Received: 27 March 2018 | Accepted: 29 May 2018

DOI: 10.1002/jso.25139

REVIEW ARTICLE

WILEY Journal of SURGICAL ONCOLOGY

Fluorescent-guided surgery for sentinel lymph node detection in gastric cancer and carcinoembryonic antigen targeted fluorescent-guided surgery in colorectal and pancreatic cancer

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Sentinel lymph node procedures for gastric cancer resections using indocyanine green (ICG) linked to Nanocoll outperformed normal ICG but did not provide information on possible lymph node metastasis. Carcinoembryonic antigen targeted fluorescent imaging using SGM-101 was successful in both pancreatic and colorectal cancer. A large phase III multicentre trial will soon be initiated in colorectal cancer patients.

KEYWORDS
lymphatic metastasis, molecular imaging, pancreatic neoplasm, rectal neoplasm

1 | FLUORESCENT-GUIDED SURGERY

Intraoperative discrimination between benign and malignant tissue is primarily based on tactile and visual examination by surgeons. Both of these subjective methods have limitations in assessing tumor margins. As a result, positive resection margins still regularly occur with local recurrence and metastases as likely consequences.

Near-infrared (NIR) photons can penetrate deeply into tissue (up to 5 to 10 mm). This is utilized by NIR fluorescence (NIRF) to provide fast and quantitative contrast images.[1,2] Fluorophores can either accumulate in tumor tissue due to either the enhanced permeability and retention (EPR) effect or by targeting a specific tumor marker. Once activated by laser, NIRF light reflected by the fluorophore can be visualized using a dedicated imaging system. NIRF-guided surgery can help distinguish benign from malignant tumor tissue and also aid in identifying lesions or metastasis outside the standard field of resection[3] and therefore might influence clinical decision making.

Recently, research has shifted from nonspecific imaging (depending on the EPR effect) toward tumor-marker specific imaging (eg, tumor receptor targeting). NIRF imaging is currently being investigated for multiple purposes in clinical trials. This review discusses two applications of targeted and nontargeted NIRF. First, the clinical and preclinical data on NIRF-guided sentinel lymph node (SLN) identification in gastric cancer using indocyanine green (ICG) is discussed. Subsequently, the available data on carcinoembryonic antigen (CEA) targeted NIRF imaging in rectal and pancreatic cancer is reviewed.

2 | SLN IDENTIFICATION IN GASTRIC CANCER

Tumor resection is imperative when treating gastric cancer with curative intent. In addition to total or partial gastric resection, a standardized lymph node dissection is performed. Lymph node metastasis in gastric cancer patients is an established prognostic factor for survival. Lymph node involvement is found in 2% to 50% of patients, increasing with tumor stage.[4] Currently, a fixed number of lymph nodes is resected, regardless of the presence of lymph node metastases. However, since extensive (D2) lymph node dissection is associated with higher morbidity (eg, anastomotic leakage) and mortality compared to D1 lymph node dissection, futile D2 lymphadenectomy in patients without metastatic lymph nodes is unfavorable.[5,6] Possibly, a SLN procedure could avoid

unnecessary lymph node dissection. During a SLN procedure only the primary draining lymph nodes (which are at the highest risk of containing tumor metastases) are identified and dissected. Whereas histological assessment on hematoxylin and eosin (H&E) stained slides can accurately identify metastases for most cancer types, it has been shown that absence of visible tumor cells on H&E slides of gastric cancer lymph nodes does not guarantee absence of micrometastases.[7-11] To overcome this diagnostic challenge, additional examination (eg, immunohistochemistry) is used to detect micrometastases.[12] Although several techniques are available, they are expensive and time-consuming.[13]

Multiple trials have been performed to investigate methods of SLN detection and showed accuracy rates up to 99% when detecting lymph nodes with a dual tracer consisting of radiolabeled tin colloid and blue dye.[14] This dual tracer provides the surgeon with visible blue dye and audible guidance based on radioactivity measured by a handheld probe. However, the disadvantage of this technique is irreversible hampering of the operating field by the blue dye.

Intraoperative NIRF can also be used to identify draining lymph nodes in real time. In contrast to the blue dye, NIRF does not alter the operating field by dark staining and allows detection of deeper situated nodes.

