

State of the Art: Precision Surgery Guided by Intraoperative Molecular Imaging

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ABSTRACT

Intraoperative molecular imaging (IMI) has recently emerged as an important tool in the armamentarium of surgical oncologists. IMI allows real-time assessment of oncologic resection quality, margin assessment, and occult disease detection during real-time surgery. Numerous tracers have now been developed for use in IMI guided tissue sampling. Fluorochromes localize to the tumor by taking advantage of their disorganized capillary milieu, overexpressed receptors, or upregulated enzymes. Although fluorescent tracers can suffer from issues of autofluorescence and lack of depth penetration, these challenges are being addressed through hybrid radioactive/fluorescent tracers and new tracers that fluoresce in the NIR-II range. IMI is already being utilized to treat numerous cancers, with demonstrated improvement in cancer recurrence and patient outcomes without incurring significant burden on either clinicians or patients. In this comprehensive review, we discuss history, mechanism, current oncologic applications, and future directions of IMI guided optical biopsy.

Keywords: Intraoperative Molecular Imaging, Optical Biopsy, Precision Guided Surgery, NIR, Fluorochrome

INTRODUCTION

Cancer is the second leading cause of death in the United States, with over 1.9 million new cases and 600 thousand deaths expected in the United States in 2022 (1). Surgery remains a mainstay in the treatment of solid tumors, with the most important predictor of postsurgical outcome being complete resection of the tumor with negative margins. Unfortunately, precise intraoperative resection still relies mostly on visual inspection under white light and tactile palpation. This makes it difficult to accurately assess margins and detect additional synchronous/metachronous lesions missed on preoperative studies (Videos 1-2). White light visualization can also make it difficult to identify vital structures that need to be preserved.

Intraoperative molecular imaging (IMI) has developed rapidly over the past decade to address these challenges. IMI utilizes optical tracers that target malignant lesions through a variety of means. These tracers fluoresce and can easily be visualized using optical imaging instrumentation, making it possible to accurately identify tumors intraoperatively, remove them with negative margins, and even identify additional lesions that were not visualized preoperatively (2). Fluorescent tracers can also help surgeons identify and avoid vital structures like nerves and vasculature (3).

Although biological imaging traces back to the 1800s with the discovery of the first fluorescent dyes, IMI is a relatively recent method of cancer detection with a history that really begins in the late 1990s (Figure 1). In 1998, Stummer first described the use of 5-ALA for the detection of malignant gliomas, and it was not until 2017, almost 20 years later, that it became the first Food and Drug Administration (FDA)-approved IMI tracer (4). The first FDA approval for an IMI-compatible imaging system, the Novadaq SPY, also came recently in 2005 (5). Although relatively new, these approvals have spurred hundreds of trials testing a wide array of IMI tracers and imaging systems (6). As of 2022, four tumor-targeted probes have reached phase III clinical trials in the US (pafolacianine, BLZ-100, LUM015, and SGM-101), and around 40 contrast agents are currently being investigated in over 85 clinical trials within the US (2,5,6).

