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Hollandsworth, Hannah M
Turner, Michael A
Hoffman, Robert M
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A Review of Tumor-Specific Fluorescence Guided Surgery for Colorectal Cancer

Hannah M. Hollandsworth, MD[1,2], **Michael A. Turner, MD**[1,2], **Robert M. Hoffman, PhD**[1,2,3], **Michael Bouvet, MD**[1,2,4]

[1]Department of Surgery, University of California San Diego, San Diego CA

[2]Moores Cancer Center, University of California San Diego, San Diego, CA

[3]AntiCancer Inc., San Diego, CA

[4]VA San Diego Healthcare System, San Diego, CA

Abstract

The present study reviews the use of tumor-specific antibodies conjugated to fluorescent dyes in preclinical and clinical studies to enhance visualization of primary tumors and metastases for fluorescence-guided surgery (FGS) in colorectal cancer (CRC). A search strategy was developed using the peer-reviewed National Center for Biotechnology Information (NCBI) database on PubMed. Studies using tumor-specific fluorescence imaging and FGS techniques on murine models of colorectal cell lines or patient-derived colorectal cancer are reviewed. A total of 24 articles were identified that met the inclusion criteria, 21 preclinical and 3 clinical trials. The most widely used target antigen in preclinical and clinical trials was carcinoembryonic antigen. Mouse studies and clinical studies have demonstrated that the use of FGS in CRC can aid in decreased residual tumor and decrease rates of recurrence. As the mainstay of colorectal cancer treatment is surgery, the addition of intraoperative fluorescence imaging can help locate tumor margins, visualize occult micro-metastases and drive surgical decision making.

Keywords

Fluorescence-guided Surgery; Colorectal Cancer; Tumor-specific Antibodies

Corresponding Author: Michael Bouvet, MD, UCSD Moores Cancer Center, 3855 Health Sciences Drive #0987, La Jolla, CA 92093-0987, mbouvet@ucsd.edu, Phone: 858-822-6191, Fax: 858-822-6192.

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INTRODUCTION

The mainstay of treatment for colorectal cancer (CRC) is largely surgical resection. Although effective, the rate of positive margins after resection has been previously reported to be 6.87%, which can lead to increased rates of distant metastases and recurrence [1]. Additional studies also demonstrated that radial margins that are positive for occult residual disease lead to a significantly worse prognosis [2]. Achieving negative margins after surgical resection is imperative for CRC.

Fluorescent-guided surgery (FGS) is an emerging technology that has been shown in numerous mouse models and increasing numbers of clinical trials to be more sensitive than direct visualization or palpation in intraoperative detection of tumors [3]. Prior studies have been successful at the creation of fluorescently-labeled monoclonal antibodies, which are useful in detecting orthotopic mouse models and metastases of pancreatic cancer [4–7].

FGS may also be effective for identifying margins in CRC surgery, to improve the rate of complete oncologic resection. In the present study, we review the literature pertaining to the use of tumor-specific antibodies conjugated to fluorescent probes to demonstrate the potential of FGS for improved visualization of micro-metastases and improved complete oncologic resection of colorectal cancer in patient-derived orthotopic mouse models and clinical trials.

MATERIALS AND METHODS

A search strategy was developed using the peer-reviewed National Center for Biotechnology Information (NCBI) database on PubMed, which was accessed February, 2020. Search phrases included “fluorescence-guided surgery AND colorectal cancer.” All studies involving antibody-based imaging for colorectal cancer in mouse models were included. Studies using indocyanine green were excluded for the purposes of this study. Prior review articles were also excluded. Studies using green-fluorescent protein (GFP) were included in the study to introduce the concept and initial preclinical work with tumor-specific fluorescence imaging in CRC.

RESULTS

Using the PubMed database, 113 studies were initially identified. After review and application of inclusion and exclusion criteria, a total of 24 studies were included in this review, 21 preclinical studies and 3 clinical studies (Figure 1).

A total of six antibody targets were identified in the included studies. These antibodies were conjugated to various fluorophores, including IRDye800CW (IR800, LI-COR, Lincoln NE), IRDye700DX (IR700, LI-COR, Lincoln NE), Cyanine 7.5, BM104 which has an emission of 700 nm and AlexaFluor 488 (Molecular Probes Inc., Eugene, OR). A summary of antibody targets, fluorophores and applications is provided in Table 1.

