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Near-infrared image guidance in cancer surgery

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Chapter 1

Introduction: The clinical use of near-infrared fluorescence imaging for image-guided oncologic procedure or treatment evaluation

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Schaafsma BE, Mieog JS, Hutteman M, van der Vorst JR, Kuppen PJ, Löwik CW, Frangioni JV, van de Velde CJ, Vahrmeijer AL

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INTRODUCTION

The identification of structures that need to be resected (e.g. tumour tissue, lymph nodes) and structures that need to be spared (e.g. nerves, ureters, bile ducts) is of paramount importance in daily oncologic surgery. However, at present, surgeons mainly rely on palpation and visual inspection. Therefore, tumour positive resection margins and surgical morbidity as result of damage to vital structures are not uncommon. Thus, there is a unmet need for new intraoperative imaging modalities that can provide real-time assessment of tumour borders and affected lymph nodes, while eliminating the risk of damaging vital structures.

Optical imaging using near-infrared (NIR) fluorescence is a new technique that can be used to visualise structures in real-time during surgery. Advantages of NIR fluorescent light (700-900 nm) include high tissue penetration (millimetres to centimetres deep) and low autofluorescence, thereby providing sufficient contrast.[1] Because the human eye is insensitive to NIR wavelengths, the use of NIR light does not alter the surgical field. Recently developed intraoperative imaging systems are able to provide simultaneous acquisition of surgical anatomy (white light, colour video) and NIR fluorescence signal.[2-4] Therefore, the use of NIR fluorescence imaging could potentially be of great value in the intraoperative detection of critical anatomical structures and oncologic targets.

In addition to NIR fluorescence imaging systems, exogenous NIR fluorescent contrast agents are necessary to visualise specific tissues. Ideally, tumour cells are labelled by targeted contrast agents. However, the only fluorescent contrast agents currently registered by the FDA and EMA for clinical applications are indocyanine green (ICG; peak emission ≈ 820 nm), methylene blue (peak emission ≈ 700 nm) and fluorescein (peak emission ≈ 520 nm, below NIR spectrum). This thesis is mainly focused on the clinical use of ICG, due to its preferable fluorescent characteristics and widespread use in clinical research. ICG provides a higher signal-to-background ratio because of lower autofluorescence and increased tissue penetration at 820 nm compared to lower wavelengths and has a greater "brightness" (quantum yield) compared to methylene blue.[5]

ICG is currently utilised in NIR fluorescence image-guided surgery for multiple indications. NIR fluorescence imaging has the potential to improve sentinel lymph node (SLN) mapping in multiple types of cancer, by real-time transcutaneous and intraoperative visualisation of lymphatic channels and subsequent detection of the SLN.[3,4,6-29] Additionally, ICG NIR fluorescence is used for endoscopic marking of colorectal tumours and intraoperative identification of certain solid tumours after intravenous injection.[30-33] Moreover, NIR fluorescence angiography using ICG can be used in intraoperative assessment of tissue perfusion in reconstructive surgery for ablative defects following oncologic surgery[34] and to lower the risk of anastomotic dehesence during various procdures.

This introduction provides a review on clinical studies using ICG in NIR fluorescence-guided cancer surgery in order to understand current applications, limitations, and future prospects, which will be further explored in this thesis.

Table I. Clinically available imaging systems

Imaging system	Excitation source	Working distance	Field of view	White light illumination of surgical field	NIR-colour overlay
PDE	LED 805 nm, power NS	15 - 25 cm	NS	No	No
SPY	Laser 806 nm, 2.0 W	30 cm	56 cm2	No	No
Fluobeam®	Laser 780 nm, 10mW/cm2	22 cm	80 cm2	Yes	No
HyperEye	LED 760 nm, power NS	30 - 50 cm	78.5 cm2	Yes	Yes
FLARETM	LED 745-779 nm, 14 mW/cm2	45 cm	3.7 cm2 - 169.5 cm2	Yes	Yes
Mini-FLARETM	LED 760 nm, 8.6 mW/cm2	30 cm	100 cm2	Yes	Yes
FDPM imager	Laser Diode 785 nm ± 10 nm, <1.9 mW/cm2	<76.2 cm	Max 900 cm2	No	No
Artemis camera system	Laser 400-1000nm, 4 mW/cm2	1.5 - 25	60	Yes	Yes
Karl Storz high definition fluorescence laparoscope	760 nm	laparoscopy	laparoscopy	Yes	No
Firefly for robotic surgery	NA	laparoscopy	laparoscopy	Yes	No
Munich / SurgOptix prototype camera system	Laser 750 nm, 300 mW	21 cm	1,5 cm2 - 107 cm2	Yes	Yes