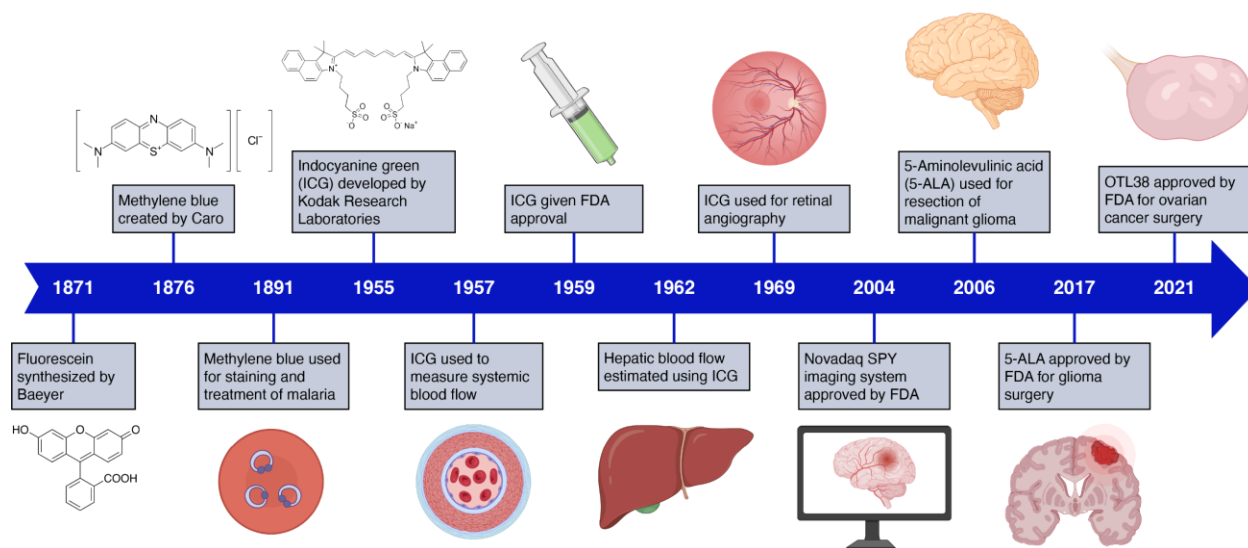


Figure 1. A timeline of notable developments in intraoperative molecular imaging.

IMI is already uniquely positioned within the perioperative continuum to intraoperatively localize lesions visualized preoperatively by nuclear medicine studies (e.g. PET). However, the marriage between IMI and nuclear medicine is even more intertwined. The forefront of research now involves developing hybrid radioactive and fluorescent tracers, which draw upon the unique advantages of fluorescent and radioactive tracers (7). For example, while fluorescence excels at real-time direct visualization, it suffers from poor tissue depth penetration and autofluorescence. In contrast, radioisotopes cannot be visualized directly, but can localize tumors deep within tissue. By combining radiotracers and fluorescent molecules, hybrid tracers can provide the best of both worlds (8).

In this review, we will provide an overview of commonly used imaging agents and surgical navigation systems, with context supplied through their use in recently completed and currently active clinical trials. With each technology, we highlight the recent advances and current challenges for clinical implementation, providing perspective on the future development of IMI.

RESULTS

Intraoperative Molecular Imaging Targeting Mechanisms and Imaging Systems

Most intraoperative optical tracers have been designed to have fluorescence maxima in the near-infrared (NIR) range. In contrast to visible light, NIR fluorescence permits deeper signal penetration, reduced tissue scatter, and decreased background interference from endogenous fluorescent chromophores, such as hemoglobin, NAD(P)H, and flavoproteins (2,9). With increased signal to background ratios (SBR), NIR agents enhance the surgical field of view and enable refinement of resection margins.

Fluorescent tracers generally act through one of three mechanisms: passive, active, and activatable (Figure 2) (5,6). Passive targeting relies on the enhanced

permeability and retention effect (EPR), which leverages the increased vascular permeability and decreased lymphatic drainage of cancerous tissue. This causes the tracer to accumulate inside the tumor milieu. Tracers that exploit EPR include methylene blue (excitation: 668 nm; emission: 688 nm), ICG (excitation: 780 nm; emission: 805 nm), and fluorescein (excitation: 494 nm; emission: 521 nm) (5).

More recently developed tracers actively target abnormally expressed receptors on malignant cells, enhancing labeling specificity and permitting higher resolution imaging. For example, pafolacianine (OTL38) is a folate-receptor alpha (FR α) targeted tracer that has allowed real-time visualization of radiologically occult ovarian and lung tumors (10). SGM-101 is an antibody-dye conjugate against carcinoembryonic antigen (CEA) being assessed for clinical translation in gastrointestinal and pulmonary cancers (11). EMI-137 (GE-137) is a c-MET targeting cyclic peptide conjugated to a cyanine dye, which has been used to improve colorectal polyp detection and facilitate penile cancer resection (2,12).

