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Krista J. Hachey, Brigham and Women’s Hospital
Christopher S. Digesu, Brigham and Women’s Hospital
Katherine W. Armstrong, Brigham and Women’s Hospital
Denis M. Gilmore, Brigham and Women’s Hospital
Onkar Vohra Khullar, Emory University
Brian Whang, Brigham and Women’s Hospital
Hisashi Tsukada, Brigham and Women’s Hospital
Yolonda L. Colson, Brigham and Women’s Hospital

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A Novel Technique for Tumor Localization and Targeted Lymphatic Mapping in Early Stage Lung Cancer

Krista J. Hachey, MD1,*, Christopher S. Digesu, MD1,*, Katherine W. Armstrong, MPH1, Denis M. Gilmore, MD1,2, Onkar V. Khullar, MD1,3, Brian Whang, MD1, Hisashi Tsukada, MD, PhD1, and Yolonda L. Colson, MD, PhD1

1Division of Thoracic Surgery, Brigham and Women’s Hospital, Boston, MA
2Department of Thoracic Surgery, Vanderbilt University Medical Center, Nashville, TN
3Division of Cardiothoracic Surgery, Emory University School of Medicine, Atlanta, GA

Abstract

Objective—To investigate safety and feasibility of navigational bronchoscopy (NB)-guided near-infrared (NIR) localization of small, ill-defined lung lesions and sentinel lymph nodes (SLN) for accurate staging in non-small cell lung cancer (NSCLC) patients.

Methods—Patients with known or suspected stage I NSCLC were enrolled in a prospective pilot trial for lesion localization and SLN mapping via NB-guided NIR marking. Successful localization, SLN detection rates, histopathologic status of SLN vs overall nodes, and concordance to initial clinical stage were measured. Ex vivo confirmation of NIR+ SLNs and adverse events were recorded.

Results—Twelve patients underwent NB-guided marking with indocyanine green (ICG) of lung lesions ranging in size from 0.4 to 2.2 cm and located 0.1 to 3 cm from the pleural surface. An NIR+ “tattoo” was identified in all cases. Ten patients were diagnosed with NSCLC and 9 SLNs were identified in 8 of the 10 patients, resulting in an 80% SLN detection rate. SLN pathologic status was 100% sensitive and specific for overall nodal status with no false negative results. Despite prior nodal sampling, one patient was found to have metastatic disease in the SLN alone, a 12.5% rate of disease upstaging with NIR SLN mapping. SLN were detectable for up to 3 hours allowing time for obtaining a tissue diagnosis and surgical resection. There were no adverse events associated with NB-labeling or ICG dye itself.

Corresponding author: Dr. Yolonda Colson MD, PhD, Division of Thoracic Surgery, Department of Surgery, Brigham and Women’s Hospital, 15 Francis St., Boston, MA 02115, Office: 617-732-6648, Fax: 617-264-5214, YCOLSON@partners.org.

*These authors contributed equally to this work.

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Disclosures: We acknowledge Novadaq for the donation of ICG dye for the initial 10 patients as proof-of-concept for NB-labeling. There has been no preview, restrictions, or direct monetary sponsorship for this trial.
**Conclusions**—NB-guided NIR lesion localization and SLN identification was safe and feasible. This minimally invasive image-guided technique may permit the accurate localization and nodal staging of early stage lung cancers.

**Introduction**

Lung cancer is the leading cause of cancer-related mortality in the U.S., with an estimated 158,080 deaths expected to occur in 2016\(^1\). Despite “curative” resection and lymphadenectomy (LAD), there is a 30% recurrence rate and a 60–80% 5-year survival in early stage non-small cell lung cancer (NSCLC)\(^2\). Attempts to improve these outcomes have led to the initiation of national lung cancer screening where an estimated 2 million Americans will be diagnosed with a new pulmonary nodule, and over 80,000 will require surgery for diagnosis and treatment\(^3\)–\(^5\).

