Image-guided cancer surgery : the value of near-infrared fluorescence imaging during oncologic and gastrointestinal procedures

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PART IV

Chapter 12

Summary and Future perspectives

SUMMARY

Intraoperative imaging using near-infrared (NIR) fluorescence is a relatively new technique that can be used to visualize tumor tissue, sentinel nodes and vital anatomical structures. This thesis is divided in three parts. In **part one** the ability to visualize surgical margins using NIR fluorescence imaging is demonstrated. Tumor visualization is established using the clinically approved contrast agent indocyanine green, as well as newly developed tumor targeted probes. The proportion of laparoscopic procedures has steadily increased over the last two decades. A challenging aspect of this conversion to minimal invasive surgery is the lack of tactile information, making it of particular interest for the development and improvement of laparoscopic NIR fluorescence imaging systems[1]. **Part two** focusses on the clinical implementation of NIR fluorescence guided sentinel lymph node mapping for several indications (e.g. breast, skin and vulvar cancer). Besides visualization of structures that need to be resected (e.g. tumor tissue or sentinel nodes), NIR fluorescence has also the potential to be of value for the identification of structures that should be spared. In **part three**, we demonstrate the first-in-human application of NIR fluorescence guided ureteral visualization, and also the optimization of bile duct imaging for routine laparoscopic cholecystectomies.

Part I: Intraoperative evaluation of surgical margins

To date Indocyanine green and methylene blue are the only two NIR fluorophores that are the approved for clinical application by the U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMA). However both fluorophores cannot be conjugated to tumor specific compounds such as antibodies or peptides. Therefore, there is a strong need for novel clinically approved tumor-targeted dyes. **Chapter 2** evaluates the use of a low dose fluorescent dye ZW800-1 conjugated to the cyclic RGD peptide (cRGD) targeting integrin for colorectal cancer imaging and ureteral visualization. cRGD-ZW800-1 was successfully used in both subcutaneous and orthotopic mouse models to identify malignant cells. Two negative control groups (cRAD-ZW800-1 and ZW800-1 alone) were included to confirm specificity of cRGD-ZW800-1 *in vivo*. Mice in the negative control groups showed no tumor signal during *in vivo* imaging using the FLARE^{tm} imaging system and *ex vivo* imaging using the Odyssey scanner. cRGD-ZW800-1 showed clear uptake in both the subcutaneous and orthotopic colon cancer mouse model. The renal clearance of cRGD-ZW800-1 also allowed visualization of the ureter. The ability to visualize both tumor demarcation and the ureters could especially be of value during (complicated) lower abdominal surgery. **Chapter 3** aims to test feasibility of using Methylene blue

as a NIR fluorescent tracer for the detection of breast cancer. A total of 24 patients were included in this study. In 83% of patients, the tumor demarcation as identified by NIR fluorescence imaging corresponded to histological presence of tumor. In addition, in one case surgical management was changed based on intraoperative NIR fluorescence findings, which avoided the need for a re-operation. This is the first study to demonstrate the use of low dose MB for the real-time identification of breast cancer using NIR fluorescence, and can be considered as a promising proof-of-concept of clinical image guided cancer surgery, especially as improved contrast agents will likely become available in the near future. In **Chapter 4** the development, current application and future prospects of NIR fluorescence imaging for hepato-pancreatobiliary surgery are evaluated. To date, the technique has already shown potential to improve the intraoperative demarcation of various liver tumors and vital structures based on physiological clearance of ICG. Although, the obtained results are promising, large randomized controlled trials are essential to define patient benefit.

