Editorial

Fluorescence-guided laparoscopic hepatectomy

Thinzar M. Lwin[1,2], Jason K. Sicklick[1], Robert M. Hoffman[1,2], Michael Bouvet[1,2]

[1]Department of Surgery, University of California San Diego, San Diego, CA, USA; [2]AntiCancer, Inc., San Diego, CA, USA

Correspondence to: Michael Bouvet, MD. University of California San Diego, Moores Cancer Center, 3855 Health Sciences Drive #0987, La Jolla, CA 92093-0987, USA. Email: mbouvet@ucsd.edu.

Comment on: Boogerd LS, Handgraaf HJ, Lam HD, et al. Laparoscopic detection and resection of occult liver tumors of multiple cancer types using real-time near-infrared fluorescence guidance. Surg Endosc 2016. [Epub ahead of print].

Received: 05 September 2016; Accepted: 27 September 2016; Published: 29 September 2016.

doi: 10.21037/ales.2016.09.04

View this article at: http://dx.doi.org/10.21037/ales.2016.09.04

Achieving negative tumor margins is critical in oncologic surgeries. This is particularly important in performing complex oncologic resections such as hepatectomy for primary and secondary hepatic malignancies. The achievement of negative resection margins has a direct impact on survival, and complete clearance of the tumor bed is the goal in laparoscopic liver resection for metastases (1). Advances in laparoscopic and robotic platforms have enabled minimally invasive approaches to liver surgeries. While determining tumor margins is difficult in an open setting, it becomes even more challenging in a laparoscopic environment, as traditional methods of direct visual inspection and palpation of the liver are limited.

Tactile feedback from tissue examination with laparoscopic instruments is extremely limited compared to manual manipulation. Laparoscopic liver surgeons are thus faced with relying on discerning subtle tissue irregularities within major anatomic landmarks, using non-specific visual cues. Pre-operative cross sectional imaging, intra-operative ultrasound (IOUS), and intra-operative frozen sections can assist in localizing the lesion, but cannot assist in achieving negative resection margins. Additionally, cross sectional imaging is difficult to translate directly into the operative field and changes with patient positioning. IOUS is limited by a steep learning curve, user variability, a two dimensional image, and a superficial blind area approximately 1 cm under the hepatic capsule (2). Laparoscopic IOUS is challenged with issues in ergonomics of access since trocar site placement limits range of motion.

Enhancement of tumor visualization via fluorescence assists in overcoming these limitations. Indocyanine green (ICG) is a hepatically-metabolized dye traditionally used to estimate cardiac output and hepatic volume (3). The dye binds to plasma proteins and has a peak absorbance at 780 nm and emits fluorescence with a wavelength of approximately 800 nm (4). ICG is preferentially retained in or around biliary malignancies due to impaired biliary excretion of hepatocytes in the affected area (5). Fluorescence-guided surgery (FGS) using ICG was first applied to hepatobiliary surgery by Ishizawa *et al.* in order to outline biliary structures, but has since been applied to hepatic malignancies (6). Gotoh *et al.* first described using ICG intra-operatively for hepatocellular carcinoma (HCC) in a series of 10 patients undergoing open hepatectomy. All ten resected lesions had strong fluorescence signals and the use of ICG detected additional lesions in four cases (40%) that were not previously detected by pre-operative imaging or IOUS (7). Ishizawa *et al.* further described different patterns of ICG fluorescence in HCC and colorectal liver metastases in their series (8). Well-differentiated HCC lesions showed complete fluorescence and moderately differentiated lesions showed partial fluorescence. Poorly-differentiated lesions and colorectal metastases showed no fluorescence, but the surrounding liver tissue fluoresced in a rim-like pattern due to increased cellular density from tumor compression. Using ICG, they were able to detect an additional 8 out of the 63 (13%) HCCs that were not evident on gross inspection.

Laparoscopic surgery is well suited for fluorescence guidance as the operator interacts with the screen image in real-time. Presence or absence of the fluorescence signal gives the surgeon immediate feedback on adequacy of tumor margins. Initial experience with laparoscopic fluorescence-guided hepatectomy was similar to open hepatectomy

with ICG. Kawaguchi *et al.* report the value of ICG fluorescence navigation in laparoscopic liver resections, especially for patients who have had scarring from previous chemotherapy and radiofrequency ablation (9). IOUS was able to identify the lesion to be resected, but was not able to assist in determining areas of recurrent tumor. However the use of ICG imaging displayed a fluorescing border which corresponded to areas of viable cancer cells on histology. In one patient, the use of ICG fluorescence allowed detection of a lesion not detected by pre-operative imaging or IOUS. Kudo *et al.* report on 17 patients undergoing laparoscopic hepatectomy for HCC, colorectal liver metastases, and uterine cancer (10). Of the 32 lesions resected, 6 were detected by visual inspection with standard laparoscopy while 23 were identified using ICG fluorescence. Similar to the open experience, laparoscopic ICG fluorescence was limited in detection of deeper lesions, >8 mm in their series. They also noted that ICG fluorescence was an especially valuable adjunct to IOUS to assist in setting dissection boundaries prior to parenchymal transection. The technology is a valuable tool to assure negative resection margins in laparoscopic hepatic resections.

