Published in final edited form as:

Semin Thorac Cardiovasc Surg. 2014 ; 26(3): 201–209. doi:10.1053/j.semtcvs.2014.09.001.

Current Innovations in Sentinel Lymph Node Mapping for the Staging and Treatment of Resectable Lung Cancer

Krista J. Hachey, MD and Yolonda L. Colson, MD, PhD

Abstract

Despite surgical resectability, early stage lung cancer remains a challenge to cure. Survival outcomes are hindered by variable performance of adequate lymphadenectomy and the limitations of current pathologic nodal staging. Sentinel lymph node (SLN) mapping, a mainstay in the management of breast cancer and melanoma, permits targeted nodal sampling for efficient and accurate staging that can influence both intraoperative and adjuvant treatment decisions. Unfortunately, standard SLN identification techniques with blue dye and radiocolloid tracers have not been shown to be reproducible in lung cancer. In more recent years, intraoperative near infrared (NIR) image-guided lung SLN mapping has emerged as promising technology for the identification of the tumor-associated lymph nodes most likely to contain metastatic disease. Additionally, the clinical relevance of SLN mapping for lung cancer remains pressing, as the ability to identify *micrometastatic* disease in SLNs could facilitate trials to assess chemotherapeutic response and the clinical impact of occult nodal disease. This review will outline the current status of lung cancer lymphatic mapping and techniques in development that may help close the gap between translational research in this field and routine clinical practice.

Introduction

Despite being the standard of care in breast cancer and melanoma, the clinical translation of blue dye and radiocolloid sentinel lymph node (SLN) mapping to lung cancer has been disappointing^{1–5}. Newer imaging technologies, however, are more adaptable to the complex anatomy and lymphatic drainage pathways in the chest, providing promising directions for lung lymphatic mapping^{6–13}. For clinical stage I/II lung cancer, complete lymphadenectomy, or resection of N1 nodes with sampling of at least 3 N2 stations, is the current recommended practice for surgical lung cancer staging¹⁴. However, development of an accurate and reproducible SLN methodology for lung cancer has the potential to propel a paradigm shift in the staging of surgically resectable disease and improve survival outcomes.

Patients with early stage lung cancer still face recurrence rates as high as 30–40% and five-year survival ranges dramatically from about 50–90%, in part due to occult disease and inadequate nodal staging^{15–19}. Therefore, the ability to better predict and analyze those N1

Dr. Yolonda L. Colson, YCOLSON@partners.org, Phone: 617-732-6824.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

and N2 nodes most likely to contain metastatic disease promises to increase disease detection through targeted nodal dissections tailored to tumor-specific lymphatic pathways and improve staging and patient outcomes. The therapeutic goals of SLN mapping for lung cancer are aimed at detecting metastatic nodal disease and then identifying those patients most likely to benefit from adjuvant treatment^{20–21}. Furthermore, with lung cancer screening now on the horizon, a reliable minimally invasive lymphatic mapping technology could improve staging and outcomes for thousands of patients with early stage lung cancers identified on low dose CT screening^{22–23}.

More recent innovative SLN detection methods, such as near infrared (NIR) imaging and computed tomography (CT) lymphography, have been shown to be feasible in early clinical trials in lung cancer^{6–13}. However, difficulties persist with the few lymphatic tracers available for human studies as well as with the limited validation of SLN predictive value that single institution studies can reach. Nonetheless, targeted lymphatic mapping for improved lung cancer staging remains the ultimate goal and this review will discuss recent advances in the translation of these technologies to clinical trial investigation.

Intraoperative SLN Imaging for Lung Cancer Staging

Following the success of blue dye and radiocolloid tracers in breast and melanoma, early lung lymphatic mapping studies revealed that these tracers were inadequate to overcome the unique technical difficulties of SLN identification in the thoracic cavity^{3–5,24–28}. Yet, work by Liptay, Little, and others have contributed immensely to an understanding of why these methods have been difficult to adopt, and how the size and composition of tracers affect lymphatic migration and signal detection within SLNs (Table 1). Small visible tracers such as methylene blue are less than 5nm in size and result in relatively fast migration through lymphatics, and even beyond SLNs if not identified within 10 to 30 minutes after injection^{4,29–30}. Furthermore, visible dyes are difficult to detect in the anthracotic pigmented nodes commonly found in the chest. Radiocolloids, on the other hand, can be up to 1000nm in size, requiring injection 6–24 hrs prior to surgery³⁰. In addition to requiring an additional procedure for the patient, radiocolloids incur the risks of radiation exposure, pneumothorax and bleeding as a result of transthoracic tracer injection. The only multicenter trial published on lung SLN identification examined the reproducibility of mapping with technetium 99m sulfur colloid, a 40nm radiocolloid injected at the time of surgery, resulting in a SLN identification rate of 61.5%, with SLNs shown to be accurate in 83.3% of cases^{5,30}. Although this study involved the intratumoral injection of radiocolloid and thus tracer migration may have been limited through compressed intratumoral lymphatics, there is considerable variability among radiocolloid studies in lung cancer patients, including those that perform peritumoral injection^{24–28,31}. “Shine-through,” signal saturation from radioactivity at the primary tumor, or around the injection site, has been a difficult technical issue to overcome with this method and significantly impairs the ability to detect smaller nearby radioactive targets such as a SLN⁵. Thus newer methods have focused on non-radioactive tracers (Table 2).