Part II: Sentinel lymph node imaging

In **Chapter 5** the diagnostic accuracy of NIR fluorescence for SLN mapping in breast cancer when used in conjunction with conventional techniques was evaluated. Patients were included at the Dana-Farber/Harvard Cancer Center and the Leiden University Medical Center. SLN identification was successful in 94 of 95 subjects (99 %) using NIR fluorescence imaging or a combination of both NIR fluorescence imaging and radioactive guidance. NIR fluorescence outperformed both radioactivity and blue dye staining in context of finding tumor-positive Sentinel Lymph nodes. Percutaneous lymphatic drainage could be visualized in 81% of patients, which facilitates in the sentinel node identification. Moreover, no adverse events related to fluorescence imaging were reported in this study, which confirms safe application of NIR fluorescence and ICG. In conclusion, this multi-center experience validates the accurate application of NIR fluorescence imaging for the identification of SLNs in breast cancer patients, but also calls into question what technique should be used as the gold standard in future studies. In **Chapter 6** the performance of a hybrid NIR fluorescence and radioactive tracer for SLN detection of breast cancer was evaluated. This study shows the ability of combining preoperative imaging and intraoperative guidance using a single tracer. The sentinel lymph nodes could be detected surgically by both γ radiation and NIR fluorescence imaging in all (N = 32) patients. Using this hybrid tracer no additional injection in the surgical theatre is necessary, intraoperative results are comparable to those provided by the use of ICG alone. In **Chapter 7** the same hybrid NIR fluorescence and radioactive tracer is evaluated in melanoma patients. This study shows successful application SLN mapping with a single tracer

for both preoperative planning as well as intraoperative guidance. The FLARE camera system used in this study was able to provide continuous "hands free" image guidance in relation to the surgical anatomy. In **Chapter 8** the ability to visualize lymphatic drainage and intraoperative SLN visualization using NIR fluorescence in bladder cancer is demonstrated. Unlike other cancers, bladder cancer requires special attention to injection technique. This study is the first to optimize NIR tracer injection. Using the optimal administration protocol NIR fluorescent lymph nodes could clearly be identified during surgery. In **Chapter 9** the implementation and added value of NIR fluorescence imaging in vulvar cancer is investigated. Premixing of ICG with human serum albumin (ICG:HSA) is advocated to improve dye retention in the SLN. In this chapter the use of different formulations of ICG for NIR fluorescence guided SLN biopsy in vulvar cancer patients are evaluated. NIR fluorescence outperformed blue dye staining in all patients. Moreover, ICG premixed with ^{99m}Tc-nanocolloid seems optimal terms of the intraoperative detection rate and has the potential to shorten operating time because no lymphatic tracer administration is needed in the operating room.

Part III: Vital structure imaging

In **Chapter 10** the first-in-human application of fluorescence guided identification of the ureters using low-dose methylene blue is demonstrated. Intraoperative detection of the ureters was successful in all patients after intravenous infusion of methylene blue. Patients were divided in three dose groups (0.25, 0.5 and 1 mg/kg). No statistical differences were observed between the dose groups; however the lowest dose group was considered optimal since the signal of the ureter in these patients was sufficient for early identification of the ureter. In **Chapter 11,** the implementation of near-infrared fluorescence cholangiography for both open and laparoscopic surgery is shown. The aim this study was to compare early- and delayed-imaging protocols using different doses of ICG to optimize NIR cholangiography using a quantitative intraoperative camera system during open hepatopancreatobiliary (HPB) surgery. These results were subsequently validated during laparoscopic cholecystectomy using a laparoscopic fluorescence imaging system. After intravenous administration, ICG is cleared by the liver and excreted into the bile. Based on this principle, the extra-hepatic bile ducts can clearly be visualized within minutes up till multiple hours after intravenous administration of ICG. This study shows that a prolonged interval between ICG administration and surgery permits optimal NIR cholangiography, as liver background signal is minimal.

FUTURE PERSPECTIVES

For centuries surgeons could only use their eyes and hands to assess the presence and extent of malignant tumors. To date, many imaging modalities are available for preoperative surgical planning such as CT, MRI and PET/SPECT. However, during surgery, visual inspection and palpation remain the most important tools to determine the extent of the disease in relation to patient's anatomy[2]. NIR fluorescence imaging has the ability to facilitate real-time image guidance and can potentially fulfill an important complementary role during cancer surgery in the near future.