Unfortunately, many very early cancers discovered with screening are too small or ill-defined, as with ground glass opacities (GGO), preventing localization for limited surgical resection or biopsy. Therefore, these small lesions are followed with imaging surveillance until they are large enough for diagnostic percutaneous biopsy or amenable to intraoperative localization. The concern of the surgeon and patient is that disease progression can occur during surveillance, thus potentially undermining the benefit of early detection. If a small or ill-defined lesion can be localized intra-operatively, limited surgical resection may not only offer a diagnosis but is also potentially curative for some of these small, peripheral lesions, if nodal disease is in fact absent.\(^6\)–\(^8\)

Furthermore, many patients with poor cardiopulmonary reserve may only be candidates for limited resection, and thus precise intra-operative localization for lung parenchyma preservation with a negative surgical resection margin is critical for maximizing long-term outcomes.

Despite negative pre-operative imaging to rule out occult locoregional lymph node metastases, pathologic upstaging found at the time of surgical resection occurs in up to 18% of patients\(^9\),\(^10\). Furthermore, nodal staging is inadequate in over 50% of limited resections performed for early stage cancers and standard hematoxylin and eosin analysis of all lymph nodes in the specimen identifies occult metastases 16–18% less often than when individual nodes are histologically scrutinized\(^11\),\(^12\). Occult nodal disease leads to understaging and missed opportunities for adjuvant therapy and may result in poor clinical outcomes\(^13\)–\(^17\).

**Thus, two major barriers facing the curative treatment of early stage lung cancer are surgical: the current challenge in identifying small, ill-defined lesions intraoperatively for parenchyma-sparing resections and the inadequate sampling and histologic scrutiny of tumor-associated lymph nodes.**

Our previous phase I dose-titration trial utilizing transpleural, peritumoral injection of the FDA-approved near-infrared (NIR) dye indocyanine green (ICG) in 41 patients demonstrated no adverse events, excellent real-time imaging via minimally invasive NIR thoracoscopy, and a dose-dependent increase in NIR signal within sentinel lymph nodes (SLNs)\(^1\). Although spillage of dye can occur following transpleural injection, SLNs were identified in 100% of patients at the optimized ICG dose. Importantly, transpleural injection
required intraoperative palpation of the lesion for peritumoral injection limiting this technique to superficial and/or relatively large lesions.

We conducted the first-in-human pilot trial of patients with suspected or known early stage NSCLC undergoing intraoperative NB-guided transbronchial, peritumoral ICG injection, subsequent NIR lesion localization, resection, and SLN mapping via minimally invasive thoracoscopic surgery. We aimed to show this comprehensive image-guided technique is both safe and feasible for both localization of early stage lung cancers and targeting of tumor-associated SLNs.

**Methods**

**Study Design and Patients**

This prospective pilot trial evaluates the feasibility of NB-guided peritumoral ICG injection for NIR “tattoo” marking of suspected or known lung cancers and NIR+ SLN retrieval for nodal staging (Figure 1). The trial protocol was approved by the Partners Internal Review Board (Boston, MA), and all cases were conducted at Brigham and Women’s Hospital (Boston, MA). Primary endpoints included visualization of the NIR+ “tattoo”, complete resection of the lesion, identification of NIR+ SLNs, and histopathologic status of SLNs vs. overall LNs in the lymphadenectomy specimen. In addition, adverse events associated with NB-labeling or to ICG dye itself were recorded.

Twelve patients with clinically suspected or biopsy-proven T1N0 NSCLC scheduled to undergo minimally invasive lung resection were enrolled between March and December 2015 following informed consent for surgery and independently for the study protocol. Exclusion criteria included age < 18 years old, pregnancy or breastfeeding, history of iodide or seafood allergy, suspected or known metastatic nodal disease, and prior neoadjuvant chemotherapy or radiation therapy to the lung. Although lobectomy is the standard of care for lung cancer, initial patients selected for this trial were those undergoing minimally invasive wedge resection for diagnosis or treatment either due to patient co-morbidity, lesion characteristics, or patient preference as the aim of this trial was to test our technique for both lesion localization and targeted lymphatic mapping. All study patients underwent standard preoperative chest CT +/- PET/CT for staging. Four patients with enlarged or suspicious lymph nodes had documented negative nodal pathology via cervical mediastinoscopy or endobronchial ultrasound prior to lung resection. All patients found to have NSCLC underwent lymphadenectomy at the time of surgery.