Near Infrared Imaging for Intraoperative Mapping

More recent intraoperative SLN identification techniques provide real-time visual mapping using near infrared (NIR) imaging of fluorescent lymphatic tracers^{6-9,32}. NIR dyes undergo excitation and emission at wavelengths of 700–1000nm with the benefit of minimal autofluorescence from in vivo tissues, thereby enhancing signal detection³³. Using a specialized thoracoscope and monitor, the NIR signal can be represented in a grayscale or color image that highlights NIR signal in green within the familiar context of a thoracoscopic image (fig 1)⁶. Studies by Yamashita, Moroga and Gilmore have demonstrated initial safety and feasibility of NIR imaging using indocyanine green (ICG), the only NIR dye currently approved by the Food and Drug Administration (FDA), and have achieved SLN detection rates ranging from 80.3% to 100%^{6-9,32}. Higher rates of SLN detection were identified when ICG was mixed with human serum albumin (HSA) in the phase I trial by Gilmore et al, as this protein carrier has been shown to increase the hydrodynamic diameter of ICG to about 7nm, facilitating retention within SLNs²⁹. While the approximate size of this tracer is not that much larger than the 5nm blue dye tracers, these studies suggest that improvements in the signal detection method using a fluorophore in the NIR range can greatly enhance SLN identification rates.

NIR studies have elucidated the variable patterns of lymphatic drainage seen in lung cancer, even when tumors are within the same lobe. In a trial of 61 patients, Yamashita et al reported an 80.3% SLN identification rate, with N2 level SLNs in 18% of cases⁸. Right upper lobe tumors with N2 SLNs were associated with the level 4R position (22.7% of RUL cases) and level 7 position (4.5%), whereas right middle and lower lobe tumors were associated with level 7 in 14.3% of cases within each lobe. Left upper lobe tumors were most commonly associated with level 4L and 5 whereas level 8 SLNs were seen with left lower lobe tumors in a minority of cases. In a more recent trial of 38 patients, Gilmore et al demonstrated a 100% SLN identification rate with an optimized ICG dose mixed with HSA⁶. In this trial, 46% of SLNs were N2 level nodes with an N2 node being the only identified SLN in 3 cases. This study demonstrated similar patterns of SLN locations based on tumor site compared to trials by Yamashita et al, although one case of a RLL tumor yielded a level 9 SLN and LUL tumors showed the greatest diversity in SLN location. Regardless of tumor location, regional level 10 and 11 N1 lymph nodes appeared to be the most common sites of SLN identification across these studies. There appears to be enough variability in SLN location and a substantial number of N2 level SLNs such that the need for intraoperative SLN mapping remains relevant to efficient and cost-effective analysis of tumor-associated SLNs.

Although false negative results were identified in 2.1% of patients in the trial by Yamashita et al⁸ in which ICG was injected without a protein carrier, use of an optimized ICG:HSA solution identified SLNs in which SLN histology was 100% predictive of the disease status of the lymphadenectomy specimen as a whole⁶. There is still controversy around whether premixing ICG with HSA enhances SLN detection, and there is only one porcine lung study directly comparing ICG with and without HSA, suggesting that either formulation is sufficient for visualization of the first tumor draining lymph node for up to 120 minutes³⁴. Randomized and double-blind human trials comparing ICG to ICG mixed with HSA, as

have been performed for NIR imaging in breast cancer,³⁵ may be required in the future to ascertain detection rates and SLN accuracy. Such assessment and the translation of NIR-guided SLN mapping to the clinical arena will be based on signal intensity in SLNs and could be further aided by the development of quantitative or semi-quantitative methods of determining signal to background ratios and signal threshold above which NIR signal within a SLN is deemed positive. Despite the above success, several NIR imaging groups have noted that a major limitation to accuracy and translation is the frequent spillage of ICG during injection into the lung via a transpleural peritumoral approach. Leakage from the site of injection into the surgical field significantly increases NIR background signal and can make SLN identification more difficult^{6,8-9}. Endoloops and endoclips have been used to temporize the problem, however a reproducible and streamlined solution has not yet been established, limiting the wider applicability of the current approach and providing an opportunity for improvement.

Computed Tomography (CT) Lymphography for SLN Imaging

Complementing intraoperative SLN mapping efforts are a handful of seminal studies examining the feasibility of pre-operative CT lymphography for lymphatic mapping in lung cancer¹⁰⁻¹³. Peritumoral CT-guided injection of a water soluble contrast dye was performed followed by interval CT images for up to 5 minutes after injection. Images were compared to a baseline CT prior to contrast exposure to identify nodal sites of contrast retention, with SLNs defined as nodes with increased attenuation by 30 Hounsfield units compared to the baseline scan¹⁰⁻¹³. Multidetector CT scanning has also been used to create 3D reconstructions defining SLN locations with respect to bronchial anatomy^{10,13}.

