Published in final edited form as:

Ann Surg Oncol. 2015 December ; 22(0 3): 1147–1155. doi:10.1245/s10434-015-4601-5.

Sentinel Lymph Node Mapping of Liver

Hideyuki Wada, MD^{1,2}, Hoon Hyun, PhD¹, Christina Vargas, MD^{1,3}, Elizabeth M. Genega, MD⁴, Julien Gravier, PhD^{1,5}, Sylvain Gioux, PhD¹, John V. Frangioni, MD, PhD^{1,6,7}, and Hak Soo Choi, PhD^{1,*}

¹Division of Hematology/Oncology, Department of Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA 02215

²Department of Gastroenterological Surgery II, Hokkaido University Graduate School of Medicine, Sapporo 060-8638, Japan

³Division of Plastic and Reconstructive Surgery, Department of Surgery, Beth Israel Deaconess Medical Center, Boston, MA 02215

⁴Department of Pathology and Laboratory Medicine, Tufts Medical Center and Tufts University School of Medicine, Boston, MA 02111

⁵INSERM, CRI, U823, Institut Albert Bonniot, 38042 Grenoble, France

⁶Curadel, LLC, 377 Plantation Street, Worcester, MA 01605

⁷Department of Radiology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA 02215

Abstract

Background—Although the sentinel lymph nodes (SLN) hypothesis has been applied to many tissues and organs, liver has remained unstudied. At present, it is unclear whether hepatic SLNs even exist. If so, they could alter management in intrahepatic cholangiocarcinoma and other hepatic malignancies by minimizing the extent of surgery while still providing precise nodal staging. We investigated whether invisible yet tissue-penetrating near-infrared (NIR) fluorescent light can provide simultaneous identification of both the sentinel lymph node (SLN) and all other regional lymph nodes (RLN) in the liver.

Method—In twenty five Yorkshire pigs, we determined whether SLNs exist in liver, and compared the effectiveness of two clinically available NIR fluorophores, methylene blue (MB)

*To whom all correspondence and reprint requests should be addressed: Hak Soo Choi, Ph.D., BIDMC, Room SL-436A, 330 Brookline Avenue, Boston, MA 02215, hchoi@bidmc.harvard.edu, Phone: 617-667-6024, FAX: 617-975-5016.

Conflict of Interest

John V. Frangioni, M.D., Ph.D.: Dr. Frangioni is currently CEO of Curadel, Curadel ResVet Imaging, and Curadel Surgical Innovations, which has licensed FLARE imaging systems and contrast agents from the Beth Israel Deaconess Medical Center.

Author's Contributions

HW, JVF, and HSC designed the study. HW, HH, CV, EMG, and JG performed the experiments. HW, SG, JVF, and HSC reviewed, analyzed, and interpreted the data. HW, JVF, and HSC wrote and revised the paper. All authors discussed the results and commented on the manuscript. JVF and HSC approved the submitted version.

**This research and paper is not presented at any communications or meetings.

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and indocyanine green (ICG), and two novel NIR fluorophores previously described by our group, ESNF14 and ZW800-3C, for SLN and RLN mapping.

Results—ESNF14 showed the highest signal-to-background ratio (SBR) and longest retention time in SLNs, without leakage to second-tier lymph nodes. ICG had apparent leakage to second-tier nodes, while ZW800-3C suffered from poor migration after intraparenchymal injection. However, when injected intravenously, ZW800-3C was able to highlight all RLNs in liver over a 4–6 h period. Simultaneous dual channel imaging of SLN (ESNF14) and RLN (ZW800-3C) permitted unambiguous identification and image-guided resection of SLNs and RLNs in liver.

Conclusion—The NIR imaging technology enables real-time intraoperative identification of SLNs and RLNs in the liver of swine. If these results are confirmed in patients, new strategies for the surgical management of intrahepatic malignancies should be possible.