Pre-clinical Trials

Green-fluorescent Protein—Initial studies compared outcomes in bright light surgery to FGS for primary colon tumors in orthotopic mouse models. One study performed by Metildi et al utilized a colon cancer cell line that expressed green-fluorescent protein (GFP) to establish orthotopic mouse models [8]. Mice were randomized into bright light surgery (BLS) or FGS. Post-operative images demonstrated higher burden of residual tumor for the BLS group and no residual tumor in the FGS mice. An additional study compared BLS to FGS using GFP in orthotopic mouse models and demonstrated improved visualization of the primary tumor as well as detection of micro-metastases that were otherwise invisible [9].

Murakami et al. performed FGS on orthotopic liver metastases models using a GFP-tagged cell line [10]. Bright-light imaging after resection was unable to identify residual disease but fluorescence imaging demonstrated detection of occult residual tumor in the resection bed [10]. In addition, mice that underwent FGS of orthotopic liver metastases had significantly reduced rate of recurrence and prolonged overall survival compared to BLS alone [10]. Yano et al. also utilized GFP-containing telomerase-dependent adenovirus (OBP-401) to successfully label orthotopic mouse models of colon cancer liver metastases [11]. Pre-operative labeling with OBP-401 allowed for detection of occult residual tumor after BLS that was able to be fully resected under FGS. Orthotopic mouse models that underwent FGS guided by telomerase-dependent GFP had a recurrence rate of 19% compared to standard BLS resection, which demonstrated a recurrence of liver metastases in 94% of mice (Figure 2) [11]. These studies proved the principle that tumor-specific fluorescence could visualize otherwise occult tumor margins and metastases and thus, FGS may be a useful adjunct to surgical resection of CRC.

Carcinoembryonic Antigen—Human carcinoembryonic antigen (CEA) is a common biomarker for gastrointestinal epithelial cancers and is expressed in most colon tumors [12, 13]. Mouse studies have demonstrated specific colon tumor detection with fluorescence imaging of patient derived orthotopic xenograft (PDOX) models using a fluorescently-labeled anti-CEA antibody [14]. After FGS with the fluorescent anti-CEA antibody, histology demonstrated negative tumor margins, indicating a complete tumor resection. Another study utilized an anti-CEA antibody with a fluorescent probe and radioisotope labelling for pre-operative PET/CT detection and intra-operative detection of colorectal tumors in mouse models [15]. Fluorescence and PET/CT imaging demonstrated specific visualization of intraperitoneal colorectal tumors in nude mice [15]. This dual-modality may be useful for pre-operative and intra-operative detection of occult tumors.

Further studies compared a chimeric anti-CEA antibody conjugated to AlexaFluor 488 to the previously used mouse anti-CEA antibody and concluded that the chimeric antibody had improved conjugation efficacy and a significantly higher tumor fluorescence signal [16]. This study then compared BLS with FGS in PDOX mouse models using the chimeric anti-CEA antibody and demonstrated an increase in the rate of negative margin resection from 86% to 96%, respectively. Although not statistically significant, this study demonstrates the use of FGS using anti-CEA antibody to decrease rates of recurrence through higher rates of

negative margins and complete oncologic resection [16]. This study confirmed that chimeric anti-CEA was efficacious for FGS and provided improved translatability to clinical studies.

Further development of anti-CEA antibodies led to the production of a humanized anti-CEA antibody conjugated to a near-infrared fluorophore, which was also been proven to successfully target colon cancer in orthotopic mouse models [17]. This provides a novel humanized antibody that could be readily translated into patient populations. Another study created a novel CEA antibody (ssSM3E) conjugated to an IR800 dye and demonstrated distinctly visualized tumor margins with a tumor to background ratio 5.1 [18].

The liver is the most common site of metastases in CRC; therefore, detection and visualization of liver metastases can aid in staging and surgical treatment of metastatic CRC. The anti-CEA antibody conjugated to a near-infrared fluorophore has also been successful for labeling and performing FGS on liver metastases in mouse models [19]. One study compared the use of fluorescence imaging with anti-CEA antibody conjugated with a near-infrared fluorophore compared to GFP imaging of colorectal liver metastases and demonstrated that the near-infrared fluorescence signal was detectable in tissues that were covered by normal liver tissue greater tissue, while GFP-labeled tumors were only visible if completely exposed on the surface of the liver parenchyma [19]. Mice that underwent FGS had significantly less residual tumor area than BLS in this study (< 1 mm^2 versus approximately 3.5 mm^2, respectively, $p < 0.001$) and significantly improved disease-free survival and overall survival [19].

