Published in final edited form as:

Semin Thorac Cardiovasc Surg. 2009 ; 21(4): 309–315. doi:10.1053/j.semtcvs.2009.11.009.

Image-Guided Sentinel Lymph Node Mapping and Nanotechnology-Based Nodal Treatment in Lung Cancer using Invisible Near-Infrared Fluorescent Light

Onkar Khullar¹, John V. Frangioni², and Yolonda L. Colson¹

¹ Division of Thoracic Surgery, Brigham and Women's Hospital, Harvard Medical School, Boston MA

² Division of Hematology/Oncology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston MA

Abstract

Current methods for sentinel lymph node (SLN) mapping and nodal treatment in lung cancer remain inadequate for routine clinical use. Here we discuss the potential for using the combination of invisible near-infrared (NIR) fluorescent light and nanotechnology for these applications. NIR fluorescence imaging has recently received significant attention for *in vivo* imaging applications because of its low tissue autofluorescence, high photon penetration into living tissue, and high signal-to-background ratio. Our large animal *in vivo* studies have been able to successfully identify sentinel lymph nodes in lung tissue and several clinical studies have examined the use of NIR fluorescence imaging systems for SLN mapping in breast and gastric cancer. Promising new nanoparticle technologies, when combined with NIR fluorescence imaging, offer the potential for image-guided treatment of lymph nodes at high risk for tumor recurrence. This review provides a theoretical and empirical framework for developing the next-generation of diagnostic and therapeutic agents for lung cancer.

Over the last twenty years, sentinel lymph node (SLN) imaging has revolutionized the treatment of several malignancies, such as melanoma and breast cancer (1–3), and has the potential to drastically improve treatment in other malignancies, including lung cancer (4). Several attempts at developing an easy, reliable, and effective method for SLN mapping in lung cancer have been unsuccessful due to unique difficulties inherent to the lung and to operating in the thoracic cavity (5–9). An inexpensive method offering rapid, intraoperative identification of SLNs, with minimal risk to both patient and provider, would allow for improved staging in patients. This, in turn, would permit better selection of patients for adjuvant therapy, thus reducing morbidity in those patients for whom adjuvant treatment is inappropriate, and ensuring that those who need this added therapy actually receive it.

Current methods for SLN identification involve the use of radioactivity-guided mapping with technetium-99m sulfur colloid and/or visual mapping using vital blue dyes (1, 2). Unfortunately these methods can be inadequate for SLN mapping in non-small cell lung cancer (NSCLC; Table 1). The use of vital blue dyes is limited *in vivo* by poor visibility, particularly in the presence of anthracotic mediastinal nodes, thereby decreasing the signal-to-background ratio (SBR) that enables nodal detection (4, 5). Similarly, results with technetium-99m sulfur colloid have been mixed when used in the thoracic cavity, where

hilar structures and aberrant patterns of lymphatic drainage make detection more difficult (8–11). Although Nomori et al. have reported an 83% nodal identification rate following a preoperative injection of technetium-99 colloid, there is an associated increased risk of pneumothorax and bleeding with this method (8). Further, the recently completed CALGB 140203 multicenter Phase 2 trial investigating the use of intraoperative technetium-99m colloid found an identification rate of only 51% with this technique (9). Clearly a technology with greater accuracy, improved SBR, and less potential risk to surgeon and patient would be welcome in the field of thoracic oncology. Near-infrared (NIR) fluorescence imaging has the potential to meet this difficult challenge.

Near-Infrared Light

NIR light is defined as that within the wavelength range of 700 to 1000 nm. Although NIR light is invisible to the naked eye, it can be thought of as “redder” than UV and visible light (Figure 1). Absorption, scatter, and autofluorescence are all significantly reduced at redder wavelengths. For example, hemoglobin and water are the major photon absorbers in living tissues. The highest absorption coefficient for hemoglobin is present within visible wavelengths and for water is present within UV and infrared wavelengths (Figure 2). In contrast, hemoglobin, water, lipids, and other endogenous chromophores, such as melanin, have their lowest absorption within the NIR spectrum, which permits increased photon depth penetration into tissues (12–16). In addition, imaging can also be affected by photon scatter, which describes the reflection and/or deflection of light when it interacts with tissue. Scatter, on an absolute scale, is often ten-times higher than absorption. However, the two major types of scatter, Mie and Rayleigh, are both reduced in the NIR, making the use of NIR wavelengths especially important for the reduction of photon attenuation.