Keywords

Sentinel lymph node biopsy; Image-guided surgery; Hepatobiliary cancer; Intrahepatic cholangiocarcinoma; Lymphadenectomy

INTRODUCTION

Sentinel lymph node biopsy (SLNB) is currently the standard of care for breast cancer and melanoma.^{1,2} SLNB provides precise nodal (N) status intraoperatively and also helps avoid unnecessary lymphadenectomies, which in turn can cause postoperative edema, postoperative bleeding, lymphatic fistulae, and tissue injury. Although there are large ongoing clinical trials testing the utility of SLNB in gastrointestinal (GI) malignancies, there is no report to date of SLNB in liver.

Intrahepatic cholangiocarcinoma (IHC) is the second most common primary hepatic malignant tumor, accounts for 4.1% of primary liver cancers, and has a very poor prognosis compared to other GI malignancies.³ Although regional lymph nodes (RLN) resection is typically recommended as a diagnostic procedure, its therapeutic benefit remains controversial,⁴ and the surgery itself has a high morbidity rate.^{5,6} The lymphadenectomy of RLN in liver is also performed in fibrolamellar hepatocellular carcinoma, which represent 0.6%–8.6% of all hepatocellular carcinomas (HCC),^{7,8} as well as extrahepatic cholangiocarcinoma and gallbladder cancer, even though they are not primary liver cancers.^{9,10} If it were possible to perform SLNB in these diseases and its feasibility were verified by a randomized clinical trial, unnecessary and invasive lymphadenectomy might be avoided while still providing the same benefit.

The liver presents one of the most complex lymphatic drainage patterns in the body. It is not possible, with any known technology, to predict the direction or rate of flow from a tumor site in liver to the nearest lymph node(s), nor to quickly and accurately identify SLNs. In this study, we exploit invisible near-infrared (NIR) fluorescent light and leverage novel chemical entities and imaging systems, to explore SLN and RLN mapping in swine liver, which has similar anatomy and size to human.

MATERIALS AND METHODS

NIR Fluorescent Contrast Agents for SLN mapping and PLN mapping

MB stock solution (10 mg/ml; 31.3 mM) was from Taylor Pharmaceuticals (Decatur, IL). ICG was from MP Biomedicals (Santa Ana, CA) and dissolved in distilled water at 2.5 mg/ml (3.2 mM). ESNF14, a pentamethine cyanine fluorophore,¹¹ and ZW800-3C, a zwitterionic (ZW) heptamethine indocyanine fluorophore, were synthesized as described in detail previously.¹² ESNF14 and ZW800-3C were dissolved in 5% dextrose in water (D5W) as 100 μ M stock solutions.

Measurement of optical properties

Optical properties were measured in fetal bovine serum (FBS) supplemented with 50 mM HEPES, pH 7.4 as described in detail previously.^{13,14} *In silico* calculations of the distribution coefficient (logD) were calculated using MarvinSketch 5.2.1 (ChemAxon, Budapest, Hungary).

Animal Models

Animal studies were performed under approved institutional protocol #034-2013 in an AAALAC-certified facility. Twenty-five female Yorkshire pigs (E.M. Parsons and Sons, Hadley, MA) averaging 35.2 kg were induced with 4.4-mg/kg intramuscular Telazol (Fort Dodge Labs, Fort Dodge, IA), intubated, and maintained with 2% isoflurane (Baxter Healthcare Corp., Deerfield, IL). Electrocardiogram, heart rate, pulse oximetry, and body temperature were monitored during the experiment.

NIR Fluorescence Imaging System

The dual-NIR channel Fluorescence-Assisted Resection and Exploration (FLARE) imaging system has been described in detail previously.^{15,16} Color image and two independent channels (700 nm and 800 nm) of NIR fluorescence images were acquired simultaneously with custom software at rates up to 15 Hz over a 15 cm diameter field of view (FOV). In the color-NIR merged images, 700 nm NIR fluorescence and 800 nm fluorescence were pseudo-colored red and lime green, respectively.