One study created a novel chimeric anti-CEA antibody conjugated to a 700 nm dye BM104 (SGM101) [20]. In this study, various mouse models were studied, including orthotopic colon, peritoneal metastases and liver metastases. In all models, tumor margins were distinctly visualized and sub-millimeter metastases were detected in the metastatic models with the fluorescent antibody [20].

Rijpikema et al also assessed mouse models of intraperitoneal CRC nodules with an anti-CEA antibody (MN-14) conjugated to IR800 and a radiolabel for radionucleotide detection [21]. An additional study considered this dual reporter in mouse models using anti-CEA antibody labetuzumab, conjugated to IR800m and labelled with a radiotracer [22]. These studies concluded that this dual approach can improve pre-operative and intraoperative detection of small colorectal tumor nodules, which may improve rates of complete resection [21, 22].

Carcinoembryonic Antigen-related Cell Adhesion Molecules—Carcinoembryonic antigen-related cell adhesion molecules (CEACAMs) are members of the CEA gene family and the immunoglobulin (Ig) superfamily [23]. These molecules play a role in cell signaling, cell adhesion and tumorigenesis in CRC [23, 24]. 6G5j is an anti-CEACAM antibody with affinity for CEACAM 1, 3, 5, 6 and 8 [25]. A study by Hollandsworth et al. demonstrated overexpression of CEACAMs in the majority of patient-derived CRC tumors via western blotting. Tumors were chosen based on high expression of CEACAM on western blotting and sensitive binding of anti-CEACAM antibody 6G5j conjugated to IR800 with detection of micro-metastases that were not visualized with bright light (Figure 2) [25]. Since varying

CEACAM's may be overexpressed in different colorectal tumors, the ability to bind multiple CEACAM's may provide improved detection if one or more CEACAM's are not over-expressed.

Claudin—Claudins are a family of tight-junction proteins that function to maintain epithelial barrier function [26]. An over-expression and deregulation of Claudin through the APC-Wnt pathway has been implicated in tumorigenesis and progression of colon cancer [27]. Claudin-1 is over-expressed in human colonic adenomas, with a 2.5-fold increase in Claudin gene expression in adenomas compared to normal colonic tissue [27]. Rabinsky et al. demonstrated specific labeling of flat and polypoid adenomas in APC knockout mice that spontaneously form colonic polyps with significantly higher target-to-background ratio [28].

Anti-claudin-1 conjugated to near-infrared fluorophore IR800 has shown to be useful for imaging of PDOX mouse models of colon cancer. A study by Hollandsworth et al. demonstrated that primary orthotopic colon cancer was readily visualized with minimal background signal and distinct tumor margins with fluorescent anti-claudin antibody [29]. Time-course imaging showed that optimal delivery of the antibody-fluorophore conjugate to the tumor occurred after 48 hours; therefore, fluorescence imaging is ideal two days after administration [29]. No toxicity was observed in any of the mouse models. Small regional metastases in orthotopic mouse models were also identified with the fluorescent anti-claudin antibody, which were otherwise invisible (Figure 3) [29]. This study also demonstrated that there is a higher expression of claudin-1 in patient-derived colon cancer metastases compared to the primary colon tumor, based on western immunoblotting [29]. Claudin-1 antibodies have yet to be studied in human patients but provide a promising target for future clinical studies on FGS in CRC.

Insulin-like Growth Factor-1—Insulin-like Growth Factor-1 (IGF-1) is a transmembrane protein that has been shown to be overexpressed in 51–100% of colon cancers [30]. In a study by Park et al, anti-IGF-1 antibody, conjugated to fluorophores with wavelengths varying from 550–650 nm, was utilized for detection of colorectal tumors in nude mice [31]. Subcutaneous, orthotopic and metastatic models were labeled with fluorescent anti-IGF-1, which enabled specific and sensitive imaging of liver and peritoneal metastases [31].

Epidermal Growth Factor—Epidermal growth factor receptor (EGFR) is overexpressed in many epithelial cancer types and prior studies have demonstrated overexpression in 69–80% of colorectal cancers [32]. Pantimumab, an anti-EGFR antibody, conjugated to IR800 dye was used to visualize colon cancer mouse models in a study by Marston et al. [33]. In this study, small tumor fragments less than 1 mm diameter were readily visualized with fluorescent imaging, which is ideal for imaging small metastases and small tumors [33].

Epithelial Cell Adhesion Molecule—Epithelial cell adhesion molecule (EpCAM) is a transmembrane glycoprotein that is involved in cell-cell signaling and plays a role in stromal adhesion [34]. EpCAM has been found to be overexpressed in various epithelial cancers, including colorectal tumors [35]. In a study by van Driel et al, an anti-EpCAM antibody conjugated to IR800 demonstrated clear tumor margins of orthotopic colon tumors in mouse models 72 hours after administration [36].