CT-guided transpleural and transbronchial injection of iopamidol and iohexol have both been shown to be feasible for CT lymphography without adverse reactions from the dye, although radiation exposure, pneumothorax and bleeding are all potential risks involved in this method¹⁰⁻¹³. Suga et al characterized the rapid lymphatic migration kinetics of iopamidol, resulting in maximum attenuation within SLNs at one minute after injection¹². Dye migration and image acquisition times are reportedly very brief, however, overall duration of the procedure and time required for collaboration with radiology, particularly if 3D reconstruction is required to delineate the exact position of SLNs, has not been reported. With the increasing availability of hybrid operating rooms, CT lymphography has the potential to be integrated into surgical staging and treatment, rendering this approach to SLN mapping a more efficient and patient-friendly process.

SLN identification rates on CT images were as high as 100% in these pilot studies, ranging from 12-15 total subjects, however SLNs were not identified in a small minority of cases¹⁰⁻¹³. It remains unclear how to manage negative CT results where no SLNs were found, such as in the trial by Sugi et al, in which a lymphatic duct was identified in one patient without lymph node enhancement¹³. While an initial negative SLN surveillance during intraoperative imaging via NIR guidance might permit a second look later on during a case, CT scan based mapping procedures inevitably must strive to minimize radiation exposure and cost. Dual modality clinical trials coupling CT lymphography with an

intraoperative SLN imaging method may shed light on the limitations of both techniques while potentially bolstering SLN identification rates.

Takizawa et al performed the most recent trial of CT lymphography and investigated the use of navigational bronchoscopy for contrast dye injection in an effort to minimize the risks involved with transthoracic injection¹⁰. The study demonstrated a 92.3% SLN identification rate with SLNs identifying two patients with metastatic disease. In one patient, SLNs were identified at the level 10 and 2R stations, with evidence of metastases on H&E staining at level 10 and 11. In the second patient, SLNs were identified at level 5 and 13 with evidence of disease at level 5. No immunohistochemical staining or molecular analysis was performed to detect micrometastatic disease.

In Sugi et al, tumor metastases was identified in the SLNs of 3 patients, and these were the only lymph nodes identified with disease¹³. In studies by Ueda and Suga, no metastatic disease was found in any cases by H&E staining^{11–12}. Encouragingly, there were no false negative results in any these studies. However, these trials have been much smaller than those conducted with technetium 99m or NIR imaging and much larger trials are needed to determine the predictive value of SLNs using CT lymphography. Limitations include potential false positive results from lymph node calcification, which can be identified on a pre-procedure CT scan, as well as difficulty identifying very small SLNs on the order of 2–3mm¹³.

A number of technical benefits render CT lymphography for lung lymphatic mapping an appealing modality. CT-guided lesion localization is theoretically beneficial for more accurate peritumoral injection of small, nonpalpable lesions or more central lesions that are otherwise difficult to palpate. Furthermore, CT lymphography has the potential to detect SLNs at any tissue depth and at any site in thoracic cavity, including the contralateral chest. Although occult contralateral nodal metastases are uncommon in early stage lung cancers, these nodes are not detectable with intraoperative radiocolloid or NIR techniques. However, although limited studies exist, no cases of contralateral SLNs were identified in these CT-guided studies, raising questions as to the real frequency of contralateral SLNs and the clinical importance of this limitation.

CT lymphography may have future use for mapping N2 level SLNs in preparation for staging procedures such as cervical mediastinoscopy or EBUS, ensuring that high risk mediastinal nodes are sampled prior to definitive resection of a cancer with the goal of improving the accuracy of pre-operative staging (table 3). While the ultimate goal for SLN mapping is a single imaging modality that can be reliably and efficiently performed at the time of surgery, the refinement of CT lymphography for lung SLN mapping has the potential to add significant value if paired with intraoperative dissection.

MRI lymphatic imaging with superparamagnetic iron oxide nanoparticles is at the cutting edge of SLN mapping technology and avoids additional radiation^{36–41}. Motoyama et al showed the feasibility of endoscopic injection of ferumoxide (a 70–140nm iron oxide particle) for MRI SLN detection in esophageal cancer, with 12% of clinical N0 patients upstaged with the aid of pre-operative MRI imaging³⁷. Studies by Harisinghani and

Nishimura have shown compelling findings with IV administration of iron oxides, resulting in differential MRI signal intensity in benign and malignant lymph nodes depending on tumor burden and particle uptake by macrophages^{38–41}. In a trial of 80 patients with prostate cancer, MRI imaging of IV iron oxide particles (ferumoxtran-10) had a sensitivity for detecting nodal of metastases of 90.5%, more than double that of conventional MRI³⁹. Follow up studies in prostate and pancreatic cancer staging using ferumoxytol showed initial safety and feasibility of this newer iron oxide derivative^{40–41}. The ability to detect tumor-bearing lymph nodes is a vital step beyond SLN prediction. While tumor burden as small as 2–3mm has been identified in some of these MRI studies, SLN mapping may remain relevant to the detection of all possible disease including micrometastases. Studies of iron oxide MRI imaging in lung cancer are needed, particularly to compare this new modality with PET/CT for clinical staging.