**Navigational Bronchoscopy-Guided ICG Peritumoral Injection**

Navigational bronchoscopy is a real-time localization system that uses 3D images generated from a pre-operativeNB-protocol chest CT or PET/CT scan with 1 × 0.8 mm slice intervals. The superDimension™ system (Covidien, Minneapolis, MN) used creates a “road map” through the airway anatomy to guide a NB catheter to the lesion of interest for intraoperative biopsy and peritumoral ICG injection. ICG (Novadaq Technologies, Bonita Springs, FL) was diluted to 2.5mg/ml using 25% human serum albumin (ICG:HSA), as albumin has been shown to increase the effective hydrodynamic diameter of ICG almost 6-fold, resulting in
increased lymphatic retention. Based on our group’s prior transpleural NIR dose escalation trial for lung cancer, a dose of 1.25mg – 2.5mg (0.5 – 1mL) ICG was used. Using the NB catheter location registration, access to within a centimeter of the lesion was obtained via the airway. A needle was then advanced towards the lesion to inject ICG just deep to the lesion of interest. This allowed ICG marking of the deep margin of the lesion ensuring the entire lesion was removed with resection of the ICG labeled parenchyma. The number of peritumoral injections used in each case ranged from 1–3 discrete injections, depending on lesion location, feasibility of multiple injection sites, and the number of lesions to be localized. The “tattoo” was visualized from the pleural surface due to the property of NIR light to penetrate tissue.

**NIR Image-Guided Resection and Lymphatic Mapping**

Following NB-guided peritumoral injection, patients were repositioned to the appropriate lateral decubitus position for resection via video-assisted thoracoscopic surgery (VATS). Real-time NIR fluorescence images were obtained intraoperatively using a minimally invasive near-infrared PINPOINT imaging system (Novadaq) including a 10 mm 30-degree NIR thoracoscopic camera. ICG signal was detected in vivo at the site of peritumoral “tattoo” in all patients, with minimal NIR autofluorescence or background signal from normal human tissue. All patients underwent lung resection and those with confirmed NSCLC underwent lymph node sampling comprising the LAD specimen with NIR imaging for SLN identification and resection. Specimens were imaged ex vivo to confirm NIR signal. The decision for definitive wedge resection vs. lobectomy was based on lesion characteristics (identification of benign disease, multifocal adenocarcinoma, or metastatic disease), limited pulmonary status, or patient preference. For diagnoses other than NSCLC (benign lesions or metastases from another primary site), LAD and NIR SLN imaging were not performed.

**Pathologic Analysis**

The surgical specimen and the individually labelled nodal specimens, including NIR+ SLNs, were sent for routine histopathologic analysis. All LNs were fixed in formalin and embedded in paraffin for hematoxylin and eosin staining. Pathologic status was determined by an experienced lung pathologist without knowledge of which node was identified as NIR+ intraoperatively.

**Statistical Analysis**

The data reported is descriptive of the events that occurred in the trial with mean and standard deviation reported where appropriate. Specificity and sensitivity of the SLN are calculated based on the pathologic status of the NIR+ SLN in comparison to all nodes sampled along with the LAD.
Results

Patient and Lesion Characteristics

Twelve patients, including 10 females and 2 males, underwent NB-guided NIR marking of lung lesions. Mean patient age was 62 years old with a standard deviation of 6.7 yrs. A total of fifteen lung lesions, all malignant, were resected ranging in size from 0.4–2.2 cm, with a depth from the pleural surface of 0.1 to 3.0 cm (Table 1). Seven lesions were characterized as solid on preoperative imaging, 6 were semi-solid, one was a pure GGO, and one was not evident on pre-operative imaging. Lesions were identified in the right upper lobe (n=4), right lower lobe (n=2), left upper lobe (n=6), and left lower lobe (n=3). Wedge resection was performed for all lesions to establish a pathologic diagnosis with completion lobectomy in one case. Two patients were diagnosed with metastatic disease from another primary and 10 patients with NSCLC.