The concept of using NIRF for the identification of draining lymph nodes in gastric cancer was first described by Soltesz et al (preclinical) and Kusano et al (clinical).[15,16] In the first study, fluorescent quantum dots were used in animal experiments and showed successful lymph node detection. The second study described the first inhuman trial using ICG (a cyanine dye which passively accumulates in tumor areas due the EPR effect, with its emission peak at 800 nm) and showed safe and accurate identification of gastric sentinel lymph nodes. Since the success of these studies, numerous other trials have proven the feasibility and safety of this method. However, more false-positive lymph nodes were found than anticipated due to spread of the contrast agent through lymphatic vessels to second-tier lymph nodes.[17-21] Hence, no additional value of the NIRF was observed, since still too many lymph nodes were unnecessarily resected.[22]

To overcome this problem of migration to second-tier lymph nodes, Tummers et al suggested using ICG adsorbed to nanocolloid.[23] This principle was first described in breast cancer and skin melanoma studies.[24,25] The adsorption of nanocolloid to ICG increases its hydrodynamic diameter from <1 nm to 20 to 80 nm. Only molecules with a hydrodynamic diameter of <10 nm can migrate to second-tier lymph nodes.[26] In this way, spreading of ICG to second-tier lymph nodes can be retained.[23]

The study by Tummers et al assessed the feasibility of ICG:Nanocoll for the identification of sentinel lymph nodes in gastric cancer and also investigated the prognostic utility of the detected sentinel lymph nodes.[23] ICG:Nanocoll was injected intraoperatively into four quadrants of the tumor after which fluorescent imaging was performed.

In this study included 22 gastric cancer patients, with varying tumor stages, undergoing partial or total gastrectomy were assessed. In 21 out of 22 patients, at least one fluorescent lymph node was visualized and a mean number of 3.1 (range 1 to 6) lymph nodes were detected. This is significantly lower compared to earlier reported means of 7.2 and 9.3 when using ICG alone.[17,20] The mean tumor-to-background ratio (TBR, strength of fluorescent signal in tumor divided by signal strength of background) was 4.4. Histological examination showed lymph node metastases in 8 out of 21 patients. In six out of these eight patients, metastatic lymph nodes could be detected with the fluorescent signal. Nonfluorescent metastatic lymph nodes (n = 7) were found in the other two patients. All 7 nonfluorescent metastatic lymph nodes, however, were completely obliterated by tumor tissue. This suggests that lymphatic functions such as lymph flow or drainage were hampered, and the fluorescent agent was not able to reach the lymph nodes. In eight patients, the initial treatment plan was altered based on fluorescent lymph nodes found outside of the standard resection plane. In two out of these eight patients, the additional lymph nodes were tumor positive.

Tummers et al conclude that NIRF can aid in identifying additional draining lymph nodes outside of the standard plane of resection (eg, skip metastasis in extraperigastric lymph nodes, which are found in up to 11% of patients) similarly to previous studies performed in breast cancer patients.[24,27,28]

Recent studies have indicated that sentinel node biopsy is most relevant in early gastric cancer cases since this subset of patients have low chances of lymph node metastasis.[29-31] A recent study by Kinami et al has demonstrated the feasibility of this technique in q72 early gastric cancer cases specifically, using ICG.[32] Only one false-negative case was observed, which was due to failure of frozen section diagnosis.

It still remains unclear if the use of ICG in SLN procedures has additional value, since it does not give any information on the presence of micrometastases in identified draining lymph nodes. Possibly, tumor-marker targeted NIRF could assist in this matter. An overview of the mentioned clinical studies is depicted in Table 1.

3 | TARGETED FLUORESCENT-GUIDED SURGERY AND RATIONALE FOR CEA IMAGING

Since nontargeted fluorophores such as ICG, have limited applicability in specifically delineating tumor tissue, the era of receptor-targeted fluorescent-guided surgery has commenced.[34] For this purpose, highly overexpressed tumor markers are required, that are not (or minimally) expressed on normal tissue. CEA is such a molecular marker. CEA is present on embryonic cells and highly expressed by many cancer types including pancreatic ductal adenocarcinoma (PDAC), colorectal cancer (CRC), and gastric cancer. CEA is also expressed under inflammatory conditions. Overexpression of CEA on tumor tissue is seen in over 90% of all CRC and PDAC patients.[35-37] After production and attachment to the cell membrane, CEA is shed into the bloodstream and is therefore a measurable protein in serum. Serum CEA is the only tumor marker that has shown to efficiently monitor therapy response in CRC patients. Consequently, monitoring of serum CEA during follow up has become standard of care.[38]