Over the last years, a great spinoff has been made in the field of NIR fluorescence guided surgery. The availability of experimental NIR fluorescence imaging systems and the clinically approved dyes Indocyanine Green and Methylene blue have allowed NIR fluorescence image-guided surgery to be introduced into clinical trials[3-6]. However, to permit this technique to become adapted as standard-of-care during cancer surgery, novel tumor targeted contrast agents are essential. In the literature, many preclinical studies have shown promising results using novel tumor specific contrast agents[7-10]. Though, the focus within the field is currently shifting towards clinical translation. For example, a clinical trial using Bevacizumab (Avastin®, Roche) conjugated to IRDye 800CW (LI-COR Biosciences, Lincoln, NE) is currently ongoing under supervision of Professor van Dam and colleagues (UMCG, Groningen, the Netherlands). Bevacizumab is a monoclonal antibody against VEGF that has been approved by the FDA for systemic therapies since 2004. It can be a good strategy to choose an antibody that has already been approved for other purposes, as safety and toxicity profiles have been broadly investigated. However, the use of antibody-based dyes has some potential drawbacks for implantation in NIR fluorescence guided surgery. First, antibodies have a half-life *in vivo* of several days; as a consequence the dye needs to be intravenously administered days before surgery. Second, due to the hepatic clearance of most antibodies high levels of background fluorescence can occur during early imaging in the peritoneal cavity and the liver. Smaller imaging agents that can be cleared by the kidneys are not exposed to those disadvantages. Chapter 2 shows an example of a recently developed fluorescent probe with minimal background uptake in the gastrointestinal tract. Nevertheless, the main hurdle for clinical translation of novel dyes is the fact that every new contrast agent has to go through a separate regulatory approval processes. This makes the clinical translation extremely costly and time consuming.

NIR fluorescence imaging has several characteristics that are advantageous during surgery, including a relatively high penetration into living tissue (up to several millimeters) and real-time, high-resolution optical guidance[11-13]. However, identification of structures lying deeper in tissue can be challenging. Combining radioactive col-

loids and NIR fluorescence has recently been introduced to overcome this shortcoming during sentinel lymph node mapping (chapter 7, 8 and 12)[14-17]. The currently used tracer for SLN mapping is based on ICG and ^{99m}Tc-nanocolloid, which are both clinically available. In the future, newly developed, fluorophores with optimized fluorescent capabilities such as IRDye 800CW or ZW800-1 (The FLARE Foundation, Wayland, MA) can potentially even facilitate easier sentinel node localization.

For many cancers, such as breast and colorectal cancer, a multidisciplinary approach, with standardized surgical, pathological, and increasingly a chemo- and radiotherapeutic workup has become the standard[18-20]. In addition, neoadjuvant therapy has become standard-of-care for selected breast and rectal cancer patients[21-23]. The shift in paradigm from adjuvant to neoadjuvant therapy has resulted in increased tissue and organ conservation (e.g. in breast and rectal cancer)[20,24]. On the other hand, areas of inflammatory tissue induced by the neoadjuvant therapy itself can make discrimination between malignant and healthy tissue during surgery even more difficult. Especially in those cases, there remains a strong need for real-time surgical guidance. On the contrary, a substantial portion of both breast and rectal cancer patients (up to 50% reported) have such a good response to neoadjuvant therapy that it leads to a complete histopathological remission of the tumor[25-28]. This means that no malignant cells can be identified during histological assessment of the resection specimen after surgery. This has raised the question as to whether surgery can be avoided in a select cohort of patients. A problem is though that there is currently no validated imaging modality to evaluate whether a tumor has undergone complete remission after systemic or locoregional therapy[29,30].

Fine-tuning the relation between tumor diagnosis, neoadjuvant treatment, preoperative evaluation and surgical planning will likely reduce surgical morbidity and improve patient outcome. Molecular imaging, both preoperatively (e.g. using targeted PET tracers / photoacoustic therapy) and during surgery (using NIR fluorescence tracers) can play a vital role in this process. Ideally, these newly designed tracers can be combined in one hybrid radioactive and fluorescent compound.

Besides visualization of structures that need to be resected (e.g. tumor tissue and sentinel nodes) is it is of paramount importance to correctly identify structures that need to be spared (e.g. nerves, bile ducts or ureters) during surgery[6,31]. Based on their biodistribution and clearance pattern, indocyanine green and methylene blue can be easily used for the identification of bile ducts and ureters respectively. However, as the incidence of iatrogenic damage to the ureter or bile duct is relatively low, it is expected that this technique mainly exposes its full potential in combination with tumor imaging. Dependent on their clearance pattern, tumor targeted dyes can not only be used to reveal tumor margins but also for the visualization of bile ducts or ureters[6]. This can be especially of value during laparoscopic liver or colorectal surgery.