Activatable probes represent the third mechanistic class, in which the specific microenvironmental and intracellular abnormalities of tumor cells activate the fluorescent probe. LUM015 (excitation: 650 nm; emission: 675 nm) fluoresces only when the quenching moiety is cleaved by cathepsin proteases upregulated in cancer tissue (13). Pegsitacianine (excitation: 780 nm; emission: 805 nm) is a pH-activatable micelle nanoprobe that exploits the metabolic acidosis of solid tumors (14). Table 1 provides an overview of some of the most common IMI imaging agents (2,6).

These three mechanisms have all been complemented by the development of hybrid tracers incorporating both a fluorescent molecule and radioisotope. Hybrid tracers address the problems of tissue penetration, autofluorescence, and photobleaching that occur with fluorescence (7,8). Because γ -radiation can be detected sensitively with little tissue attenuation, the radioisotope enables intraoperative localization even with deeper tumors, while the fluorescent tracer allows for direct visual examination and localization. Furthermore, β -emitting radioisotopes allow for preoperative surgical planning (similar to ^{18}F -FDG PET) and provide quantitative information on biodistribution. Many radioisotopes, including ^{111}In , ^{68}Ga , and ^{89}Zr , have been conjugated to fluorescent molecules for use in IMI, with $^{99\text{m}}\text{Tc}$ emerging as a particularly strong candidate given its favorable decay parameters and low cost (8).

The recent explosion in the development of new experimental molecules for IMI has been paralleled by a similarly exponential growth in the number of imaging systems available in the market. While the discussion of individual systems is beyond the scope of this review, Supplemental Table 1 provides an overview of several popular systems, and Supplemental Figure 1 depicts components common to many imaging systems. Although most IMI imaging systems have been designed for open surgical procedures, many systems have also been adapted for laparoscopic and robotic use, including the Intuitive Surgical Firefly and the Quest Artemis. Most available imaging systems have been optimized for use with ICG, and development of new systems operating in other light wavelengths is required.

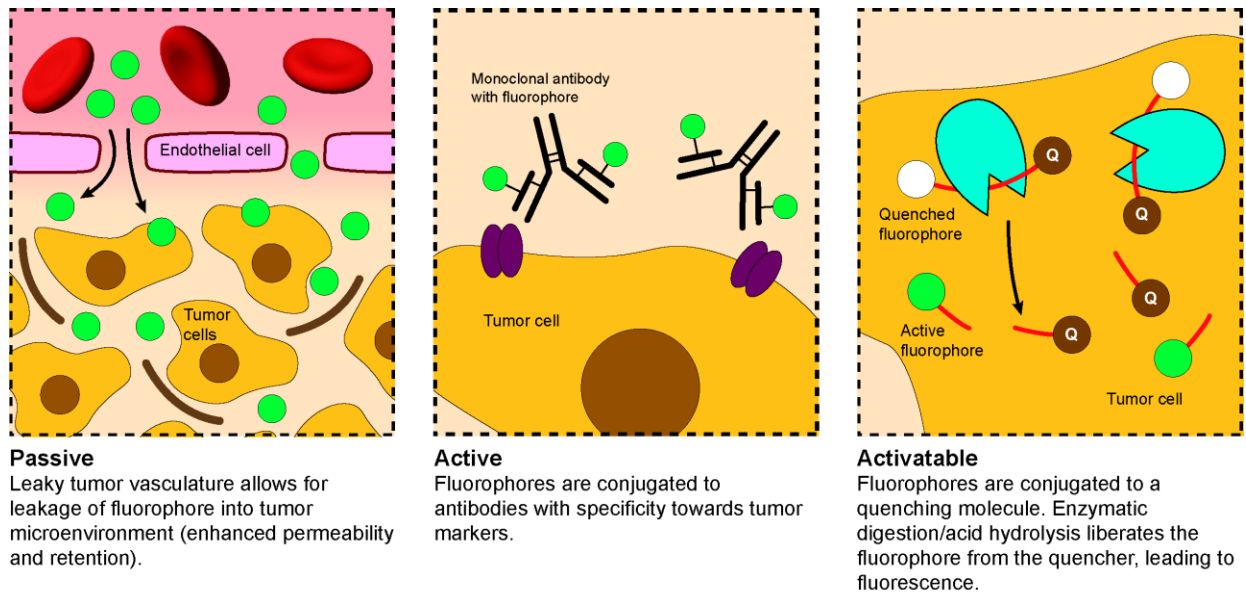


Figure 2. Fluorophores used in IMI generally employ one of three mechanisms to facilitate specific targeting.