Navigational Bronchoscopy-Guided NIR Lesion Localization and Resection

NB-guided NIR “tattoo” marking was performed under general anesthesia with the patient supine, and immediately prior to lung resection in all 12 cases with all 14 known lesions NIR marked and intra-operatively identified. The size of the visualized pleural “tattoo” depended on the depth and location of injection of ICG. Two separate lesions were marked with 0.5cc of ICG:HSA per lesion in patients 5 and 12. These patients underwent wedge resections for diagnosis of potential multifocal disease. Patient 12 was discovered on final pathology to have a third focus of adenocarcinoma not palpable or identified on preoperative imaging, but was removed within the wedge resection of an adjacent NIR marked tumor.

Total NB time for peritumoral injection, which included biopsy and on-site cytologic interpretation as requested by the surgeon in 7 cases, averaged 34.5 minutes with approximately 20 minutes being for the entire navigation, dye preparation, and lesion “tattoo” if biopsy was not required. Total fluoroscopy time for injection of ICG alone ranged from 3–20 seconds, and for patients that also underwent NB-biopsy, ranged from 19.7–26 seconds with a total dose of 0.93–3.94 mGy.

Immediately upon placement of the NIR thoracoscope, the NIR “tattoo” was successfully identified on the pleural surface corresponding to each lesion in all cases. The average time to visualization following injection was 65 minutes with a standard deviation of 31 minutes. Given the depth of the lesion in Patient 6, needle localization under CT-guidance following ICG injection was unsuccessfully attempted as a backup strategy prior to VATS delaying the assessment of NIR marking which was still readily visible at 161 minutes (Figure 2). For each patient, the site of NIR⁺ “tattoo” co-localized with the primary nodule identified on all frozen section analyses and was associated with negative surgical margins in all cases (mean margin distance 0.9 cm +/- 0.7 cm). The average lesion to lesion:margin ratio was 1.3 +/- 0.8. No clinically significant adverse events following navigational bronchoscopy and the injection of ICG were noted including bleeding, pneumothoraces, or bronchospasm.
**NB-Guided NIR imaging for SLN Identification**

Ten of the 12 patients were found to have primary lung cancer for which SLN mapping and subsequent LAD were performed. SLN mapping was not performed in two patients without primary lung cancer. At least one NIR$^+$ SLN was identified in 8 of the 10 lung cancer cases, for an 80% detection rate (Table 2). Notably, 75% of SLNs identified were in N2 stations increasing the likelihood of finding disease that can upstage patients to stage III, as occurred in patient 1. SLNs were not identified in patients 6 and 8. As mentioned, patient 6 was delayed due to an unsuccessful attempt at needle localization. Although the NIR “tattoo” readily aided in tumor localization, the delay resulted in greater than 3 hours elapsing between the time of injection and examination of LN stations. Patient 8 also had a visible NIR$^+$ “tattoo” of a peripheral lesion, but ICG was noted to have extravasated through the visceral pleura resulting in a smaller actual dose delivered to the lung parenchyma and no NIR$^+$ SLN was identified. In addition, this patient had a diagnosis of sarcoidosis with significant fibrosis involving the lymph nodes and associated lung parenchyma which may have also contributed to this failure. Patient 7 underwent concurrent methylene blue injection of the lesion which was visible at the site of the NIR$^+$ “tattoo”, however the SLN was only visible with ICG and no methylene blue was present within the LAD specimen. Representative cases in which a SLN was identified are shown in Figure 3.