Another important factor for clinical acceptance of this technique will be the continued development of imaging systems, as a large proportion of currently used imaging systems are still in the experimental phase. To create a substantial impact on patient care it is essential that NIR fluorescence imaging can be easily applied and imaging systems are affordable. Novel systems have shown improved image quality and resolution, better sensitivity, and increased usability. Over the last decade laparoscopic surgery has become standard-of-care for an increasing number of indications. As palpation is hampered during these procedures, there is an increased need for additional intraoperative imaging modalities making it of particular interest for the development and improvement of laparoscopic fluorescence imaging systems. One of the major limiting factors of NIR fluorescence imaging is its limited tissue penetration depth. Current studies report a penetration depth ranging from several millimeters to, at most, one centimeter. To improve depth penetration, it is expected that future research will be more focused on the integration of several imaging modalities to overcome the shortcomings of a single modality. For example, by combining the beneficial characteristics of radioactive and fluorescence guidance, it is possible to create an imaging modality with superior depth penetration but also accurate real-time localization.

In conclusion, novel image-guided modalities have the potential to play an important role in the surgical management of future cancer patients. However, to assess true patient benefit the optimization of contrast agents and imaging systems is essential. Despite the very promising results already obtained, the next decade will show if Image-Guided Cancer Surgery using NIR fluorescence imaging delivers benefits to surgical outcome in day-to-day patient care.

REFERENCES

1. Verbeek FP, van der Vorst JR, Schaafsma BE et al. Image-guided hepatopancreatobiliary surgery using near-infrared fluorescent light. J Hepatobiliary Pancreat Sci 2012; 19:626-637.
2. Vahrmeijer AL, Frangioni JV. Seeing the invisible during surgery. Br J Surg 2011; 98:749-750.
3. Gioux S, Choi HS, Frangioni JV. Image-guided surgery using invisible near-infrared light: fundamentals of clinical translation. Mol Imaging 2010; 9:237-255.
4. Schaafsma BE, Mieog JS, Hutteman M et al. The clinical use of indocyanine green as a near-infrared fluorescent contrast agent for image-guided oncologic surgery. J Surg Oncol 2011; 104:323-332.
5. van der Vorst JR, Schaafsma BE, Verbeek FP et al. Intraoperative Near-Infrared Fluorescence Imaging of Parathyroid Adenomas using Low-Dose Methylene Blue. Head Neck 2013.
6. Verbeek FP, van der Vorst JR, Schaafsma BE et al. Intraoperative near infrared fluorescence guided identification of the ureters using low dose methylene blue: a first in human experience. J Urol 2013; 190:574-579.
7. Luo S, Zhang E, Su Y et al. A review of NIR dyes in cancer targeting and imaging. Biomaterials 2011; 32:7127-7138.
8. Oliveira S, van Dongen GA, Stigter-van WM et al. Rapid visualization of human tumor xenografts through optical imaging with a near-infrared fluorescent anti-epidermal growth factor receptor nanobody. Mol Imaging 2012; 11:33-46.
9. Choi HS, Gibbs SL, Lee JH et al. Targeted zwitterionic near-infrared fluorophores for improved optical imaging. Nat Biotechnol 2013; 31:148-153.
10. Bunschoten A, Buckle T, Visser NL et al. Multimodal Interventional Molecular Imaging of Tumor Margins and Distant Metastases by Targeting alpha(v) beta(3) Integrin. ChemBioChem 2012; 13:1039-1045.
11. Frangioni JV. In vivo near-infrared fluorescence imaging. Curr Opin Chem Biol 2003; 7:626-634.
12. Vahrmeijer AL, Hutteman M, van der Vorst JR et al. Image-guided cancer surgery using near-infrared fluorescence. Nat Rev Clin Oncol 2013.
13. Verbeek FP, Troyan SL, Mieog JS et al. Near-infrared fluorescence sentinel lymph node mapping in breast cancer: a multicenter experience. Breast Cancer Res Treat 2014; 143:333-342.
14. van der Poel HG, Buckle T, Brouwer OR et al. Intraoperative Laparoscopic Fluorescence Guidance to the Sentinel Lymph Node in Prostate Cancer Patients: Clinical Proof of Concept of an Integrated Functional Imaging Approach Using a Multimodal Tracer. Eur Urol 2011; 60:826-33.
15. Schaafsma BE, Verbeek FP, Rietbergen DD et al. Clinical trial of combined radio- and fluorescence-guided sentinel lymph node biopsy in breast cancer. Br J Surg 2013; 100:1037-1044.
16. van Leeuwen AC, Buckle T, Bendle G et al. Tracer-cocktail injections for combined pre- and intraoperative multimodal imaging of lymph nodes in a spontaneous mouse prostate tumor model. J Biomed Opt 2011; 16:016004.
17. Brouwer OR, van den Berg NS, Matheron HM et al. A hybrid radioactive and fluorescent tracer for sentinel node biopsy in penile carcinoma as a potential replacement for blue dye. Eur Urol 2014; 65:600-609.

