The role of ICG NIRL fluorescence imaging in the surgical treatment of digestive system tumors (Review)

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Abstract. Indocyanine green (ICG) is a relatively non-toxic fluorescent dye with a history of safe use, which has fueled the development of new applications for ICG. Research on the use of ICG near-infrared light (NIRL) fluorescence imaging during oncologic surgery has increased, revealing its role in tumor identification and localization, lymph node navigational resection and blood perfusion assessment. The purpose of the present review was to provide a comprehensive overview of advances in the clinical application of ICG NIRL fluorescence imaging during gastrointestinal tumor surgery. The present review discusses the techniques, outcomes, limitations and key considerations necessary for clinical practice, aiming to provide a valuable resource for professionals in the field.

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1. Introduction

The development of new drugs is challenging, as it requires lengthy research and development cycles, and incurs high costs. This makes explorations of new clinical applications for existing drugs advantageous. One such example is the use of indocyanine green (ICG) in near-infrared light (NIRL) fluorescence imaging, which involves repurposing an existing drug for innovative applications. ICG is a water-soluble, relatively non-toxic iodide dye with a molecular weight of 776 Da (1). After intravenous injection, ICG binds to plasma proteins, remains in the vascular compartment and is rapidly excreted into bile, resulting in rapid hepatic clearance (2). These properties allow its effective application even at lower doses. The United States Food and Drug Administration approved the use of ICG in the medical field in 1959 (3), and it has been safely utilized since the mid-1950s. The established safety and efficacy of ICG have paved the way for new applications in various fields.

Initially, ICG was used to quantitatively measure liver and cardiac function (4), with a focus on its concentration in the body. Subsequent research revealed that the absorption spectrum of ICG lies in the infrared region. When excited by NIRL at 700-900 nm, ICG fluoresces at 820 nm, allowing the dye to be detected by specialized imaging systems (5-7). This fluorescence capability enables the visualization of anatomical structures where the dye is localized. ICG has been used in intraoperative angiography to assess superficial ocular vessels, coronary artery grafts, peripheral vascular disease and solid organ transplants (8-11). Furthermore, ICG can act as a photosensitizer, generating reactive oxygen species and heat to target and destroy cancer cells through photodynamic and photothermal therapy (12,13). The peak absorption of ICG in plasma or blood occurs at 800-810 nm, with peak emission at 835 nm, allowing tissue penetration to a depth of ~1 cm; thus, ICG is ideal for visualizing tissue within a depth of 8 mm (14) (Fig. 1). In oncologic surgery, NIRL fluorescence imaging guided by ICG has enhanced tumor localization and the identification of structures that should be preserved, such as lymph nodes (3,15). This technique has expanded the visual field of the surgeon beyond traditional methods, improving accuracy in identifying surgical margins. The fluorescent properties of ICG have significantly advanced targeted surgical procedures. Although numerous studies have focused on the role of ICG in the surgical treatment of tumors, there is no comprehensive

summary of specific techniques, limitations and best practices. The present review aims to fill this gap by providing a detailed analysis of the methods, outcomes, limitations and clinical considerations of the use of ICG NIRL fluorescence imaging during the surgical treatment of gastrointestinal tumors, offering a valuable resource for clinicians.

