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Near-infrared fluorescence imaging in colorectal cancer and its metastases

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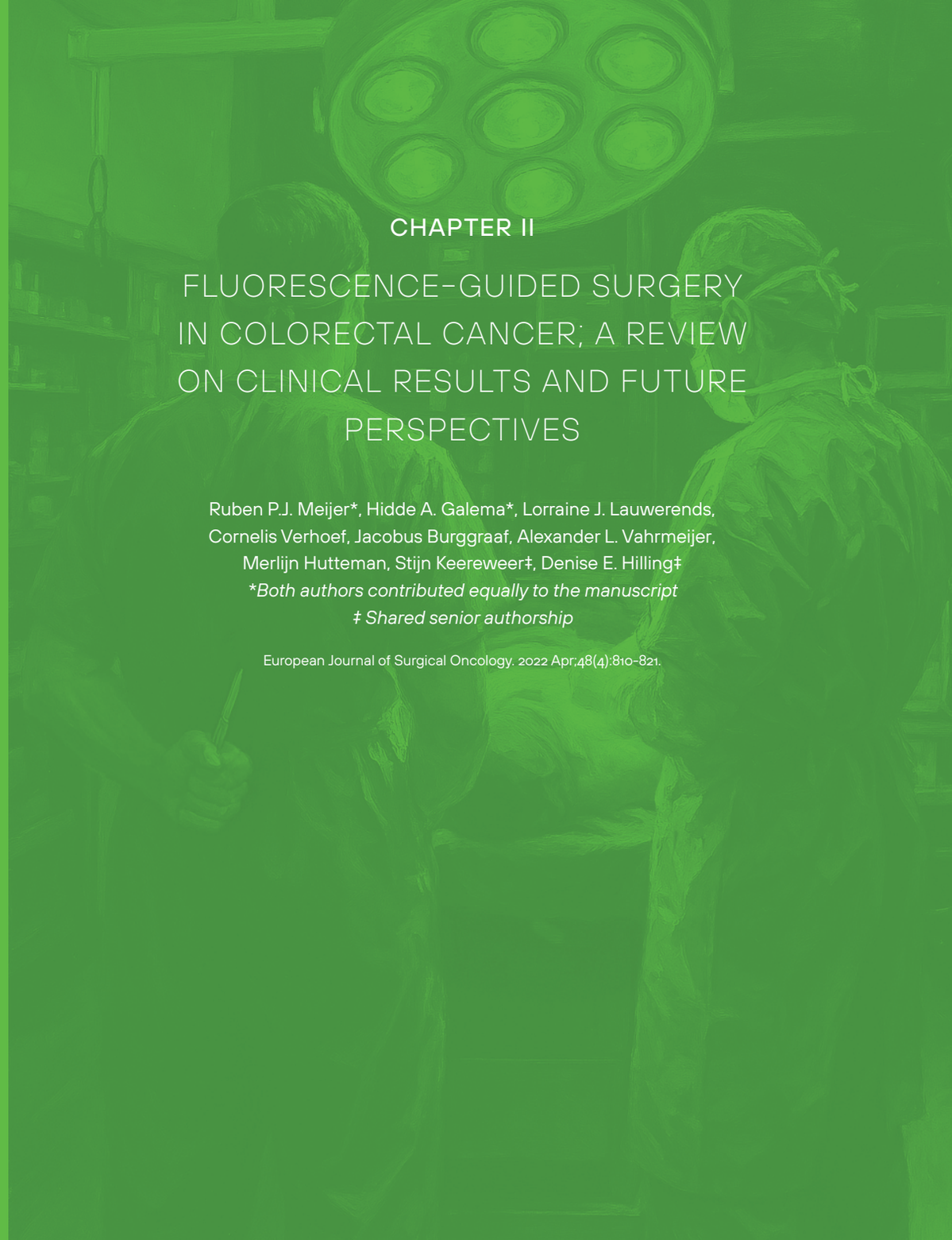
PART 1

NIR FLUORESCENCE IMAGING IN COLORECTAL CANCER SURGERY



CHAPTER II

FLUORESCENCE-GUIDED SURGERY IN COLORECTAL CANCER; A REVIEW ON CLINICAL RESULTS AND FUTURE PERSPECTIVES

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Abstract

BACKGROUND Colorectal cancer is the fourth most diagnosed malignancy worldwide and surgery is one of the cornerstones of the treatment strategy. Near-infrared (NIR) fluorescence imaging is a new and upcoming technique, which uses an NIR fluorescent agent combined with a specialised camera that can detect light in the NIR range. It aims for more precise surgery with improved oncological outcomes and a reduction in complications by improving discrimination between different structures.

METHODS A systematic search was conducted in the Embase, Medline and Cochrane databases with search terms corresponding to 'fluorescence-guided surgery', 'colorectal surgery', and 'colorectal cancer' to identify all relevant trials.

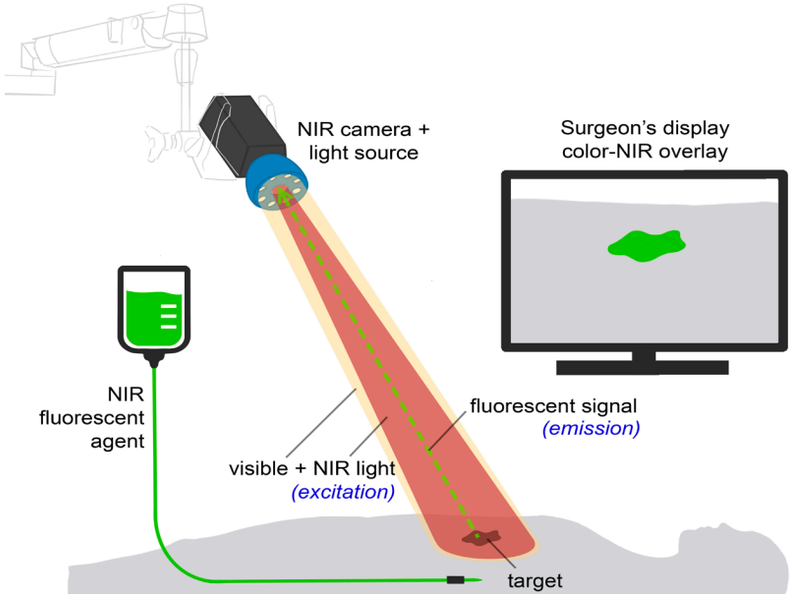
RESULTS The following clinical applications of fluorescence guided surgery for colorectal cancer were identified and discussed: (1) tumour imaging, (2) sentinel lymph node imaging, (3) imaging of distant metastases, (4) imaging of vital structures, (5) imaging of perfusion. Both experimental and FDA/EMA approved fluorescent agents are debated. Furthermore, promising future modalities are discussed.

CONCLUSION Fluorescence-guided surgery for colorectal cancer is a rapidly evolving field. The first studies show additional value of this technique regarding change in surgical management. Future trials should focus on patient related outcomes such as complication rates, disease free survival, and overall survival.

Introduction

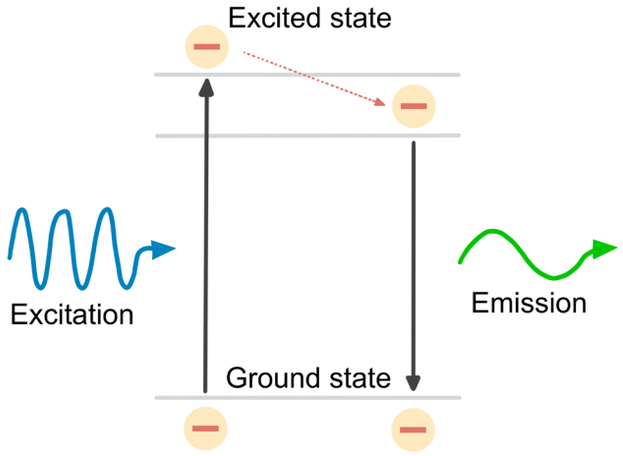
Colorectal cancer (CRC) is globally the fourth most common malignancy and the second cause of cancer related mortality with over 550 000 deaths annually.[1] In most CRC patients, surgery remains the cornerstone of treatment. Complete surgical resection of the tumour is associated with better overall survival and lower recurrence rates.[2,3] Minimal invasive surgery, laparoscopic or robot-assisted, is increasingly used in the last two decades. Despite it its advantages, this application also brought new technical challenges as it lacks tactile feedback for tumour identification and identification of vital structures. These challenges sparked the interest in novel intraoperative visualisation techniques, such as near-infrared (NIR) fluorescence imaging.