While tumor fluorescence patterns for HCC, metastatic colorectal adenocarcinoma, and cholangiocarcinoma are well described, other hepatic malignancies are not (11,12). Boogerd *et al.* in their series of laparoscopic fluorescence-guided hepatectomies, describe additional hepatic malignancies visualized with ICG such as metastatic uveal melanoma and breast cancer (13).

Although there are only 22 patients included in their series, due to the limited availability of surgeons performing laparoscopic hepatectomies using ICG fluorescence guidance during their procedures, Boogerd's case series is the largest currently describing the experience. Previous studies report on fewer patients and Boogerd's study broadens limited literature available in this emerging field (9,10,14).

Boogerd *et al.* additionally compared the sensitivity of pre-operative imaging modalities with ICG-FGS and noted that of the 26 resected malignancies, 20% were missed by CT, 16% by MRI, 38% by inspection, 12% by laparoscopic IOUS, and 8% by ICG fluorescence imaging. Lesions missed by laparoscopic IOUS tended to be more superficial whereas the lesions missed by ICG fluorescence imaging tended to be deeper (>8 mm). When combining the two modalities of laparoscopic IOUS and ICG fluorescence imaging, the sensitivity for tumor detection reached 100%. Other studies have examined the rate of hepatic tumor detection using ICG in an open laparotomy (2). Peloso *et al.* compared pre-operative CT with IOUS and with ICG fluorescence imaging in patients undergoing open hepatectomy and reported that the combined use of IOUS and ICG fluorescence imaging was useful in identifying lesions, particularly <3 mm. Boogerd's study is the first to apply these questions of comparative efficacy of modalities used to identify hepatic lesions to the laparoscopic ICG-FGS setting. Their work thoroughly compares ICG fluorescence imaging with other pre-operative and intra-operative imaging modalities in a wide variety of primary and secondary lesions in the liver.

Although ICG has a high sensitivity in contrast enhancement and improved visualization, a major limitation of the dye is that it lacks specificity. Its non-specific plasma protein binding is a drawback. In Boogerd's study, a positive-predictive value for ICG-FGS was 75% and 70.3% when combined with laparoscopic IUOS. Although the data are not stratified by presence or absence of cirrhosis, 14 of their patients had cirrhosis. In cirrhotic patients, regenerative hepatic nodules are known to take up ICG and fluoresce as discrete nodules, leading to false positives. A positive predictive value of ICG for malignancy in one series was 5.4% in cirrhotic livers *vs.* 100% in healthy livers (15).

Tumor-targeted fluorescent dyes are being developed to overcome limitations of non-specific ICG binding. Our lab has shown that the use of tumor-specific fluorescent organ labeling in laparoscopy aids in rapid and accurate identification of tumors, especially at the sub-millimeter resolution (16). We have also studied FGS with tumor-specific fluorescence in a colon-cancer liver-metastasis model (17,18). Fluorescence was delivered via an anti-CEA antibody or tumor-specific adenovirus vectors expressing GFP. Both modalities labeled the tumor well and highlighted microscopically-positive margins (*Figure 1*). Mice undergoing FGS had a significantly lower volume of residual tumor and improved overall and disease-free survival (*Figure 2*) (17,18). Other tumor specific-fluorescence labeling modalities are being developed such as pH-sensitive probes, activatable cell-penetrating peptides and fluorescent nanoparticles (19-22).

Clinical applications of tumor-specific fluorescent dyes under trials currently include fluorescent folate to label ovarian cancer and fluorescent cetuximab to label head and neck cancers, but data on survival advantage and rate of tumor recurrence are not yet available (23,24). The number