2. ICG NIRL fluorescence imaging and liver cancer surgery

Primary liver cancer is the 6th most common cancer worldwide and the second leading cause of cancer-related mortality, and 80% of cases are attributed to hepatocellular carcinoma (HCC) (16). Additionally, secondary liver metastases are frequently observed in patients with common types of cancer, including breast, lung, colorectal, pancreatic, esophageal, gastric and small intestine cancer, among others (17). In previous years, ICG NIRL fluorescence imaging has been integrated into hepatobiliary surgery to enhance the identification of HCC lesions during operations. In one study, 273 out of 276 HCC lesions were successfully identified using fluorescence imaging, with a sensitivity of 99%. However, 16 false-positive lesions were detected, primarily including large regenerative nodules, heterogeneous hyperplastic nodules, bile duct hyperplasia and necrosis, leading to a positive predictive value of 94% (18). The fluorescence pattern of HCC is closely associated with its pathological features; well- and moderately differentiated HCCs display complete or partial fluorescence, whereas poorly differentiated HCCs, often with microvascular invasion, predominantly show marginal fluorescence (18-20). Furthermore, Abo *et al* (21) demonstrated that among 12 patients with intrahepatic cholangiocarcinoma, 10 (83%) exhibited ICG fluorescence. Of these, 60% displayed marginal staining and 30% showed complete or partial staining. This study also revealed the potential of ICG fluorescence imaging combined with portal vein injections to assess the extent of portal vein tumor thrombosis in patients with HCC while also identifying benign lesions, such as hepatic hemangiomas, cholangiocarpal adenomas and cavernous hemangiomas (21). Therefore, ICG fluorescence imaging has been demonstrated to be a reliable tool for surgical navigation, enhancing the safety and precision of HCC resection.

For injection protocols, previous studies have suggested that administering ICG intravenously at a dose of 0.5 mg/kg body weight within 2 weeks prior to surgery enables the effective intraoperative visualization of liver tumors (19,22) (Table I; Fig. 2). In the surgical treatment of colorectal cancer (CRC) liver metastasis, ICG fluorescence imaging provides real-time feedback regarding tumor margins and yields higher rates of complete tumor resection in patients who received a single intravenous infusion of 10 mg of ICG 24 h before surgery (23) (Fig. 2). This guideline is based on protocols that were originally designed for preoperative liver function assessments rather than direct tumor detection. In patients with cirrhosis or compromised liver function due to chemotherapy, the administration of ICG the day before surgery should be avoided to minimize background liver signals and reduce the likelihood of false positives (24). These injection protocols may need to be tailored for specific clinical scenarios on the basis of emerging evidence.

Despite the high sensitivity and positive predictive value of ICG fluorescence imaging, false-positive rates remain a concern, reaching up to 40% in certain cases (18,19). The factors that contribute to high false-positive rates include cirrhosis, dysplastic nodules, brief intervals between ICG injection and surgery (<24 h), bile duct hyperplasia, necrosis, cysts, hemangiomas and atypical non-malignant lesions. As a result, new lesions that are detected by fluorescence should undergo additional evaluation through examination, palpation or intraoperative ultrasound (IOUS). In response to these false-positive situations, nanoprobe fluorescence imaging is more targeted, more feasible and safer when compared with ICG fluorescence imaging (25). Occasionally, ICG fluorescence imaging may also yield false-negative results, particularly when the time between ICG injection and surgery exceeds 24 days, requiring careful consideration in clinical decision-making.

ICG NIRL fluorescence imaging improves intraoperative visualization and the resection rate of liver tumors, and it is a good navigation tool in the surgical treatment of gastrointestinal and esophageal cancer, with improved recognition and safety of tumor resection.

3. ICG NIRL fluorescence imaging and gastric cancer surgery

Gastric cancer is the 5th most prevalent malignancy worldwide and a leading cause of cancer-related mortality due to its advanced stage at diagnosis; gastric cancer is the third most common cause of cancer-related mortality (26). Currently, surgical treatment remains the cornerstone of treatment for both early and advanced gastric cancer (26). In previous years, laparoscopic surgery has been validated as a safe and effective option for gastric cancer management in both Eastern Europe and Western nations (27-39). These findings suggest that minimally invasive approaches for gastric cancer treatment may dominate future surgical trends.