Next Generation Tracers for Lymphatic Mapping

Among the challenges faced by SLN mapping in lung cancer is the limited number of available lymphatic tracers and the need for signal optimization and tracer retention within SLNs without migration to second tier nodes. In NIR technology, Indocyanine Green is currently the only near infrared fluorophore approved by the FDA, and has been investigated as a lymphatic tracer in breast, melanoma, lung and gastrointestinal cancer staging^{6–9,42–45}. ICG is a small tracer with a hydrodynamic diameter of only about 1.2nm, and optimal SLN detection with ICG alone has been reported to occur within 10–20 minutes after injection^{8,29,46}. Dilution of ICG in human serum albumin (HSA) results in a noncovalent interaction, increasing the diameter to about 7nm with a threefold higher quantum yield compared to ICG alone²⁹. There have been no direct comparisons of ICG alone and ICG:HSA in human clinical trials for lung lymphatic mapping, although SLN identification rates in the study by Gilmore et al that used ICG:HSA, were higher than in those using ICG alone by Yamashita's group and detected up to about two hours after injection^{6–9}. Nonetheless, human clinical trials have shown that strong NIR signal detection within the thoracic cavity at tissue depths greater than several centimeters remains a challenge, and innovative tracers are needed^{6,30}.

NIR quantum dots, fluorescent semiconductor nanocrystals with a metal core and organic coating, have been shown to achieve hydrodynamic diameters of 15–20nm³⁰. Soltesz et al demonstrated in a large animal model that NIR QDs were retained within lung SLNs at four hours, a more clinically relevant time point compared to the more rapid ICG transport. Unfortunately, QDs are limited in terms of their clinical translation as they are in part comprised of heavy metals such as cadmium and selenide. Nonetheless, this study demonstrates the potential significant improvement that an organic tracer of larger hydrodynamic diameter could achieve if designed with minimal risk to patients.

Along these lines, next generation NIR lymphatic tracers in development include a nanocolloidal albumin carrier, comprised of albumin aggregates ranging from a total of 7–30nm in size, coupled with NIR dyes such as IRDye 800CW as well as mannose receptor-specific dyes that target lymph node macrophages^{29,46–51}. In the nanocolloidal albumin-IRDye 800CW approach investigated by Heuveling et al, albumin aggregates serve as a

scaffold for approximately 14 covalently bound dye particles with an average total particle size of 15nm, similar to the size of nanocolloidal albumin by itself and about twice the size of ICG:HSA⁴⁶. The quantum yield of nanocolloidal albumin-IRDye 800CW is similar to ICG:HSA, suggesting that the binding of multiple dye particles to a carrier scaffold does not result in a reduction of signal intensity from fluorescence quenching. However, it does not appear to enhance signal intensity either. In a rabbit metastatic model of head and neck squamous cell cancer, SLNs were readily identified with both nanocolloidal albumin-IRDye 800CW and ICG:HSA within 5 mins of injection. As expected, the larger diameter of the nanocolloid correlated with increased SLN retention. There was no decrease in SLN signal for up to 24 hours with nanocolloidal albumin-IRDye 800CW, whereas there was a significant decrease in signal intensity with ICG:HSA over the same time frame. As both dyes appear to perform well within five minutes after injection, a key determinate of whether the covalent coupling of NIR dyes to carriers such as nanocolloidal albumin is better than ICG:HSA and practical in the clinical setting is whether there is a difference in SLN signal detection within SLNs about 2–3 hours after injection, during which lung resection and lymphadenectomy would be performed.

The development of tracers specific to the lymph node microenvironment, such as macrophage targeting tracers, is a nascent concept with some pilot clinical studies in lung cancer SLN mapping^{48–52}. Several animal studies have characterized mannosylated human serum albumin (MSA) using in vivo biodistribution and receptor blocking studies to show that MSA-based particles injected into the bloodstream accumulate in macrophage-rich organs such as the liver and spleen^{51–53}. These observations have been supported by confocal microscopy showing kupffer cell uptake of MSA-based particles in the liver⁵². Examining SLN mapping capabilities, Jeong et al demonstrated that 99mTc-MSA particles showed improved lymph node uptake and sustained activity when compared to 99mTc-HSA in a murine model with subcutaneous injection⁵³. Thus, it has been hypothesized that MSA-linked tracers would preferentially associate with lymph node macrophages when injected intraparenchymally into lung tissue, prolonging retention in SLNs^{47,49–53}.

Clinical trials of Technetium-99m and indocyanine green coupled with MSA for lung cancer SLN mapping demonstrate the initial safety and feasibility of this new carrier, however, these early studies do not compare MSA to HSA, and thus it is unclear if MSA is an improvement upon current methods^{47,49–50}. Future studies would benefit from demonstrating direct colocalization of MSA with lymph node macrophages to illustrate the presumed mechanism.

Using a high throughput screen of over 1,100 organic fluorescent small molecules, Yoo et al identified a novel MFP (macrophage-specific fluorescent probe) and demonstrated macrophage-specific interaction via live cell confocal microscopy and flow cytometry, reinforcing these findings with lymph node histology from in vivo mouse studies⁴⁸. In a mouse metastatic lung cancer model, MFP signal intensity was significantly diminished in SLNs positive for disease compared to negative nodes, which correlated with the infiltration of these SLNs by tumor lesions seen on histology. While similar in concept to using pre-operative MRI detection of iron oxides to differentiate benign from malignant nodes, identifying malignant nodes in vivo based on the relative absence of fluorescence signal may

be technically challenging intraoperatively. Future studies are needed to determine if this is a potential limitation of macrophage-specific tracers in general.