**Pathologic Analysis**

Both the lung and SLN specimens were imaged ex vivo prior to pathologic analysis to confirm NIR$^+$ status. Pathologic analysis of resected lesions revealed primary lung adenocarcinoma (n=9), squamous cell carcinoma (n=1), atypical spindle cell neoplasm (n=1) and metastatic leiomyosarcoma (n=1). In all cases where LAD was performed, a negative SLN was reflective of the status of all other nodes in the LAD specimen. Importantly, the NIR$^+$ level 7 SLN identified in Patient 1 was the only pathologic positive lymph node identified within the LAD specimen, resulting in upstaging from a negative nodal status (despite preoperative cervical mediastinoscopy) to stage IIIa disease. Based on these findings, the pathologic status of the 9 identified SLN was 100% sensitive and specific for overall nodal disease.

**Discussion**

This is the first reported human pilot trial to demonstrate the safety and feasibility of NB-guided peritumoral NIR marking of lung lesions and mapping of tumor-associated SLNs. Twelve patients successfully underwent NB-guided marking, allowing for lung lesion localization and resection with a negative margin in all patients. SLNs were identified in 80% of the ten patients found to have NSCLC. This minimally invasive technique allows for the accurate localization and staging of small, ill-defined pulmonary nodules and GGO's representing a significant advancement in the way we manage, treat, and stage early lung cancers.

Nodal status is a significant prognostic indicator in lung cancer, yet many patients still do not undergo proper nodal staging leading to potentially under-staged and under-treated disease which may contribute to poor overall survival and relatively high recurrence.

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rates\textsuperscript{2,12–17}. Although pre-operative imaging, in particular CT and PET-CT, is necessary for clinical staging, histologic diagnosis is still required to ensure N0 status given the relatively low sensitivity and specificity for detecting metastatic nodal disease in early stage lung cancers\textsuperscript{21,22}. The high false negative rate is evident by the nearly 18\% incidence of pathologic upstaging reported in early stage lung cancers, and in fact, our study resulted in the upstaging to stage III disease in 12.5\% (1/8) of patients in which SLNs were analyzed\textsuperscript{9,10}. Although nodal sampling has been shown to be equivalent to radical lymphadenectomy, SLN mapping offers distinct advantages including targeting the tumor-draining node(s) that are at increased risk of harboring metastatic disease and also permitting focused in-depth histologic scrutiny\textsuperscript{23}. In addition, patients with early stage NSCLC are prone to developing a second primary NSCLC at a rate of 1–2\%/yr. In the future, targeted nodal assessment could potentially allow for subsequent nodal staging of a second ipsilateral lung cancer, an opportunity which is currently not possible if a full lymphadenectomy has previously been performed\textsuperscript{24}.

Under the current NCCN guidelines, patients with small, peripheral semi-solid or slow growing lesions may not require a lobectomy (though an anatomic resection is still preferred)\textsuperscript{6}, and some studies are indicating favorable long-term outcomes with limited resection\textsuperscript{7,8}. These small ill-defined lesions may be difficult to localize intra-operatively, however with this technique, they can easily be identified and staged in a directed fashion and in a single operative setting. This is important for patients undergoing sub-lobar resection as traditional rates of nodal sampling with sub-lobar resection have been highly variable\textsuperscript{11}. Furthermore, this technique for targeted lymphatic mapping may offer an advantage for poor surgical candidates that cannot tolerate a large anatomic resection, but would receive better staging than a patient undergoing focused definitive radiation such as stereotactic body radiation therapy.

In this pilot study, the first patient evaluated was found to have nodal disease solely in the N2 level NIR\textsuperscript{+} SLN, which was not previously detected on PET/CT or cervical mediastinoscopy despite multiple LNs sampled including several nodes in the same station. This change in clinical stage significantly changed the prognosis and treatment of the patient resulting in the administration of adjuvant chemotherapy. This disease may have remained occult without the focused direction of the NIR signal as all of the other NIR\textsuperscript{−} nodes in the same nodal station were negative for metastatic disease. Similar upstaging was noted in 2 patients in our prior transpleural NIR trial reinforcing the importance of targeting specific tumor-associated SLNs for focused pathologic analysis\textsuperscript{18}.