Initially, ICG NIRL fluorescence imaging was used for sentinel lymph node detection in early gastric cancer (40,41). Its use has since expanded to include preoperative endoscopic marking for tumor localization, ensuring negative surgical margins (42) and facilitating comprehensive lymph node mapping (43). Lymph node retrieval is key for accurate staging, with most guidelines recommending the examination of at least 16 regional nodes, whereas the retrieval of 30 or more nodes is considered optimal (44,45). Several studies have demonstrated that ICG NIRL fluorescence imaging increases the efficiency of regional lymph node dissection during laparoscopic surgery, increasing the yield of nodes at key anatomical sites (45,46-49). Compared with traditional lymphadenectomy methods, ICG-guided laparoscopic lymphadenectomy is both safe and effective in improving survival outcomes for patients with resectable gastric cancer (50). Additionally, a randomized controlled trial revealed that ICG markedly increases the quality of lymph node dissection in patients with locally advanced gastric cancer undergoing laparoscopic radical gastrectomy following neoadjuvant chemotherapy (51). For early mucosal tumors without ulceration or ulcer scarring, endoscopic mucosal resection (EMR) is typically recommended. The decision to carry out additional radical gastric cancer surgery

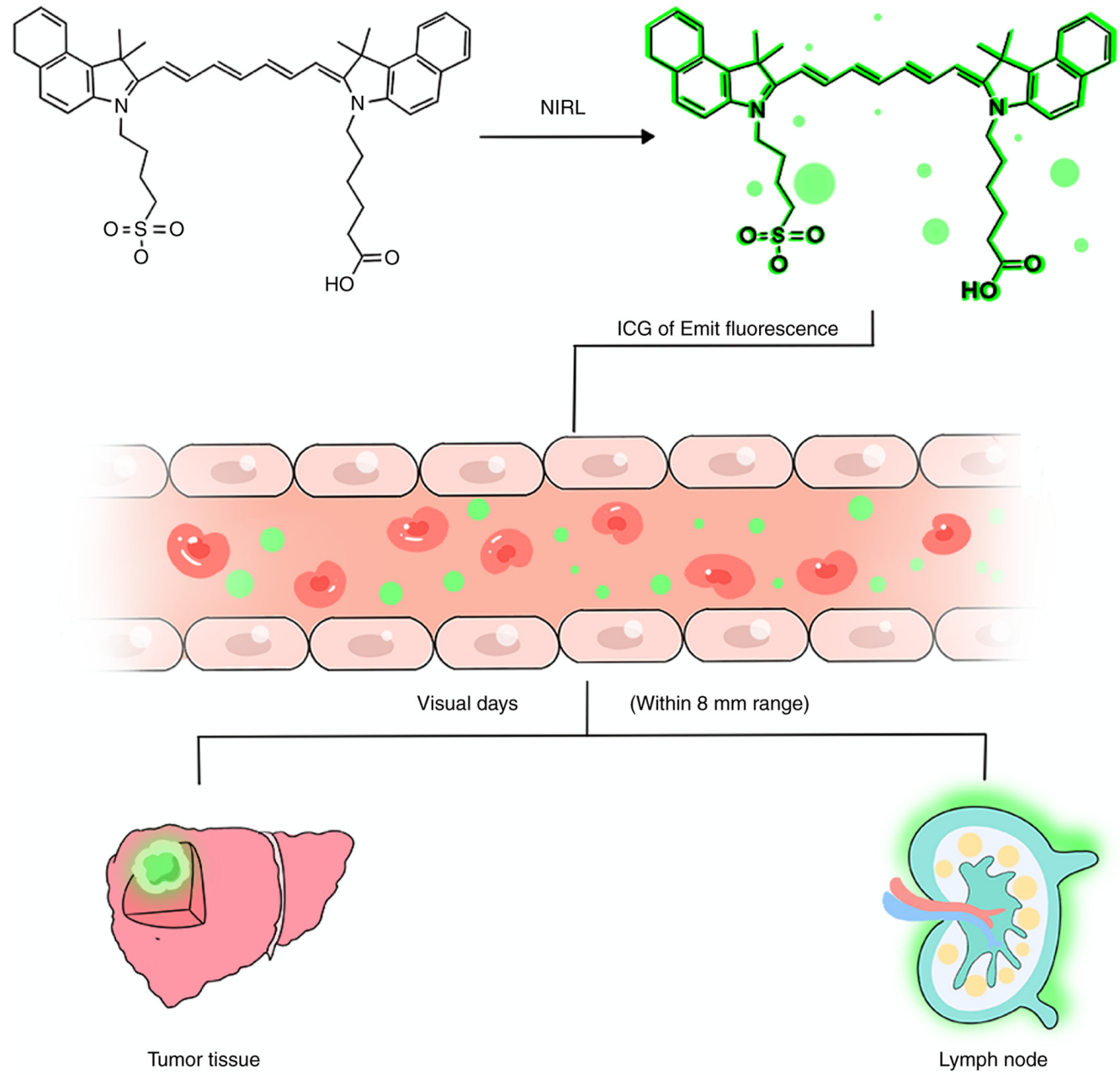

Figure 1. Schematic of the fluorescence properties and clinical applications of ICG. Spectral absorption peak of ICG in plasma or blood is between 800 and 810 nm, with an emission peak at 835 nm. These wavelengths enable tissue penetration up to a depth of ~1 cm, making it suitable for visualizing structures within 8 mm of tissue depth. Consequently, ICG is an effective visual dye for NIRL fluorescence imaging-guided tumor surgery, facilitating the identification and precise localization of structures that require removal or preservation, such as tumor tissues and lymph nodes. ICG, indocyanine green; NIRL near infrared light.