NIR Imaging of Sentinel Lymph nodes in Liver and Pan Lymph Nodes in Pigs

A midline laparotomy and a right transrectus incision were performed. Swine liver contains six lobes: right lateral, right medial, left medial, left lateral, quadrate, and caudate. The right lateral and the right medial lobe are equivalent to the human right posterior segment and the right anterior segment, and the left medial and the left lateral lobe to the human left medial segment and the left lateral segment, respectively. For SLNB experiments, NIR fluorescence images were acquired at 0, 1, 3, 5, 10, 15, and 30 min post-injection. At 30 min post-injection all NIR hotspots were resected and analyzed microscopically. For pan lymph nodes (PLN) mapping experiments, images were acquired at 0, 15, 30, 60, 90, 120, 180, 240, 360, and 480 min post-injection.

Classification of Regional Lymph Nodes in Liver

Regional lymph nodes in human are classified as hilar (H-LN), periduodenal and peripancreatic (PP-LN) in right liver, H-LN and gastrohepatic lymph nodes (G-LN) in left liver, and celiac and/or periaortic and caval lymph node (PA-LN), with the latter defined as distant metastasis in IHC.^{17,18} Since swine anatomy is similar, we classified the RLN of swine liver as H-LN, PP-LN, and G-LN. After the resection of identified SLNs, N = 6 pigs underwent the resection of all RLN (H-LN, PP-LN, G-LN) and PA-LNs regardless of the injection lobe. Kocherization was not performed in all cases because the duodenum and the pancreas were not fixed to the retroperitoneum in pig. The common bile duct and the hepatic artery in the hepatoduodenal ligament were resected with these nodes without reconstruction during lymphadenectomy to minimize operative time.

Immunohistochemical Analysis and NIR Fluorescence

Microscopy A board-certified pathologist reviewed all resected tissue samples. In addition, anti-CD79a antibody (HM47/A9) and anti-Bcl-6 antibody (N-3) were purchased from Abcam (Cambridge, MA) and Santa Cruz Biotechnology (Dallas, TX), respectively. These antibodies were used for immunohistochemical staining to further confirm that resected tissue was lymph nodes. NIR fluorescence microscopy was performed on a 4-filter Nikon Eclipse TE300 epifluorescence microscope as previously described.^{19,20} To detect ESNF14, we used 650 ± 22 nm excitation filter and 710 ± 25 nm emission filter. To detect ZW800-3C, we used a custom filter set (Chroma Technology Corporation, Brattleboro, VT) composed of a 750 ± 25 nm excitation filter, a 785 nm dichroic mirror and an 810 ± 20 nm emission filter.

Quantitation and Statistical Analysis

The fluorescent intensity (FI) of a region of interest (ROI) over the LN, rectus abdominis muscle, pancreas, and liver were quantified using custom FLARE software. The performance metric for this study was the signal-to-background ratio (SBR). $SBR = FI \text{ of ROI} / \text{background (BG) intensity}$. The rectus abdominis muscles, pancreas, and liver were used as BG for this calculation to yield SBR (LN/Mu), SBR (LN/Pa), and SBR (LN/Li), respectively. Results were presented as mean $\pm$ standard deviation (SD). The statistical analysis was performed using the Unpaired T between two groups and a one-way ANOVA between multiple groups. A **P* value of less than 0.05 was considered significant.

RESULTS

Optical Properties of NIR Fluorescent Contrast Agents

The chemical structure, absorbance spectra, and fluorescence spectra for the MB, ICG, ESNF14, and ZW800-3C are shown in Supplementary Information Fig. S1. Additionally, Table S1 details the optical properties of these agents in FBS, i.e., in the microenvironment they encounter after injection into the body. ESNF14 has a 2-fold higher extinction coefficient and more than a 4-fold higher QY compared to MB, resulting in a total brightness over 8-times higher. Similarly, optical properties of ZW800-3C result in a 4-fold higher brightness compared to ICG. ESNF14 and ZW800-3C have ideal separation in their

absorbance and fluorescence spectra, which matches the two channels (700 nm and 800 nm) of the FLARE imaging system and thus enables simultaneous dual-channel NIR fluorescence imaging.