Early porcine studies of NB-guided NIR imaging by Anayama and Wada demonstrated the significant potential for clinical translation of a transbronchial ICG injection technique\textsuperscript{19,25,26}. Anayama et al. reported the feasibility of NB-guided ICG delivery adjacent to porcine agar pseudotumors, with NIR signal detection up to a depth of 2.4 cm and lasting as long as 6 hours in an inflated porcine lung\textsuperscript{25}. In the current study, the NIR “tattoo” was readily identifiable in all 12 patients with peritumoral NIR signal detection at depths of up to 3 cm from the pleural surface. Premixing ICG with HSA, which we have previously shown to be critical for successful SLN identification in humans, also resulted in a stable NIR\textsuperscript{+} signal without significant parenchymal diffusion for over 4.5 hours following ICG injection.
In the Wada et al. study, attenuated nodal signal was noted with transbronchial ICG injection using ICG concentrations as low as 10μg for lung marking, though previous studies have shown that these low ICG doses are inadequate for human translation of NIR techniques\(^{18,19}\). Thus, using our previously established dose of ICG:HSA, the current trial demonstrates the feasibility of SLN identification after transbronchial injection with persistence of NIR signal in SLN for at least 3 hours following injection, allowing sufficient time for completion of intraoperative diagnosis, resection, and staging.

NB-guided NIR marking and directed surgical resection allows for diagnosis and treatment to be carried out in a single operative setting with the NIR “tattoo” placed via the same NB working channel used for biopsy of a lesion, thus streamlining care, reducing time to therapy, and potentially reducing costs. This is in contrast to CT-guided percutaneous biopsy which typically requires coordination with an interventional radiologist and access to an imaging suite prior to surgery. In addition, patients are exposed to radiation and are at risk of pneumothorax and possible chest tube placement for management of complications that may delay definitive diagnosis and treatment\(^{27,28}\). This risk is mitigated with NB as biopsy and ICG injection do not require traversing the pleural surface, and may be irrelevant when performed immediately before surgical entry into the chest. The current study demonstrated that NB-guided ICG injection was safe without associated adverse events. Total procedure time including NB-guided peritumoral injection was similar to previous reports adding on average 20 minutes to the operative time for both diagnosis, when indicated, and labeling of the lesions\(^{29}\). Importantly, other approaches to intraoperative detection of pulmonary nodules have been described, including lesion localization with blue dyes, microcoils, percutaneous wire placement and intravenous ICG, but none of these techniques permit concurrent SLN mapping for the lesion of interest\(^{30–35}\).

Blue dyes and radiocolloid tracers have not demonstrated reproducible or accurate SLN detection even in multicenter studies, in large part due to pigmented anthracotic thoracic LNs, high tracer background signal, and rapid migration due to small particle size\(^{36–37}\). The one patient in this trial who underwent concurrent methylene blue injection did demonstrate equivalent localization with lesional “tattoo”, however there was no discernible migration to LNs and the SLN was identified only by ICG. The translational success of other tumor-targeted approaches to fluorescence labeling, such as antibody-conjugated and enzyme-activated probes, appears to rely on the ability to predict tumor biology and histology, which can be difficult when evaluating ill-defined lung nodules, particularly when lesions are very small and pre-operative histology or genomic sequencing is not available\(^{38–39}\).