PDE, Photodynamic Eye; LED, Light emitting diode; NS, not specified; FLARE, Fluorescence-Assisted Resection and Exploration; FDPM, Frequency Domain Photon Migration

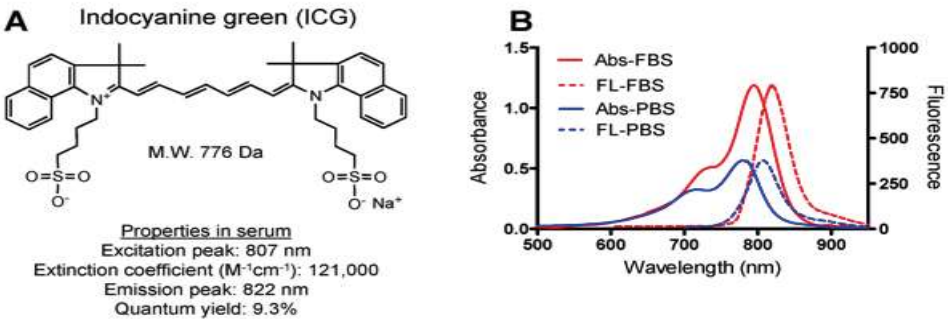

Fig. 1. Chemical and optical characteristics of ICG: A. Chemical structure and key optical properties (in serum). **B.** Absorption and emission of 10 mM ICG dissolved in phosphate-buffered saline (PBS) and fetal bovine serum (FBS).

NIR FLUORESCENCE IMAGING

Clinically available NIR imaging systems

Several NIR fluorescence imaging systems have been described for intraoperative clinical use (reviewed in Gioux et al.[35]). Although differing in their technical specifications, all of these systems provide the surgeon with an image of the NIR fluorescence signal that would otherwise be invisible to the human eye (Table I). The majority of clinical studies published to date use the commercially available Photodynamic Eye (PDE, Hamamatsu Photonics, Hamamatsu, Japan) imaging camera system[18]. Other commercially available systems are the SPY system (Novadaq Technologies, Concord, ON, Canada), the Fluobeam® (Fluoptics, Grenoble, France), Artemis (Quest Medical Imaging, Middenmeer, The Netherlands)[91], Karl Storz high definition fluorescence laparoscope (Karl Storz, Tuttlingen, Germany) and integrated in the DaVinci Firefly system (Intuitive Surgical, Sunnyvale, CA, USA). Several others imaging systems have been used in clinical studies but are not commercially available: HyperEye[2] (Kochi Medical School, Kochi, Japan), the FLARE™ and Mini-FLARE™[3] (Beth Israel Deaconess Hospital, Boston, MA, USA), the FDPM imager[36] (Texas Medical Center, Houston, TX, USA), and a prototype camera system from Munich[4] (Technical University Munich, Munich, Germany and SurgOptix Inc., Redwood Shores, CA, USA).

Indocyanine green

ICG is a negatively charged, amphiphilic, water-soluble, tricarbocyanine with a molecular mass of 776 Da.[37,38] ICG has been registered for several decades to determine cardiac output, hepatic function, and ophthalmic perfusion. Rapid registration was attributable to favourable characteristics such as the confinement to the vascular compartment by binding to plasma proteins, the fast and almost exclusive excretion into the bile, and the very low toxicity of ICG.[39,40] ICG is safe to use, as the number of allergic reactions is very low (1: 10 000, as reported by manufacturer). The dose used for standard diagnostic procedures lies between 0.1 and 0.5 mg/kg. Above 0.5 mg/kg, the incidence of immediate allergic reactions increases.[41]

In plasma, ICG has an absorption peak around 807 nm and an emission peak around 822 nm, which is within the NIR window (Fig. 1). After intravenous administration, ICG has a short half-time of 150-180 seconds and is cleared exclusively by the liver.[42] ICG molecules bind rapidly and almost completely to serum proteins. The binding to relative large serum proteins prevents the unwanted interaction between the ICG molecules and thereby improves its brightness (quantum yield increases 3.5x) and increases its hydrodynamic diameter.[39,43-45] Hydrodynamic diameter has

important implications for distribution and transport of ICG for tumour visualisation and retention in the SLN as will be discussed below.[6,46,47]

