Supplementary Online Content

Quan YH, Oh CH, Jung D, et al. Evaluation of intraoperative near-infrared fluorescence visualization of the lung tumor margin with indocyanine green inhalation. *JAMA Surg*. Published online June 24, 2020. doi:10.1001/jamasurg.2020.1314

This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods 1.

Organ Distribution of the Inhaled ICG in a Normal Mouse Model

Twenty 6-week-old C57BL/6 mice (10 females and 10 males) weighing 20–25 g were used for this study. The mice were divided into 5 groups according to the time of ICG inhalation (10 min and 1, 3, 6, and 24 h post inhalation, n = 4 per group). The inhalation of 0.25 mg/mL of ICG for 11.9 min using a nebulizer (Expose; Scireq) resulted in the delivery of 2.5 mg/kg ICG to the mice. The lungs, kidneys, liver, spleen, and brain were harvested at different time points, and the ICG distribution was investigated using the NIR fluorescence imaging system (LI-COR, Biosciences). The fluorescence signal in each organ was quantified using Image J software.

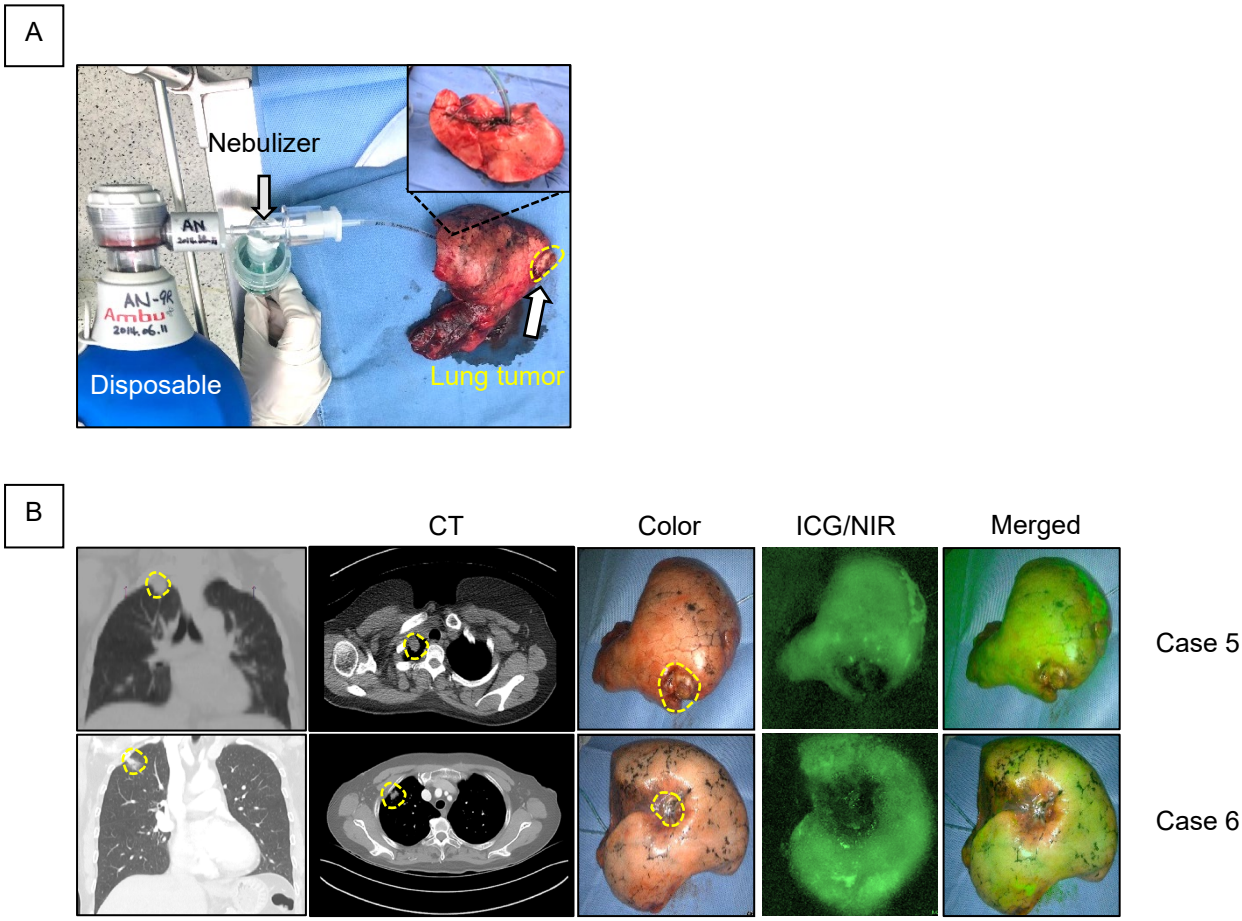
eMethods 2

Human study

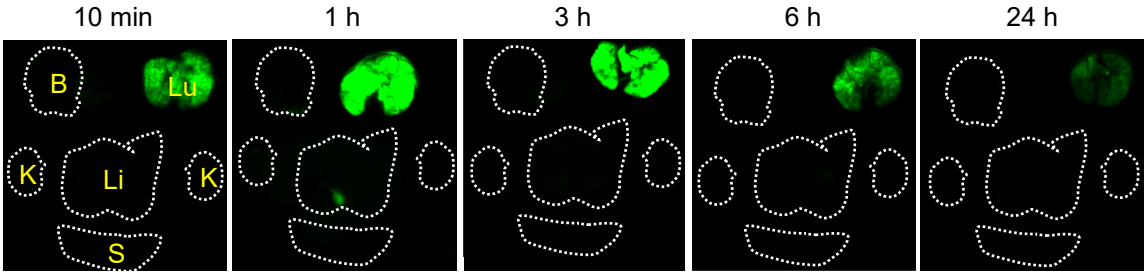
This prospective study was approved by the Korea University Medicine (IRB-2017GR0023) in 2017. Consent was requested for all study interventions and assessments. Informed consent of patients was obtained by the participating surgeons at least 24 hours before the intervention. The patients (≥ 19 years) who had agreed to participate (oral or written) and were scheduled for lobectomy between May 2017 and March 2019 in Korea University Guro Hospital were