Urokinase Receptor—Urokinase receptor (uPAR) has been implicated in tumorigenesis and development of metastases in CRC [37]. One study utilized an anti-uPAR antibody conjugated to both radionucleotide and ZW800, a near-infrared fluorophore that fluoresces at 800 nm [38]. In this study, PDOX models imaged and demonstrated visualization of 1–2 mm tumors 72 hours after administration of the antibody-fluorophore conjugate. In addition, PET imaging allowed for improved pre-operative detection of small tumor nodules [38].

Additional Applications—The use of fluorescence in early detection during colonoscopy has also been assessed in other rodent models. Jones et al. studied the use of tumor-targeting peptide (LS301) conjugated to near-infrared dye Cy7.5 for colonoscopy detection of polyps in rat models [39]. Flat polyps that were not detectable under bright light colonoscopy could be detected with near-infrared imaging. The polyp was removed at the time of procedure and recurrence was assessed with near-infrared imaging five weeks later. In all animals, there was no recurrence of disease [39].

Clinical Trials—Tjalma et al utilized a fluorescently labeled anti-vascular endothelial growth factor (VEGF)-A antibody bevacizumab to evaluate patients for residual tumor during endoscopy after neoadjuvant chemotherapy for rectal cancer [40]. Fluorescence endoscopy with bevacizumab was able to detect residual tumor that was not visualized with standard bright-light endoscopy [40].

A study by Haarlar et al also utilized bevacizumab conjugated to IR800 for detection of tumor in patients with colorectal peritoneal metastases [41]. Out of the seven patients enrolled in this study, surgical decision making was altered in two patients due to the use of intra-operative fluorescent imaging. Surgeons were able to detect small peritoneal implants. However, histopathology demonstrated a high rate of false positives, likely due to the lack of specificity of VEGF for CRC [41]. No adverse effects were noted in the patients. Multiple clinical trials also studied intraoperative imaging of colorectal peritoneal metastases using SGM-101, the chimeric anti-CEA antibody mentioned above [42, 43]. One study enrolled 17 patients and a total of 43 peritoneal implants were identified with fluorescence imaging that were not visible intraoperatively or identified on preoperative imaging [42]. In another study, a change in peritoneal carcinomatosis index, which is an indicator for extent of disease, was modified in 14 patients due to visualization of additional tumors with fluorescent imaging using SGM-101 [43].

DISCUSSION

Colorectal cancer is an important application for FGS, especially in the setting of liver and peritoneal metastases. Fluorescence imaging can aid in detection of micro-metastases otherwise not visualized at the time of surgery. Tumor-specific antigens targeted in the reviewed studies include CEA, CEACAM, Claudin, IGF-1, EGFR, EpCAM and uPAR. These various antibodies readily bound to colorectal tumors in mouse models and may be useful in clinical trials of FGS for CRC. The most widely used antibody for colorectal tumor targeting in mouse models and clinical studies are anti-CEA antibodies. The advances to humanized versions to allow anti-CEA antibodies to be readily translatable in clinical studies.

Mouse models of CRC, especially orthotopic models of liver metastases, have demonstrated proof of principle that FGS is beneficial for complete resection of this disease. We have demonstrated in orthotopic mouse models, including PDOX models, that tumor-specific fluorescence, whether with GFP or labelled antibodies, provides improved detection of tumor margins and micro-metastases.

Initial clinical studies have demonstrated improvement in preoperative and intraoperative imaging of peritoneal metastases from colorectal cancer. In these trials, surgeons were able to identify a significantly higher number of peritoneal implants that were otherwise invisible under bright-light surgery [42, 43]. The ability to identify occult peritoneal metastases could aid in achieving a complete oncologic resection in the setting of CRC peritoneal carcinomatosis. There are currently no clear guidelines on the role of surgery in CRC peritoneal carcinomatosis. Further clinical trials on the use of FGS for peritoneal metastases in CRC may lead to improved outcomes and a larger role for surgical resection in this disease. The most common colorectal-specific antigen targeted in clinical trials was CEA, which is overexpressed in the majority of CRC and proven to be effective in many preclinical trials.