SENTINEL LYMPH NODE MAPPING

NIR fluorescence imaging provides new opportunities to improve and extend the indications of the SLN procedure. Gamma ray-emitting radiotracers and blue dyes are currently used as standard of care in clinical practice. However, the use of gamma ray-emitting radiotracers requires involvement of a nuclear medicine physician, localisation of the SLN can be difficult using a handheld gamma probe, and preoperative access to the injection site is required. Blue dyes cannot be easily seen through the skin and fatty tissue. Additionally, the learning curve for the standard SLN procedure using these techniques is estimated to be 60 required cases for technical proficiency when working with breast cancer patients.[48]

NIR fluorescence imaging using ICG has been shown to visualise superficial lymphatic channels transcutaneously.[14] Thereby, it could potentially reduce time of surgery and improve localisation of the SLN so that a small incision can be made, while maintaining a high identification rate. Moreover, the NIR fluorescence signal could aid the pathologist in both preparing and analysing the tissue specimen.[13,18] It should be noted, however, that NIR fluorescence detection is in the millimetre to centimetre range, far less than radioactive tracers, which requires caution when examining thick tissues.

ICG has been used as lymphatic tracer in SLN procedures in breast, skin, gastro-intestinal, non-small cell lung, oropharyngeal and gynaecological cancer.[3,4,7-29] Differences in imaging systems, ICG doses and injection sites prevent direct comparison of the results. In the next sections, the results will be discussed for each tumour type separately.

Breast cancer

Most studies using ICG as NIR fluorescent SLN tracer have been in breast cancer patients.[3,12-21] Before the introduction of NIR fluorescence imaging systems, Motomura et al.[49] used only the intrinsic green colour of ICG and identified the SLN in 73.8% of the patients. After the introduction of intraoperative NIR fluorescence imaging systems, higher identification rates of 87.5% to 100% (aggregate 98.6%) were obtained and an average of 3.4 (range 1.5 to 5.4) SLNs were identified.3,12-21 Two studies performed an axillary dissection irrespective of the SLN status and found an aggregate false-negative rate of 7.7% in 39 patients with a negative SLN.12,14 Additionally, as a result of the capability of NIR fluorescence light to penetrate tissue, ICG offers non-invasive imaging of lymphatic flow (Fig. 2). Upon injection of ICG, travel time to the axilla

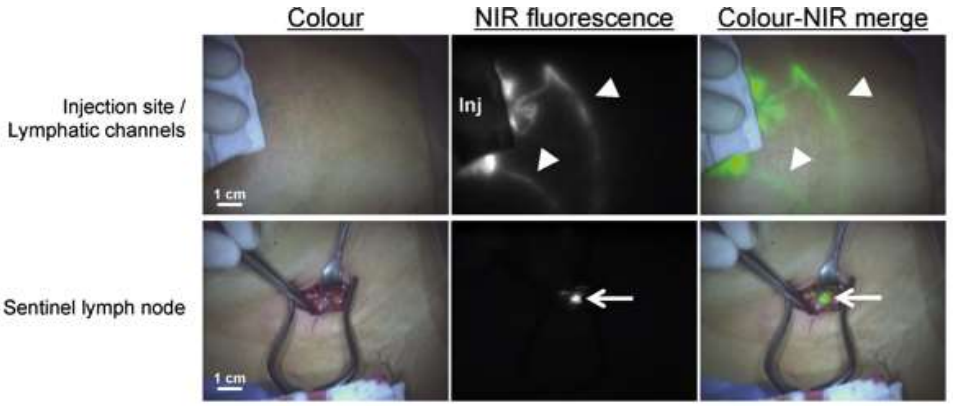

Fig. 2. Near-infrared fluorescence sentinel lymph node mapping: SLN mapping after injection of 600 mM ICG adsorbed to human serum albumin (ICG:HSA) in a breast cancer patient. Images acquired using the Mini-FLARETM imaging system (Frangioni laboratory, Boston, MA). Shown are colour images (left), NIR fluorescence images (middle), and pseudo-coloured (lime green) merge of the two images (right).In the upper panel, the periareolar injection site (Inj) of ICG:HSA is shown. In the NIR fluorescence image, the injection site (partially covered) and lymphatic channels (arrowheads) of the breast running into the axilla are clearly visualised. In the lower panel, identification of the SLN (arrow) with NIR fluorescence imaging is demonstrated. Camera exposure times were 67 msec (top row) and 20 msec (bottom row). Scale bars = 1 cm.