Lastly, living tissue has extremely high “autofluorescence” in the UV and visible wavelength ranges due to endogenous fluorophores, such as NADH and the porphyrins. Therefore, UV/visible fluorescence imaging of the intestines, bladder, and gallbladder is essentially precluded. However, in the NIR spectrum, autofluorescence is extremely low (13, 14), providing the black imaging background necessary for optimal detection of a NIR fluorophore within the surgical field. As a result of these three special properties, NIR light has received considerable attention in biomedical imaging applications including intraoperative real-time image-guided surgery (13, 14, 17–19).

Additionally, optical imaging techniques, such as NIR fluorescence, eliminate the need for ionizing radiation. This, combined with the availability of a NIR fluorophore already FDA-approved for other indications and having extremely low toxicity (discussed below), make this a potentially safe imaging modality. To summarize, the properties of NIR light and NIR fluorescence help to optimize the SBR of SLN detection and permit real-time imaging without the use of ionizing radiation (Box 1).

NIR Fluorescence Imaging Systems

Although NIR light has significant advantages for image-guided surgery, it is also invisible to the human eye. On one hand, this is a major advantage, because there is no change in the appearance of the surgical field. On the other hand, special imaging systems are required to “see” the NIR fluorescent light that is present. Currently there are three intraoperative NIR imaging systems in various stages of development. The Spy™ system from Novadaq utilizes laser light excitation in order to obtain fluorescent images. The Spy™ system has been studied for imaging patency of vascular anastomoses following CABG and organ transplantation (20, 21). A second system, the Photodynamic Eye™ (PDE) from Hamamatsu is presently available only in Japan. A third system, the Fluorescence-Assisted Resection and Exploration (FLARE™) system developed by our laboratory utilizes NIR light-emitting

diode (LED) excitation, eliminating the need for a potentially harmful laser. Additionally, the FLARE™ system has the advantage of being able to provide simultaneous color imaging, NIR fluorescence imaging, and color-NIR merged images, allowing the surgeon to simultaneously visualize invisible NIR fluorescence images within the context of surgical anatomy. FLARE™ is completely self-contained, has a working distance of 18" above the patient, which means there is no need for patient contact, and has a sterile drape and shield to maintain sterility (Figure 3). Further details of the FLARE™ system have been described in detail previously (22, 23).

Near-Infrared Fluorescent Nanoparticle Contrast Agents

The ideal contrast agent for SLN mapping would be anionic and within 10–50 nm in size in order to facilitate rapid uptake into lymphatic vessels with optimal retention within the SLN (24–26). Due to the lack of endogenous NIR tissue fluorescence, exogenous contrast agents must be administered for *in vivo* studies. The most important contrast agents that emit within the NIR spectrum are the heptamethine cyanines fluorophores, of which indocyanine green (ICG, Figure 4) is the most widely used, and fluorescent semiconductor nanocrystals, also known as quantum dots (QDs).

ICG is an extremely safe NIR fluorophore, with its only known toxicity being rare anaphylaxis. The dye was FDA approved in 1958 for systemic administration for indicator-dilution studies including measurements of cardiac output and hepatic function. Additionally, it is commonly used in ophthalmic angiography. When given intravenously, ICG is rapidly bound to plasma albumin and cleared from the blood via the biliary system. Peak absorption and emission of ICG occur at 780 nm and 830 nm respectively, within the window where *in vivo* tissue absorption is at its minimum (17). ICG has a relatively neutral charge, has a hydrodynamic diameter of only 1.2 nm, and is relatively hydrophobic. Unfortunately, this results in rapid transport out of the SLN and relatively low fluorescence yield, thereby decreasing its efficacy in mapping techniques. However, noncovalent adsorption of ICG to human serum albumin (HSA), as occurs within plasma, results in an anionic nanoparticle with a diameter of 7.3 nm and a three-fold increase in fluorescence yield markedly improving its utility in SLN mapping (26).