The most commonly utilized fluorescent probes include IRDye800CW and IRDye800DX. Current laparoscopic and robotic platforms are equipped for visualization of near-infrared probes with 700 and 800 nm wavelength, therefore, monoclonal antibodies conjugated to these probes can be readily translated into clinical intraoperative practices. Clinical studies have demonstrated safety of near-infrared dye IR800 in patients [44]. A phase II trial is also being conducted on the use of IR700 dye for photodynamic treatment of inoperable head and neck cancers. Therefore, the use of these near-infrared probes has been proven to be safe in patients, increasing the clinical translatability of these preclinical mouse trials for CRC.

Pre-operative imaging is imperative to the staging of CRC. Preclinical studies utilizing antibodies labelled with a radionucleotide tracer demonstrated improved pre-operative detection of small tumor nodules in mouse models. Clinical adaptation of this technology can help to improve staging of colorectal cancer, which guides decision making for appropriate treatment. In addition to pre-operative staging, FGS can be useful for rectal tumors, where it can be difficult to obtain negative margins in a relatively small operative field. It can also help to clearly delineate tumor margins for intra-operative planning.

While surgery is the mainstay of treatment for CRC, there remain challenges in treatment strategies for hepatic and peritoneal metastases. For liver metastases, treatment options include surgical resection, only if complete resection of metastases is possible [45]. In this review, we show with multiple studies that the use of FGS may help to detect hepatic metastases not visualized on pre-operative imaging and improve complete resection of metastases for improved survival. This is especially useful for staging purposes when occult liver metastases are not visualized with standard pre-operative imaging, such as computed tomography or MRI. Peritoneal carcinomatosis in colorectal cancer is also a condition without clear treatment guidelines [46]. Cytoreductive surgery is occasionally employed, which may be improved with the use of intra-operative fluorescence imaging. Clinical studies have demonstrated that the use of intraoperative fluorescence imaging can lead to

improved detection of peritoneal metastases that cannot be seen with the naked eye. FGS may improve treatment options for these difficult to treat conditions in patients with colorectal cancer.

CONCLUSION

Mouse studies and clinical studies have demonstrated that fluorescence labelling with either genetic reporters or tumor-specific antibodies enables detection of otherwise occult tumor margins and micro-metastases. FGS has resulted in decreased residual tumor and reduced rates of recurrence, including cures not possible with bright light surgery. As the mainstay of colorectal cancer treatment is surgery, the addition of intraoperative fluorescence imaging can help guide resection, visualization of occult metastases and drive surgical decision making. Continuing preclinical and clinical studies will help to determine the most advantageous uses for FGS in CRC surgery.

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- Fluorescence imaging decreases positive margin in mouse models of colorectal cancer
- Fluorescence imaging improves complete resection rates of colorectal liver metastases
- Fluorescence guided surgery allows detection of occult colorectal metastases

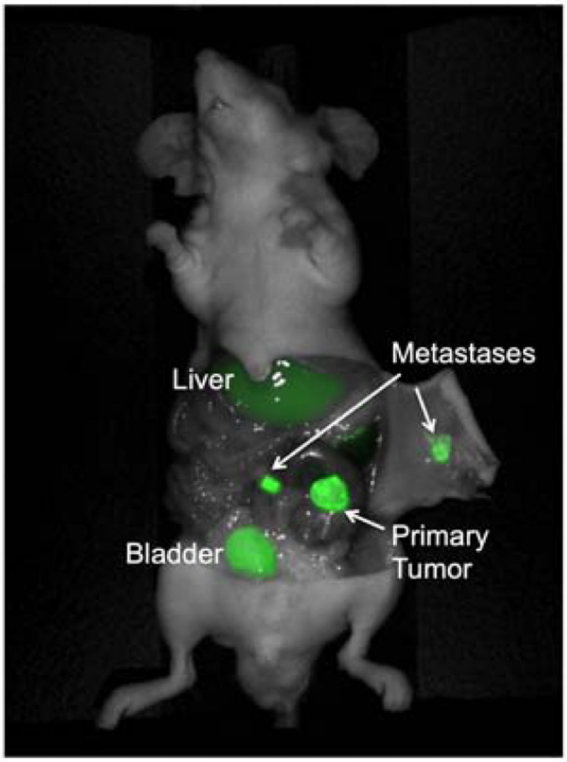

Figure 1:
Schematic representation of search methods.