consecutively enrolled in this study. These patients, irrespective of whether they had received neoadjuvant therapy, had been diagnosed with primary lung cancer by CT scan of the chest and biopsy, with the tumor being located less than 1 cm from the surface of the pleura. Patients who did not agree to participate in this study, or patients who had lung tumor more than 1 cm from the pleural surface, or tumor larger than 5 cm as detected by the CT scan, were excluded from this study. All surgeries were performed using the video-assisted thoracic surgery (VATS) approach, which is the standard method used in our hospital. Once the lung lobe with tumor was removed, the distal end of the endotracheal tube was inserted into the lobar bronchus of the resected lobe and ligated tightly, and an ambu-bag was connected to the proximal end of the endotracheal tube to manage the air flow in the lung lobe. After the air leak point of the lung was secured via suture ligation, the nebulizer was connected to the endotracheal tube. Next, the ICG (0.75 mg/mL for 4 min) was delivered into the resected lung lobe (eFigure 5). The distribution of ICG in the lung lobe was visualized in real-time using NIR fluorescence thoracoscopy (Pinpoint, Stryker). All specimens were sent to the pathology department to be evaluated by pathologists.

eFigure 1. Detection of TM in the Resected Lung Tissue of Patients by ICG Nebulization.

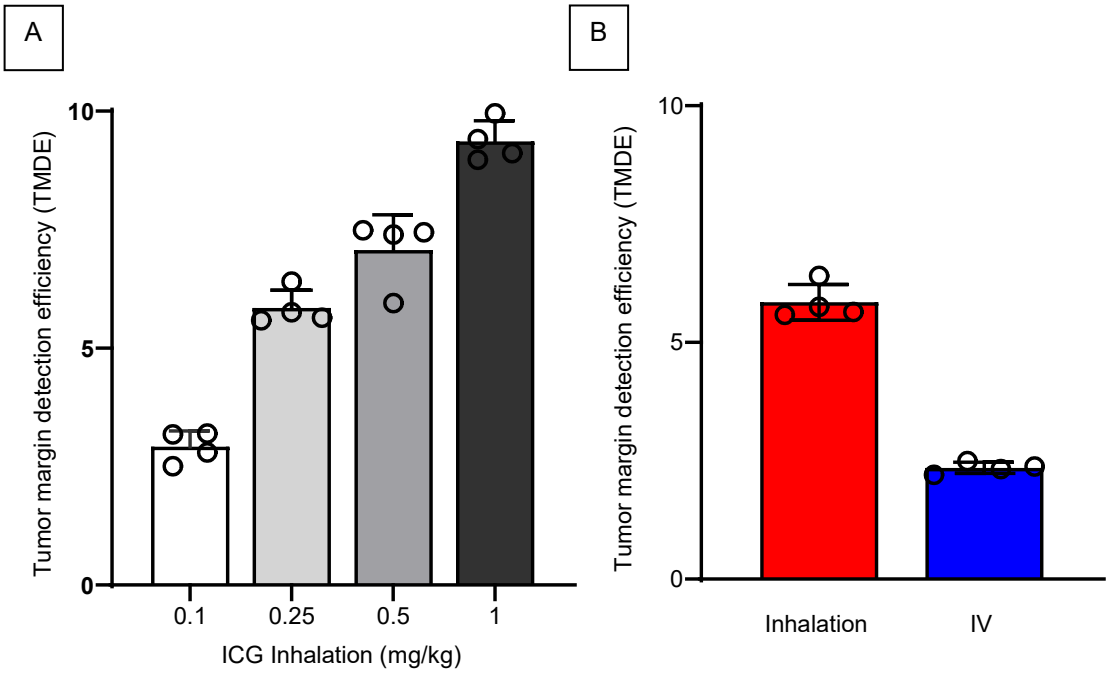
In vivo lung images in color, near-infrared (NIR) (green), and merged (NIR with color) in the rabbit lung tumor model that inhaled indocyanine green (ICG).



