ICG doses of 2 to 3 mg/kg. All malignant tumors were fluorescent with no background fluorescence from the heart or ribs at a dose of 2 mg/kg. However, no occult tumors were detected at this dose, and the TBR was lower than at 3 mg/kg. At 3 mg/kg, there was a significant decrease in background fluorescence in the heart and ribs compared with 4 to 5 mg/kg, but still excellent sensitivity for very small nodules. Two of the colorectal cancer metastases detected at 3 mg/kg were 2 and 3 mm in size and were detected only with NIR imaging. We ultimately decided on a dose of 3 mg/kg rather than 2 mg/kg for the confirmation cohort due to concern that we would miss small nodules at 2 mg/kg. The findings in this cohort were similar to those from the dose de-escalation: all tumors were fluorescent, and there was background fluorescence from the heart or ribs in 2 of 5 cases. From these results, we feel confident that 3 mg/kg with imaging 1 day later is

effective for NIR imaging of thoracic metastases, thymoma, and mesothelioma. Dosing could be further optimized by examining additional time points after an infusion of 2 to 3 mg/kg in future studies.

Observations from this study generated 2 major hypotheses regarding the mechanism of optimal fluorescence. First, we believe that tumors that have a true capsule or a pseudocapsule are more likely to fluoresce at 1 to 3 mg/kg of ICG. In previous sarcoma studies, we found the pseudocapsule retained ICG and prevented dye leakage into the surrounding tissues.[27] Second, we believe the vascularity of the structures surrounding the tumor has a major impact on background fluorescence, which affects the TBR. For example, tumors in the anterior mediastinum are typically surrounded by adipose tissue, which is avascular, thereby eliminating background noise and raising the TBR.

The finding that tumor imaging is possible and effective with ICG doses lower than 5 mg/kg could be promising for NIR imaging of nonthoracic malignancies. For example, nonspecific fluorescence in the wound bed limited the clinical utility of NIR imaging, with a second window ICG dose of 5 mg/kg for breast cancer.[22] It was hypothesized that fluorescence in the wound bed was related to ICG spillage and that this nonspecific fluorescence could be decreased with a lower ICG dose. This study suggests that a lower ICG dose does, in fact, decrease background fluorescence while still permitting detection of solid tumors, albeit in a different type of cancer. It would be worthwhile

to test a lower ICG dose for clinical breast cancer cases in the future.

Based on our findings, we make the following recommendations. If NSCLC is either biopsy confirmed or highest on the differential, we recommend giving a second window ICG dose of 4 to 5 mg/kg, with imaging 24 hours later. If pulmonary metastasis, thymoma, or mesothelioma is biopsy confirmed or highest on the differential, we recommend giving a dose of 2 to 3 mg/kg, with imaging 24 hours later. If a pulmonary nodule is truly undifferentiated, then the dose depends on the reason for imaging. If the primary goal of imaging is localization of a small undifferentiated pulmonary nodule using video-assisted thoracoscopic surgery (VATS), then we recommend using 4 to 5 mg/kg to allow localization of both primary lung cancers and pulmonary metastases. If the primary goal of imaging is optical biopsy, then a lower dose of 2 to 3 mg/kg will allow rapid discrimination of tumor histology.

There are several limitations to this study. First, it included a very heterogeneous group of tumors; there is assuredly additional nuance based on tumor histology that we were unable to uncover with such a heterogeneous group. However, one of the major benefits of second window ICG is that it can be used for so many different types of tumors. We believe that his study has captured most thoracic tumor histologies for which this imaging technique will be used. Second, specificity was suboptimal because 2 of 5 benign nodules were fluorescent. However, a general principle of intraoperative NIR imaging is that sensitivity is more critical than specificity. We believe that sacrificing some specificity (ie fluorescence in some benign nodules) is acceptable for near perfect sensitivity (ie detection of all malignant tumors). This limitation can potentially be overcome with continued development of new targeted NIR imaging tracers.

This study also begs the question of whether other parameters, most importantly, timing between dye infusion and imaging, can also be optimized. Future studies need to explore this variable. Finally, with time and more data, we should be able to refine the nomogram to confirm our findings.

CONCLUSIONS

We demonstrate for the first time that the optimal dose for NIR imaging with second window ICG is histology dependent. Intraoperative NIR imaging is an emerging technology that will be increasingly integrated into clinical practice. Many targeted NIR imaging agents are in various stages of development, but ICG is a cheap, commercially available agent that is effective for NIR imaging of many solid tumors. Refinement of the second window ICG technique may improve the clinical effectiveness of this technology, and ultimately, patient outcomes.

Author Contributions

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Drafting of manuscript: Newton, Predina, Corbett, Frenzel-Sulyok, Xia, Petersson, Tsourkas, Nie, Delikatny, Singhal

Critical revision: Newton, Predina, Corbett, Frenzel-Sulyok, Xia, Petersson, Tsourkas, Nie, Delikatny, Singhal

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