with lymph node dissection is made on the basis of the pathologic assessment of the EMR specimen, particularly regarding infiltration depth and ulceration status (52). While no reports have specifically addressed the use of fluorescence imaging to guide tumor localization during EMR, a previous study suggests that ICG fluorescent lymphography-administered via endoscopic injection around the postoperative scar-can allow effective visualization of lymphatic vessels and assessment of the sensitivity and negative predictive value for detecting lymph node metastasis (53). There is no standardized method in terms of specific injections when ICG fluorescence imaging is used to identify lymph nodes in gastric cancer. For identification of sentinel lymph nodes in gastric cancer, 0.5-2.5 mg/ml of ICG solution was endoscopically injected into 4-8 sites in the submucosal layer surrounding the tumor (a total of 2-4 ml) during surgery for gastric cancer (54). During surgical treatment of advanced gastric cancer, 0.125 mg/ml ICG solution was endoscopically injected into four sites in the submucosal layer surrounding the tumor, 0.5 ml per site, during surgery for gastric cancer (55) (Fig. 2).

In addition to lymph node localization, ICG NIRL fluorescence imaging has been demonstrated to be valuable for marking tumor margins. In laparoscopic gastrectomy, ICG was endoscopically injected into the submucosal layer of the stomach, ~1 cm from the tumor edge, to delineate early-stage cancer boundaries. The recommended dose was 0.1 ml of

Table I. Applications of ICG fluorescence imaging in various tumor surgeries.