Detection of Micrometastasis

In 2011, Rusch et al published the first multicenter trial demonstrating the prognostic importance of occult micrometastatic disease in early stage lung cancer⁵⁴. In a cohort of 1,047 patients, the ACOSOG Z0040 trial demonstrated that 22.4% of patients previously deemed N0 on routine histologic analysis were found to have occult micrometastases using immunohistochemical (IHC) staining for cytokeratins. Most importantly, the presence of nodal micrometastases conferred a significantly worse prognosis with decreased disease-free (hazard ratio 1.63, $P=.009$) and overall survival (hazard ratio 1.59, $P=.007$), when compared to N0 patients without micrometastases. This study highlights the importance and need for efficient and accurate methods of identifying SLNs for focused immunohistochemical (IHC) and molecular analysis.

Regarding the detection of micrometastatic disease in lung SLNs, there is considerable variation in the literature in terms of both the detection method utilized (i.e. IHC, RT-PCR or both) as well as the specific markers detected. Common molecular markers include cytokeratins (with some studies using pan anti-cytokeratin antibodies such as AE1/AE3, whereas others have looked only at specific cytokeratins such as 7,9 and 19), p53, Ber-EP4 and CEA antigen^{5–6,8,13,54–59}. Most recently, Dai et al used RT-PCR to detect fragile histidine triad (FHIT) and CDKN2A (p16INK4a) mRNA deletions, resulting in a 32.7% identification rate, which was associated with a significantly reduced disease-free and overall survival⁵⁵. Key questions remain regarding the impact of adjuvant chemotherapy on patients with detected micrometastatic disease as well as the most relevant markers for histologic subtypes such as squamous cell lung cancer, which has been underrepresented in many studies.

Future Directions in SLN Mapping for Lung Cancer

The field of SLN mapping in lung cancer is moving toward minimally invasive techniques with more sophisticated lymphatic tracers in the pipeline. Future multicenter trials examining NIR mapping, CT lymphography or other methods may benefit from “hands-on” training by the lead investigative team prior to accrual, such that the learning curve and nuances of these novel surgical techniques do not impede the ability to truly examine their reproducibility and accuracy. Further studies of next generation tracers will require direct comparisons to the few tracers currently in use and multimodality studies may aid in the validation of SLN identification if both approaches are able to identify the same lymphatic pathways. Now with multicenter trial evidence of the prognostic importance of micrometastatic disease, future trials are needed to determine which molecular markers are indispensable to the pathologic staging of SLNs and to evaluate the efficacy of adjuvant chemotherapy for the treatment of micrometastatic disease.