Oncologic Applications of Intraoperative Molecular Imaging Brain Cancer

Surgical treatment for high-grade gliomas (HGG) made a remarkable advance with the 2017 FDA approval of 5-ALA (Gleolan) for the identification of HGGs (15). Although 5-ALA has revolutionized HGG treatment, its 635 nm emission wavelength is problematic in that many other anatomical structures are also visible at this shorter wavelength, detracting from the contrast provided by the fluorochrome. Towards this end, NIR agents such as ICG are being tested as alternatives.

Second-Window ICG, also known as TumorGlow, involves administering ICG at a high concentration and waiting 24 hours for the ICG to clear out of normal tissue. A phase I trial by Lee et al. (NCT02280954) found that TumorGlow had an average signal to background ratio (SBR) of 7.5 and sensitivity and positive predictive value both around 85% (16). TumorGlow could visualize tumors through the dura to a depth of 13 mm (17).

In pediatric neurosurgery, tozuleristide, a chlorotoxin and ICG conjugated tracer, has been studied with success in four phase I clinical trials (9). The cohorts in these studies demonstrated a >95% malignant lesion localization ex vivo and 89% specificity in a wide variety of neurologic malignancies. There is currently a PNOC012 (Pacific Pediatric Neuro-oncology Consortium) sponsored randomized controlled trial ongoing (NCT03579602) (18).

Lung Cancer

IMI has demonstrated strong success in identifying lung adenocarcinomas by facilitating tumor removal and enabling visualization of nearby vital structures (Figure 3). However, the high smoking prevalence in this patient population renders unique challenges, as anthracosis generates significant autofluorescence that can reduce IMI

efficacy (19). Nevertheless, several clinical trials have provided strong evidence for the value of IMI in thoracic surgery.

Based on the high expression of FR α by lung adenocarcinomas, fluorescent tracers have been conjugated to folic acid for thoracic surgery. Pafolacianine (OTL38), a cyanine based-NIR dye conjugated to folic acid, has demonstrated success in identifying positive margins and occult synchronous lesions. A phase I trial conducted at the University of Pennsylvania found that pafolacianine identified all (21/21) preoperatively identified lung adenocarcinomas and could also detect subcentimeter pulmonary nodules (SPN) and ground glass opacities (20). In a subsequent phase II trial of 110 patients, pafolacianine found 24 previously undetected nodules, 9 of which were cancers (10%), and detected 8 positive margins (9%). These findings changed clinical staging for 7 patients and improved outcome for 26% of patients (21). A phase III trial is currently ongoing (NCT04241315). Pafolacianine fluorescence quantification by TBR measurements generally do not provide information about the histopathologic nature of the tumor (22).