QDs consist of an inorganic heavy metal core and shell which emit within the NIR spectrum. This structure is then surrounded by a hydrophilic organic coating which facilitates aqueous solubility and lymphatic distribution. QDs have been extensively studied and are ideal for SLN mapping as their hydrodynamic diameter can be customized to the appropriate size within a narrow distribution (15–20 nm), they can be engineered to have an anionic surface charge, and exhibit an extremely high SBRs with significant photostability (15, 27). Unfortunately, safety concerns due to the presence of heavy metals within the QDs so far have precluded clinical application (28).

Preclinical Animal Studies

As a proof of concept for intrathoracic SLN mapping, our lab has previously investigated the use of QDs and ICG:HSA for SLN mapping of the pleural space, lung, and esophagus with great success (29–31). NIR QDs have been injected into the pleural space, lung, or esophagus of both rats and pigs with good result. Animals were imaged after injection of the NIR contrast agent within the lung parenchyma using the FLARE™ NIR fluorescence imaging platform and SLN(s) was identified. In all 16/16 experiments in pigs the SLN was identified within 5 minutes of injection (Figure 5), 14 of which were located in the ipsilateral mediastinum and 2 of which were hilar intraparenchymal nodes (29). In a similar experiment 16 rats and 6 pigs were injected with QDs into the pleural space, following which the SLN was identified in the superior mediastinum in all cases (30). Finally,

following submucosal injection of QDs into the esophagus, a SLN was identified in 6 of 6 pigs (31). Further large animal studies of NIR SLN mapping with QDs have been conducted in several large animal organ systems, including skin, stomach, colon, and bladder, providing further validation of this system (27, 32–36). While NIR fluorescent QDs may be ideal for lymphatic mapping given their diameter and anionic charge, they consist of a potentially toxic heavy metal core making them problematic for human use until further toxicology studies are completed (28).

Given these concerns, several animal studies have investigated the use of indocyanine green for lymphatic imaging (23, 26, 37–40). While ICG:HSA may not have the ideal charge or size for lymphatic uptake, it has the advantage of being extremely safe and composed of drugs already FDA-approved for other indications. Of published studies, three have investigated the use of ICG:HSA for sentinel lymph node mapping in large animals (23, 26, 37). Similar to the previously described QDs, the ICG:HSA formulation reliably identified the draining sentinel node following subcutaneous injections. The clinical relevancy of this powerful approach was demonstrated by the study from Tanaka, et al. in which both QDs and ICG:HSA were tested as lymphatic tracers in a spontaneous melanoma model in Sinclair pigs. Both agents resulted in rapid lymphatic migration from injection sites around melanoma lesions with easy identification of the SLN in 4 of 4 experiments for each (37). Of great importance was the subsequent identification of micrometastatic disease in the SLN removed under NIR guidance, with absence of disease in non-NIR positive nodes within the same regional basin.

Human Clinical Studies of NIR SLN Mapping

Several studies have investigated the clinical use of indocyanine green without adsorption to HSA for NIR fluorescence-guided SLN mapping in breast and gastric cancer with good success (41–45). Kitai et al. first examined this technique in 2005 in breast cancer patients, and was able to identify a SLN node in 17 of 18 patients using NIR fluorescence rather than the visible green color of ICG (41). Sevick-Muraca et al. reported similar results using significantly lower microdoses of ICG (10 – 100 µg), successfully identifying the SLN in 8 of 9 patients (43). Similar to these subcutaneous studies, 56 patients with gastric cancer underwent endoscopic ICG injection into the submucosa around the tumor 1 to 3 days preoperatively or injection directly into the subserosa intraoperatively with identification of the SLN in 54 patients (45). Interestingly, this study showed that preoperative injection was associated with identification of a higher number of NIR-positive nodes and a higher accuracy rate compared to intraoperative injection. This suggests that more ICG can migrate further down the lymphatic pathways over time, reaching several draining lymph nodes all at risk for metastatic disease. This is likely the result of the small diameter and hydrophobicity of ICG. For this reason, ICG:HSA may prove to be a more accurate contrast agent for SLN mapping.