is one to ten minutes.[13,16] The small size of the ICG particle is probably responsible for this relatively high velocity, which has logistical advantages compared to relatively larger gamma ray-emitting radiotracers. Hojo et al.[15] compared ICG to patent blue in 113 patients and showed that ICG had a higher identification rate (100%) than patent blue (93%). Three studies compared the method of SLN detection by ICG fluorescence (71 out of 73 nodes) and the radiotracer (70 out of 73 nodes).[12,15,17] Though both identification rates were similar, as both techniques are used simultaneously in all studies, no comparison can be made whether one is superior to the other.

Several factors influence the success of the SLN procedure using ICG. In the reported clinical trials, various doses of ICG have been used ranging from 0.01 mM to 6.4 mM. Sevick-Muraca et al.16 found that a minimal dose of 0.01 mM ICG is required for successful SLN mapping. Mieog et al.[17] allocated patients in groups of escalating ICG concentrations from 0.05 mM to 1.0 mM diluted in albumin and obtained the highest brightness of the SLN using a concentration between 0.4 mM to 0.8 mM ICG (1.6 ml injection volume). Additionally, because of its relatively small hydrodynamic diameter, ICG is able to pass through the sentinel node to second-tier nodes and eventually spread through the subcutaneous tissue.[18] To surpass this effect, imaging should to be performed shortly after ICG administration. Furthermore, as a result of the limited tissue penetration of the fluorescent signal, visualisation is limited once ICG has reached the axillary fossa, particularly in patients with a high body mass index.[13,19,50]

Skin cancer

ICG has successfully been introduced as an NIR fluorescent lymphatic tracer in the SLN procedure in skin cancer patients.[22-25,93] The NIR fluorescence-guided SLN procedure resulted in identification of at least one SLN in over 97% of the patients (total number of SLNs identified was not reported).[22-25,93] This is concordant with recent trials using conventional techniques, which showed a 93% to 100% identification rate.[51,52] Upon intradermal injection, ICG enables easy visualisation of the subcutaneous lymphatic drainage, which takes approximately 15 minutes after injection to reach the SLN and stays visible for at least three hours.[22] The results of these studies are promising. However, larger trials are needed to assess patient benefit.

Gastro-intestinal cancer

Nodal status is one of the most important prognostic factors in gastric and colorectal cancer. It is hypothesised that the SLN procedure in gastro-intestinal cancer patients can improve nodal staging.[53] Currently, prophylactic lymphadenectomy is considered standard-of-care in these patients. Several studies have assessed the use of the SLN procedure using radiotracers or blue dye, or both.[53-55] However, these studies show varying lymphatic drainage patterns and report high rates of skip metastases, preventing the introduction of the SLN procedure in general clinical practice.

In early gastric cancer, multiple studies reported the use of ICG as NIR fluorescent lymphatic tracer in the SLN procedure.[26-29] ICG was injected during surgery or at one to three days before surgery. After both preoperative subserosal and preoperative submucosal injection of ICG, lymphatic vessels draining the tumour could be visualised.[26-29] The identification rates ranged from 90.9% to 96.4% (aggregate 94.9%) with an average number of SLNs identified of 3.0 to 7.5.[26-29] The false-negative rates reported in these studies ranged from 14.3% to 33.3% in T1 tumours, which increased with tumour stage up to 75% in T3 gastric tumours.[26,28,29] However, the number of patients in these tumour stages with tumour positive lymph nodes is small (range 3-10).

Several factors influence the success of SLN procedure in gastric cancer. Frequent leakage was observed from lacerated lymphatic vessels during the SLN mapping in patients with intraoperative ICG injection.[28] Preoperative endoscopic ICG injection results in a higher number of fluorescent lymph nodes and lower false negative rate compared to intraoperative injection.[28] Due to the longer interval between injection and imaging, it is expected that ICG passes through the SLN to the higher-tier nodes. Additionally, next to fluorescence imaging, absorption by ICG can be used for SLN detection in early gastric cancer by infrared ray electronic endoscopy.[27,55-58] Miyashiro et al.[27] compared both fluorescent imaging and infrared ray imaging in 3 patients after

laparotomy and was able to visualize the individual nodes more clearly by fluorescent imaging.