SLN Mapping in Liver

In preliminary experiments (data not shown), we optimized the injection dose of each agent. To validate the theory of SLNB in liver, we injected 16 μmol of MB, 50 nmol of ESNF14, 1.6 μmol of ICG, or 50 nmol of ZW800-3C in 0.5 ml of D5W directly into the liver parenchyma of either the right lateral lobe, the right medial lobe, the left medial lobe, or the left lateral lobe ($N = 14$ pigs total). Due to their anatomy and therefore technical difficulty, injections were not attempted in the quadrate or the caudate lobes. MB was injected in two cases (Fig. 1a). MB showed a faint NIR hotspot in one case and no NIR hotspots in another case. MB was also absorbed systemically and resulted in detectable NIR fluorescence signal in the pancreas.²¹ Because of this poor performance, we excluded MB from subsequent experiments.

The results of SLN mapping in liver using the remaining NIR fluorophores are shown in Tables 1a, 1b and Supplementary Table S2. After injection into the liver parenchyma, we could identify NIR hotspots promptly using ESNF14, ICG, and ZW800-3C (Fig. 1b, 1c, and 1d). ESNF14 showed a high signal in each hotspot and was retained for at least 30 min (Fig. 1b). In two cases of ICG, however, we observed efflux from the first draining node (i.e., SLN) into second tier nodes within 5 min (Fig. 1c). ZW800-3C did not migrate well from the injection site and identified hotspots were not as bright and exhibited lower SBR than other agents, although the signal was retained for at least 30 min (Fig. 1d). Interestingly, the common bile duct (CBD) was visualized in all cases of injected NIR fluorophores due to excretion from the liver parenchyma into the biliary system. In addition, there were no highlighted lymphatic pathways toward the diaphragm.

Resected NIR hotspots underwent a rigorous 3-step process to confirm the presence of lymph nodes. First, the gross appearance, anatomic location, and texture was recorded, and in all cases was consistent with lymphatic tissue. Second, a board-certified pathologist reviewed every tissue sample. In all cases, the pathologist confirmed the presence of lymph node tissue. Finally, random samples (20% of total) were subjected to immunohistochemical staining of consecutive tissue sections using two independent biomarkers. As shown in Fig. 1e, resected NIR hotspots not only had an H&E appearance consistent with lymph nodes, but also stained for CD79a (mantle zone) and Bcl-6 (germinal center) antigens in a pattern that unambiguously confirmed the presence of lymph nodes.

The performance of SLN mapping was compared among NIR fluorophores and among lobes (Table 1). Average time from injection to detection was 3.5 min. Average number of NIR hotspots detected *in vivo* and resected was 2.0 and 2.1, respectively (Table 1a). A NIR hotspot was sometimes found to harbor more than one resected NIR hotspot. All NIR hotspots were found in either H-LN or PP-LN (Table 1b). There was no statistical difference in detection rates among fluorophores or among lobes except for a lower number of resected NIR hotspots identified in the left vs. right lobes of H-LN ($P = 0.032$).

The best performing 700 nm NIR SLN tracer, ESNF14, was then compared to ICG, which is the only clinical 800 nm NIR fluorophore. 50 nmol of ESNF14 ($N = 3$) and 1.6 μmol of ICG ($N = 3$) were injected into the liver parenchyma, including both sides of lateral and medial lobes. The average number of NIR hotspots was 2.7 and 2.0, and the average number of resected NIR hotspots was 2.7 and 2.3 for ICG and ESNF14, respectively. In this experiment, RLN was resected in a conventional manner, i.e., without the use of real-time image guidance. The average number of resected RLN was 22.3 and 19.0 and average time of lymphadenectomy was 66.2 min and 64.8 min for ICG and ESNF14, respectively (Table 2). All resected lymph nodes were examined for NIR fluorescence *ex vivo* using the FLARE imaging system. When using ICG, an additional 5.0 NIR fluorescent nodes on average were identified after resection. When using ESNF14, no additional NIR fluorescent nodes were found ($P = 0.001$). ESNF14 was therefore chosen as the optimal SLN tracer for dual-NIR imaging studies.