Recently, Troyan et al. have completed a pilot phase I clinical trial examining the utility of NIR imaging the ICG:HSA nanoparticle fluorophore for SLN mapping/biopsy in breast cancer using the FLARE™ system (23). In this study, 6 patients received both ^{99m}Tc-sulfur colloid lymphoscintigraphy along with ICG:HSA at micromolar doses. SLNs were identified in all patients using both methods. In 4 of 6 patients the SLNs identified were the same, while in the remaining two, lymphoscintigraphy identified an additional node in one patient and ICG:HSA identified an additional SLN in the other. Irrespective, this study demonstrates that NIR SLN mapping with low dose ICG:HSA is a viable method for intraoperative SLN identification.

These promising preclinical and clinical trial results suggest that NIR imaging may provide an easy, reliable method for SLN mapping in lung cancer. For this reason, we have recently begun a phase I clinical trial at Brigham and Women's Hospital to study the utility of such a technique in human NSCLC (Figure 3b). This study is currently in the early dose-escalation phases and is actively enrolling patients. Given the potential impact of SLN identification in NSCLC, we are eagerly awaiting the results of this trial.

Nanotechnology and Drug Delivery in Lung Cancer

In addition to its uses in molecular imaging, nanotechnology has received much interest for drug delivery. Polymer nanotechnology has been an area of significant research over the past decade as polymer nanoparticle drug delivery systems offer several advantages over traditional methods of chemotherapy delivery. As nanoparticles are easily modified, they can be customized for both the type of drug and target site, thereby increasing specificity of drug delivery while reducing systemic toxicity (46–49). As the importance of micrometastatic lymphatic spread of tumor becomes clearer, there has been much interest in the use of nanoparticles for lymphatic drug delivery. The considerable focus on developing an effective method for SLN mapping for lung cancer is indicative of the importance of nodal spread on overall survival. Further, a recent study from Chen, et al. showed approximately 200-fold greater levels of chemotherapy in regional lymph nodes following subcutaneous injection compared with intravenous administration in patients with breast cancer (50). A method to guide, concentrate, and keep chemotherapy within these regional lymph nodes could have a major impact on survival after resection (51).

Several investigators have shown delivery of nanoparticles to lymph nodes in small animal systems (52–58). A recent study by Liu, et al. has shown the feasibility of translymphatic delivery of paclitaxel to mediastinal lymph nodes following intrapleural implantation of a gelatin sponge impregnated with polylactide-co-glycolide paclitaxel microspheres (57). Our lab is investigating the use of image-guided nanoparticles engineered for lymphatic drug delivery. We have previously described the synthesis of novel, pH-responsive methacrylate nanoparticle systems (59). Following a simple subcutaneous injection of NIR fluorophore-labeled nanoparticles 70 nm in size, we have shown that we can deliver paclitaxel loaded within the particles to regional draining lymph nodes in several organ systems of Yorkshire pigs while simultaneously confirming nodal migration using NIR fluorescent light (Figure 6). Future studies will need to investigate the ability of nanoparticles to treat and prevent nodal metastases in animal cancer models. Additionally, the development of tumor specific nanoparticles will potentially allow for targeting of chemotherapy to small groups of metastatic tumor cells further limiting systemic toxicities by narrowing the delivery of cytotoxic drugs (60).

Summary and Conclusion

Regional lymphatic spread and recurrence of NSCLC remains a significant cause of patient morbidity. SLN mapping is currently the standard of care for several solid organ malignancies. Unfortunately, for various reasons SLN mapping has not been readily adopted for treatment and staging of NSCLC. As an imaging modality ideal for real-time, intraoperative use *in vivo*, NIR fluorescence imaging has the potential to become a relatively simple and reliable method for SLN mapping. Several studies have validated this technique in human trials in breast and gastric cancer. Our currently active Phase I clinical trial will investigate its clinical efficacy and determine the feasibility of intraoperative NIR SLN mapping in lung cancer.