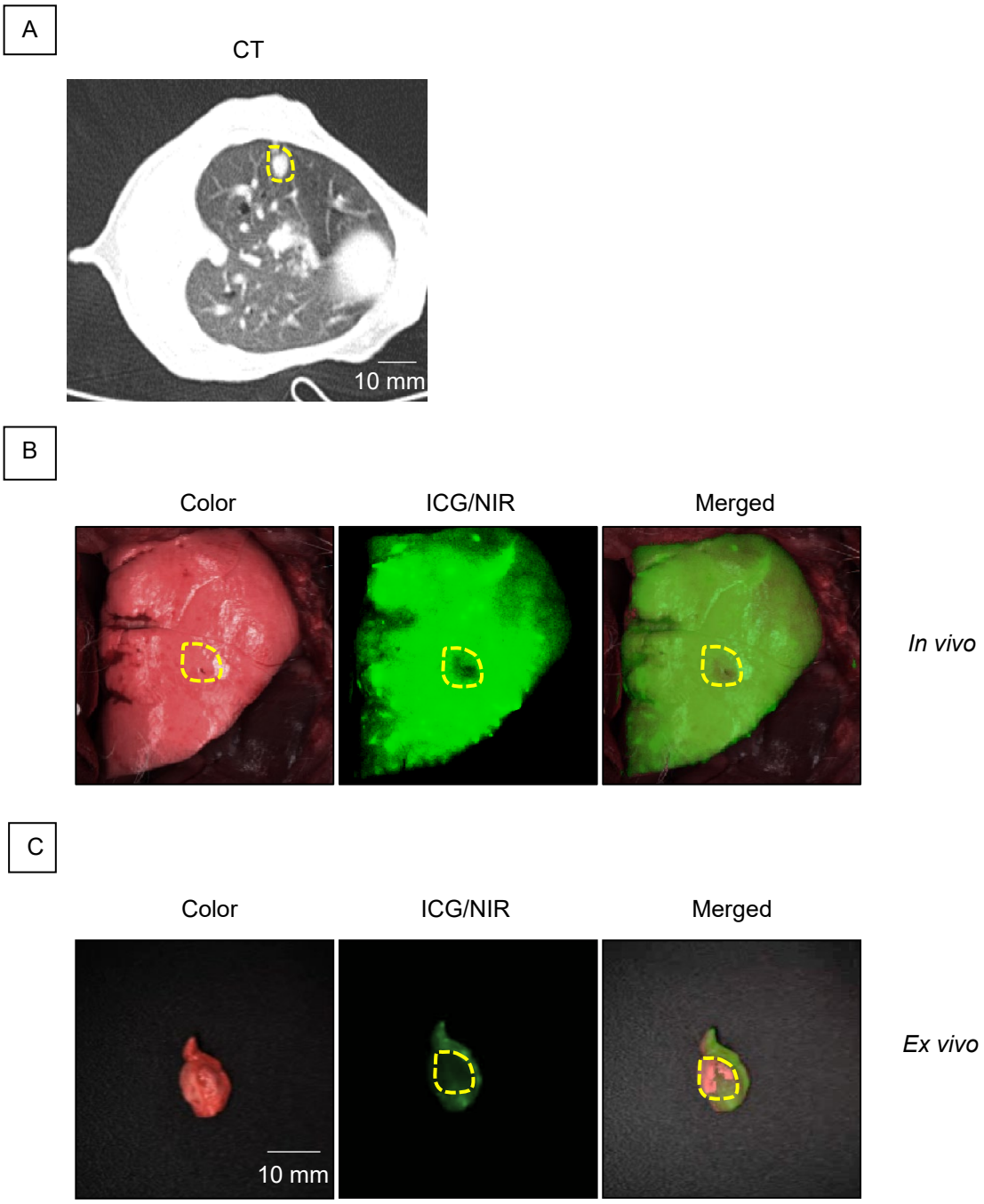
eFigure 2. Biodistribution of ICG in Different Mouse Organs after ICG inhalation.
The near-infrared (NIR) fluorescence images of the brain, lungs, kidneys, liver, and spleen of a normal mouse after indocyanine green (ICG) inhalation. The green color represents the NIR fluorescence signal.