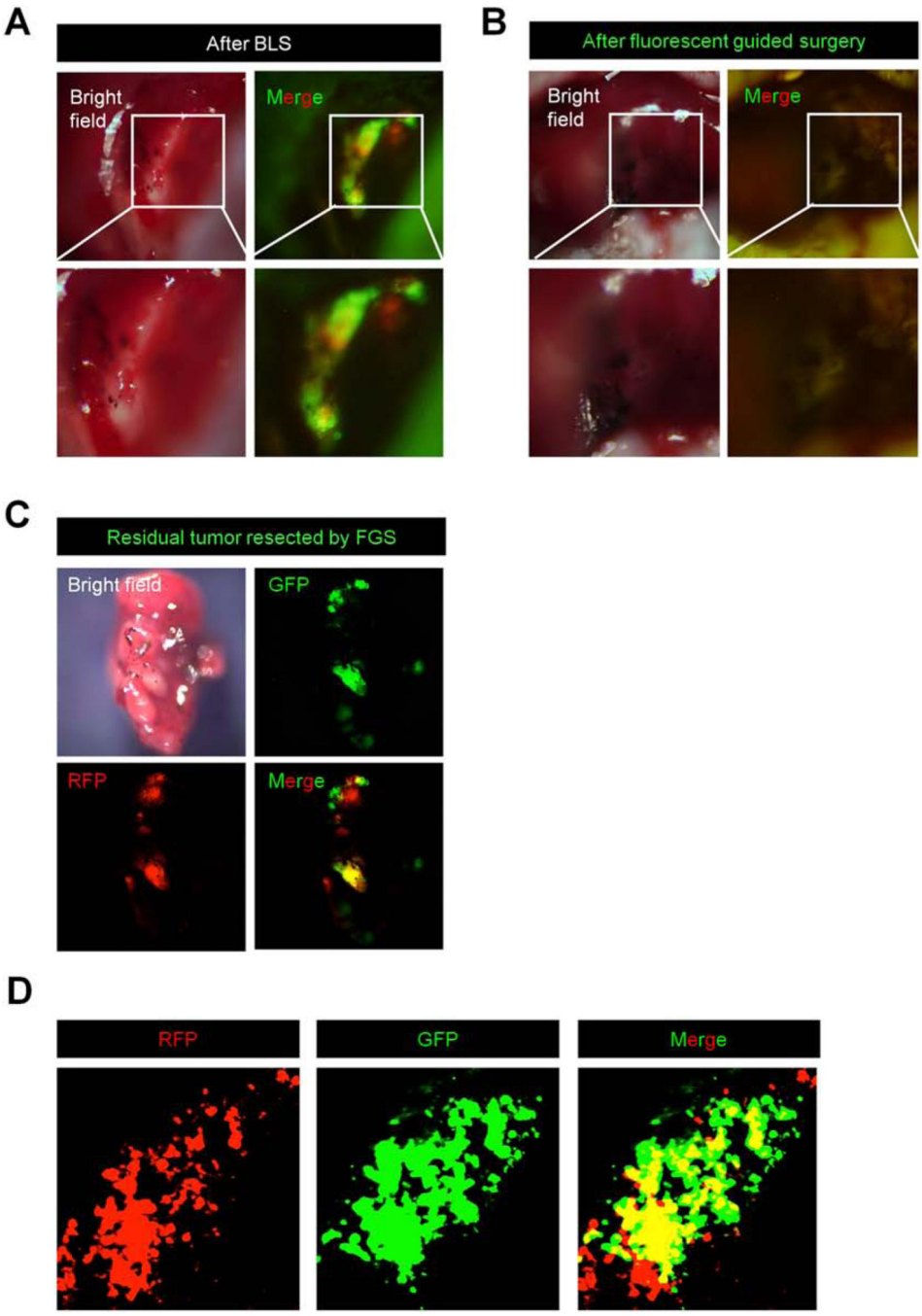

Figure 2:

Fluorescent detection of residual tumor after BLS of orthotopic liver metastases. (A)Fluorescent detection of residual tumor after BLS (B) Complete resection after FGS, compared to incomplete resection in panel A under BLS. (C). Confirmation of residual tumor (D) Single cell imaging of residual tumor. From Yano et al. PLOS, 2016 [11]