TABLE 1 Overview of clinical trials and their main outcomes

Author	Patients	Main outcomes
Sentinel lymph node navigation using ICG in gastric cancer		
Kusano et al[16]	22	The detection rate, accuracy, and false-negativity rates were 90.9%, 88.9%, and 33.3% in T1 stage patients, but lower in higher tumor stages. A mean of 3.6 nodes per patients was detected.
Lee et al[87]	22	The sensitivity, specificity, and false-negativity were respectively 100%, 94.4%, and 0.0%.
Tajima et al[20]	56	A mean of 7.2 sentinel nodes was found. A higher accuracy rate was established in T1 stage cancers compared to higher tumor stages.
Yano et al[88]	130	All (100%) lymph nodes were identified and 100% sensitivity was established using ICG. All metastatic lymph nodes were fluorescent.
Kinami et al[32]	72	The sensitivity, specificity, and accuracy of ICG sentinel node mapping were, respectively, 90.1%, 100%, and 98.6%. A median of six fluorescent nodes was found.
Tummers et al[23]	22	Sentinel nodes outside the standard plane of resection were identified using ICG:Nanocoll. In 8 of 22 patients, the initial treatment plan was altered based on the fluorescent imaging.
CEA-targeted fluorescent-guided surgery in colorectal and pancreatic cancer		
Boogerd et al[33]	26	Optimal dosing was set at 10 mg of SGM-101, best imaged 96 hours postinjection. Primary cohort: seven of nine colorectal tumors visualized using fluorescence (two nonfluorescent lesions were pathological complete responders). Expansion cohort: in 6 of 17 patients the treatment strategy was altered based on fluorescence. Sensitivity 98%, specificity 62%, and accuracy 84%.
Hoogstins (unpublished data)	12	Optimal dosing was set at 10 mg of SGM-101, best imaged 96 hours postinjection. All pancreatic tumors were visible using NIR fluorescence, with a mean TBR of 1.6. Also all four metastatic lesions were visible using NIR fluorescence.

Since CEA is highly expressed by many tumor types and absent on healthy tissue, it could serve as a target for tumor-specific imaging and therapy. Previous research has already investigated CEA as a target for therapy of solid tumors including T cell-specific antibodies and radioimmunotherapy.[39-41]

An imaging modality in which CEA could be used as a target is fluorescence-guided surgery. Recently, SGM-101, a CEA-targeted fluorescent probe (emission peak at 700 nm) was developed (SurgiMab, Montpellier, France) and is currently being tested in clinical trials.[42] The BM104 dye (a fluorescent carbocyanine dye) was conjugated to an anti-CEA chimerized monoclonal antibody and extensive preclinical research has been performed.[42] Other CEA-targeted fluorescent probes have been developed and tested preclinically although they have not been used in-human clinical trials yet.[43-46] Boonstra et al[46] have successfully developed a CEA-targeting fluorescent probe using single-chain antibody fragments (ssSM3E/800CW), but have not translated this into clinical trials. DeLong et al[45] have developed anti–CEA-IR-Dye800CW. However, this was also only tested in mouse models so far. SGM-101 was first evaluated in mouse models with peritoneal carcinomatosis. This study showed that SGM-101 allowed identification and resection of very small tumors that would otherwise not have been detected.[42] Next, experiments using SGM-101 in orthotopic colon cancer grafts, liver metastases, and pancreatic tumor cell xenografts were performed.[42] All three experiments showed great promise in detecting and demarcating tumor nodules that were previously not visible. Histological assessment of tumor tissue showed colocalization of tumor cells and fluorescent signal.