Finally, nanomedicine is an exciting new field that offers the opportunity to customize and target delivery of chemotherapy to areas of interest. Nanotechnology provides a potential method to deliver chemotherapy via the lymphatic system and isolate drug delivery to regional draining lymph nodes. By avoiding systemic delivery this method may significantly reduce toxicity, while still concentrating chemotherapeutic agents in lymph nodes at levels that cannot be currently achieved. Further, with the future development of tumor specific nanoparticles, drugs may be targeted to small numbers of tumor cells providing a new modality for treatment of, at least regionally, metastatic disease. By combining these two innovative technologies we may one day be able to target both contrast agents and cytotoxic agents to tumor cells that currently evade existing cancer therapies.

References

1. Jakub JW, Pendas S, Reintgen DS. Current Status of Sentinel Lymph Node Mapping and Biopsy: Facts and Controversies. *Oncologist*. 2003; 8:59–68. [PubMed: 12604732]
2. Chen SL, Iddings DM, Scheri RP, et al. Lymphatic mapping and sentinel node analysis: current concepts and applications. *CA Cancer J Clin*. 2006; 56:292–309. [PubMed: 17005598]
3. Morton DL, Thompson JF, Cochran AJ, et al. Sentinel-node biopsy or nodal observation in melanoma. *N Engl J Med*. 2006; 355:1307–17. [PubMed: 17005948]
4. Liptay MJ. Sentinel node mapping in lung cancer: the holy grail? *Ann Thorac Surg*. 2008; 85:S778–9. [PubMed: 18222216]
5. Little AG, DeHoyos A, Kirgan DM, et al. Intraoperative lymphatic mapping for non-small cell lung cancer: the sentinel node technique. *J Thorac Cardiovasc Surg*. 1999; 117:220–4. [PubMed: 9918960]
6. Schmidt FE, Woltering EA, Webb WR, et al. Sentinel nodal assessment in patients with carcinoma of the lung. *Ann Thorac Surg*. 2002; 74:870–4. discussion 4–5. [PubMed: 12238853]
7. Sugi K, Fukuda M, Nakamura H, et al. Comparison of three tracers for detecting sentinel lymph nodes in patients with clinical N0 lung cancer. *Lung Cancer*. 2003; 39:37–40. [PubMed: 12499092]
8. Nomori H, Ikeda K, Mori T, et al. Sentinel node navigation segmentectomy for clinical stage IA non–small cell lung cancer. *J Thorac Cardiovasc Surg*. 2007; 133:780–5. [PubMed: 17320584]
9. Liptay MJ, D'amico TA, Nwogu C, et al. Intraoperative Sentinel Node Mapping with Technitium-99 in Lung Cancer: Results of CALGB 140203 Multicenter Phase II Trial. *J Thorac Oncol*. 2009; 4:198–202. [PubMed: 19179896]
10. Liptay MJ, Masters GA, Winchester DJ, et al. Intraoperative radioisotope sentinel lymph node mapping in non-small cell lung cancer. *Ann Thorac Surg*. 2000; 70:384–9. discussion 9–90. [PubMed: 10969649]
11. Liptay MJ. Sentinel node mapping in lung cancer. *Ann Surg Oncol*. 2004; 11:271S–4S. [PubMed: 15023767]
12. Chance B. Near-infrared images using continuous, phase-modulated, and pulsed light with quantitation of blood and blood oxygenation. *Ann N Y Acad Sci*. 1998; 838:29–45. [PubMed: 9511793]
13. Weissleder R. A clearer vision for in vivo imaging. *Nat Biotechnol*. 2001; 19:316–7. [PubMed: 11283581]
14. Frangioni JV. In vivo near-infrared fluorescence imaging. *Curr Opin Chem Biol*. 2003; 7:626–34. [PubMed: 14580568]
15. Lim YT, Kim S, Nakayama A, et al. Selection of quantum dot wavelengths for biomedical assays and imaging. *Mol Imaging*. 2003; 2:50–64. [PubMed: 12926237]
16. Conway JM, Norris KH, Bodwell CE. A new approach for the estimation of body composition: infrared interactance. *Am J Clin Nutr*. 1984; 40:1123–30. [PubMed: 6507337]
17. Sevcik-Muraca EM, Houston JP, Gurfinkel M. Fluorescence-enhanced, near infrared diagnostic imaging with contrast agents. *Curr Opin Chem Biol*. 2002; 6:642–50. [PubMed: 12413549]
18. Frangioni JV. New technologies for human cancer imaging. *J Clin Oncol*. 2008; 26:4012–21. [PubMed: 18711192]