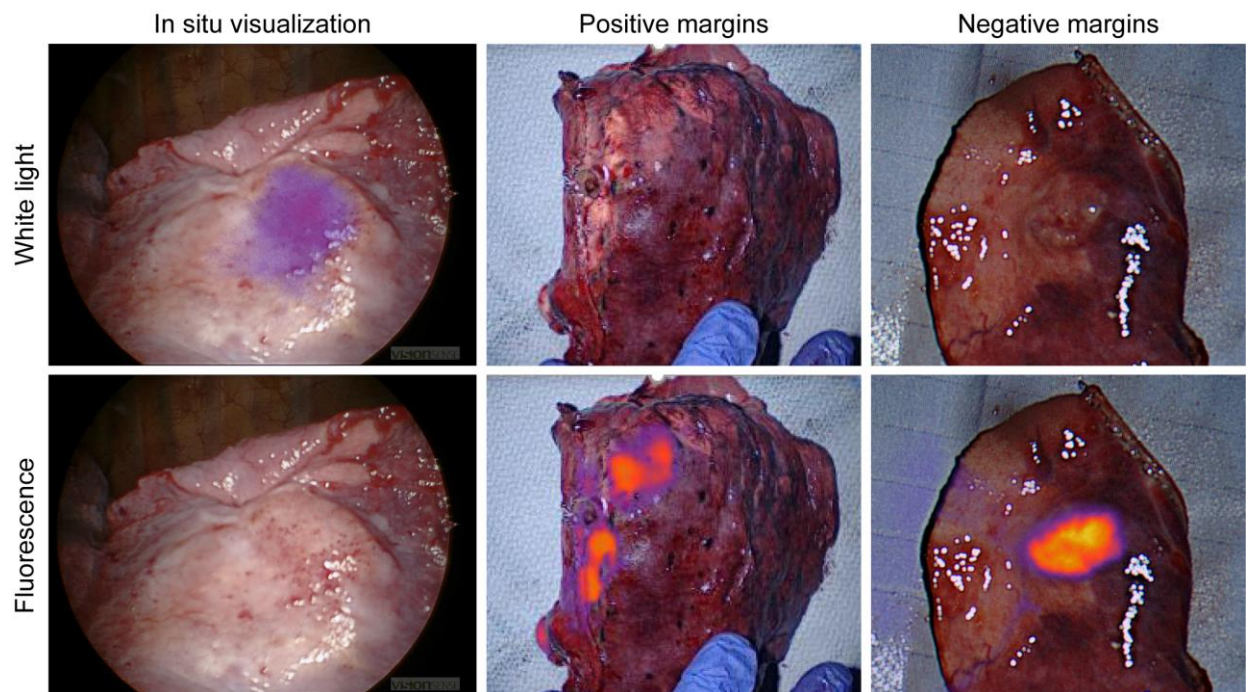


Figure 3. IMI has been used with great success in thoracic surgery, where it can clearly delineate tumor boundaries and facilitate complete R0 resection.

TumorGlow (second-window ICG) has also been used in the operative evaluation of primary and metastatic lung nodules. Okusanya et al. found that TumorGlow detected 16/18 primary nodules previously identified with manual palpation and visual inspection, and also identified 5 additional lesions, including some as small as 0.2 cm (23). Although TumorGlow has a high success rate in identifying lesions, it is limited by dye accumulation in areas of inflammation and consequently may overestimate the size of the tumor (24). TumorGlow has also been used to identify pulmonary sarcoma metastases with a sensitivity of 93.1% (25). In addition, TumorGlow can locate occult

synchronous metastases, leading to increased surveillance, higher rates of adjuvant systemic chemotherapy, and overall improved progression-free survival (26).

As a result of these successes, several fluorescent tracers are in preclinical development for intraoperative detection of lung tumors. JAS239, a fluorescent carbocyanine that competitively inhibits choline kinase, is currently in veterinary clinical trials for the intraoperative detection of early-stage adenocarcinomas in canines (27). Additionally, DDAO-arachidonate, a cytosolic phospholipase A2 activatable probe, is scheduled to enter veterinary trials in late 2022 (28).

Sentinel Lymph Node Biopsy

Sentinel lymph node (SN) biopsy is of critical importance in the surgical management of nearly all cancers, particularly breast cancer and melanoma. The use of fluorescence-guided SN biopsy is rising, but this field has benefited strongly from nuclear medicine; radiotracer-dye combinations for SN biopsy have existed since the 1990s with the use of lymphoscintigraphy together with patent blue dye. Since then, refinements in technology and dyes (e.g. introduction of ICG) have increased SN biopsy efficiency, with greater numbers of SNs identified without increasing overall operation time (29).