Currently, there are two ongoing clinical trials using SGM-101. The first is performed by our group (Clinicaltrials.gov: NCT02973672). SGM-101 is administered 2 to 6 days before surgery with a dose escalation of 5 to 15 mg in patients with pancreatic cancer, primary CRC, recurrent rectal cancer or colorectal peritoneal metastases. The second trial includes patients with peritoneal carcinomatosis from digestive cancers (FLUOCAR-1 trial) and is performed by the group of Dr Francois Quenet in Montpellier, France (Clinicaltrials.gov: NCT02784028). In this trial, SGM-101 is administered 1 to 2 days before surgery with a dose escalation of 5 to 15 mg. The main goal of this study is to determine the optimal dose for phase II studies.

4 | CEA AND CRC

The cornerstone of CRC treatment is resection of the tumor with clear margins (R0 resection). Despite neoadjuvant treatment protocols for rectal cancer, up to 17.2% of rectal cancer patients and 5.3% of colon cancer patients still have positive resection margins (R+).[47,48] In rectal cancer patients with threatened or involved circumferential resection margins, R1 resections are seen in up to 25% of the patients.[49,50] Positive resection margins are correlated with higher rates of local recurrence (up to 40%) and worse disease-free and overall survival.[51-53]

CEA serum measurements are currently used during follow-up and can indicate local recurrence or metastatic disease when values are rising.[38] Interestingly, a recent histopathological study by Boogerd et al[54] showed lack of correlation between serum CEA

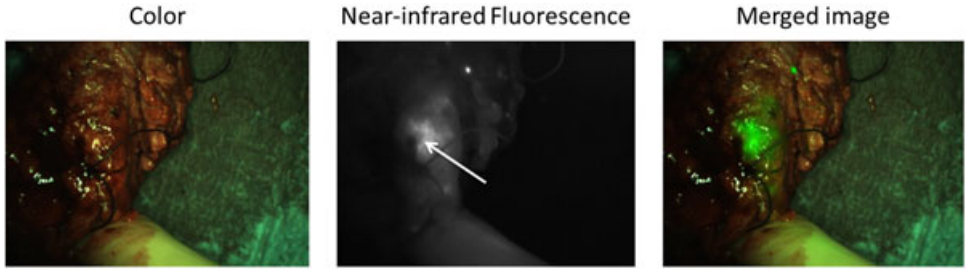

FIGURE 1 Ex vivo fluorescence imaging of a recurrent rectal tumor using SGM-101 (injected 4 days before surgery, 10 mg). The remaining fluorescence (arrow) was confirmed tumor-positive. Color (left column), fluorescence (middle column), and merged (right column) images [Color figure can be viewed at wileyonlinelibrary.com]

and tumor CEA expression in rectal cancer. Most important for fluorescent-guided surgery, a great difference is seen in expression levels of CEA between CRC tissue and healthy tissue (60-fold).[55] The antigenic density of CEA on CRC cells is 10^5 to 10^6 antigens per cell.

Synchronous peritoneal metastases are seen in 4.3% of primary CRC patients, and in 20% to 50% of patients with recurrent CRC.[56-61] With the introduction of cytoreductive surgery (CRS) in combination with hyperthermic intraperitoneal chemotherapy, overall median survival has risen from 12 to 32 months.[62] Since the peritoneal cancer index and the extent of resection in CRC are associated with survival, maximal resection of all peritoneal cancer tissue is crucial.[63] As fluorescent-guided surgery can detect (often small) metastatic nodules, it could help detect and resect metastatic disease, thereby increasing local control and survival.

The combination of a good level of contrast between healthy and cancer tissue and high antigenic density makes CEA an excellent candidate for fluorescent-guided surgery in CRC. Furthermore, CEA expression does not seem to alter after neoadjuvant therapy.[64]

Several CEA-targeting fluorescent probes have been tested preclinically in colon cancer models. Anti–CEA-IRDye800CW has been tested by DeLong et al[45] in orthotopic mouse models injected with HT-29 colon cancer cells. This study demonstrated effective labeling of CEA-expressing human colon cancer in mouse models during laparotomy at both 24 and 48 hours after injection. Boonstra et al[46] developed a single-chain antibody fragment (ssSM3E/800CW) to visualize CRC lesions. Animal experiments showed a mean TBR of 2.37 at 24 hours postinjection, which is sufficient for clear identification of tumor tissue. At histological assessment, CEA expression clearly correlated with the NIRF signal using light and fluorescence microscope.