Limitations of the current prospective pilot trial include a small sample size, limited histologic and radiographic variability in tumor type, and restriction to NB-accessible lesions. Pathologic findings demonstrated the predictive ability of identified NIR+ SLNs scrutinized by routine pathologic analysis to identify occult nodal disease, and in the future, in-depth immunohistochemical evaluation may further improve the sensitivity of this approach. Additionally, long-term recurrence and survival outcomes are necessary in larger multi-center trials to determine if cases identified as N0 based on SLN status behave as true node negative cases and exhibit better overall survival than patients deemed node-negative by standard hematoxylin and eosin analysis of routine lymphadenectomy specimens.
This novel first-in-human prospective pilot trial demonstrates that the NB-guided NIR technique for tumor localization and targeted lymphatic mapping is safe and feasible even for small ill-defined, stage I lung cancers. With continued development, this technology has the potential to significantly extend surgical resection and staging to include non-palpable lesions in patients that are unable to tolerate a “blind lobectomy” and may alter the way early stage NSCLC is identified and staged intra-operatively. Under a single anesthetic, patients may have an early stage NSCLC accurately biopsied for diagnosis, definitively resected and accurately staged allowing surgeons to truly achieve the curative benefits purported with early detection CT screening programs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Glossary of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>NIR</td>
<td>near-infrared</td>
</tr>
<tr>
<td>LN</td>
<td>lymph node</td>
</tr>
<tr>
<td>SLN</td>
<td>sentinel lymph node</td>
</tr>
<tr>
<td>ICG</td>
<td>indocyanine green</td>
</tr>
<tr>
<td>NB</td>
<td>navigational bronchoscopy</td>
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<tr>
<td>HSA</td>
<td>human serum albumin</td>
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<tr>
<td>LAD</td>
<td>lymphadenectomy</td>
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<td>PET</td>
<td>positron emission tomography</td>
</tr>
<tr>
<td>CT</td>
<td>computed tomography</td>
</tr>
<tr>
<td>GGO</td>
<td>ground glass opacity</td>
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References


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Perspective Statement

Two major barriers to curative treatment of early stage lung cancer are surgical: 1) challenge in identifying small, ill-defined lesions intraoperatively for resection and 2) inadequate sampling and histologic scrutiny of tumor-associated lymph nodes. Navigational bronchoscopy-guided near-infrared marking is a novel technique that permits tumor localization and targeted staging of early lung cancers.
Central Message

Navigational bronchoscopy-guided near-infrared tumor marking is feasible and allows localization and nodal staging of early stage lung cancers.
Central picture.
Level 7 NIR$^+$ SLN Following NB-guided Peri-lesional “Tattoo” with Indocyanine Green
Figure 1. NB-guided NIR Imaging Technique
NB-guided lesion localization for NIR⁺ "tattoo", NIR lymphatic migration, and in situ NIR⁺ SLN identification for in-depth pathologic analysis.
Figure 2. NB-guided NIR “tattoo” of lesion 2.1cm from the pleural surface
A. Navigational bronchoscopy guides the injection of 1cc of ICG. B. Axial CT image demonstrating a 1.1 cm lesion (yellow marker) that is 2.1 cm from the pleural surface (blue arrows). C. NIR+ “tattoo” is easily identifiable from the pleural surface nearly 4 hours after ICG injection.
Figure 3. NB-guided NIR “tattoo” and SLN identification
A. NB-guided lesion localization for transbronchial, peritumoral ICG injection
B. NIR+ “tattoo” on pleural surface ~1hr after ICG injection (3 injections used)
C. In vivo NIR+ level 7 SLN identification ~2hrs after ICG injection
D. Axial CT of a 1.2 cm LLL lesion
E. NIR+ ICG “tattoo” is nearly identical to a concomitant methylene blue marking (blue arrow)
F. In vivo NIR+ level 7 SLN (white arrow) identified in situ by ICG alone with no methylene blue.
G. Axial CT of a 1.5 cm ill-defined, pure ground glass opacity
H. In vivo NIR lymphatic track from RLL peri-tumoral “tattoo” (blue arrow) to level 11R nodal station, RUL peri-tumoral “tattoo” (white arrow)
I. In vivo NIR+ level 11R SLN (white arrow)
Table 1

Characteristics of Pulmonary Nodules and NB-guided NIR “Tattoo” Marking.