FIGURE 1 The basic principles of fluorescence-guided surgery.
NIR fluorescent agents are administered intravenously or locally. Imaging of the agent is performed using a fluorescence imaging system. Besides a white light source and camera, this system includes a dedicated NIR excitation light, collection optics and filtration, and a camera dedicated to NIR fluorescence emission light. NIR fluorescence output is displayed on a screen in the operating theatre. A simultaneous visible light image, which can be merged with the NIR fluorescence image, is desirable.



fluorescent agent with a specialised imaging system (figure 1). These systems can capture light emitted by a fluorescent agent after excitation with an appropriate light source (figure 2). NIR light (650-900 nm) is favourable for intraoperative imaging compared to visible light because of its better depth penetration in tissue (up to 10 mm). Moreover, the fluorescent agents will not interfere with the standard surgical field, as the human eye is unable to detect light within these NIR wavelengths.

FIGURE 2 The basic principles of fluorescence.
NIR fluorescent agents are administered intravenously or locally. Imaging of the agent is performed using a fluorescence imaging system. Besides a white light source and camera, this system includes a dedicated NIR excitation light, collection optics and filtration, and a camera dedicated to NIR fluorescence emission light. NIR fluorescence output is displayed on a screen in the operating theatre. A simultaneous visible light image, which can be merged with the NIR fluorescence image, is desirable.



NIR fluorescent agents are predominantly injected intravenously and can be divided into two groups: targeted (binding to a specific ligand or activated by the tumour-specific environment) and non-targeted. Currently, various targeted fluorescent agents are tested in phase I-III clinical trials.[4] In the group of non-targeted agents, indocyanine green (ICG) and methylene blue (MB) are approved by the United States Food and Drug Administration (FDA) and the European Medicines Agent (EMA), for other purposes. ICG was first used in 1957 to determine hepatic function, but its fluorescent properties (excitation peak around 800 nm), and hence other applications, became known decades later.[5] MB on the other hand, is predominantly cleared renally and has its excitation peak around 700 nm.[6] Both agents have been proven to be safe for fluorescence utilisation.

There are many applications for NIR fluorescence imaging during colorectal surgery. This review provides an overview of the currently available clinical applications and promising future modalities of fluorescence-guided surgery in the treatment of CRC patients.

Methods

Due to heterogeneity in available literature and study phases between the several subjects, this study was not fully conducted according to the PRISMA guidelines.

LITERATURE SEARCH AND SELECTION CRITERIA

A systematic search was conducted in the Embase, Medline and Cochrane databases with search terms corresponding to 'fluorescence-guided surgery', 'colorectal surgery', and 'colorectal cancer'. The search strategy was expanded with terms to identify articles reporting on vital structure imaging and colorectal metastases. Supplement 1 shows the search strategies per database and its corresponding hits. The last search was conducted on December 21st, 2020. All articles were independently screened based on title and abstract by two authors (HG and RM). Next, full article screening and reference screening was performed. Inconsistencies were discussed with an additional author (DH). Regarding experimental fluorescent agents, all clinical studies were included in the final reference list. Regarding ICG and MB, the final reference list was generated based on the quality of the article and the amount of scientific evidence available per subject. Articles on the following subjects were included: fluorescence-guided surgery for CRC for the imaging of the primary tumour, lymph nodes, metastases (peritoneal, liver, extra-abdominal), vital structures (nerve, ureter, urethra), and perfusion (anastomosis, omentoplasty). Only articles in English and published after the year 2000 were considered.

DATA EXTRACTION

The following data was extracted: tumour type, fluorescent agent, fluorescence imaging application, (optimal) dose, (optimal) dosing interval, optimal tumour-to-background (TBR), sensitivity, specificity, change in surgical management, and other outcomes.

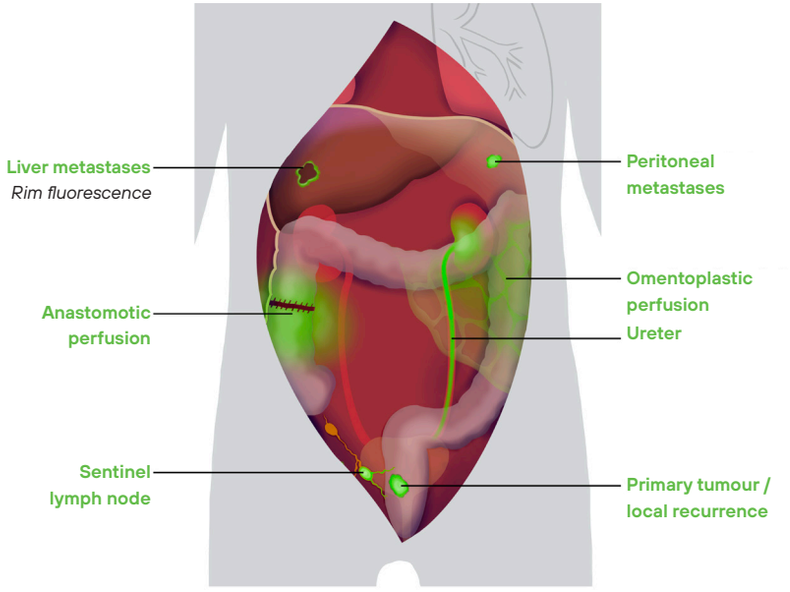
QUALITY ASSESSMENT

Quality assessment was performed for all studies assessing experimental fluorescent agents. The Methodological index for non-randomized studies (MINORS score) was used for quality assessment. A total score of 16 (for non-comparative studies) or 24 (for comparative studies) could be obtained.[7]

Results

A total of 14 completed clinical studies (supplement 2) and 8 ongoing trials (supplement 3) assessing experimental fluorescent agents for CRC surgery were identified. Figure 3 gives an overview of all clinical applications of fluorescence-guided surgery (FGS) for CRC, with the assessed fluorescent agents (table 1). All 14 clinical studies regarding experimental fluorescence agents had a MINORS score of 11 or higher (supplement 4).

FIGURE 3 A schematic overview of all clinical applications of fluorescence guided surgery for colorectal cancer.



Abbreviations: ICG Indocyanine Green | MB: Methylene blue

IMAGING OF THE PRIMARY TUMOUR AND LOCAL RECURRENCE

Achieving tumour-negative resection margins is of utmost importance in the surgical treatment of CRC patients, as tumour-positive resection margins are associated with a significant decrease in overall survival.[2,8] Tumour-positive resection margins are reported in 5% of all colon cancer cases, but occur more frequently with increasing tumour stage, with an occurrence of up to 14% in T4 colon cancer.[2] Moreover, in primary locally advanced rectal cancer the proportion of tumour-positive resection margins is reported up to 28%.[8] This rate is even higher in patients with recurrent rectal cancer, where up to 50% tumour-positive resection margins are reported.[9] These high rates in recurrent rectal cancer are likely a consequence of the distorted anatomy and treatment related fibrosis after previous resection and (re-)neoadjuvant treatment. Tumour identification is challenging in these cases due to the difficult distinction (both visual and tactile) between fibrosis and residual tumour tissue.