First author/s, year	Tumor	Effect	Injection method	(Refs.)
Ishizawa *et al*, 2009; Terasawa *et al*, 2017	Liver cancer	Detection of hepatocellular carcinomas, cholangiocarcinoma	0.5 mg/kg, iv, 2 weeks before surgery	(19,22)
Achterberg *et al*, 2024	Liver cancer	Detection of colorectal cancer liver metastasis tumor	10 mg ICG, iv, 24 h before surgery	(23)
Miyashiro *et al*, 2011	Gastric cancer	Show sentinel lymph nodes in early gastric cancer	1.25 mg/ml ICG, 2-4 ml total, inject into the submucosa in the four quadrants around the tumor.	(54)
Lombardi *et al*, 2022	Gastric cancer	Show lymph nodes in advanced gastric cancer	0.125 mg/ml ICG, 0.5 ml per site, inject into the submucosa in the four quadrants around the tumor.	(55)
Tanaka *et al*, 2020	Gastric cancer	Mark gastric cancer margins	0.5 mg/ml ICG, 0.1 ml, inject into the submucosa, 1 cm from the tumor edge	(56)
Chen *et al*, 2021	Gastric cancer	Mark gastric cancer margins	1.25 mg/ml ICG, 0.5 ml, inject into the submucosa, in the four quadrants around the tumor.	(57)
Rho *et al*, 2021	Esophageal cancer	Angiography Detecting esophageal tumors	2 mg/kg ICG, iv, 12 h before operation	(72)
van Manen *et al*, 2018	Colorectal cancer	Evaluate intestinal perfusion	0.05 mg/kg ICG, iv, 2-20 mg total, for 60 sec, in surgery.	(80)
Watanabe *et al*, 2021	Colorectal cancer	Evaluate intestinal perfusion	0.25 mg/kg ICG, iv, before intestinal anastomosis.	(84)
De Nardi *et al*, 2020	Colorectal cancer	Evaluate intestinal perfusion	0.3 mg/kg ICG, iv, before colonic transection and after anastomosis	(85)
Ahn *et al*, 2022	Colorectal cancer	Standard lymph node testing	0.5-1 mg ICG, 12-18 h, colonoscopy submucosal injection before surgery	(90)
Su *et al*, 2023; Kim *et al*, 2020	Colorectal cancer	Lateral pelvic lymph node dissection	1.0-1.5 ml (25 mg/10 ml) ICG, colonoscopy submucosal injection before surgery	(91,92)
Park *et al*, 2018	Colorectal cancer	Colorectal cancer recognition	12.5 mg/ml ICG, 0.5-1 ml, inject into the submucosa in the four quadrants around the tumor 1 day before surgery	(96)

ICG, indocyanine green; iv, intravenous.

0.5 mg/ml ICG (Fig. 2), which ensured clear margins without excessive blurring (56). Despite the lack of a standardized submucosal injection protocol, a study have utilized higher concentrations (1.25 mg/ml), injecting 0.5 ml around the primary tumor in four quadrants, equivalent to 2.5 mg of ICG (57) (Table I) (Fig. 2).

While extensive research supports the role of ICG-guided lymph node dissection during laparoscopic gastrectomy, its practical advantages remain under debate, with certain studies highlighting potential limitations (58,59), but they have small sample sizes, necessitating larger, randomized clinical trials. A previous single-center study published in The Journal of the American Medical Association Surgery, which increased the sample size to 133 cases, revealed that only 56.3% of metastatic lymph nodes were detected by fluorescence, suggesting a notable risk of false-negative results when ICG NIRL fluorescence imaging was used for metastatic lymph node identification (60). These false-negative results may occur due to extensive cancer cell infiltration or lymphatic obstruction (61), resulting in the failure of ICG to accumulate in positive lymph nodes. This may also be the result of insufficient learning time for the operator and incomplete histological evaluation of frozen sections. A small prospective study by Shoji *et al* (62) revealed that when ICG was injected around the primary tumor during surgery, followed by a one-step nucleic acid amplification assay, the expression of the epithelial protein CK19 could be rapidly determined. The detection rate of sentinel lymph nodes using this method was 85%, with a false-negative rate as low as 0% (62).

4. ICG NIRL fluorescence imaging and esophageal cancer surgery

Esophageal cancer is associated with high morbidity and mortality rates, surpassing several other malignancies in terms of severity (63). Radical esophagectomy remains