SGM-101 is a clinically translated fluorescent agent targeting CEA. Recently, Boogerd et al[33] have published the first in-human clinical trial with CEA-targeted fluorescent-guided surgery in CRC patients. This study assessed the tolerability and pharmacokinetics of ascending doses of SGM-101 and determined the optimal dose and timing for fluorescent-guided surgery (10 mg, 96 hours postinjection). In the primary cohort seven of nine tumors showed in vivo or ex vivo fluorescent signal (mean TBR 1.8). The two nonfluorescent rectal tumors were confirmed as pathological complete responders, thus no tumor tissue was present. An expansion cohort of 17 patients with recurrent rectal cancer or peritoneal metastasis of CRC was set up. In 6 of 17 patients, the original treatment strategy was altered based on fluorescent imaging findings. It was concluded that SGM-101 showed a sensitivity of 98%, specificity of 62% and an accuracy of 84% in detecting rectal cancer. Figure 1 shows the ex vivo fluorescence imaging of a recurrent rectal tumor using SGG-101.

CEA-targeted fluorescent-guided surgery could aid in better resection of primary colorectal tumors, colorectal recurrence, and metastases (eg, liver, lung, peritoneal). Particularly in local recurrent rectal cancer, fluorescent-guided surgery could be useful since distinguishing fibrosis from tumor tissue can be challenging. Also, during the follow up period after CRC surgery, tumor-targeted fluorescent imaging could be of additional value. Currently, conventional endoscopy is used to assess the anastomosis and possible other newly developed or missed tumors. Fluorescent-guided endoscopy could possibly better indicate tumor remnants or local regrowth. Fluorescent-guided endoscopy targeting VEGF (bevacizumab) and c-Met is currently being investigated in clinical trials at the University Medical Centre Groningen in The Netherlands (Clinicaltrials.gov: NCT03205501).[65,66] CEA also shows great promise to be a successful imaging target for endoscopy. For example, in patients with a clinical complete response (cCR) after neoadjuvant chemoradiotherapy in whom surgery is omitted and are followed in a Watch-and-Wait protocol. In the same way, CEA-targeted positron emission tomography (PET) imaging could aid in detecting tumors. By targeting tumor cells, the radioactive signal could indicate remaining or recurrent tumors. This concept is already routinely performed in prostate cancer with a prostate-specific membrane antigen PET tracer but could also be useful in CRC cancer staging and follow up using a CEA-targeted tracer.

5 | CEA AND PANCREATIC CANCER

Compared to the relative good overall survival of CRC cancer patients, pancreatic cancer still has a dismal prognosis. Pancreatic cancer accounts for 7% of all cancer related deaths in the United States, with the vast majority of neoplasms being PDACs.[67] The overall 5-year survival rate is 8%, whereas patients diagnosed with metastatic disease only have a 3% five-year survival rate.[67,68] The ESPAC-4 trial underlines the importance of radical resection of pancreatic cancer considering the median overall survival time of

39.5 months after R0 resection versus 23.7 months after R1 resection (both observed in patients treated with adjuvant gemcitabine plus capecitabine).[69] For the eligible patients, pancreatic resection is associated with serious morbidity (eg, pancreatic fistula and hemorrhage; 19.9%), perioperative mortality (2%) and up to 30% disease-related mortality in the first year after resection.[70-73] CT is the preferred modality and has shown to be able to predict resectability in >75% of patients.[74] Intraoperative distinction between normal (often inflamed) tissue and tumor tissue is challenging, resulting in up to 70% irradical resections.[75] CT is insufficient in detecting tumor downstaging after neoadjuvant therapy and is also inadequate in distinguishing viable tumor cells from scar tissue. Thus, neoadjuvant treatment can complicate the assessment of resectability on CT even more.[76,77]

Amongst others, fluorescent-guided surgery could potentially aid in distinguishing noncancerous pancreatic tissue from tumor tissue and thus might lead to a higher rate of radical resections, resulting in increased overall survival. Additionally, this method could help identify (occult) metastases during staging laparoscopy.[78] The role of staging laparoscopy is currently under debate, since only 15% to 51% of occult metastasis cases can be identified using this modality.[79] A study investigating staging laparoscopy using contrast-enhanced ultrasound and fluorescence (ICG) has been performed by our group.[80] Twenty-five patients were included in this study. Patients received 10 mg of ICG 1 day prior to surgery to detect possible liver metastases. Intraoperative fluorescence imaging and laparoscopic ultrasound of the liver were performed. Of every lesion suspect for metastasis a biopsy or resection was performed. This study concluded that intraoperative use of ultrasound has limited value. However, laparoscopic fluorescence imaging using ICG showed the highest accuracy in detecting liver metastases compared to inspection and laparoscopic ultrasound.