BIBLIOGRAPHY

1. Kern KA. Concordance and validation study of sentinel lymph node biopsy for breast cancer using subareolar injection of blue dye and technetium 99m sulfur colloid. *J Am Coll Surg*. 2001; 195(4): 467–475. [PubMed: 12375751]
2. Morton DL, et al. Final Trial Report of Sentinel-Node Biopsy versus Nodal Observation in Melanoma. *NEJM*. 2014; 370(7):599–609. [PubMed: 24521106]
3. Little AG, et al. Intraoperative lymphatic mapping for non-small cell lung cancer: the sentinel node technique. *JTCVS*. 1999; 117:220–224.
4. Rzyman W, et al. Blue-dye intraoperative sentinel lymph node mapping in early non-small cell lung cancer. *EJSO*. 2006; 32(4):462–465. [PubMed: 16504458]
5. Liptay MJ, et al. Intraoperative sentinel node mapping with technitium-99 in lung cancer: results of CALGB 140203 multicenter phase II trial. *J Thorac Oncol*. 2009; 4(2):198–202. [PubMed: 19179896]
6. Gilmore DM, et al. Identification of metastatic nodal disease in a phase 1 dose-escalation trial of intraoperative sentinel lymph node mapping in non-small cell lung cancer using near-infrared imaging. *J Thorac Cardiovasc Surg*. 2013; 146(3):562–570. [PubMed: 23790404]
7. Yamashita S, et al. Video-assisted thoracoscopic indocyanine green fluorescence imaging system shows sentinel lymph nodes in non-small cell lung cancer. *JTCVS*. 2011; 141:141–143.
8. Yamashita S, et al. Sentinel node navigation surgery by thoracoscopic fluorescence imaging system and molecular examination in non-small cell lung cancer. *Ann Surg Oncol*. 2012; 19(3):728–733. [PubMed: 22101727]
9. Moroga T, et al. Thoracoscopic segmentectomy with intraoperative evaluation of sentinel nodes for stage I non-small cell lung cancer. *Ann Thorac Cardiovasc Surg*. 2012; 18(2):89–94. [PubMed: 22082811]
10. Takizawa H, et al. Computed tomography lymphography by transbronchial injection of iopamidol to identify sentinel nodes in preoperative patients with non-small cell lung cancer: a pilot study. *JTCVS*. 2012; 144:94–99.
11. Ueda K, et al. Preoperative Imaging of the Lung Sentinel Lymphatic Basin with Computed Tomographic Lymphography: A preliminary Study. *Ann Thorac Surg*. 2004; 77:1033–1038. [PubMed: 14992921]
12. Suga K, et al. Computed Tomography Lymphography with Intrapulmonary Injection of Iopamidol for Sentinel Lymph Node Localization. *Investigative Radiology*. 2004; 39:313–324. [PubMed: 15167097]
13. Sugi K, et al. New Method of Visualizing Lymphatics in Lung Cancer Patients by Multidetector Computed Tomography. *J Comput Assist Tomogr*. 2005; 29:210–214. [PubMed: 15772539]
14. Ettinger DS, et al. Non-small cell lung cancer. *J Natl Compr Canc Netw*. 2010; 8:740–801. [PubMed: 20679538]
15. Kelsey CR, et al. Local Recurrence After Surgery for Early Stage Lung Cancer: an 11 year experience with 975 patients. *Cancer*. 2009; 115(22):5218–5227. [PubMed: 19672942]
16. Rusch VW. The IASLC Lung Cancer Staging Project: proposal for the revision of the N descriptors in the forthcoming seventh edition of the TNM classification for lung cancer. *J Thorac Oncol*. 2007; 2(7):603–612. *J Thorac Oncol* (2007). [PubMed: 17607115]
17. El-Sherif A, et al. Outcomes of sublobar resection versus lobectomy for stage I non-small cell lung cancer: a 13-year analysis. *Ann Thorac Surg*. 2006; 82(2):408–415. *Ann Thorac Surg*. [PubMed: 16863738]
18. Little AG, et al. Patterns of surgical care of lung cancer patients. *Ann Thorac Surg*. 2005; 80:2051–2056. [PubMed: 16305843]
19. Verhagen AF, et al. Completeness of lung cancer surgery: is mediastinal dissection common practice? *Eur J Cardiothorac Surg*. 2012; 41:834–838.
20. Roth JA, et al. Long-term follow up of patients enrolled in a randomized trial comparing perioperative chemotherapy and surgery with surgery alone in resectable stage IIIA non-small-cell lung cancer. *Lung Cancer*. 1998; 21(1):1–6. [PubMed: 9792048]

21. Pignon JP, et al. Lung Adjuvant Cisplatin Evaluation: A Pooled Analysis by the LACE Collaborative Group. *J of Clin Oncol.* 2008; 26(21):3552–3559. [PubMed: 18506026]
22. National Lung Screening Trial Research, T., et al. Reduced lung-cancer mortality with low-dose computed tomographic screening. *N Engl J Med.* 2011; 365(5):395–409. [PubMed: 21714641]
23. National Lung Screening Trial Research T. et al. Results of initial low-dose computed tomographic screening for lung cancer. *N Engl J Med.* 2013; 368(21):1980–1991. [PubMed: 23697514]
24. Liptay MJ, et al. Intraoperative sentinel lymph node mapping in non-small cell lung cancer improves detection of micrometastases. *J Clin Oncol.* 2002; 20:1984–1988. [PubMed: 11956256]
25. Rzyman W, et al. Intraoperative, Radio-Guided Sentinel Lymph Node Mapping in 110 Nonsmall Cell Lung Cancer Patients. *Ann Thorc Surg.* 2006; 82:237–242.
26. Nomori H, et al. Sentinel node navigation segmentectomy for clinical stage IA non-small cell lung cancer. *JTCVS.* 2007; 133:780–785.
27. Tiffet O, et al. Feasibility of the detection of the sentinel lymph node in peripheral non-small cell lung cancer with radioisotopic and blue dye techniques. *Chest.* 2005; 127(2):443–448. [PubMed: 15705980]
28. Meyer A, et al. Successful Migration of three tracers without identification of sentinel nodes during intraoperative lymphatic mapping for non-small cell lung cancer. *Interact Cardiovasc Thorac Surg.* 2007; 6(2):214–218. [PubMed: 17669814]
29. Ohnishi S, et al. Organic Alternatives to Quantum Dots for Intraoperative Near-Infrared Fluorescent Sentinel Lymph Node Mapping. *Molecular Imaging.* 2005; 4(3):172–181. [PubMed: 16194449]
30. Soltesz EG, et al. Intraoperative Sentinel Lymph Node Mapping of the Lung Using Near-Infrared Fluorescent Quantum Dots. *Ann Thorac Surg.* 2005; 79:269–277. [PubMed: 15620956]
31. Padera T, et al. Pathology: Cancer cells compress intratumor vessels. *Nature.* 2004; 427:695. [PubMed: 14973470]
32. Khullar OV, et al. Preclinical study of near-infrared guided sentinel lymph node mapping of the porcine lung. *Ann Thorac Surg.* 2013; 95(1):312–318. [PubMed: 23103009]
33. Khullar OV, et al. Image-Guided Sentinel Lymph Node Mapping and Nanotechnology-Based Nodal Treatment in Lung Cancer Using Invisible Near-Infrared Fluorescent Light. *Semin Thorac Cardiovasc Surg.* 2009; 21:309–315. [PubMed: 20226343]
34. Oh Y, et al. Intraoperative combined color and fluorescent images-based sentinel node mapping in the porcine lung: Comparison of indocyanine green with or without albumin premixing. *JTCVS.* 2013; 146:1509–1515.
35. Hutteman M, et al. Randomized, double-blind comparison of indocyanine green with or without albumin premixing for near-infrared fluorescence imaging of sentinel lymph nodes in breast cancer patients. *Breast Cancer Res. Treat.* 2011; 127:163–170. [PubMed: 21360075]
36. Bashir M, et al. Emerging applications for ferumoxytol as a contrast agent in MRI. *J Magn. Reson. Imaging.* 2014 Epub ahead of print:
37. Motoyama S, et al. Preoperative mapping of lymphatic drainage from the tumor using ferumoxide-enhanced magnetic resonance imaging in clinical submucosal thoracic squamous cell esophageal cancer. *Surgery.* 2007; 141:736–747. [PubMed: 17560250]
38. Nishimura H, et al. Preoperative Esophageal Cancer Staging: Magnetic Resonance Imaging of Lymph Node with Ferumoxtran-10, an Ultrasmall Superparamagnetic Iron Oxide. *J Am Coll Surg.* 2006; 202:604–611. [PubMed: 16571430]
39. Harisinghani MG, et al. Noninvasive Detection of Clinically Occult Lymph-Node Metastases in Prostate Cancer. *NEJM.* 2003; 348(25):2491–2499. [PubMed: 12815134]
40. Harisinghani M, et al. Utility of a new bolus-injectable nanoparticle for clinical cancer staging. *Neoplasia.* 2007; 9(12):1160–1165. [PubMed: 18084623]
41. McDermott S, et al. Accurate Prediction of Nodal Status in Preoperative Patients with Pancreatic Ductal Adenocarcinoma Using Next-Gen Nanoparticle. *Translational Oncology.* 2013; 6:670–675. [PubMed: 24466369]
42. Gilmore DM, et al. Effective low-dose escalation of indocyanine green for near-infrared fluorescent sentinel lymph node mapping in melanoma. *Ann Surg Oncol.* 2013; 20(7):2357–2363. [PubMed: 23440551]