Preoperative endoscopic tattooing with India ink, a permanent marker injected distal to the tumour, is the current standard of care for intraoperative tumour identification in CRC, with an accuracy rate of 70-88%.[10,11] However, India ink can leak into the abdominal cavity and thereby interfere with the surgical procedure.

TABLE 1 Overview of all fluorescent agents used for colorectal cancer surgery and their optical properties.

Fluorescent Agent	Molecular target	Fluorophore	~Peak absorbance wavelength	~Peak emission wavelength	Reference
Bevacizumab-800CW	VEGF-A	IRDye-800CW	778 NM	794 NM	21
cRGD-ZW800-1	Integrins (αvβ6, αvβ3, αvβ5)	ZW800-1	785 NM	805-850 NM	69
HSA800	NA	IRDye-800CW	778 NM	795 NM	21
ICG	NA	ICG	780 NM	830 NM	5
IRDye-800BK	NA	IRDye-800BK	774 NM	790 NM	72
IS-001	NA	IS-001	780 NM	815 NM	70
LUM015	Cathepsins (K,L,S,B)	Cy5	650 NM	675 NM	22
MB	NA	MB	667 NM	685 NM	6
ONM-100	Metabolic acidosis*	ICG	780 NM	830 NM	5
SGM-101	CEA	BM-104	685 NM	705 NM	19
ZW800-1	NA	ZW800-1	785 NM	805-850 NM	69

*Activated in a tumour-specific pH-environment
Abbreviations: VEGF-A vascular endothelial growth factor alpha | NM nanometre | ICG indocyanine green | MB methylene blue | CEA carcinoembryonic antigen | NA not applicable

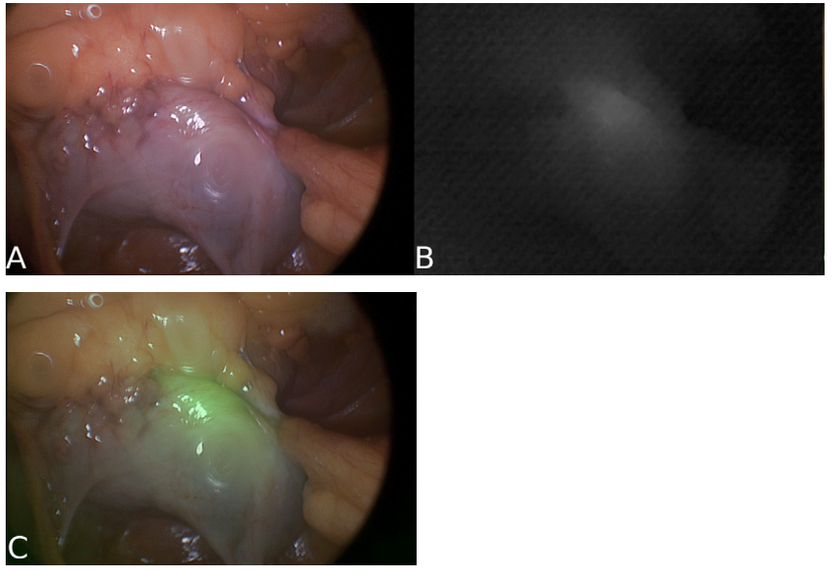
In 2009, the first NIR fluorescence imaging technique to identify the primary tumour in CRC was introduced by injecting ICG peritumoural via endoscopy. It has a high tumour identification rate (100%) and minimal adverse events.[12,13] However, a major drawback is the relatively rapid clearance of ICG, as detection rates tend to decrease two to seven days after injection.[12] Therefore, patients must undergo an additional endoscopy in the week before surgery, in contrast to the conventional injection of India ink that can be administered at the initial, diagnostic colonoscopy. Because of these drawbacks, peritumoural ICG injection has not been widely implemented for tumour identification. Moreover, this technique will not improve the tumour-negative resection margin rates because it does not differentiate between tumour tissue and benign surrounding tissue, nor does it enable the detection of additional lesions. A potential solution is the use of fluorescent agents that specifically bind to tumour cells.

The number of tumour-targeted fluorescent agents has substantially increased in the past two decades. The use of these agents is aimed to achieve complete tumour resection. This should lead to a decrease in the number of tumour-positive resection margins, detection of additional lesions and avoid unnecessary removal of benign tissue. To quantify fluorescence intensity, most studies use the signal-to-background ratio (SBR) or TBR. This is a ratio of the mean fluorescence intensity of the tumour and the surrounding tissue (background). A TBR of at least 1.5 and preferably 2.0 is deemed sufficient for tumour identification. Currently, four tumour-targeted fluorescent agents have been tested in early phase clinical studies for CRC and have shown promising results: SGM-101, cRGD-ZW800-1, bevacizumab-800CW, and ONM-100.[14–18]

SGM-101 consists of a monoclonal antibody targeting the carcinoembryonic antigen (CEA) bound to the fluorophore BM-104.[19] A phase II study of 37 patients showed an intraoperative TBR of 1.9. Importantly, based on fluorescence assessment, the surgical plan was changed in 9 (24%) patients. In 7 patients, fluorescence led to resection of malignant lesions that were not identified with white light only. In two patients, clinically suspected but non-fluorescent tissue was proven to be benign, which resulted in a less extensive resection (18). These promising results have led to the initiation of 2 phase III trials using SGM-101 (NCT03659448, NCT04642924).

cRGD-ZW800-1 is a cyclic pentapeptide (cRGD) conjugated to the 800 nm zwitterionic NIR fluorophore ZW800-1. It targets various integrins that have been shown to be overexpressed on colorectal tumour cells. In the first-in-human study, 12 colon cancer patients were included. Intraoperative fluorescence imaging of the primary tumour was feasible in both open and minimal invasive surgeries (figure 4). The highest mean TBR of 1.6 was found in the highest dosing of 0.05 mg/kg.[15]

FIGURE 4 Fluorescence imaging results of primary colon cancer.
Intraoperative fluorescence imaging result of an adenocarcinoma of the ascending colon (TBR 1.6) using cRGD-ZW800-1. Imaging was performed using the Quest Spectrum laparoscopic imaging system. It shows an image in white light (A), near-infrared (B) and merge of A and B (C).



Bevacizumab-800CW consists of a monoclonal antibody targeting vascular endothelial growth factor A (VEGF-A), bound to IRDye800CW.[20,21] Bevacizumab-800CW has been studied in eight rectal cancer patients.[16] During back table fluorescence assessment of the resection margins on the surgical specimen, a tumour-positive margin was correctly identified in one out of two patients. In the six patients with a tumour-negative resection margin, one (17%) showed a false-positive signal.

ONM-100 is a pH-activatable fluorescent agent that exploits the metabolic microenvironment of solid tumours.[17] It does not bind to specific tumour receptors but is activated in the acidic tumour environment. It is a conjugation of a pH-sensitive nanoparticle to ICG, which becomes fluorescent in environments with a pH below 6.9. Thirty patients were studied with this agent, of which three

underwent surgery for CRC. All three CRC patients showed a sharply demarcated fluorescent signal during back table imaging. Currently, a phase II study using ONM-100 is ongoing in which patients undergoing surgery for CRC are also included (NCT03735680).

A phase I/II trial will be conducted using LUM015, a novel PEGylated protease-activated fluorescent imaging agent targeting cathepsins, which play a crucial role in mammalian cell turnover.[22] The first results of this study, which focuses on intraoperative imaging of CRC, are expected in 2021 (NCT02584244).

Altogether, these early phase clinical trials have shown that tumour-targeted fluorescence imaging is a feasible addition to CRC surgery. The fluorescent agents detected most of the known tumours and SGM-101 even detected additional lesions, which were not detected in white light. To assess the impact on patient related outcomes, future studies should focus on clinical endpoints like tumour-negative resection margin rate and change in surgical management.