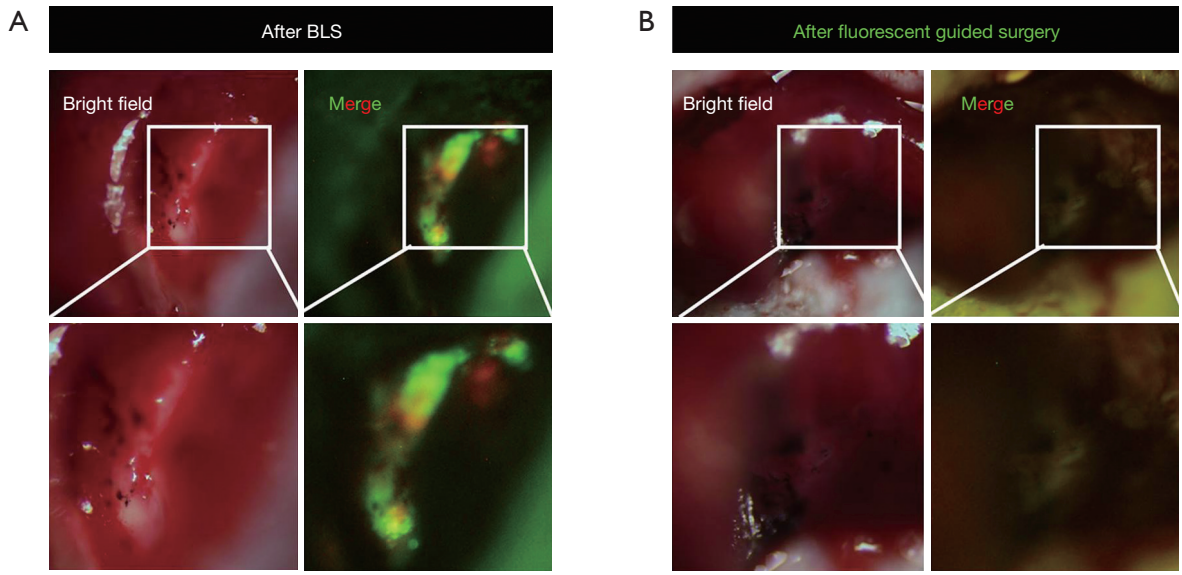

Figure 1 Tumors labeled with a telomerase-dependent adenovirus (OBP-401) expressing green fluorescent protein undergo bright-light surgery and fluorescence-guided surgery 3 days post infection. Residual cancer cells were detectable in the resection bed for the BLS group, but not the FGS group (18). BLS, bright light surgery; FGS, fluorescence-guided surgery.

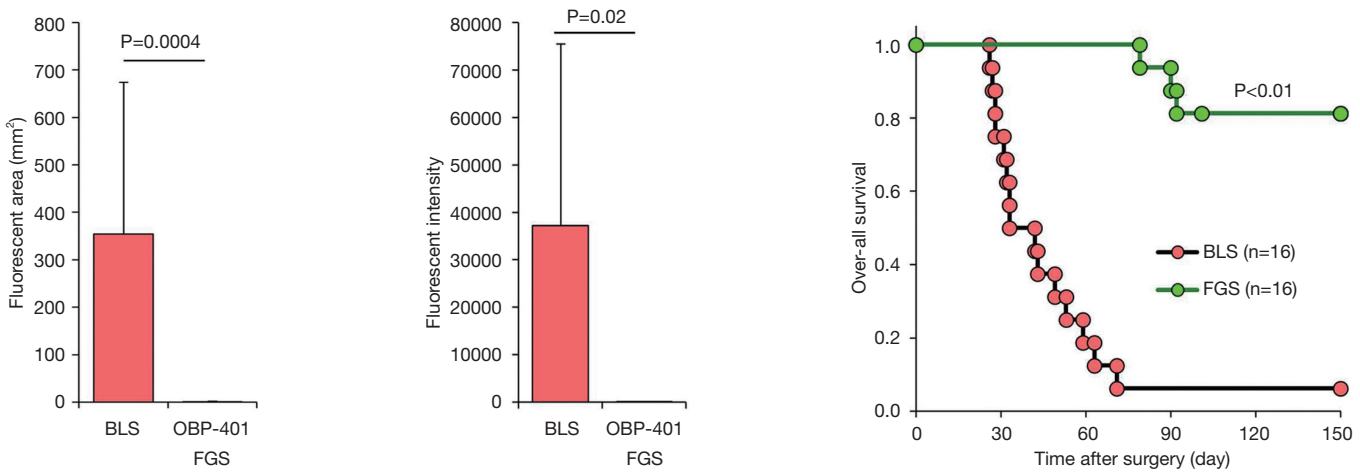

Figure 2 Comparison of fluorescence intensity of locally-recurrent tumors after bright light surgery (BLS) or FGS with adenovirus vector expressing GFP (OBP-401) in a colon cancer liver metastasis model (18). Kaplan Meyer shows improved overall survival after FGS with adenovirus vector compared to BLS. FGS, fluorescence-guided surgery.

of fluorescence labeling platforms will greatly increase in the future along with further data on their impact of oncologic outcomes.

Minimally-invasive hepatectomy is a promising area where FGS can potentially impact oncologic outcomes. Combining ICG and laparoscopic IOUS enhances surgical detection of lesions, superficial and deep, but are limited by a lack of specificity in determining malignancy. Newly-developing tumor-specific fluorophores will play a major role in addressing this issue. Enhanced contrast helps clearly delineate tumor margins and identify lesions that would otherwise be missed, increasing rates of complete resection.

The goal of FGS, in both laparoscopic and open surgery, is to increase rates of curative cancer surgery. FGS with

tumor specific-probes, effected with fluorescent antibodies or viral labeling with a genetic reporter has increased the survival in orthotopic mouse models. A second step after FGS, such as an intra-operative UV irradiation of the resection bed could be used to kill any residual cancer cells (25). Such combined strategies hold great promise for curative cancer surgery.

Acknowledgments

Funding: TM Lwin was funded by the NIH/NCI Award T32CA121938.

Footnote

Provenance and Peer Review: This article was commissioned by the editorial office, *Annals of Laparoscopic and Endoscopic Surgery*. The article did not undergo external peer review.

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/ales.2016.09.04). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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doi: 10.21037/ales.2016.09.04
Cite this article as: Lwin TM, Sicklick JK, Hoffman RM, Bouvet M. Fluorescence-guided laparoscopic hepatectomy. Ann Laparosc Endosc Surg 2016;1:10.