43. Mitsumori N, et al. Sentinel lymph node navigation surgery for early stage gastric cancer. *World J Gastroenterol.* 2014; 20(19):5685–5693. [PubMed: 24914329]
44. Yuasa T, et al. Sentinel lymph node biopsy using intraoperative indocyanine green fluorescence imaging navigated with preoperative CT lymphography for superficial esophageal cancer. *Ann Surg Oncol.* 2012; 19(2):486–493. [PubMed: 21792510]
45. Troyan SL, et al. The FLARE Intraoperative Near-Infrared Fluorescence Imaging System: A First-in-Human Clinical Trial in Breast Cancer Sentinel Lymph Node Mapping. *Ann Surg Oncol.* 2009; 16:2943–2952. [PubMed: 19582506]
46. Heuveling DA, et al. Nanocolloidal albumin-IRDye 800CW: a near infrared fluorescent tracer with optimal retention in the sentinel lymph node. *Eur J Nucl Med Mol Imaging.* 2012; 39:1161–1168. [PubMed: 22349719]
47. Oh Y, et al. Thoracoscopic Color and Fluorescence Imaging System for Sentinel Lymph Node Mapping in Porcine Lung Using Indocyanine Green-Neomannosyl Human Serum Albumin: Intraoperative Image-Guided Sentinel Nodes Navigation. *Ann Surg Oncol.* 2014; 21:1182–1188. [PubMed: 24310791]
48. Yoo JS, et al. A Macrophage-Specific Fluorescent Probe for Intraoperative Lymph Node Staging. *Cancer Res.* 2014; 74:44–55. [PubMed: 24322984]
49. Kim S, et al. Intra-operative sentinel lymph node identification using a novel receptor-binding agent (technetium-99m neomannosyl human serum albumin, 99mTc-MSA) in stage I non-small cell lung cancer. *Euro J of Cardiothorac Surg.* 2010; 37:1450–1456.
50. Kim HK, et al. Comparison between Preoperative Versus Intraoperative Injection of technetium-99m Neomannosyl Human Serum Albumin for Sentinel Lymph Node Identification in Early Stage Lung Cancer. *Ann Surg Oncol.* 2012; 19:1343–1349. [PubMed: 22143575]
51. Choi JY, et al. Development of 68Ga-labeled mannosylated human serum albumin (MSA) as a lymph node imaging agent for positron emission tomography. *J Nuclear Medicine and Biology.* 2011; 38:371–379.
52. Kim S, et al. Differential receptor targeting of liver cells using 99mTc-Neoglycosylated Human Serum Albumins. *Arch Pharm Res.* 2008; 31(1):60–66. [PubMed: 18277609]
53. Jeong JM, et al. Development of 99mTc-neomannosyl human serum albumin (99mTc-MSA) as a novel receptor binding agent for sentinel lymph node imaging. *Nuclear Medicine Communications.* 2004; 25:1211–1217. [PubMed: 15640781]
54. Rusch VW, et al. Occult metastases in lymph nodes predict survival in resectable non-small-cell lung cancer: report of the ACOSOG Z0040 trial. *J Clin Oncol.* 2011; 29(32):4313–4319. [PubMed: 21990404]
55. Dai C-H, et al. Molecular diagnosis and prognostic significance of lymph node micrometastasis in patients with histologically node-negative non-small cell lung cancer. *Tumor Biol.* 2013; 34:1245–1253.
56. Maeda J, et al. Detection of occult tumor cells in lymph nodes from non-small cell lung cancer patients using reverse transcription-polymerase chain reaction for carcinoembryonic antigen mRNA with the evaluation of its sensitivity. *Lung Cancer.* 2006; 52:235–240. [PubMed: 16510209]
57. Nwogu C, et al. Lung cancer lymph node micrometastasis detection using real-time polymerase chain reaction: Correlation with vascular endothelial growth factor expression. *JTCVS.* 2013; 145:702–708.
58. D'Cunha J, et al. Molecular staging of lung cancer: real-time polymerase chain reaction estimation of lymph node micrometastatic tumor cell burden in stage I non-small cell lung cancer- preliminary results of Cancer Leukemia Group B Trial 9761. 2002; 123(3):484–491.
59. Gu C-D, et al. Detection of Micrometastatic Tumor Cells in pN0 lymph nodes of patients with completely resected nonsmall cell lung cancer. *Ann Surg.* 2002; 235(1):133–139. [PubMed: 11753052]