19. Rasmussen JC, Tan I-C, Marshall MV, et al. Lymphatic imaging in humans with near-infrared fluorescence. *Curr Opin Biotechnol.* 2009; 20:74–82. [PubMed: 19233639]
20. Reuthebuch O, Häussler A, Genoni M, et al. Novadaq SPY: intraoperative quality assessment in off-pump coronary artery bypass grafting. *Chest.* 2004; 125:418–24. [PubMed: 14769718]
21. Sekijima M, Tojimbara T, Sato S, et al. An intraoperative fluorescent imaging system in organ transplantation. *Transplant Proc.* 2004; 36:2188–90. [PubMed: 15518796]
22. Gioux S, Kianzad V, Ciocan R, et al. High-power, computer-controlled, light-emitting diode-based light sources for fluorescence imaging and image-guided surgery. *Mol Imaging.* 2009; 8:156–65. [PubMed: 19723473]
23. Troyan S, Kianzad V, Gibbs-Strauss S, 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 [Epub ahead of print].
24. Swart PJ, Beljaars L, Kuipers ME, et al. Homing of negatively charged albumins to the lymphatic system: general implications for drug targeting to peripheral tissues and viral reservoirs. *Biochem Pharmacol.* 1999; 58:1425–35. [PubMed: 10513986]
25. Josephson L, Mahmood U, Wunderbaldinger P, et al. Pan and Sentinel Lymph Node Visualization Using a Near-Infrared Fluorescent Probe. *Mol Imaging.* 2003; 2:18–23. [PubMed: 12926234]
26. Ohnishi S, Lomnes SJ, Laurence RG, et al. Organic alternatives to quantum dots for intraoperative near-infrared fluorescent sentinel lymph node mapping. *Mol Imaging.* 2005; 4:172–81. [PubMed: 16194449]
27. Frangioni JV, Kim SW, SO, et al. Sentinel lymph node mapping with type-II quantum dots. *Methods Mol Biol.* 2007; 374:147–59. [PubMed: 17237537]
28. Hauck TS, Anderson RE, Fischer HC, et al. In vivo Quantum-Dot Toxicity Assessment. *Small.* 2009 [Epub ahead of print].
29. Soltesz EG, Kim S, Laurence RG, et al. Intraoperative sentinel lymph node mapping of the lung using near-infrared fluorescent quantum dots. *Ann Thorac Surg.* 2005; 79:269–77. discussion –77. [PubMed: 15620956]
30. Parungo CP, Colson YL, Kim SW, et al. Sentinel lymph node mapping of the pleural space. *Chest.* 2005; 127:1799–804. [PubMed: 15888861]
31. Parungo CP, Ohnishi S, Kim SW, et al. Intraoperative identification of esophageal sentinel lymph nodes with near-infrared fluorescence imaging. *J Thorac Cardiovasc Surg.* 2005; 129:844–50. [PubMed: 15821653]
32. Kim S, Lim YT, Soltesz EG, et al. Near-infrared fluorescent type II quantum dots for sentinel lymph node mapping. *Nat Biotechnol.* 2004; 22:93–7. [PubMed: 14661026]
33. Soltesz EG, Kim S, Kim SW, et al. Sentinel lymph node mapping of the gastrointestinal tract by using invisible light. *Ann Surg Oncol.* 2006; 13:386–96. [PubMed: 16485157]
34. Parungo CP, Soybel DI, Colson YL, et al. Lymphatic Drainage of the Peritoneal Space: A Pattern Dependent on Bowel Lymphatics. *Ann Surg Oncol.* 2007; 14:286–98. [PubMed: 17096058]
35. Knapp DW, Adams LG, Degrand AM, et al. Sentinel lymph node mapping of invasive urinary bladder cancer in animal models using invisible light. *Eur Urol.* 2007; 52:1700–8. [PubMed: 17646044]
36. Ballou B, Ernst LA, Andreko S, et al. Imaging vasculature and lymphatic flow in mice using quantum dots. *Methods Mol Biol.* 2009; 574:63–74. [PubMed: 19685300]
37. Tanaka E, Choi HS, Fujii H, et al. Image-guided oncologic surgery using invisible light: completed pre-clinical development for sentinel lymph node mapping. *Ann Surg Oncol.* 2006; 13:1671–81. [PubMed: 17009138]
38. Reynolds JS, Troy TL, Mayer RH, et al. Imaging of spontaneous canine mammary tumors using fluorescent contrast agents. *Photochem Photobiol.* 1999; 70:87–94. [PubMed: 10420847]
39. Kwon S, Seveck-Muraca EM. Noninvasive quantitative imaging of lymph function in mice. *Lymphat Res Biol.* 2007; 5:219–31. [PubMed: 18370912]
40. Ogata F, Azuma R, Kikuchi M, et al. Novel lymphography using indocyanine green dye for near-infrared fluorescence labeling. *Ann Plast Surg.* 2007; 58:652–5. [PubMed: 17522489]