IMAGING OF THE SENTINEL LYMPH NODE

Adequate lymph node staging in CRC patients is crucial; it is an important prognostic feature and determines the need for (neo)adjuvant chemotherapy and/or radiotherapy. The sentinel lymph node (SLN) may be crucial in nodal staging, as it is defined as the first lymph node draining the tumour and is believed to be the first place for lymphogenic metastases. Moreover, one in three patients with stage I and II colon cancer, who are staged as lymph node-negative, still develop distant metastases.[23] This might be a consequence of understaging by histopathology, due to lymph nodes with occult malignant cells and micrometastases. Currently, a single paraffin embedded slide per lymph node is reviewed during routine histopathological analysis, increasing the chance of missing tumour cells away from the slide's cutting edge. More extensive histopathological analysis of all resected lymph nodes would improve nodal staging, but this process is time-consuming and expensive.[24]Extensive analysis of only the SLN is feasible, and thus unfolds a niche for SLN mapping in CRC. Moreover, tumour-negative SLNs create an opportunity for endoscopic or local resection of early stage tumours.[25]

A reason for the absence of SLN mapping in the routine treatment of CRC patients might be a consequence of so-called skip metastases that are reported in up to 22% the patients.[26] In these cases, malignant cells are absent in the SLN, but present in other regional lymph nodes. Moreover, the use of blue dye for SLN mapping in CRC appears limited due to its minimal depth penetration in the mesocolic and mesorectal fat.[27] Therefore, the interest in fluorescent dyes, especially the peritumoural injection of ICG, has increased. These fluorescent dyes have already shown to be of additional value for the identification of complete lymph drainage patterns, including aberrant flow.[28,29] Nevertheless, the identification of only the SLN would be a valuable addition.

Various techniques have been used in studies assessing fluorescence-guided SLN mapping in CRC. Agent administration and SLN mapping can be performed before or during the procedure (*in vivo*) or after resection (*ex vivo*). Although *ex vivo* imaging might be easier to adapt in the current surgical or pathological workflow, it has drawbacks. Most importantly, *ex vivo* injection of an agent and identification of the SLN lacks the possibility of finding SLNs in patients with aberrant lymph node drainage patterns.[30] Another technical consideration is the site of injection. For *in vivo* SLN mapping, submucosal injection is done endoscopically, prior to surgery. Alternatively, subserosal injection can be performed during surgery, which in laparoscopic surgery demands transcutaneous needle placement. Submucosal injection is preferred over subserosal injection because of better accuracy of injection near the tumour and easier endoscopic needle positioning.[31]

ICG is the only fluorescent dye that has been reported for *in vivo* SLN mapping in CRC with cohorts up to 48 patients and success rates of SLN detection ranging from 65.5 to 100%.[31–35] The accuracy of this technique seems to diminish with increasing tumour stage.[34,36] Which is most likely a result of the distorted drainage patterns caused by transmural growth of advanced tumours.

Ex vivo SLN mapping facilitates the use of experimental agents, like HSA800 (IRDye 800CW conjugated to human serum albumin). HSA800 has shown a potential advantage over ICG, due to its bigger hydrodynamic diameter that results in better retention in the SLN.[37] HSA800 has demonstrated successful identification of the SLN in 95-100% of 96 patients.[38–40]

Despite high fluorescence-guided SLN identification rates, the SLN itself was associated with a relatively low negative predictive value (74-100%) in general, mainly as a result of high false-negative rates (when the SLN did not contain tumour tissue, but other regional lymph nodes did).[31–35,38,39] This can be a result of the occurrence of skip metastases.[26]

Correct staging is essential for treatment planning in CRC patients and may be improved with SLN mapping. A patient is upstaged when no tumour deposits were seen during conventional histopathology of all lymph nodes, but the SLN showed malignant cells at advanced histopathological analysis using serial sectioning and immunohistochemistry. SLN mapping with ICG and subsequent advanced histopathology resulted in upstaging in 6-23% of the patients.[31–33]

Although plausible, it is yet unknown whether upstaged patients with micrometastatic lymph nodes will benefit from subsequent neoadjuvant treatment.

Overall, it can be concluded that fluorescence-guided identification of the SLN is feasible and potentially of additional clinical value. However, a wide variety of techniques for fluorescence-guided SLN identification are currently used. It is recommended to first determine the optimal agent, injection technique and patient population. The high false-negative rate (tumour-negative SLN with tumour-positive regional nodes) remains a major drawback for SLN mapping in CRC in general. Nevertheless, its value in terms of upstaging and the consequence of adjuvant treatment seems enough reason to further explore this field.

IMAGING OF DISTANT METASTASES

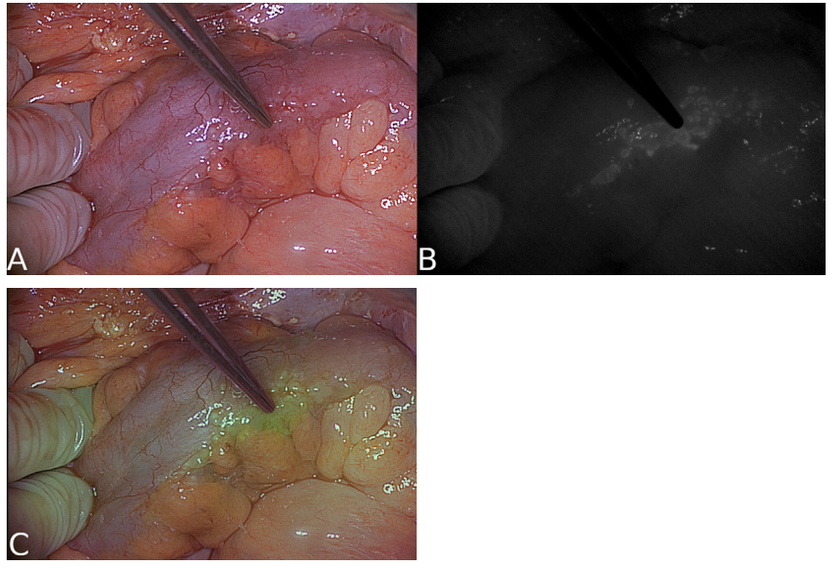
PERITONEAL METASTASES

Approximately 10% of all CRC patients develop peritoneal metastases during the course of the disease.[41] In the past, this diagnosis was considered non-curable with a median overall survival of approximately 12 months.[42] These survival rates have improved with the introduction of cytoreductive surgery followed by intraoperative hyperthermic intraperitoneal chemotherapy (HIPEC).[3,43] Studies have shown that in particular complete cytoreduction plays a major role as it prolongs the long-term survival of patients with peritoneal metastases.[3] However, identification of small peritoneal lesions can be challenging, especially after previous abdominal surgery or neoadjuvant therapy with subsequent fibrosis. An accurate peritoneal cancer index (PCI) is essential as this score plays a crucial role in the decision to perform a HIPEC procedure or not.[44] Fluorescence imaging can potentially lead to more complete cytoreductive surgery by more accurately identifying peritoneal lesions.