RLN Mapping in Liver

We previously reported that intravenous injection of ZW800-3C provided high contrast between all lymph nodes in the body and nearby tissues and organs, such as muscle, kidney, and liver, but did not explore RLN in the liver itself.¹¹ An ideal agent for hepatobiliary surgery needs to provide high contrast in LNs relative to both liver and pancreas. ZW800-3C was injected intravenously into $N = 4$ pigs at a dose of 1 μmol and mesenteric lymph nodes (M-LN), H-LN, and PP-LN were observed over the next 8 h post-injection (Fig. 2a). As shown in Fig. 2b, the SBRs of LN/Mu and LN/Pa were high, with peak SBR occurring between 4 and 6 h post-injection while the SBR of LN to liver (LN/Li) varied considerably over time because ZW800-3C is partially cleared by liver.

Simultaneous Dual-NIR Imaging of SLN and PLN in Pigs

To permit dual-channel imaging of RLN and SLN simultaneously, we injected 1 μmol of ZW800-3C (800 nm emission; for RLN mapping) intravenously 4 h prior to imaging and 50 nmol of ESNF14 (700 nm emission; for SLN mapping) into the liver subcapsular parenchyma of the right medial lobe 30 min prior to imaging ($N = 1$ pig). The SLNs were stained immediately and the dual-channel imaging of SLN and RLN were obtained simultaneously (Fig. 3a). We performed SLN and RLN resection under direct FLARE image guidance and examined the fluorescence of the resected lymph nodes *ex vivo*. As expected, the SLNs exhibited both 700 nm and 800 nm fluorescence whereas the RLNs exhibited only 800 nm fluorescence. As a negative control, we resected a groin lymph node prior to injection of NIR fluorophore, which exhibited no endogenous NIR fluorescent signal (Fig. 3b). Microscopically, RLN had a bright 800 nm signal in the cortex, adjacent to vasculature structures. The SLN, on the other hand, exhibited a more focal and stronger 700 nm signal, along with a diffuse 800 nm signal, in the cortex (Fig. 3c).

DISCUSSION

Our study proves the feasibility of SLN mapping of liver provided that the appropriate contrast agents are employed. Because MB and ICG are already available clinically, they are attractive candidates for future clinical translation. However, MB performed poorly and ICG

exhibited escape from SLNs to second tier nodes and additional NIR fluorescent nodes in resected RLN, as has been seen in previous reports,^{22,23} and these phenomena lower accuracy of the SLNB. Interestingly, the 800 nm NIR fluorophore ZW800-3C also performed poorly for SLN mapping because it did not efficiently enter lymphatic channels after injection, likely due to its high ionic charge. Of all agents tested, the 700 nm NIR fluorophore ESNF14 was optimal for SLNB, while the 800 nm NIR fluorophore ZW800-3C was optimal for pan lymph node mapping. 800 nm is also the preferred wavelength to maximize tissue penetration while minimizing autofluorescence, which is needed for pan lymph node mapping.

Recently, a large molecule weight, polymeric mannose derivative (technetium Tc 99m tilmanocept; Lymphoseek) was approved for lymph node mapping using radioactive gamma scintigraphy^{24,25}. Unlike Lymphoseek, ESNF14 is a small molecule with ultrarapid flow from injection site to lymph nodes, doesn't exposure caregivers or patients to ionizing radiation, and provides high resolution, real-time image guidance using NIR light. Its major disadvantage, of course, is the depth of penetration of NIR light (millimeters versus centimeters), but at the very least complements radioactive lymphatic tracers.