41. Kitai T, Inomoto T, Miwa M, et al. Fluorescence navigation with indocyanine green for detecting sentinel lymph nodes in breast cancer. *Breast Cancer*. 2005; 12:211–5. [PubMed: 16110291]
42. Ogasawara Y, Ikeda H, Takahashi M, et al. Evaluation of breast lymphatic pathways with indocyanine green fluorescence imaging in patients with breast cancer. *World journal of surgery*. 2008; 32:1924–9. [PubMed: 18330628]
43. Sevick-Muraca EM, Sharma R, Rasmussen JC, et al. Imaging of lymph flow in breast cancer patients after microdose administration of a near-infrared fluorophore: feasibility study. *Radiology*. 2008; 246:734–41. [PubMed: 18223125]
44. Miyashiro I, Miyoshi N, Hiratsuka M, et al. Detection of sentinel node in gastric cancer surgery by indocyanine green fluorescence imaging: comparison with infrared imaging. *Ann Surg Oncol*. 2008; 15:1640–3. [PubMed: 18379850]
45. Tajima Y, Yamazaki K, Masuda Y, et al. Sentinel node mapping guided by indocyanine green fluorescence imaging in gastric cancer. *Ann Surg*. 2009; 249:58–62. [PubMed: 19106676]
46. Duncan R. Polymer conjugates as anticancer nanomedicines. *Nat Rev Cancer*. 2006; 6:688–701. [PubMed: 16900224]
47. Torchilin VP. Multifunctional nanocarriers. *Adv Drug Deliv Rev*. 2006; 58:1532–55. [PubMed: 17092599]
48. Kabanov AV. Polymer genomics: An insight into pharmacology and toxicology of nanomedicines. *Adv Drug Deliv Rev*. 2006; 58:1597–621. [PubMed: 17126450]
49. Davis ME, Chen ZG, Shin DM. Nanoparticle therapeutics: an emerging treatment modality for cancer. *Nat Rev Drug Discov*. 2008; 7:771–82. [PubMed: 18758474]
50. Chen J, Wang L, Yao Q, et al. Drug concentrations in axillary lymph nodes after lymphatic chemotherapy on patients with breast cancer. *Breast Cancer Res*. 2004; 6:R474–7. [PubMed: 15217515]
51. Xie Y, Bagby TR, Cohen M, et al. Drug delivery to the lymphatic system: importance in future cancer diagnosis and therapies. *Expert Opin Drug Deliv*. 2009
52. Nishioka Y, Yoshino H. Lymphatic targeting with nanoparticulate system. *Adv Drug Deliv Rev*. 2001; 47:55–64. [PubMed: 11251245]
53. Moghimi SM, Hawley AE, Christy NM, et al. Surface engineered nanospheres with enhanced drainage into lymphatics and uptake by macrophages of the regional lymph nodes. *FEBS Lett*. 1994; 344:25–30. [PubMed: 8181558]
54. Hawley AE, Illum L, Davis SS. Preparation of biodegradable, surface engineered PLGA nanospheres with enhanced lymphatic drainage and lymph node uptake. *Pharm Res*. 1997; 14:657–61. [PubMed: 9165539]
55. Reddy ST, Rehor A, Schmoekel HG, et al. In vivo targeting of dendritic cells in lymph nodes with poly(propylene sulfide) nanoparticles. *J Control Release*. 2006; 112:26–34. [PubMed: 16529839]
56. Liu J, Meisner D, Kwong E, et al. A novel trans-lymphatic drug delivery system: implantable gelatin sponge impregnated with PLGA-paclitaxel microspheres. *Biomaterials*. 2007; 28:3236–44. [PubMed: 17434581]
57. Liu J, Meisner D, Kwong E, et al. Translymphatic Chemotherapy by Intrapleural Placement of Gelatin Sponge Containing Biodegradable Paclitaxel Colloids Controls Lymphatic Metastasis in Lung Cancer. *Cancer Res*. 2009; 69:1174–81. [PubMed: 19176391]
58. Lu HX, Li B, Kang Y, et al. Paclitaxel nanoparticle inhibits growth of ovarian cancer xenografts and enhances lymphatic targeting. *Cancer Chemother Pharmacol*. 2007; 59:175–81. [PubMed: 16718469]
59. Griset AP, Walpole J, Liu R, et al. Expansile nanoparticles: synthesis, characterization, and in vivo efficacy of an acid-responsive drug delivery system. *J Am Chem Soc*. 2009; 131:2469–71. [PubMed: 19182897]
60. Farokhzad OC, Langer R. Impact of nanotechnology on drug delivery. *ACS Nano*. 2009; 3:16–20. [PubMed: 19206243]