Intravenous injection of ICG and subsequent fluorescence imaging of peritoneal metastases is primarily based on the enhanced permeability and retention (EPR) effect. The EPR effect is dependent on the porous nature of tumour vasculature and the extended circulation of the fluorescent agent, leading to accumulation in the tumour.[45] ICG is administered at the start of the surgical procedure and has shown good intraoperative imaging of peritoneal metastases, which has led to a modification of the surgical plan in 4 out of 14 patients (29%) solely based on the fluorescence assessment.[46] Nevertheless, the authors reported limited ability to assess fluorescence in areas with high physiological ICG accumulation such as the liver, as well as a sensitivity of 0% in patients with mucinous tumours (46). Moreover, neoadjuvant treatment resulted in a higher false-negative rate (53.8% vs. 42.9%) and lower sensitivity (65.0% vs 76.3%) compared to patients who did not receive neoadjuvant treatment.[47]

To date, two tumour-targeted agents have been used for *in vivo* detection of peritoneal metastases in CRC: bevacizumab-800CW and SGM-101. Bevacizumab-800CW was the first tumour-targeted fluorescent agent that was reported to yield promising results, identifying additional peritoneal metastases in two out of seven (29%) patients.[48] Similar results were achieved in a study with SGM-101, where fluorescence imaging led to a change in PCI in 5 out of 12 (42%) patients (figure 5). Four patients had a higher PCI and one patient a lower PCI, all confirmed by histopathology.[49] It is noteworthy that both studies reported a high false-positive rate (38% and 47%, respectively). This could be a result of non-specific localisation of the fluorescent agent or autofluorescence of collagen-rich structures and calcifications.[50]

FIGURE 5 Fluorescence imaging result of colorectal peritoneal metastases.
Intraoperative fluorescence imaging result of peritoneal metastases of a mucinous carcinoma with signet ring cell differentiation (TBR 1.8) using SGM-101. Imaging was performed using the Quest Spectrum open imaging system. It shows an image in white light (A), near-infrared (B) and merge of A and B (C).



One clinical trial is currently ongoing using LUM015, including patients with peritoneal metastases of gastrointestinal cancer, ovarian cancer, and mesothelioma (NCT03834272). The aforementioned phase III study with SGM-101 will also include patients with peritoneal metastases (NCT03659448).

The feasibility of fluorescence-guided detection of peritoneal metastases has been demonstrated, allowing for detection of peritoneal deposits and potentially also of occult lesions. This is especially valuable knowing that treatment success is primarily determined by complete cytoreduction.

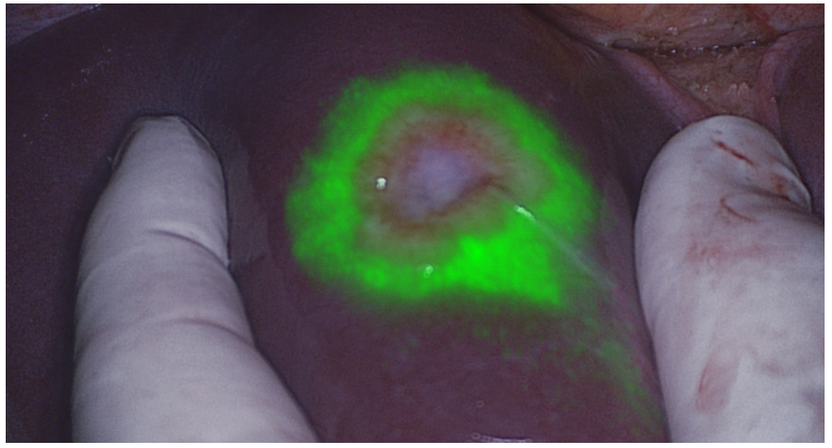
LIVER METASTASES

Over the course of the disease, 20-30% of CRC patients develop liver metastases (CRLM).[51] Complete resection of these metastases is an important treatment option, with positive resection margins being associated with a two- to three-fold decrease in 5-year survival compared to negative margins. However, positive resection margins occur in approximately 13% of patients.[52] In the past, the hepatic surface was palpated for superficial lesions during surgery. With minimal invasive surgery this has become challenging. Nowadays, preoperative magnetic resonance imaging (MRI), computed tomography (CT) and intraoperative ultrasound (IOUS) are the most frequently used imaging modalities for the identification of CRLM.[53] Fluorescence imaging offers surgeons another tool for detecting CRLM. It is suitable for detecting small superficial metastases (up to eight mm deep), but also for resection margin assessment. Intravenous ICG doses between 10-50 mg are described with injection windows of 1-14 days prior to surgery.[54] After intravenous injection, ICG is exclusively cleared by the liver. Immature hepatocytes, located at the transition zone between healthy and malignant liver cells, are unable to excrete ICG into bile due to down-regulation of anion transporters, resulting in an accumulation of ICG. This causes CRLM to show a rim of fluorescence (figure 6).[55]

Various studies have reported on fluorescence imaging with ICG for the detection of occult CRLM, but none were randomized.[55-57] In one systematic review, six out of nine studies reported a sensitivity exceeding 94%.[54] Furthermore, when fluorescence imaging was added to conventional imaging, extra metastases were found and resected in 20 out of 148 patients (13.5%). Tumour-targeted fluorescence identification of CRLM was previously reported in one study using SGM-101. SGM-101 provided visualisation of all 12 malignant lesions in eight patients with a mean *in vivo* TBR of 1.7.[58]

It is widely debated if additional resection of small superficial CRLM improves overall survival. One study has retrospectively assessed (disease-free) survival of 86 patients after ICG-guided resection of CRLM.[59] Significantly more additional lesions were found when fluorescence-guided resection with ICG was added compared to standard care (25% vs. 13%, p=0.04). However, this was not associated with a significant decrease in local recurrence-free survival (HR: 0.74; 95% CI: 0.42-1.28), and overall survival (HR: 0.94; 95% CI: 0.50-1.76).

FIGURE 6 Fluorescence imaging result of a liver metastasis.
A colorectal liver metastasis showing clear 'rim fluorescence' after intravenous injection of 10 mg indocyanine green 24 hours prior to surgery. Imaging was performed using the Quest Spectrum open imaging system.



assessment and aiding in tumour delineation for CRLM resection. Two small case series with a total of 52 patients achieved 100% tumour-negative resection margins using ICG to determine the precise tumour border.[56,57] Recently, a systematic workflow was proposed to detect or prevent tumour positive margins in CRLM surgery.[60] In a selected group of eight patients with initial tumour positive resection margins, the surgeons were able to correctly identify seven out of eight positive margins by using the proposed surgical workflow. The currently ongoing prospective MIMIC-trial will assess whether this surgical workflow can lead to a decrease of tumour-positive resection margins in 186 patients with CRLM (Netherlands trial register: NL7674).

Fluorescence imaging with ICG has been demonstrated to improve intraoperative detection of CRLM. It can also be used for margin assessment and aiding in tumour delineation for CRLM resection. Future trials must confirm this potential and demonstrate whether this technique will improve patient survival.

EXTRA-ABDOMINAL METASTASES

A feasibility study with SGM-101 to identify colorectal lung metastases is currently recruiting (NCT04737213). A similar study with SGM-101 to identify colorectal brain metastases will be conducted soon (NCT04755920).

IMAGING OF VITAL STRUCTURES

URETERS

Iatrogenic ureteral injury is a severe complication in abdominal surgery and has an incidence of up to 5.7% in colorectal surgery. Surgeries on the distal colon and rectum bear the highest risk for ureteral injury.[61] Depending on the time of diagnosis, location, and extent of the injury, treatment ranges from minimal invasive transurethral procedures to complex surgical reconstruction. Consequences of (undiagnosed) iatrogenic ureteral injury include kidney failure, sepsis, ureteral stenosis, urinoma, and fistulas.[62] The ureter is usually identified through visual inspection and palpation, which can be difficult due to its retroperitoneal location. The introduction of minimal invasive surgery in the last decades has further increased this challenge.[61] Intraoperative fluorescence imaging can guide the surgeon in identification of the ureter, which could result in less ureteral injury. ICG and MB have both been studied for ureteral identification. Due to the hepatic clearance of ICG, retrograde intra-ureteral injection is needed, which makes ureteral identification with ICG a complex procedure.[63] Successful ureteral identification using ICG is reported in 94-100% of procedures.[63-65] As MB is cleared renally, intravenous injection is possible for intraoperative identification of ureters. Outcomes of intravenous MB administration for ureteral identification show variable results.[66-68] Fluorescence of the ureters is reported in 50-100% of cases and usually between 10-90 minutes after injection of MB. Optimal visualisation is achieved with doses between 0.5 mg/kg and 1.0 mg/kg. Most important, in most cases, the ureter could only be identified with fluorescence after it was already adequately identified in white light, thus the clinical benefit was minimal. Overall, MB appears to be suboptimal for ureteral identification.