18. Kapiteijn E, Marijnen CA, Nagtegaal ID et al. Preoperative radiotherapy combined with total mesorectal excision for resectable rectal cancer. N Engl J Med 2001; 345:638-646.
19. van Gijn W, Marijnen CA, Nagtegaal ID et al. Preoperative radiotherapy combined with total mesorectal excision for resectable rectal cancer: 12-year follow-up of the multicentre, randomised controlled TME trial. Lancet Oncol 2011; 12:575-582.
20. Mieog JS, van der Hage JA, van de Velde CJ. Preoperative chemotherapy for women with operable breast cancer. Cochrane Database Syst Rev 2007;CD005002.
21. Peeters KC, van de Velde CJ. Surgical quality assurance in breast, gastric and rectal cancer. J Surg Oncol 2003; 84:107-112.
22. Oehler C, Ciernik IF. Radiation therapy and combined modality treatment of gastrointestinal carcinomas. Cancer Treat Rev 2006; 32:119-138.
23. Jin HL, Zhu H, Ling TS et al. Neoadjuvant chemoradiotherapy for resectable esophageal carcinoma: a meta-analysis. World J Gastroenterol 2009; 15:5983-5991.
24. Mieog JS, van der Hage JA, van de Velde CJ. Neoadjuvant chemotherapy for operable breast cancer. Br J Surg 2007; 94:1189-1200.
25. Glynne-Jones R, Wallace M, Livingstone JI et al. Complete clinical response after preoperative chemoradiation in rectal cancer: is a "wait and see" policy justified? Dis Colon Rectum 2008; 51:10-19.
26. O'Neill BD, Brown G, Heald RJ et al. Non-operative treatment after neoadjuvant chemoradiotherapy for rectal cancer. Lancet Oncol 2007; 8:625-633.
27. Buzdar AU, Ibrahim NK, Francis D et al. Significantly higher pathologic complete remission rate after neoadjuvant therapy with trastuzumab, paclitaxel, and epirubicin chemotherapy: results of a randomized trial in human epidermal growth factor receptor 2-positive operable breast cancer. J Clin Oncol 2005; 23:3676-3685.
28. Gavila J, Guerrero A, Climent MA et al. Efficacy and safety of neoadjuvant chemotherapy with concurrent liposomal-encapsulated doxorubicin, paclitaxel and trastuzumab for human epidermal growth factor receptor 2-positive breast cancer in clinical practice. Int J Clin Oncol 2014.
29. Rosen EL, Blackwell KL, Baker JA et al. Accuracy of MRI in the detection of residual breast cancer after neoadjuvant chemotherapy. AJR Am J Roentgenol 2003; 181:1275-1282.
30. Franklin JM, Anderson EM, Gleeson FV. MRI features of the complete histopathological response of locally advanced rectal cancer to neoadjuvant chemoradiotherapy. Clin Radiol 2012; Jun;67(6):546-52.
31. Verbeek FP, van der Vorst JR, Schaafsma BE et al. Image-guided hepatopancreatobiliary surgery using near-infrared fluorescent light. J Hepatobiliary Pancreat Sci 2012; 19:626-637.