Figure 1.
Electromagnetic spectrum

Figure 2.

Between 700 and 900 nm (the “NIR Window”), the sum of the oxygenated hemoglobin (OxyHb), deoxygenated hemoglobin (DeoxyHb), water, and lipid spectra reaches a minimum, thereby helping to make this window ideal for *in vivo* imaging. Composite figure adapted with permission from Chance, Ann NY Acad Sci... and Conway, Am. J. Clin. Nutr. 1984;40:1125, American Society for Nutrition.

Figure 3. FLARE™ imaging system fully extended, undraped (a) and fully draped, in intra-operative use (b). The camera head is fully enclosed and sits 18 cm above the field of view (arrow). A satellite monitor (arrowhead) is positioned in the operating room to allow for easy viewing by the surgeon.

Figure 4.
Molecular structure of indocyanine green, a commonly used NIR fluorescent contrast agent.

Figure 5.

SLN mapping of the lung with NIR quantum dots. Color video (left), NIR fluorescence (middle), and color-NIR merge (right) are presented. The SLN (arrow) was identified 45 seconds after QD injection into the RUL (arrowhead). Figure adapted with permission from Ann Thorac Surg, 79, Soltesz et. al., Intraoperative sentinel lymph node mapping of the lung using near-infrared fluorescent quantum dots, 272. Copyright Elsevier (2005).

Figure 6. 24 hours following a subcutaneous injection into the hindleg of a Yorkshire pig (arrowhead), NIR labeled methacrylate nanoparticles are seen migrating to the draining SLN (arrow) through a discrete lymphatic vessel.

Table 1

SLN Mapping in NSCLC

Authors	Year	N	Tracer	% Success	Institution
Little et al. (5)	1999	36	Isosulfan blue dye	47	Single
Schmidt et al. (6)	2002	31	Tc-99/isosulfan blue dye	81	Single
Sugi et al. (7)	2003	16	Indocyanine green	6	Single
Nomori et al. (8)	2007	52	Tc-99 sulfur colloid preoperatively	83	Single
Liptay et al. (11)	2004	148	Tc-99 sulfur colloid	70	Single
Liptay et al. (9)	2009	39	Tc-99 sulfur colloid	51	Multi

Table 2Advantages of NIR Fluorescence for *In Vivo* Imaging

<ul style="list-style-type: none">• Low autofluorescence• Minimal photon absorption and scatter• Improved tissue depth penetration• Non-radioactive• Non-invasive• Relatively inexpensive• Portability
--