The concept of fluorescent-guided surgery in pancreatic cancer has already been tested with ICG. Unfortunately, insufficient contrast between benign and malignant tissue was achieved and no additional value of optical fluorescent-guided surgery was found.[81]

In search of other tumor-specific imaging targets, de Geus et al found that CEA, integrin αvβ6, epithelial growth factor receptor and urokinase plasminogen activator receptor seem suitable targets for pancreatic imaging.[82] In this study, 158 of 165 (96%) of pancreatic and periampullary adenocarcinomas could be identified using CEA immunohistochemistry.

Tummers et al[83] observed membrane-bound, heterogeneous expression of CEA in PDAC and a significantly lower expression on chronic pancreatitis compared to PDAC. CEA is present in PDAC, but not in normal acini and healthy pancreatic tissue. Unfortunately, loss of expression after neoadjuvant treatment was seen and is disadvantageous since no distinction between vital and necrotic tumor tissue can thus be made after neoadjuvant treatment based on CEA expression.[83]

As mentioned before, SGM-101 is also being investigated for intraoperative imaging in pancreatic cancer patients. Gutowski et al[42] showed that in orthotopic mouse models injected with BxPC3 cells (pancreatic cancer), SGM-101 showed clear tumor delineation with a TBR of 3.5. Experiments using other CEA-targeted fluorescent agents (anti–CEA-Alexa Fluor 488) on BxPC3 cells in mouse models were performed by Metildi et al[84] and Cao et al.[85] In these studies, fluorescent-guided surgery resulted in less tumor recurrence ($P = 0.01$), higher cure rates (45% vs 40%, $P = 0.01$) and higher 1-year survival rates (0% vs 28%, $P = 0.01$) when compared to conventional bright light surgery.

Following the successful preclinical experiments, our group included 12 PDAC patients in a clinical trial (Clinicaltrials.gov: NCT02973672, Hoogstins, unpublished data). No clear difference was observed in TBR between the three dosing groups and 96 hours postinjection was established as the preferred time of imaging. The study demonstrated that the use of SGM-101 in pancreatic cancer patients is safe. All tumors were visible with intraoperative fluorescent imaging, with a mean TBR of 1.6 in primary tumors and 1.7 in metastatic lesions. All metastatic lesions (three liver and one peritoneal metastasis) were visible using fluorescence. Additionally, eight nonfluorescent but clinically suspect lesions (not primary tumors) were resected, of which two contained tumor cells.

Of the initial 12 patients, in five patients the resection was abandoned due to irresectability or metastases. These five patients also had higher serum CEA levels preoperatively. This possibly indicates a predictive role for CEA in resectability of pancreatic tumors.

The TBR as seen in intraoperative setting was significantly lower than expected from the mouse models (TBR 3.5). A possible explanation for this can be the poor vascularization of pancreatic tumors and presence of desmoplastic stroma in pancreatic tumors, resulting in poor delivery of SGM-101.[86] However, the fact that fluorescent signal was observed in most patients demonstrates that the contrast agent was undoubtedly able to reach the tumor cells. The limitations of this “far red” part of the NIR spectrum are more autofluorescence and lower depth penetration compared to the higher NIR wavelengths.[89]

Currently, fluorescent agents targeting the endothelial growth factor receptor and the vascular endothelial growth factor (VEGF; PENGUIN trial) are also being clinically tested for pancreatic cancer imaging (Clinicaltrials.gov: NCT03384238, NCT02743975).

6 | DISCUSSION

This review outlines the available literature on SLN procedures in gastric cancer using ICG and provides an overview of CEA-targeted NIRF imaging. An SLN procedure using ICG:Nanocoll has shown to be feasible and safe; however, the clinical applicability is still unclear. Only one CEA-targeted NIRF agent, SGM-101, has yet been tested in clinical trials. Successful studies using SGM-101 have been described in pancreatic cancer, CRC, and peritoneal metastasis of digestive cancers.