To date, three experimental fluorophores have been used in clinical studies to image the ureter: ZW800-1, IS-001, and IRDye800BK.[69-71] These experimental fluorophores are all fluorescent dyes with peak emission around 800 nm. ZW800-1 is a zwitterionic molecule that shows low non-specific binding and is exclusively renally cleared. ZW800-1 was intravenously administered during abdominopelvic surgery in 12 patients. Using ZW800-1, all ureters became fluorescent within 10 minutes, without dissecting the peritoneum.[69] The SBR was 2.7 in the group with 2.5 mg throughout the first hour. The ureters remained visible with NIR during the whole procedure, with the longest procedure being over 3.5 hours. The first clinical study assessing the safety and efficacy of IS-001 included 24 patients who underwent laparoscopic gynaecological surgery[70]. The ureters could be identified in all patients, the highest SBR (3.6) was observed with a dose of 20 mg. Signal intensity decreased rapidly over time, with the peak SBR occurring 30 minutes after injection. The third experimental fluorescent dye that was studied for ureter identification is IRDye800BK, a hydrophilic dye.[72] In this trial, the optimal dose of 0.06 mg/kg was administered in 25 patients.[71] In all patients, the ureter was visualised within ten minutes. After 90 minutes the ureter was still visible in 89% of the patients. Currently, another clinical trial is ongoing using IRDye800BK, including 40 patients undergoing laparoscopic surgery (NCT03387410).

ZW800-1, IS-001, and IRDye-800BK appear suitable for ureter identification with NIR fluorescence imaging and have advantages over MB and ICG. Future clinical trials are needed to confirm the promising early results of these experimental fluorescent agents. However, large sample sizes are required for such studies due to relatively low incidence of iatrogenic ureteral injury. Therefore, phase III studies should focus on patients with high risk for intraoperative ureteral injury.

URETHRA

Besides ureteral injury, the urethra is also at risk for injury during pelvic surgery. Perineal dissection in (low) rectal surgery is an especially high-risk step for urethral injury. One clinical study with urethral administration of ICG during prostatectomies in 12 patients has been published.[73] No intraoperative urethra injury occurred. In another study, ICG was injected in the urethra during a transanal total mesorectal excision in one patient, resulting in successful identification of the urethra.[74]

NERVES

Sexual and urological dysfunction due to iatrogenic nerve injury are complications of rectal surgery, significantly affecting quality of life. Up to 79% of the patients

undergoing rectal surgery acquire some sort of sexual or urological dysfunction.[75] The hypogastric, splanchnic, and levator ani nerves are at risk during (colo) rectal surgery.[76] Nerve targeted fluorescence-guided surgery has the potential to improve nerve identification, and therefore prevent injury. Although promising pre-clinical results of nerve specific fluorescence imaging have been reported, the translation to clinical studies has yet to be made.[77-79] The main difficulties include fluorescent agents not being able to pass the nerve-blood barrier, and relatively high nonspecific uptake of nerve targeted agents by fat and muscle.[79]

IMAGING OF PERFUSION

ANASTOMOTIC PERFUSION

Anastomotic leakage is one of the most severe complications in CRC surgery. It often requires additional surgical or radiological intervention, leading to a prolonged hospital stay. Anastomotic leakage is reported up to 13% of patients undergoing CRC surgery with subsequent mortality rates of up to 27%.[80,81] Poor bowel perfusion is thought to play an important role in anastomotic leakage. ICG fluorescence-angiography can provide real-time feedback of bowel perfusion and aid the surgeon in determining the optimal location for the anastomosis. ICG doses between 2-20 mg have been reported.[82,83] In general, bowel perfusion can be assessed within 60 seconds after intravenous injection.

Over the years, several cohort studies have been published on the effect of ICG fluorescence angiography use on anastomotic leakage. Studies specifically addressing colonic anastomoses are sporadic and fail to show a significant decrease in anastomotic leakage rates when using ICG fluorescence angiography.[84,85] More data has been reported on rectal surgery. Song *et al*. published the most recent and complete meta-analysis on rectal anastomoses including 2088 patients from nine retrospective studies.[86] Their pooled analysis showed an odds ratio for anastomotic leakage of 0.34 (95% CI: 0.22-0.52) in favour of ICG fluorescence angiography over standard of care.

Recently, the first randomised controlled trials (RCTs) on ICG fluorescence angiography have been published. One study included 240 patients undergoing left-sided colon or rectal resection and failed to show a significant difference in anastomotic leakage rate between the ICG fluorescence angiography group and the control group (5% vs 9%; p = 0.2).[87] The second study investigated the value of ICG fluorescence angiography on the occurrence of anastomotic leakage in 377 patients undergoing sigmoid or rectal resection. A significantly lower anastomotic leakage rate was found in the ICG fluorescence angiography group (9.1% vs 16.3%; p = 0.04).[88] However, this difference was predominantly based on the occurrence of anastomotic leakage grade A, which does not alter patient management.[89] Thus, minimal clinical benefit was demonstrated as no difference was observed in the number of re-operations or the length of postoperative hospital stay. The third RCT also failed to report a significant decrease of anastomotic leakage in the ICG fluorescence angiography group compared to the control group (9.0% vs 9.6%; p = 0.37).[90] It should be noted that the pre-determined sample size was not achieved due to a decrease in accrual rates. More RCTs have been registered that will include similar or higher amount of patients (NCT02598414, NCT04012645). Noteworthy are the INTACT-trial and the AVOID-trial, both planning to include up to 1000 patients (ISCRN: 13334746, NCT04712032). Also, the prospective IMARI trial is assessing a series of interventions, including ICG fluorescence angiography, and its influence on anastomotic leakage in rectal cancer surgery (Netherlands trial register: NL8261).

In conclusion, ICG fluorescence angiography has potential in the prevention of anastomotic leakage in a safe and simple way. Pooled analysis of cohort studies has demonstrated that ICG fluorescence angiography reduces anastomotic leakage, but high-quality evidence is currently lacking. RCTs with inclusion up to 1000 patients are currently ongoing and might provide with the answer if ICG fluorescence angiography prevents anastomotic leakage in CRC surgery.

OMENTOPLASTIC PERFUSION

Perineal wound bed complications occur in almost 50% of the patients undergoing abdominoperineal resection (APR) and carry major morbidity.[91] Omentoplasty can be performed for the prevention and management of these complications. It is hypothesised that the transferred omentum prevents dead space formation, has an anti-inflammatory and antibacterial effect, and provides excellent vascularisation to the wound bed.[92] However, its clinical benefit in rectal cancer surgery has been disputed. A meta-analysis of 1894 patients showed that omentoplasty did not reduce the risk of postoperative presacral abscesses or perineal complications.[93] ICG fluorescence angiography of the transferred omentum was recently assessed in a pilot study.[94] Remarkably, ICG fluorescence angiography led to a change in surgical management in 80% of the patients. A follow up study by the same group showed a decrease in pelviperineal non-healing in the ICG group compared to the control group (22% vs 42%; p = 0.051).[95] However non-significant, this study showed a trend towards improved outcomes after ICG

fluorescence angiography guided omentoplasty. The reported alteration of the surgical plan in 80% of cases suggests that 'standard' omentoplasty is vulnerable to poor omental perfusion. Further research on ICG fluorescence angiography for omentoplasty is therefore warranted.