In colorectal cancer, the use of ICG as an NIR fluorescent lymphatic tracer resulted in an identification rate of 88.5% and 92%, with an average number of identified SLNs of 2.1 and 2.6.[7,26] The false negative rate in these studies was relatively high (44%) in patients with tumour positive lymph nodes.

When the SLN procedure is used for nodal staging to determine prognosis and possible adjuvant therapy, as has been suggested for colorectal cancer, an ex vivo approach can be considered.[59] Using an ex vivo approach, more optimised NIR fluorescent dyes can be used, which are not yet approved for in vivo administration, such as IRDye 800CW (LI-COR, Lincoln, NE, USA). These dyes can be coupled to albumin or nanocolloid to increase lymph node retention.[43,60] This ex vivo strategy was successfully applied in a recent clinical study.[6]

Conclusions sentinel lymph node mapping

NIR fluorescence SLN mapping has shown excellent results to date in breast and skin cancer. Therefore, the use of ICG should be particularly attractive to hospitals unable to work with radioactive isotopes as an adjunct or possible replacement to the use of blue dye alone.[15,17] Though, lack of studies in which a direct comparison between ICG fluorescence and radiotracers is made prohibits drawing conclusions between these two methods. In gastro-intestinal cancer, SLN procedures using ICG obtain high identification rates, although the high false negative rates in the small patient samples require further assessment. Additionally, the feasibility of NIR fluorescence SLN mapping using ICG has also been assessed in single studies in cervical, vulvar, anal, oropharyngeal and non-small cell lung cancer.[4,8-11]

As the available data on ICG fluorescence in the sentinel lymph node procedure is relatively limited, conclusion on direct patient benefit and clinical outcome cannot yet be drawn. Currently, several groups are performing clinical trials using NIR fluorescence imaging and ICG in the SLN procedure in multiple malignancies.

TUMOUR IMAGING

The main goal of cancer surgery is the complete and 'en-bloc' excision of tumours with adequate tumour-free margins while minimising surgical morbidity. Presently, though, intraoperative assessment of tumour margins relies on palpation and visual inspection. NIR fluorescence imaging is a promising technique for intraoperative tumour identification. NIR fluorescent probes that specifically target tumour cells could aid the surgeon in determining resection margins and possibly reduce the risk of locoregional recurrence.[62,63] Although ICG is a non-targeted probe, it can provide

NIR fluorescence tumour localisation in a limited number of hepatobiliary cancer patients[31,64-67], either due to physiological uptake in well-differentiated tumours or rim uptake as a result of leakage and retention in poorly-differentiated tumours and colorectal metastases.[32,68]