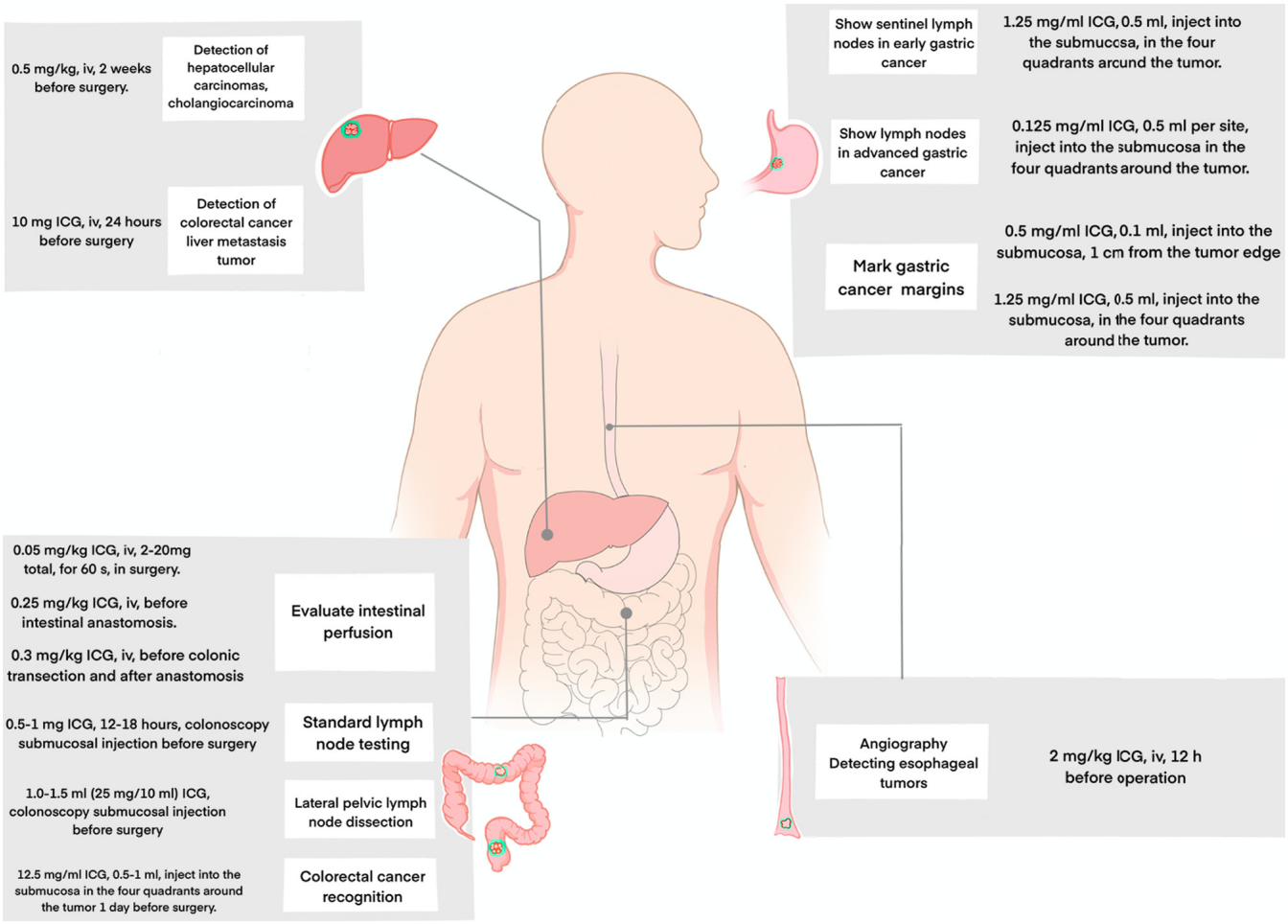

Figure 2. ICG NIRL fluorescence imaging in various tumor surgeries. Illustration delineates the application of ICG NIRL fluorescence imaging in surgeries for liver cancer, gastric cancer, esophageal cancer and colorectal cancer, encompassing details such as the injection dosage, ICG concentration, timing of injection and the site of injection. ICG, indocyanine green; iv, intravenous; NIRL, near infrared light.

the primary treatment option for early-stage esophageal cancer. However, despite surgical intervention, ~80% of patients experience tumor recurrence, with >40% of recurrences attributed to lymph node metastasis (64). Thus, the precision of lymph node dissection during radical esophagectomy is key for improving the outcomes of patients. ICG NIRL fluorescence imaging has demonstrated promise in identifying sentinel lymph nodes across various types of cancer (65,66). In esophageal cancer surgery, several studies have demonstrated the feasibility of ICG NIRL fluorescence-guided lymphography, which has achieved high detection rates for sentinel lymph nodes (67-69). In addition to lymph node detection, recent research has revealed that ICG NIRL fluorescence imaging can reduce the risk of anastomotic leakage by allowing the assessment of blood flow during surgery (70,71). This technique involves intravenous injection of ICG (2 mg/kg) 12 h before surgical resection of the esophagus (Fig. 2). Both animal models and clinical studies investigating patients with esophageal cancer have demonstrated the efficacy of this dosage in accurately localizing tumors (72). Importantly, no adverse reactions related to intravenous ICG administration have been reported in these patients, highlighting the safety of the procedure (Table I).