Fig. 1.
Figures for Review on NIR Lung Lymphatic Mapping
Colson/Hachey

Table 1

Advantages and Disadvantages of Lymphatic Mapping Techniques

Technique	Advantages	Disadvantages
Blue Dye	<ul style="list-style-type: none"> Minimal risk to patients 	<ul style="list-style-type: none"> Difficult to detect in anthracotic (pigmented) nodes Small particle size = rapid migration beyond SLN
Radiocolloid	<ul style="list-style-type: none"> Can be covalently labeled with larger carriers such as a nanocolloidal albumin 	<ul style="list-style-type: none"> Large particle size = may require injection up to 24 hrs in advance Radiation exposure “Shine through effect”
Near Infrared Imaging	<ul style="list-style-type: none"> Minimal risk to patients Minimal background signal from surrounding tissues 	<ul style="list-style-type: none"> Leakage from injection site with current transpleural approach
CT lymphography	<ul style="list-style-type: none"> Preoperative or intraoperative detection High resolution SLN localization 	<ul style="list-style-type: none"> Radiation exposure Risk of pneumothorax, bleeding (transthoracic injection)
MR lymphography	<ul style="list-style-type: none"> Preoperative or intraoperative detection Distinguish benign from suspicious nodes (IV iron oxide injection) No radiation 	<ul style="list-style-type: none"> Risk of pneumothorax, bleeding (transthoracic injection) Has not been evaluated in lung cancer patients

Table 2

Lung SLN Mapping: Methods, Detection Rates and Histopathologic Predictive Capability

Trial	Tracer	Approach	#pts	SLN ID rate	SLN Predictive?	
					Accuracy	False Neg Rate
Blue Dye						
Rzyman et al ⁴	Patent blue V	transpleural	42	36%	-	37
	Methylene blue	transpleural	68	22%	-	25
Radiocolloid						
Liptay et al ⁵	Tc99m sulfur colloid	transpleural	46	61.5%	83.3%	-
Near Infrared Imaging						
Yamashita et al ⁸	ICG alone	transpleural	61	80.3%	78.7%	2.1%
Moroga et al ⁹	ICG alone	transpleural	83	80%	-	0%
Gilmore et al ⁶	ICG:HSA	transpleural	38	100% (at optimized dose)	100%	0%
CT Lymphography						
Takizawa et al ¹⁰	iopamidol	transbronchial	13	92.3%	100%	-
Ueda et al ¹¹	iopamidol	trans thoracic	11	91%	100% [*]	-
Sugi et al ¹³	iohexol	trans thoracic	15	93.3%	-	-
Suga et al ¹²	iopamidol	trans thoracic	9	100%	100% [*]	-

* No metastatic nodes identified in this study

Table 3

Potential Applications for Lung Lymphatic Mapping

<ul style="list-style-type: none">▪ <u>Clinical staging of Lung Cancer</u><ul style="list-style-type: none">○ Identify N2 SLNs for targeted cmed/EBUS○ Distinguish benign from suspicious LNs based on differences in tracer uptake by tumor bearing and uninvolved nodes.
<ul style="list-style-type: none">▪ <u>Pre operative planning</u><ul style="list-style-type: none">○ Localize SLNs prior to surgery to perform efficient intraoperative staging
<ul style="list-style-type: none">▪ <u>Intraoperative SLN identification</u><ul style="list-style-type: none">○ Real time intraoperative SLN mapping with visual guidance
<ul style="list-style-type: none">▪ <u>Pathologic Staging</u><ul style="list-style-type: none">○ Targeted immunohistochemical and molecular analysis of SLNs
<ul style="list-style-type: none">▪ <u>Treatment</u><ul style="list-style-type: none">○ Novel drug delivery options targeted to tumor draining lymphatics