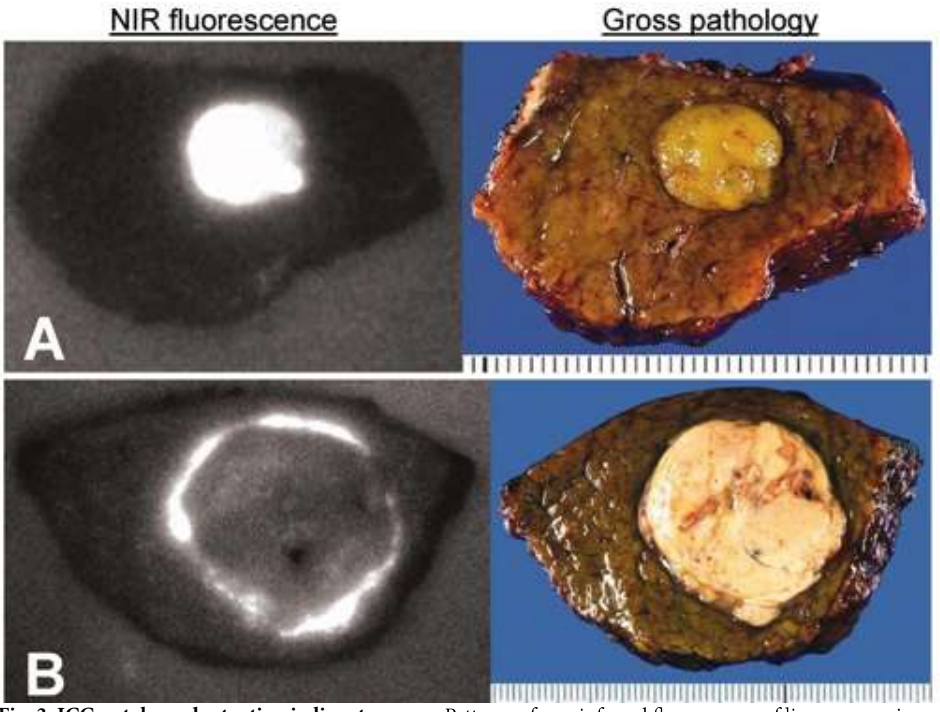

Fig. 3. ICG uptake and retention in liver tumours: Patterns of nearinfrared fluorescence of liver cancers in surgical specimens using the PDE (Hamamatsu Photonics, Hamamatsu, Japan) (left) and their gross appearances (right). A. Well-differentiated hepatocellular carcinoma, 7 mm in diameter, displaying uniform fluorescence. B. Poorly differentiated hepatocellular carcinoma, 30 mm in diameter, displaying rim fluorescence. Adapted from Ishizawa et al. (200915) and reprinted with permission from John Wiley & Sons, Inc.

Imaging of hepatobiliary cancer

Liver resection is the only curative option in the treatment of hepatobiliary cancer. Intrahepatic recurrence rates after resection of colorectal cancer metastases range from 11% to 37.5% and the majority of these recurrences appear within two year after resection.[69-73] A possible explanation for this high intrahepatic recurrence rate is that these hepatic metastases were present at time of resection of the liver metastases but were undetected by preoperative imaging and intraoperative ultrasound. NIR fluorescence detection is a new technique to intraoperatively visualise hepatobiliary cancer.

ICG is excreted exclusively into the bile, which allows real-time NIR fluorescence cholangiography of biliary anatomy during cholecystectomy and other hepatobiliary surgery.[74-76] This technique provides a reliable roadmap of the biliary

tree, which enables the surgeon to avoid injuring the bile duct.[74] In hepatobiliary cancer, it is hypothesised that the NIR fluorescent signal in or around the tumour is caused by passive accumulation due to hampered biliary excretion, which, in the case of a colorectal liver metastasis, results in a fluorescent rim around the tumour (Fig. 3).[31] Several studies have reported the use of ICG in NIR fluorescence imaging of hepatobiliary cancer including colorectal metastasis, hepatocellular carcinoma and cholangiocarcinoma.[31,64-67] To identify liver tumours, the best time window is beyond 24 hours after injection, when most ICG is washed out of the healthy liver parenchyma and is still present in and around the tumour tissue.[31]

In patients with hepatocellular carcinoma or colorectal liver metastases, 98.1% to 100% of the lesions were detected using NIR fluorescence in the resection tissue specimen.[31,65,67] However, due to limited penetration of the NIR fluorescent signal, intraoperative detection of deeper located tumours was not possible (Ishizawa et al.[31] reported a maximal detection depth of 8 mm). Tumours located at the liver surface provide a bright fluorescent signal and are easily detected, which is especially useful for colorectal liver metastases as these are mostly located on the surface of the liver parenchyma. In these studies this resulted in detection of new small superficial lesions by NIR fluorescence imaging that could not be detected by intraoperative ultrasonography or by visual inspection.[31,67]

In the case of cholangiocarcinoma, Harada et al.[66] showed ICG fluorescence on the liver surface in the regions of liver with cholestasis caused by bile duct tumour invasion or thrombi. Although the tumour itself was not fluorescent, the information provided by NIR fluorescence imaging can help to estimate the extent of the bile duct tumour infiltration.

In conclusion, ICG fluorescence might be of value during hepatobiliary surgery when used as an adjunct to intraoperative ultrasound, and could be particularly useful in the intraoperative identification of small superficially located liver tumours. However, to identify deeper tumours, intraoperative ultrasound imaging is still required. Additionally, ICG fluorescence can aid in the identification of tumour lesions during pathological examination.