5. ICG NIR fluorescence imaging and CRC surgery

CRC is the 3rd most commonly diagnosed cancer worldwide and the second leading cause of cancer-related mortality (73). Despite advances in treatment, surgical resection remains the primary approach for achieving a complete response (74). A considerable postoperative concern is anastomotic leakage, which not only increases morbidity and mortality rates but also negatively impacts long-term oncological outcomes and reduces quality of life (75). Adequate blood supply to the anastomotic site is an important factor influencing successful healing. ICG NIRL fluorescence imaging has become a valuable tool for evaluating intestinal perfusion during anastomosis, aiming to reduce leakage rates (76-78). ICG NIRL fluorescence imaging perfusion assessment has the advantages of safety, simplicity and short adjustment time, and it is a tool that should be considered for reducing the incidence of anastomotic leakage after colorectal surgery (79). This method is known for its safety, simplicity and rapid assessment, with typical intraoperative ICG doses ranging from 2 to 20 mg (Fig. 2). Intestinal blood perfusion can generally be evaluated within 60 sec following intravenous ICG administration (80,81). For standard ICG preparation, 25 mg of ICG was dissolved in 10 ml of distilled water, corresponding to 0.05 mg/kg (82) (Table I).

The optimal distance between the near infrared camera and the colon is 4-5 cm for accurate visualization (83). A study by Watanabe *et al* (84) revealed that anastomotic fistula and reoperation rates were markedly reduced when patients were given 0.25 mg/kg ICG (Fig. 2) intravenously prior to intestinal anastomosis, and a study by De Nardi *et al* (85) demonstrated a reduction in anastomotic fistula and reoperation rates when patients were given 0.3 mg/kg ICG intravenously before colonic transection and after anastomosis (Fig. 2).

In addition, sentinel node identification using ICG fluorescence imaging has been reported as a viable adjunct for use in CRC surgery (86). This approach enables the precise identification of metastatic and lateral lymph nodes, as well as peritoneal metastases, supporting complete lymph node dissection during cancer resection (87-89). While effective, the accuracy of this technique heavily relies on the expertise and experience of the surgeon. Ahn *et al* (90) demonstrated favorable results with submucosal injection of 0.5-1 mg of ICG for standard lymph node testing for colonoscopy 12-18 h before surgery (90) (Fig. 2). Su *et al* (91) and Kim *et al* (92) demonstrated the effectiveness of a colonoscopy submucosal injection of 1.0-1.5 ml (25 mg/10 ml) ICG for lateral pelvic lymph node detection (metastasis of rectal cancer) before surgery (91,92) (Fig. 2). Fluorescence imaging also holds promise for the management of early-stage CRC, particularly in the context of minimally invasive surgery. Accurate tumor localization without tactile feedback is essential, particularly for small lesions or tumors in resectable colon segments (93-95). There is potential for ICG NIRL fluorescence imaging to facilitate precise tumor resection in endoscopic procedures, although further research is needed to validate its efficacy. Park *et al* (96) revealed that 0.5-1 ml of ICG at a concentration of 12.5 mg/ml can be injected into each of the four sites around the tumor 1 day before surgery (96) (Table I; Fig. 2). The field has also seen innovations, such as ICG-liposome conjugates, which improve tumor specificity and enhance visibility during surgery (97,98).