The lymphadenectomy in hepatobiliary cancer, and especially IHC, is one of the most difficult techniques in GI surgery. Resection is complex and serious postoperative complications are common. Nevertheless, lymphadenectomy is still recommended to precisely define N status.⁴ In the present study, we demonstrated that NIR fluorescence can help find RLNs quickly and with high sensitivity, thus minimizing the extent of exploration. When combined with a SLN contrast agent and a dual-NIR imaging system, both SLNs and RLNs can be identified and resected under real-time image guidance. This technology may make it possible to someday omit lymphadenectomy when it is confirmed that no SLN metastases are present.

Importantly, future clinical translation of ESNF14 and ZW800-3C is feasible. The anticipated dose of ESNF14 falls under the microdosing guidelines of the FDA, making it eligible for an exploratory investigational new drug application (eIND), and even the dose of ZW800-3C is 25 times lower than the typical dose of ICG. Nevertheless, appropriate toxicology studies must be performed prior to human testing. The approval of these novel compounds by FDA is strongly expected for the clinical translation of these promising techniques since NIR fluorescence imaging systems are now widely available for both open and minimally-invasive surgery.

There are important limitations to our study. First, although we used a large animal (pig) with similar anatomy to human, there are some differences in the lymphatic system between pig and human. For example, pigs lack lymph nodes around liver, especially the H-LN, where lymph node metastases appear frequently in hepatobiliary cancer. And, humans may have alternative lymphatic drainage pathways, not only to the diaphragm, but also to distant LNs such as para-aortic for example, which aren't seen in the present study. Second, there is no IHC tumor model in pigs so we could not assess this technology in the setting of tumor and lymphatic involvement, which might alter lymphatic flow and SLN identification. Third, NIR fluorescence is only capable of finding targets approximately 5–8 mm below the

tissue surface, making deeper LNs invisible to the technique. And, finally, ZW800-3C is partially cleared by hepatic transport to bile, causing a transiently high background in liver and duodenum, which could interfere with LN identification.

In conclusion, we proved the feasibility of SLNB and complete RLN resection in liver using a human-sized large animal model system and novel NIR fluorescent contrast agents optimized for SLN and pan lymph node mapping. Although it is necessary to verify the feasibility of SLNB in human clinical study, this technology should enable both precise intraoperative staging and minimal invasive surgery in hepatobiliary cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Financial Support This study was supported by the following grants from the National Institutes of Health: NCI BRP grant #R01-CA-115296 (JVF), NIBIB grant #R01-EB-010022 (JVF and HSC), and NIBIB grant #R01-EB-011523 (HSC and JVF).

We thank Rita G. Laurence for assistance with animal surgery, David J. Burrington, Jr. for editing, and Eugenia Trabucchi for administrative assistance, and Frank Kettenring and Florin Neacsu for assistance with development and maintenance of the FLARE imaging system and software.

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Synopsis

We demonstrate that the sentinel lymph node hypothesis applies to the liver and that dual-NIR fluorescence imaging permits real-time identification of sentinel and regional lymph nodes. This technology may someday reduce unnecessary lymphadenectomy and improve prognostication in oncologic hepatobiliary surgery.

e.