Discussion and future perspectives

NIR fluorescence-guided surgery is a rapidly evolving technique with various clinical applications in CRC surgery. This review provides an overview of the clinical applications of all fluorescent agents for CRC surgery. ICG, the nonspecific FDA/EMA approved fluorescent agent is already used in a variety of clinical applications of which CRLM resection and ICG fluorescence angiography show the most potential. However, no unequivocal benefits in relevant outcome measures have yet been reported. Over the past years, promising experimental fluorescent agents (targeted and non-targeted) have been investigated. These agents could potentially improve intraoperative fluorescence imaging, ultimately leading to improved detection of tumour tissue, vital structures, and vascularisation. Improving intraoperative detection of tumour could not only lead to more complete resections, but can also lead to better patient selection, as unnecessary surgery could be prevented if the disease is found to be too advanced. On the other hand, false-positive lesions would lead to unnecessary resection of healthy tissue which makes tumour-binding specificity of the fluorescent agent crucial.

Quantification of the fluorescence signal is challenging, with numerous factors such as scattering, absorption, camera angulation and distance, and background light influencing the signal intensity.[96] The latest studies on ICG fluorescence angiography for the prevention of anastomotic leakage focus on less subjective perfusion assessment by analysing time-dependent inflow parameters.[97,98] Real-time quantification of the fluorescence signal of tumour-targeted agents, aiding surgeons in deciding whether tissue is malignant or not, has not been reported yet. Most clinical studies report the SBR (or TBR) and change in surgical management as the main parameters in early phase studies. Eventually, trials should report on clinically significant events such as the tumour-negative resection margin rate, detection of occult lesions, surgical complications, and (disease free) survival.[99]

Nowadays, a variety of fluorescence camera systems is available in clinical practice. It is important to keep in mind that these camera systems can influence imaging results.[21] This also counts for the difference between open- and laparoscopic cameras. Most laparoscopes that are currently clinically available are optimised for ICG at 830 nm. This wavelength is slightly too high for optimal imaging of most experimental fluorescent agents, which have peak emission wavelengths around 800 nm (table 1). Therefore, there is a need for high-quality laparoscopes that are optimised for imaging of specific tumour-targeted fluorescent agents. Fluorescence imaging could account for the lack of tactile feedback in minimal invasive surgery, as it has the potential to improve visualisation of vital structures (e.g. the ureter, nerves) and tumours. Moreover, fluorescence imaging can be integrated in the laparoscopic field with an overlay view, which is an advantage over open surgery, where an additional handheld camera is needed. Especially in rectal surgery, in the conically shaped (male) pelvis, difficulties are experienced with optimal positioning due to the size of most open cameras. A laparoscope is much smaller and therefore easier to manoeuvre towards an optimal imaging angle.

Conclusion

In conclusion, the field of fluorescence-guided surgery is rapidly evolving with already several clinical applications in CRC surgery. ICG is widely used, and its use appears to be beneficial in specific applications. Many experimental fluorescent agents have been developed and several of these agents are currently being assessed in late phase clinical studies. The most promising applications of these experimental fluorescent agents in CRC surgery are distinguishing between fibrotic and tumour tissue after neo-adjuvant treatment, improving the rate of tumour-negative resection margins in locally advanced and recurrent rectal cancer, detection of occult metastases in cytoreductive surgery for peritoneal metastases, and ureteral imaging in high-risk cases. An essential next step for the implementation of these agents in clinical practice is to show direct patient benefit in terms of change in surgical management, surgical complications, recurrence-free survival, and overall survival.

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Supplementaries

SUPPLEMENT 1 Search strategies per database and the corresponding hits after removal of duplicates.

Database	Full search strategy
Embase (1123 articles)	('fluorescence guided surgery'/de OR (('fluorescence angiography'/de OR 'fluorescence imaging system'/de OR 'indocyanine green angiography'/de OR 'indocyanine green'/de OR 'near infrared spectroscopy'/exp OR 'near infrared imaging system'/de OR 'fluorescence imaging'/de) AND ('surgery'/exp OR surgery:lnk OR 'laparoscope'/exp)) OR (((fluorescen* OR indocyanine-green* OR ICG OR near-infrared* OR near-infra-red*) NEAR/9 (surg* OR operat* OR intraoperat* OR resect* OR microsurg* OR laparoscop*))):ab,ti,kw) AND ('large intestine tumour'/exp OR 'large intestine cancer'/exp OR 'large intestine carcinoma'/exp OR 'colorectal surgery'/exp OR 'ureter'/de OR 'ureter injury'/de OR 'ureter surgery'/exp OR 'urethra'/exp OR 'urethra injury'/de OR 'urethra surgery'/exp OR 'peripheral nerve'/de OR (((rect* OR colorect* OR colon* OR appendi* OR anal* OR anus OR cecum OR caecum OR large-intestin* OR sigmoid* OR bowel*) NEAR/3 (carcinoma* OR neoplas* OR tumour* OR tumour* OR cancer* OR surger* OR resect*)) OR ureter* OR urethr* OR (nerve* NEAR/3 (imag* OR detect* OR locali*))):ab,ti,kw) NOT (conference abstract)/lim AND (english)/lim
Medline (198 articles)	(((Fluorescein Angiography/ OR Optical Imaging/ OR Indocyanine Green/ OR Spectroscopy, Near-Infrared/) AND (exp Surgical Procedures, Operative/ OR surgery.fs. OR Laparoscopy/)) OR (((fluorescen* OR indocyanine-green* OR ICG OR near-infrared* OR near-infra-red*) ADJ9 (surg* OR operat* OR intraoperat* OR resect* OR microsurg* OR laparoscop*))).ab,ti,kf.) AND (exp Colorectal Neoplasms/ OR Colorectal Surgery/ OR Urethra/ OR Ureter/ OR Peripheral Nerves/ OR (((rect* OR colorect* OR colon* OR appendi* OR anal* OR anus OR cecum OR caecum OR large-intestin* OR sigmoid* OR bowel*) ADJ3 (carcinoma* OR neoplas* OR tumour* OR tumour* OR cancer* OR surger* OR resect*)) OR ureter* OR urethr* OR (nerve* ADJ3 (imag* OR detect* OR locali*))).ab,ti,kf.) NOT (letter* OR news OR comment* OR editorial* OR congres* OR abstract* OR book* OR chapter* OR dissertation abstract*).pt. AND english.lg.
Cochrane (73 articles)	((((fluorescen* OR indocyanine-green* OR ICG OR "near-infrared*" OR "near-infra-red*") NEAR/9 (surg* OR operat* OR intraoperat* OR resect* OR microsurg* OR laparoscop*))):ab,ti,kw) AND ((((rect* OR colorect* OR colon* OR appendi* OR anal* OR anus OR cecum OR caecum OR large-intestin* OR sigmoid* OR bowel*) NEAR/3 (carcinoma* OR neoplas* OR tumour* OR tumour* OR cancer* OR surger* OR resect*)) OR ureter* OR urethr* OR (nerve* NEAR/3 (imag* OR detect* OR locali*))):ab,ti,kw)

SUPPLEMENT 2 Overview of all clinical studies on colorectal cancer surgery assessing experimental fluorescent agents.