The use of fluorescence imaging with ICG during SLN procedures in gastric cancer remains controversial. Since no specific receptor target is used, the fluorescent signal is not specific for lymph node

metastases. As shown by Tummers et al[23] distinguishing tumor-positive lymph nodes from tumor negative lymph nodes is impossible using ICG:Nanocoll. Also, as discussed above, completely obliterated lymph nodes cannot be reached with ICG and are thus not visible with fluorescence, underlining the need for tumor-specific fluorescent tracers.

During CRC surgery intraoperative fluorescence imaging was successful. Definitely, in the expansion cohort with recurrent rectal cancer and peritoneal metastasis of rectal cancer, an additional value of SGM-101 has been observed. Following the recent successful trial with SGM-101 in CRC, a multicentre, phase III clinical trial will soon be initiated. A large cohort of CRC patients will be included in up to 10 centers in Europe and the United States. The rationale for this study is that SGM-101 fluorescence imaging can offer clear delineation of tumor masses, identify subclinical carcinomatosis or aid in the assessment of residual disease and thus lead to more radical resections with hopefully less local recurrence or metastatic disease.

Furthermore, CEA-targeted imaging could be helpful during the follow up of rectal cancer patients with a cCR after neoadjuvant chemoradiotherapy. Patients with a cCR might enter a follow-up period of watchful waiting (in clinical studies) and surgery will be omitted. Considering that only vital tumor cells express CEA, CEA-targeted fluorescent signals in routine follow up endoscopy could show remaining tumor cells after therapy or local regrowth during follow up.

In spite of the poor vascularization and presence of stroma in PDAC, molecular imaging of PDAC targeting CEA was feasible. The fluorescent signal was observed in all pancreatic tumors. This study demonstrates the feasibility of antibody-targeted fluorescent imaging using SGM-101 in pancreatic cancer. Considering the much higher TBR's measured ex vivo versus in vivo, there might be a need for more sensitive intraoperative imaging systems. In addition to CEA, integrin αvβ6 also shows great promise for targeted imaging and will be investigated for both optical fluorescence and PET tracer purposes by our group. Possible applications for tumor-targeted pancreatic imaging could be in detecting primary and metastatic disease, but also in assessing vascular involvement. Particularly after neoadjuvant chemotherapy, NIRF could aid in distinguishing chemotherapy induces fibrosis from viable tumor tissue.

Because CEA expression on the cell surface of tumors is variable, patient selection before surgery would be beneficial. However, since Boogerd et al[54] described a lack of correlation between tumor CEA expression and serum CEA levels in rectal cancer, serum CEA levels seem inadequate for patient selection. In contrast to rectal cancer, the study did find a significant correlation between serum CEA and the percentage of CEA-expressing tumor cells in PDAC patients. A possible explanation for this could be the different vascularization of both tumor types (rectal tumors are usually well vascularized whereas pancreatic tumors show poor vascularization and drug delivery). In PDAC however, predicting CEA tumor expression using serum CEA levels seems to be more accurate.

In addition to the described CEA-targeted antibody, various other methods of tumor targeting are available. Specific targeting using smaller tumor-targeting particles (eg, nanobodies, antibody fragments, peptides) can increase tumor specificity and enable injection at the day of surgery. When combining this with a fluorophore emitting light at higher wavelengths, lower background signal could be achieved.

In conclusion, this review shows that SLN procedures for gastric cancer resections using ICG:Nanocoll outperformed procedures using normal ICG. However, the clinical relevance can be argued since the fluorescent signal only indicates lymph node presence, but gives no information as for the presence of lymph node metastasis. Tumor-specific targeting by CEA-targeted fluorescent imaging using SGM-101 was successful in both pancreatic and CRC patients. A large phase III trial will soon be initiated in CRC patients.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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How to cite this article: Vuijk FA, Hilling DE, Mieog JSD, Vahrmeijer AL. Fluorescent-guided surgery for sentinel lymph node detection in gastric cancer and carcinoembryonic antigen targeted fluorescent-guided surgery in colorectal and pancreatic cancer. *J Surg Oncol*. 2018;118:315–323. https://doi.org/10.1002/jso.25139