Figure 1. SLN mapping in liver using NIR fluorophores

16 μmol of MB (a), 50 nmol of ESNF14 (b), 1.6 μmol of ICG (c), or 50 nmol of ZW800-3C (d) was injected directly into the liver parenchyma and images acquired at time 0 (Injection) and over the next 30 min in N = 14 pigs total. Arrow = lymphatic tract. Arrowheads = NIR hotspots. Dotted red circle = second tier hotspot. All NIR fluorescence images for each condition have identical exposure times and normalizations. 700 nm and 800 nm NIR fluorescence images were pseudo-colored red and lime green, respectively on the Color-NIR merge. Scale bars = 1 cm. (e) Immunohistochemical analysis of paraffin-embedded pig lymph nodes. Hematoxylin and eosin staining (H&E; left column), staining with anti-CD79a antibody (middle column), and staining with anti-Bcl-6 antibody (right column). The 10X images are magnified from the dashed boxes in the 4X images. Scale bars = 100 μm . Abbreviations used are: CBD: common bile duct; Du: duodenum; GB: gallbladder; GC: germinal center; H-LN: hilar lymph nodes; In: intestine; Li: liver; LF: lymphoid follicle; MZ: mantle zone; PP-LN: periduodenal and peripancreatic lymph nodes; Pa: pancreas; St: stomach.

Figure 2. PLN mapping in pigs using ZW800-3C over 8 h

1 μ mol of ZW800-3C was injected intravenously into 35 kg Yorkshire pigs, and their mesenteric lymph nodes observed over 8 h. (a) NIR hotspots in M-LN (1st row), P-LN (2nd row), and H-LN (3rd row) were imaged at 4 h post-injection. Arrow = common bile duct; arrowheads = lymph nodes. NIR fluorescence images have identical exposure times and normalizations. 800 nm fluorescence images were pseudo-colored in lime green in the Color-NIR merge. Scale bars = 1 cm. Abbreviations used are: CBD: common bile duct; Du:

duodenum; H-LN: hilar lymph nodes; In: intestine; Li: liver; M-LN: mesenteric lymph nodes; Mu: muscle; PP-LN: periduodenal and peripancreatic lymph nodes; Pa: pancreas; St: stomach. **(b)** SBR (LN/Mu), SBR (LN/Pa), and SBR (LN/Li) (mean $\pm$ SD) of the H-LN and PP-LN were measured over the course of 8 h post-injection in N = 4 pigs.

Figure 3. Simultaneous Dual-NIR Fluorescence Imaging of SLN and RLN

1 μ mol of ZW800-3C (800 nm) was injected intravenously into 35 kg Yorkshire pigs 4 h prior to imaging, and 50 nmol of ESNF14 (700 nm) was injected into the liver parenchyma of the right medial lobe 30 min prior to imaging. **(a)** Dual-channel imaging of SLN (ESNF14; 700 nm) and RLN (ZW800-3C; 800 nm). Arrow = lymphatic tract; arrowheads = NIR hotspots found in the SLN; red dotted circle = RLN. Scale bars = 1 cm. Abbreviations used are: CBD: common bile duct; Du: duodenum; H-LN: hilar lymph nodes; In: intestine; Li: liver; PP-LN: periduodenal and peripancreatic lymph nodes; Pa: pancreas; St: stomach. **(b)** Dual-channel Imaging of SLN and RLN *ex vivo*: Arrow = groin lymph node (control); arrowheads = SLN; red dotted square = RLN. **(c)** Histological analysis of frozen-sectioned SLN and RLN: control lymph nodes before injection of the agents (1st row), PLN (2nd row), and SLN (3rd row). The color images show the lymphatic structure stained by hematoxylin and eosin (H&E). Scale bars = 100 μ m. Abbreviation used are: LF: lymphoid follicle; S: subcapsular sinus; Ve: vessel.

Table 1**Table 1a. SLN mapping performance as a function of NIR fluorophore.**

	Total (N = 12)	ICG (N = 5)	ESNF-14 (N = 5)	ZW800-3C (N = 2)	P
Detection of NIR hotspots	12/12	5/5	5/5	2/2	
# of NIR hotspots, mean $\pm$ SD	2.0 $\pm$ 0.7	2.2 $\pm$ 0.8	2.0 $\pm$ 0.7	1.5 $\pm$ 0.7	0.572
Detection time (min) of NIR hotspot, mean $\pm$ SD	3.5 $\pm$ 1.5	4.2 $\pm$ 2.0	3.0 $\pm$ 1.0	3.3 $\pm$ 0.7	0.469
# of resected NIR hotspots, mean $\pm$ SD	All	2.2 $\pm$ 0.8	2.2 $\pm$ 0.8	1.5 $\pm$ 0.7	0.568
	H-LN	0.8 $\pm$ 0.7	1.0 $\pm$ 0.6	1.0 $\pm$ 1.0	0.679
	PP-LN	1.3 $\pm$ 1.1	1.2 $\pm$ 1.0	0.5 $\pm$ 0.5	0.499
	G-LN	0	0	0	0
	PA-LN	0	0	0	0