PRIMARY TUMOUR

First author	Tumour type	Year	Fluorescent agent	Study design	Fluorescence imaging application	Nr of patients	(Optimal) dose	(Optimal) adm. interval	Optimal TBR (mean)	Sensitivity	Specificity	Change in surgical management	Other outcomes
Boogerd (14)	CC + RC	2018	SGM-101	Open-label, dose escalation	*In vivo* and PEARL MSI	26*	10 mg	4 days	1.64 (*in vivo*) 6.1 (*ex vivo*: PEARL MSI)	98%	62%	35%	ppv: 81% | npv: 94% | acc: 84%
De Jongh (16)	RC	2020	Bevacizumab-800CW	Open-label, fixed dose, pilot study	1 = Back table imaging for CRM evaluation 2 = Odyssey imaging to determine sens / spec for tumour detection	1 = 8 2 = 17	4.5 mg	2-3 days	4.7 (*ex vivo*: Odyssey)	96.19%**	80.39%**	na	Tumour positive correctly identified in 1 out of 2 patients (50%)
De Valk (15)	CC	2020	cRGD-ZW800-1	Open-label, dose escalation	*In vivo* and PEARL MSI	12	0.05 mg/kg	18 hours	1.6 (*in vivo*) 6.2 (*ex vivo*: PEARL MSI)	nr	nr	nr	Lymph nodes: sens 100% | spec: 87% | ppv: 33% | npv: 100% | acc: 88%
De Valk (18)	CC + RC	2020	SGM-101	Open-label, dose escalation	*In vivo* and back table	37*	10 mg	4 days	1.9 (*in vivo*) 3.5 (*ex vivo*: back table)	96%	63%	24%	ppv: 70% | npv: 94% | acc: 78%
Voskuil (17)	nr***	2020	ONM-100	Open-label, dose escalation	PEARL MSI	3	1.2 mg/kg	24 hours	nr***	nr***	nr***	nr***	-

(B) SENTINEL LYMPH NODE

First author	Year	Fluorescent agent	Study design	Fluorescence imaging application	Nr of patients	(Optimal) dose	(Optimal) adm. interval	SBR	Sensitivity	Specificity	Change in surgical management	Other outcomes
Hutteman (39)	2011	HSA800	Pilot study	Ex vivo	24	1cc 50 µM	post-operative in specimen	nr	nr	na	nr	SLN's detected: 100% | avg SLN's: 3
Schaafsma (38)	2013	HSA800	Pilot study	Ex vivo	22	1cc 50 µM	post-operative in specimen	nr	80%	na	nr	SLN's detected: 95% | avg SLN's: 3.5
Weixler (40)	2017	HSA800	Prospective single-centre	Ex vivo	50	1cc 50 µM	post-operative in specimen	nr	64%	na	10%	SLN's detected: 100% | avg SLN's: 4.4

(C) PERITONEAL METASTASES

First author	Year	Fluorescent agent	Study design	Fluorescence imaging application	Nr of patients	(Optimal) dose	(Optimal) adm. interval	Optimal TBR (mean)	Sensitivity	Specificity	Change in surgical management	Other outcomes
Harlaar (48)	2016	Bevacizumab-800CW	Open-label, feasibility study	*In vivo* and back table	7	4.5 mg	2 days	6.92 (*ex vivo*: back table)	nr	nr	29%	ppv: 53% | npv: 100%
Schaap (49)	2020	SGM-101	Open-label, pilot study	*In vivo* and back table	14	10-15 mg	4-6 days	nr	98.5%	62.2%	50%	ppv: 82.3%| npv: 95.8%| acc: 85.4%

(D) LIVER METASTASES

First author	Year	Fluorescent agent	Study design	Fluorescence imaging application	Nr of patients	Optimal dose	(Optimal) adm. interval	Optimal TBR (median)	Sensitivity	Specificity	Change in surgical management	Other outcomes
Meijer (58)	2020	SGM-101	Open label, dose finding	*In vivo* (occult lesions and resection margin)	8	10 mg	4 days	2.0 (*in vivo*)	nr	nr	0%	ppv: 89% | positive : 9%

(E) URETER

First author	Year	Fluorescent agent	Study design	Fluorescence imaging application	Nr of patients	Optimal dose	Adm. interval	Optimal SBR	Ureters identified	Duration of visualisation
De Valk (69)	2019	ZW800-1	Open label, dose finding	In vivo	12	2.5 mg	per-operative	2.7	100%	3.5 hours
Farnam (70)	2019	IS-001	Open label, dose finding	In vivo	24	20-40 mg	per-operative	3.6	100%	nr
Huh (71)	2020	IRDye-800BK	Open label, dose finding	In vivo	41	0.06 mg/kg	per-operative	nr	100%	1.5 hours

* 21 out of 26 patients from Boogerd et al were also included in the study by de Valk *et al.*

** Based on microscopy determined optimal mean fluorescence intensity cut off values for tumour detection

*** Results of interest not separately specified for colorectal cancer patientsw

SUPPLEMENT 3 overview of all ongoing clinical trials on colorectal cancer surgery assessing experimental fluorescent agents.

Principal investigator	Fluorescent agent	Study phase	Imaging target	Tumour type	Planned inclusion FLI (n)	Control group (n)	Recruitment status	(Estimated) study completion date	NCT number
Barnes, T.G.	IRDYe-800BK	Phase I/II	Ureter	na	40	na	Completed	nov-18	NCT03387410
Chan, A.T.	LUM015	Phase I	Primary tumour	All stage colorectal cancer	11	na	Recruiting	dec-20	NCT02584244
Cusack, J.C.	LUM015	nr	Peritoneal metastases	Peritoneal metastases of CRC	nr*	na	Not yet recruiting	feb-21	NCT03834272
OncoNano Medicine, Inc.	ONM-100	Phase II	Primary tumour	Colorectal cancer, not specified	nr*	na	Recruiting	dec-20	NCT03735680
Vahrmeijer, A.L.	SGM-101	Phase III	Primary tumour and peritoneal metastases	LARC & LRRC, T4 colon cancer, locally recurrent colon cancer, and peritoneal metastases of CRC	240	60	Recruiting	dec-21	NCT03659448
Vahrmeijer, A.L	SGM-101	Phase II	Extra abdominal metastases	Colorectal lung metastases	10	na	Recruiting	dec-21	NCT04737213
Vahrmeijer, A.L	SGM-101	Phase II	Extra abdominal metastases	Colorectal brain metastases	10	na	Not yet recruiting	dec-22	NCT04755920
Vahrmeijer, A.L	SGM-101	Phase III	Primary tumour	LARC & LRRC	203	na	Recruiting	oct-23	NCT04642924

* Study assessing various tumour types, amount of patient undergoing surgery for colorectal cancer is not specified.

SUPPLEMENT 4 MINORS score per study assessing experimental fluorescent agents.

First author	MINORS score	Maximum MINORS score	Clearly stated aim	Consecutive patients	Prospective data collection	Appropriate endpoint	Unbiased evaluation of endpoints	Appropriate follow-up	Loss to follow-up	Prospective calculation of sample size	Gold Standard control	Contemporary groups	Baseline equivalence of groups	Statistical analysis adapted to study design
Boogerd (14)	14	16	2	2	2	2	0	2	2	2	NA	NA	NA	NA
De Jongh (16)	11	16	2	2	1	2	0	2	2	0	NA	NA	NA	NA
De Valk (15)	14	16	2	2	2	2	2	2	2	0	NA	NA	NA	NA
De Valk (18)	14	16	2	2	2	2	0	2	2	2	NA	NA	NA	NA
Voskuil (17)	14	16	2	2	2	2	2	2	2	0	NA	NA	NA	NA
Hutteman (39)	11	16	1	2	2	2	0	2	2	0	NA	NA	NA	NA
Schaafsma (38)	18	24	2	2	2	2	0	2	2	0	2	2	0	2
Weixler (40)	13	24	2	0	1	2	0	2	0	0	2	0	2	2
Harlaar (48)	14	16	2	2	2	2	2	2	2	0	NA	NA	NA	NA
Schaap (49)	12	16	2	2	2	2	0	2	2	0	NA	NA	NA	NA
Meijer (58)	12	16	2	2	2	2	0	2	2	0	NA	NA	NA	NA
De Valk (69)	14	16	2	2	2	2	2	2	2	0	NA	NA	NA	NA
Farnam (70)	14	16	2	2	2	2	0	2	2	2	NA	NA	NA	NA
Huh (71)	12	16	2	2	2	2	0	2	2	0	NA	NA	NA	NA