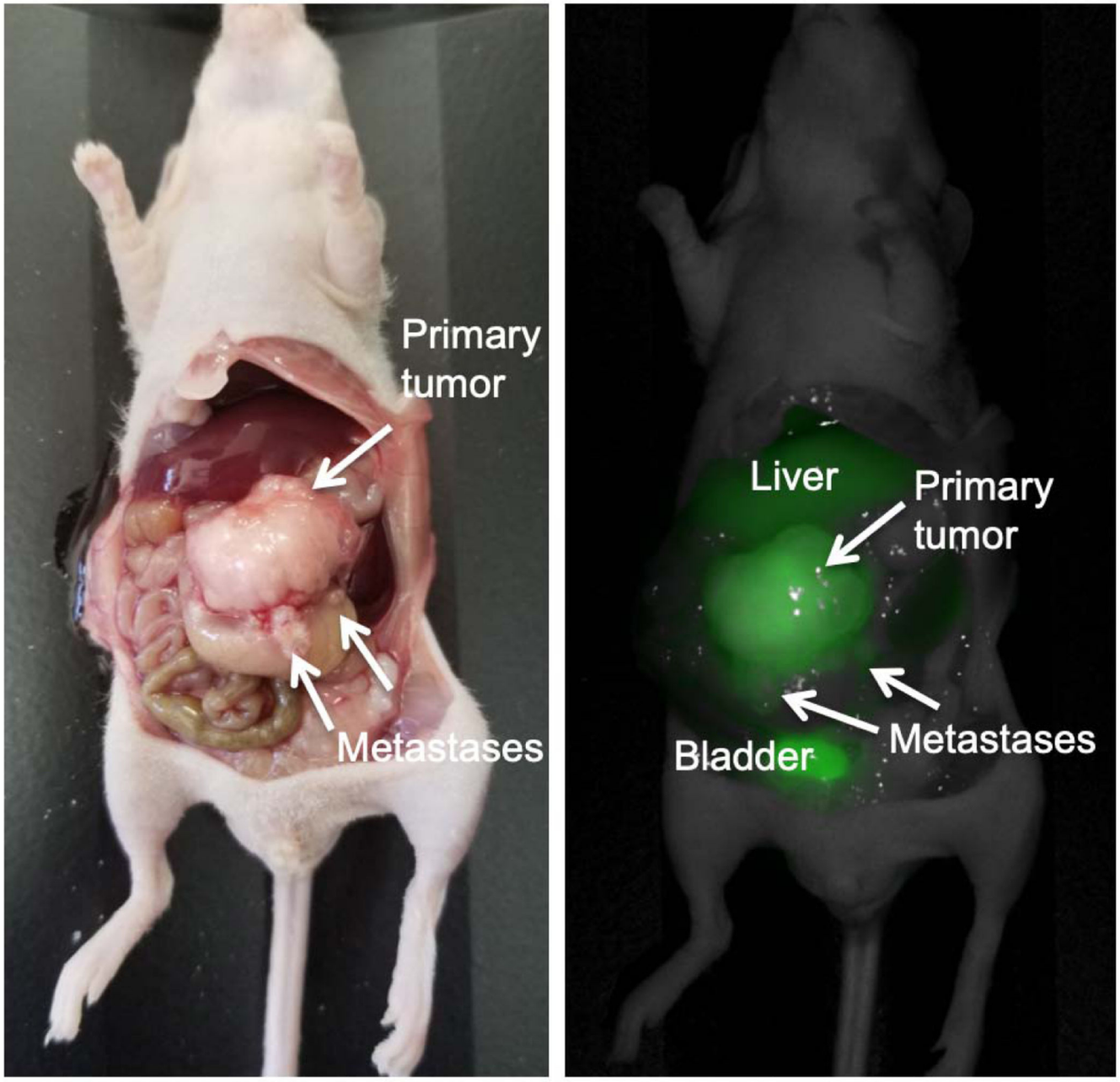

Figure 3:
Anti-CEACAM antibody 6G5j conjugated to near-infrared dye IR800 for detection of occult metastases. From Hollandsworth et al. Oncotarget, 2020 [25]