<table>
<thead>
<tr>
<th>Lesion Location</th>
<th>Final Pathologic Size (cm)</th>
<th>Lesion Type</th>
<th>Depth from Pleural Surface (cm)a</th>
<th>Fluoro Time for ICG Injection (sec)</th>
<th>Time from ICG Injection to NIR tattoo ID (min)</th>
<th>Margin Distance of Initial Wedge Resection (cm)b</th>
<th>Lesion Diagnosis</th>
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<td>–</td>
<td>43</td>
<td>Adenocarcinoma</td>
</tr>
<tr>
<td>LUL</td>
<td>2.2</td>
<td>Solid</td>
<td>0.1</td>
<td>6</td>
<td>14</td>
<td>66</td>
<td>Squamous Cell Carcinoma</td>
</tr>
<tr>
<td>LUL</td>
<td>0.9</td>
<td>Mixed GGO/solid</td>
<td>0.1</td>
<td>12</td>
<td>12</td>
<td>48</td>
<td>Adenocarcinoma</td>
</tr>
<tr>
<td>LUL</td>
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<td>Mixed GGO/solid</td>
<td>1.6</td>
<td>3</td>
<td>10</td>
<td>73</td>
<td>Adenocarcinoma</td>
</tr>
<tr>
<td>RUL</td>
<td>2.1</td>
<td>Mixed GGO/solid</td>
<td>0.3</td>
<td>–</td>
<td>–</td>
<td>64</td>
<td>Adenocarcinoma</td>
</tr>
<tr>
<td>RLL</td>
<td>1.5</td>
<td>GGO</td>
<td>1.0</td>
<td>–</td>
<td>–</td>
<td>81</td>
<td>Adenocarcinoma</td>
</tr>
</tbody>
</table>

aMeasured from lesion to nearest pleural surface on pre-operative CT

bIntra-operative margin
cNot recorded
dPatient did undergo completion lobectomy
eTwo separate lesions were NIR marked with 1.25mg of ICG (in 0.5cc of ICG:HSA) per lesion. Patient 12 also had an incidentally found third focus of adenocarcinoma on final pathology.

fCase included needle localization under CT-guidance following NIR “tattoo” and before conversion to VATS
Table 2

Sentinel Lymph Node (SLN) Yield and Specimen Pathology.

<table>
<thead>
<tr>
<th>Pt #</th>
<th>SLN Station (# nodes)</th>
<th>Time to SLN Resection (min) (from injection)</th>
<th>SLN Pathology</th>
<th>Other LN Stations (# nodes)</th>
<th>Other LN Pathology</th>
<th>Pathologic Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Level 7 (1)</td>
<td>124</td>
<td>Positive</td>
<td>Level 4 (1); Level 7 (1); Level 10 (1); Level 11 (5)</td>
<td>Negative</td>
<td>T1aN2</td>
</tr>
<tr>
<td>2</td>
<td>Level 10 (1)</td>
<td>180</td>
<td>Negative</td>
<td>Level 5 (1); Level 10 (4); Other hilar (6)</td>
<td>Negative</td>
<td>T1aN0</td>
</tr>
<tr>
<td>4</td>
<td>Level 5 (1)</td>
<td>155</td>
<td>Negative</td>
<td>Level 11 (2); Level 3 (1)</td>
<td>Negative</td>
<td>T1aN0</td>
</tr>
<tr>
<td>6</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Level 5 (2); Level 7 (3)</td>
<td>Negative</td>
<td>T1aN0</td>
</tr>
<tr>
<td>7</td>
<td>Level 7 (3)</td>
<td>64</td>
<td>Negative</td>
<td>Level 9 (3)</td>
<td>Negative</td>
<td>T1aN0</td>
</tr>
<tr>
<td>8</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Level 5 (1); Level 7 (1); Level 10L (1)</td>
<td>Negative</td>
<td>T1aN0</td>
</tr>
<tr>
<td>9</td>
<td>Level 5 (1)</td>
<td>134</td>
<td>Negative</td>
<td>Level 10L (1)</td>
<td>Negative</td>
<td>T1bN0</td>
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<td>Negative</td>
<td>Level 5 (1); Level 10 (1)</td>
<td>Negative</td>
<td>T1aN0</td>
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<td>Level 5 (1) Level 7 (1)</td>
<td>102</td>
<td>Negative</td>
<td>–</td>
<td>Negative</td>
<td>T1aN0</td>
</tr>
<tr>
<td>12</td>
<td>Level 11R (1)</td>
<td>92</td>
<td>Negative</td>
<td>Level 4R (1); Level 7 (1)</td>
<td>Negative</td>
<td>T1bN0</td>
</tr>
</tbody>
</table>

*Additional nodes removed as part of the routine lymphadenectomy*