Marking tumours

Endoscopic marking of intestinal lesions is essential in laparoscopic surgery or when difficulty in locating the lesion during resection is anticipated.[30,68] India ink is a frequently used dye, but is associated with complications and side effects and alters the surgical field.[68] ICG could be a more suitable dye for tattooing, because of fewer side effects, relatively long absorption time (up to 14 days), and potential increased detection using NIR fluorescence compared to macroscopic colour perception.[30,77,78]

Watanabe et al.[30] and Handgraaf et al.[92] showed accurate and clear NIR fluorescence tumour localization after preoperative and intraoperative peritumoural injection of ICG. In all patients, the NIR fluorescence signal was detected in the colon or rectal tumour and could be visualised clearly. In the preoperative injected patients the signal was visible for at least 72 to 120 hours, whereas the marked location detection based on the intrinsic green colour of ICG was possible in only two patients. Moreover, this technique allows for simultaneous SLN mapping.[33,92]

Other solid tumours

It has been proposed that ICG can be used in intraoperative imaging of solid tumours other than hepatobiliary cancer. The "enhanced permeability and retention" (EPR) effect can potentially be used for tumour imaging. Due to newly formed, more porous blood vessels, molecules can passively accumulate in tumour tissue. Furthermore, a poorly developed tumoural lymphatic system results in increased retention.[79-82]

Exploiting the EPR effect, multiple clinical studies with breast tumours used ICG for tumour identification in an outpatient, mammography-like setting.[32,82-86] These studies used optical tomography, which has higher depth penetration and potentially higher specificity, albeit with much lower resolution. During the first 10 minutes, ICG was retained in the breast tumour tissue and provided contrast to the surrounding healthy tissue.[82,83] Hagen et al.[32] and Poellinger et al.[86] used a prototype fluorescence mammographic imaging system and showed the ability to discriminate between malignant and benign lesions after intravenous administration of ICG. However, in an intraoperative setting a higher tumour-to-background ratio would be needed to provide sufficient tumour demarcation.

Additionally, ICG can be useful as a diagnostic tool to estimate the invasiveness of early gastric cancer during endoscopy. Multiple Japanese studies used NIR fluorescence endoscopy using ICG as contrast agent to differentiate between mucosal and submucosal or more invasive tumours, and obtained a diagnostic accuracy of 85 % up to 93 %.[87-90] The NIR fluorescence signal was visible up to 3 minutes in tumour tissue compared to several seconds in healthy tissue.[87] Therefore, ICG might be useful to distinguish mucosal cancer from submucosal and deeper cancers, which is a risk factor for lymph node metastasis. However, the short duration of the signal limits its use in a surgical setting. Therefore, the lack of direct tumour targeting properties prevents the introduction of ICG as NIR fluorescent probe in most tumour types in an intraoperative setting.

CONCLUSION AND OUTLINE OF THE THESIS

The recent introduction of NIR fluorescence image guidance provides new opportunities for cancer surgery. Currently, ICG and methylene blue are the only clinically available NIR fluorescent probes. Clinical experience with ICG for intraoperative NIR fluorescence imaging is rather extensive and shows a favourable safety profile. Though, conclusions on direct patient benefit and clinical outcome cannot yet be drawn. This thesis further explores the possibilities of NIR imaging for SLN mapping and tumour and is divided in two parts: Part I focuses on the optimization and exploration of added value of NIR fluorescence imaging for SLN mapping, Part II describes the use of NIR imaging for tumour identification.

Part I, chapter 2 and 3 explore whether premixing with human serum albumin is indeed beneficial for SLN mapping in a randomized controlled trial in patients with vulvar and cervical cancer. In chapter 4 the added value of NIR fluorescence SLN mapping in breast cancer is explored using a novel hybrid radioactive and fluorescent ICG-based SLN tracer. In chapter 5 the additional value of human serum albumin linked to fluorescent tracer 800CW is evaluated for SLN mapping in patients with colon cancer and compared to results obtained with blue dye.

In Part II, chapter 6 the use of NIR light without NIR contrast agents is explored for tumour detection and neoadjuvant treatment monitoring by diffuse optical spectroscopy in patients with locally advanced breast cancer. In chapter 7, the optimal dose and timing of administration of ICG is assessed for the NIR fluorescence detection of liver metastases from colorectal cancer and the added value of ICG is evaluated.

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