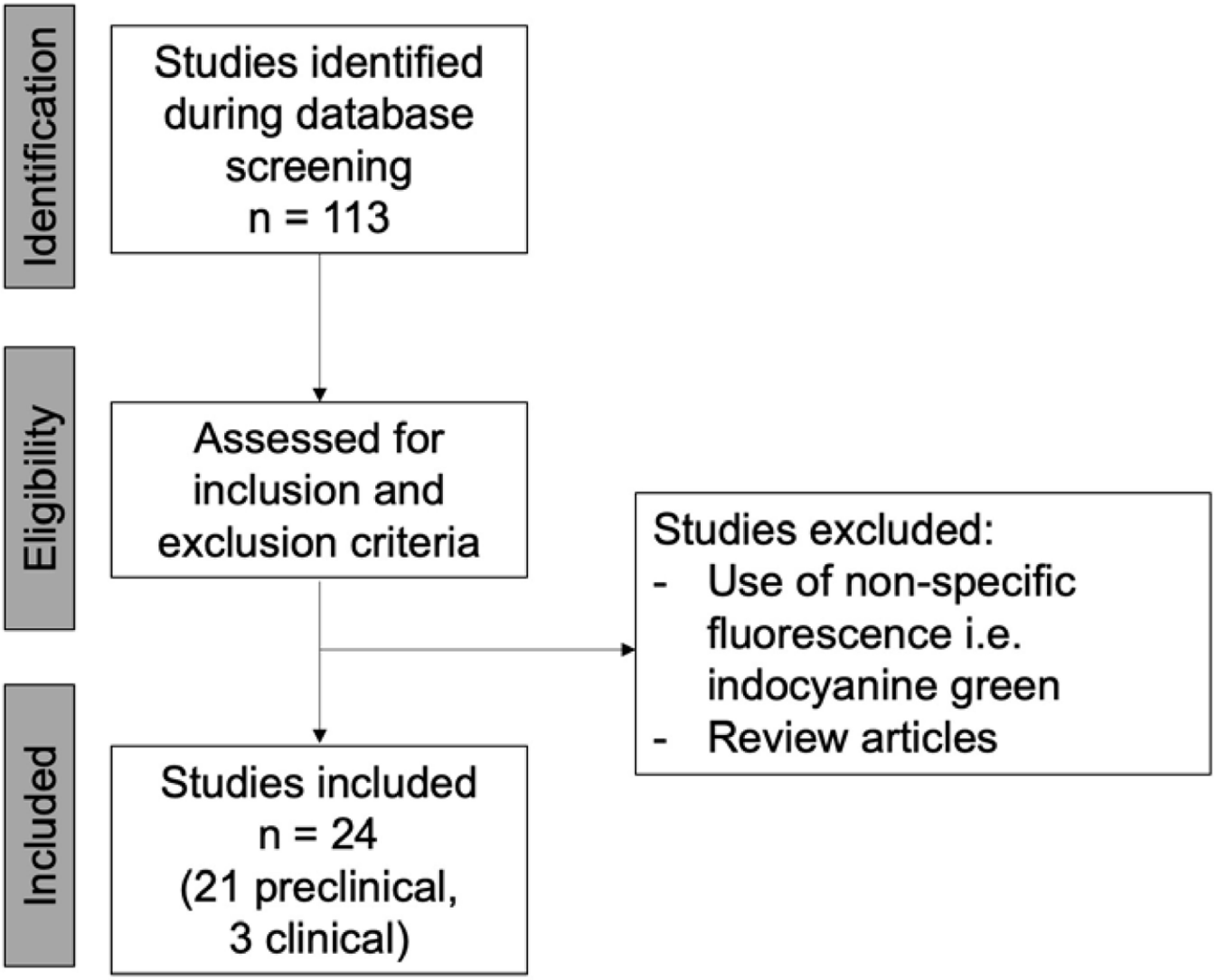

Figure 4:
Orthotopic mouse model with regional metastases. (a) Bright light imaging. (b) Regional metastases visualized with fluorescent anti-claudin-IR800 antibody. From Hollandsworth, et al. J Surg Research [29]

Table 1:

Targeted Antibodies for FGS in Colorectal Cancer

Applications	Target	Fluorescent Agent	Study Phase
Early Diagnosis (Colonoscopy)	Tumor-targeting Peptide LS301 (39)	Cy7.5	Preclinical
	Claudin-1 [28]		Preclinical
	VEGF-A [40]		Clinical
Tumor Imaging		GFP [8,9]	Preclinical
	CEA [14,15,17]	IRDye700DX	Preclinical
	CEA [16]	AlexaFluor488	Preclinical
	CEA [18]	IRDye800CW	Preclinical
	CEA (SGM101) [20]	BM104 (700 nm)	Preclinical
	CEACAM [25]	IRDye800CW	Preclinical
	Claudin-1 [29]	IRDye800CW	Preclinical
	EGFR [33]		Preclinical
	EpCAM [36]	IRDye800CW	Preclinical
	uPAR [38]	ZW800 (800 nm)	Preclinical
Metastases Imaging		GFP [10,11]	Preclinical
	CEA [19]	IRDye700DX	Preclinical
	CEA (SGM101) [20]	BM104 (700 nm)	Preclinical
	CEA [21, 22]	IRDye800CW	Preclinical
	CEACAM [25]	IRDye800CW	Preclinical
	Claudin-1 [29]	IRDye800CW	Preclinical
	IGF-1 [31]	650 nm Dye	Preclinical
	VEGF-A [41]	IRDye800CW	Clinical
	CEA (SGM101) [42,43]	BM104 (700 nm)	Clinical