Table 1b. SLN mapping performance as a function of liver lobe.

	Total (N = 12)	Right Lateral (N = 3)	Right Medial (N = 3)	Left Medial (N = 3)	Left Lateral (N = 3)	P
Detection of NIR hotspots	12/12	3/3	3/3	3/3	3/3	
NIR fluorophores		ICG x 2, ESNF14	ICG, ESNF14, ZW800-3C	ESNF14 x 2, ZW800-3C	ICG x 2, ESNF14	
# of NIR hotspots, mean $\pm$ SD	2.0 $\pm$ 0.7	1.7 $\pm$ 1.2	2.0 $\pm$ 0	2.0 $\pm$ 1.0	2.3 $\pm$ 0.6	0.702
Detection time (min) of NIR hotspot, mean $\pm$ SD	3.5 $\pm$ 1.5	3.3 $\pm$ 1.3	2.8 $\pm$ 0.8	3.7 $\pm$ 0.7	4.4 $\pm$ 2.8	0.531
# of resected NIR hotspots, mean $\pm$ SD	All	1.7 $\pm$ 1.2	2.3 $\pm$ 0.6	2.0 $\pm$ 1.0	2.3 $\pm$ 0.6	0.702
	H-LN	0.8 $\pm$ 0.7	1.3 $\pm$ 0.6	0 $\pm$ 0	0.7 $\pm$ 0.6	0.032
	PP-LN	1.3 $\pm$ 1.1	0.3 $\pm$ 0.6	1.0 $\pm$ 1.0	1.7 $\pm$ 1.2	0.227
	G-LN	0	0	0	0	0
	PA-LN	0	0	0	0	0

Abbreviations used are: SLN, sentinel lymph node; SD, standard deviation; H-LN, the hilar lymph nodes; NIR, near-infrared; PP-LN, the periduodenal and peripancreatic lymph nodes; G-LN, the gastrohepatic lymph nodes; PA-LN, the celiac and/or periaortic and caval lymph nodes;

Table 2

Results of SLN and RLN Resection.

	Total (N = 6)	ICG (N = 3)	ESNF14 (N = 3)	P
# of NIR hotspots, mean $\pm$ SD	2.3 $\pm$ 0.5	2.7 $\pm$ 0.6	2.0 $\pm$ 0.0	0.116
Detection time (min) of NIR hotspots, mean $\pm$ SD	3.9 $\pm$ 2.0	4.6 $\pm$ 2.7	3.3 $\pm$ 1.2	0.479
# of resected NIR hotspots, mean $\pm$ SD	2.5 $\pm$ 0.5	2.7 $\pm$ 0.6	2.3 $\pm$ 0.6	0.518
# of resected RLN, mean $\pm$ SD	20.7 $\pm$ 5.7	22.3 $\pm$ 4.9	19.0 $\pm$ 7.0	0.537
Additional NIR fluorescent nodes, mean $\pm$ SD	2.5 $\pm$ 2.8	5.0 $\pm$ 1.0	0.0 $\pm$ 0.0	0.001
Total time (min) of lymphadenectomy, mean $\pm$ SD	70.5 $\pm$ 20.3	66.2 $\pm$ 21.6	74.8 $\pm$ 22.5	0.66

Abbreviations used are: SLN, sentinel lymph node; RLN, regional lymph node; NIR, near-infrared; SD, standard deviation;