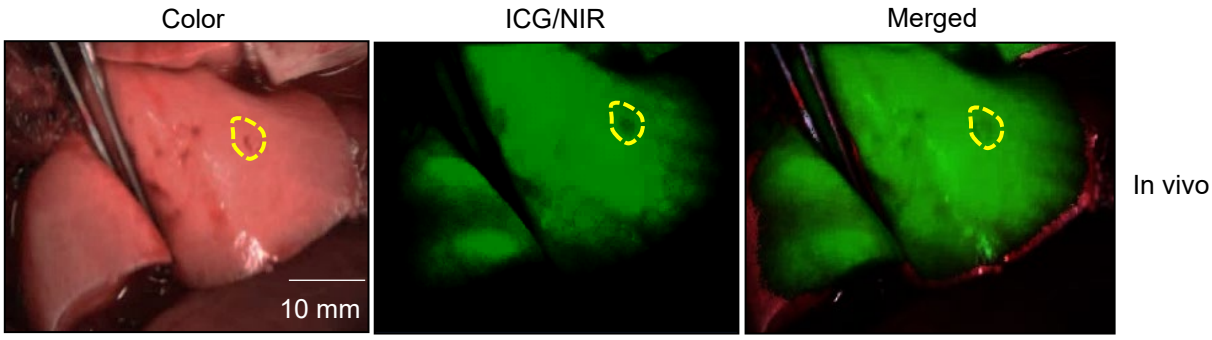
eFigure 3. Fluorescent Assessment of Lung TM with Inhaled and Intravenously Injected ICG. (A) Quantification of the tumor margin detection efficiency (TMDE) in rabbit models after the inhalation of indocyanine green (ICG) at different doses. (B) Quantification of TMDE after inhalation (0.25 mg/kg) and intravenous injection (5 mg/kg) of ICG. Error bars represent the median and interquartile ranges (*** *P* <.001).



eFigure 4. ICG Inhalation Can Detect Tumors at a Depth of 0.5 cm. (A) Representative images of computed tomography (CT) scan of the rabbit model (B) Representative *in vivo* lung images in color, near-infrared (NIR) (green), and merged (NIR with color) in the rabbit lung tumor model that inhaled indocyanine green (ICG). (C) *Ex vivo* lung images in color, NIR (green), and merged (NIR with color) in the rabbit lung tumor model that inhaled ICG. The yellow circular dotted line indicates the tumor site.



eFigure 5. A 0.2 cm Tumor could be Detected with ICG Inhalation. (A) The overall process of tumor margin detection in the lung tumor tissues via indocyanine green (ICG) nebulization. (B) The computed tomography (CT) image and Color, NIR and Merged *ex vivo* images of a lung from patients with lung cancer. The yellow circular dotted line indicates the tumor site.



eTable. Clinical and demographic characteristics of patients with lung cancer.

Abbreviations: RLL, right lower lobe; LUL, left upper lobe; RUL, right upper lobe; RML, right middle lobe; TMDE, tumor margin detection efficiency.

Case	Sex	Age	pTNM	Tumor	Pathology Type	Depth	Surgical Procedure	TMDE
1	M	56	pT1bN2 M0	2.2	Adenocarcinoma	1.0	RLL lobectomy	2.8
2	M	64	pT2N0M0	2.4	Adenocarcinoma	1.0	LUL lobectomy	2.7
3	F	52	pT3N2 M0	1.3	Adenocarcinoma	0.2	RUL lobectomy	3.3
4	F	65	pT2aN0 M0	3.1	Adenocarcinoma	0.5	RML lobectomy	2.6
5	F	48	pT2aN0 M0	2.1	Pleomorphic carcinoma	0	RUL lobectomy	3.4
6	F	64	pT2aN0 M0	2.8	Adenocarcinoma	0.2	RUL lobectomy	2.8