6. Summary and outlook

ICG NIRL fluorescence imaging has been extensively studied not only in the surgical treatment of liver cancer and gastrointestinal tumors but also in the surgical treatment of gynecological tumors (99), breast cancer (100) and brain tumors, and it has also been studied in the context of transplantation surgery (101). ICG NIRL fluorescence imaging aids in lesion localization and portal vein tumor thrombosis detection during liver cancer surgery. Its applications in gastrointestinal oncology include tumor identification, localization, lymph node navigation and blood perfusion assessment, enabling more precise resections of tumors and lymph nodes. This precision has contributed to more accurate tumor staging, reduced postoperative complications and improved patient outcomes. The localization of ICG in certain regions of tumor tissue appears to be the result of increased tissue permeability and retention, but is not tumor cell-specific (102). While several specific methods and dosages for ICG application have been explored in small-scale clinical case studies, large-scale clinical trials are still needed to establish standardized protocols for clinical practice. Due to the penetrating ability of near infrared light, fluorescence imaging can visualize tumors up to 8 mm from the surface of the liver or 8 mm from the surface of the parenchymal cut. For deeper liver lesions, IOUS can be used simultaneously (19). However, ICG can only be used to localize liver tumors, and some false-positive and false-negative rates were observed in the present review; additionally, ICG does not inhibit tumor growth. The combination of ICG and nanotechnology markedly increases the photostability and tumor-targeting ability of ICG and achieves liver tumor clearance (103,104). In addition, ICG-based targeted radiopharmaceutical therapy has also been proposed (105). In early gastric cancer, ICG can be used to identify the location of the lesion as well as sentinel lymph nodes and specimen lymph nodes, and it can aid in tumor resection and complete lymph node dissection during surgery. In advanced gastric cancer, the application of ICG can minimize the extent of lymph node dissection. These applications and advantages should be validated in more cases. The false-negative and false-positive rates involved should also be emphasized. The reasons for the occurrence of these false-negative and false-positive results may be the obstruction of lymphatic vessels caused by cancer cells and inappropriate injection doses; furthermore, the targeting of ICG needs to be improved. ICG in combination with other molecules, tracers or monoclonal antibodies also has great potential to detect metastasis and can be detected by a variety of diagnostic tools (magnetic resonance imaging; near infrared and multimodal imaging using a variety of novel fluorophores) (106). An increasing number of devices are also being developed with the aim of making fluorescence quantifiable, overcoming dual tracer methods (107) or assessing perfusion quality (108). ICG NIRL fluorescence imaging perfusion evaluation has the advantages of safety, simplicity and short adjustment time to reduce the incidence of anastomotic fistula after CRC surgery. It can also identify sentinel lymph nodes, accurately identify metastatic and lateral lymph nodes as well as peritoneal metastases, support complete lymph node clearance during cancer resection and locate the tumor site to enable further precise resection. However, there is still a lack of high-quality evidence from randomized controlled trials for the present review. Since ICGs do not bind specifically to tumor cells, further reduction of the resection margin distance is not possible. Therefore, the study of tumor-targeted fluorophores, which promote the precise localization of tumors, is valuable and may be a solution to effectively shorten the margin distance. Certain tumor-targeted fluorophores, such as SGM-101 and IR-783, have achieved good results in clinical studies (109,110). In addition, quantification of the fluorescence signal is challenging. The selection of appropriate quantification parameters is a major issue and fluorescence intensity may be affected by a variety of factors, such as ambient light, the fluorescence emission source and the distance between the camera and the colorectum (85,111).

Notably, minimally invasive treatment for early gastrointestinal cancer is rapidly advancing. Considering the effectiveness of ICG NIRL fluorescence imaging in laparoscopic surgery, there is a possibility that this technology can be applied to gastrointestinal endoscopic procedures, such as endoscopic submucosal dissection and EMR. This would increase the precision of surgical resection and minimize the risks of insufficient or excessive excision. Such advances could considerably

improve the techniques that are used in the management of early gastrointestinal cancer and further enhance the capabilities of minimally invasive endoscopic procedures.

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Availability of data and materials

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Authors' contributions

YH contributed to the studyconception and manuscript drafting. TW contributed to critical revisions of the intellectual content. BT made contributions to the study conception and design. All authors read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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