The use of radiotracers mitigates the current inability of fluorescent markers to image deeply in the tissue, while the fluorescent molecule adds the benefit of direct visualization. Several recent trials have used ICG-^{99m}Tc-nanocolloid for SN biopsy with strong results. In a trial of 495 patients, KleinJan et al. used ICG-^{99m}Tc-nanocolloid to identify 1643 sentinel lymph node specimens. Radiation identified >98% of SNs, and fluorescence identified >95% of SNs. For nodes deeper than 0.5-1 cm, the radioactive signature allowed for accurate localization and removal (7). Another study of 404 patients with penile cancer found that ICG-^{99m}Tc-nanocolloid identified 98% SNs with the gamma probe and 96% with fluorescent imaging, with 100% of histologically tumor positive SNs visualized intraoperatively by fluorescence (30).

Fluorescent tracers have also been tested in combination with photoacoustic imaging guidance, which can image deep tumors with high resolution (31). Many novel molecules have been tested for dual photoacoustic-fluorescent imaging, including various nanoparticles doped with fluorescent molecules. Recently, a tri-modal imaging system has been developed that utilizes fluorescence, photoacoustic, and ultrasound guidance to identify SNs and reduce false-negative rates (32). Some of these multimodal systems have entered clinical trials. For example, Nishio et al. showed that panitumumab-IRDye800CW could be used for dual photoacoustic and fluorescence imaging (NCT02415881). They found that metastatic nodes had a photoacoustic signal 5x and fluorescence signal double that of benign nodes (33).

Breast Cancer

Breast cancer therapy is being revolutionized by the Lumicell (LUM) Imaging System, which utilizes a far-red fluorescent, PEGylated, protease-activated molecule (LUM015). LUM015 requires only one second to image a 2.6 cm diameter area, allowing examination of the entire lumpectomy cavity in less than a minute (13). A study of 45 patients and 570 cavity margin images found a sensitivity of 84% for tumor detection and specificity of 73%. In the 8 patients with positive margins, LUM015 could

detect residual tumor with a sensitivity of 100% (34). A follow-up multisite phase II study (NCT03321929) enrolling 234 patients showed that LUM015 can detect residual disease missed by histopathologic evaluation in 10% of patients, sparing them from reoperation (35).

Numerous other fluorescent molecules and imaging systems are currently being studied for breast cancer treatment. For example, bevacizumab-IRDye800CW can detect microscopic margins in 10% of excised masses (36). A nanoparticle-based formulation of ICG (ONM-100) has shown strong ability to identify occult lesions and positive margins in a phase II study (14). Other fluorescent molecules like gGlu-HMRG and AVB-620 are also being studied. The Smart Goggle system, a wearable, stereoscopic imaging modality with a handheld microscopy unit, is being used to study ICG accumulation in sentinel lymph nodes of patients with breast cancer (NCT02802553). IMI has also been used in breast reconstruction to assess tissue perfusion during nipple-sparing mastectomies.

Several hybrid tracers have been studied in preclinical trials for breast cancer. Sampath et al. labeled the HER2 antibody trastuzumab with ^{64}Cu and IRDye800, generating $(^{64}\text{Cu-DOTA})_n\text{-trastuzumab-(IRDye800)}_m$. The radioactive signature could detect lung metastases, and ex vivo fluorescence measurements could detect malignant cells in the lung, muscle, skin, and lymph nodes (37). Two trials of ICG- $^{99\text{m}}\text{Tc}$ nanocolloid also recently showed that fluorescence and lymphoscintigraphy could be used together to detect SNs and identify additional SNs that were not detected preoperatively (38,39).

Head and Neck Malignancies

IMI may be particularly important in the surgical management of head and neck cancers due to the difficulty of orienting resected masses, the need to remove all lymph node metastases, and the proximity to neurovascular structures. Hybrid tracers are of great importance given the need for lymph node dissection. Stoffels et al. evaluated ICG- $^{99\text{m}}\text{Tc}$ -nanocolloid in a prospective trial of 40 patients with head and neck cancer (DRKS00004622), finding that the hybrid tracer could identify sentinel nodes in 100% of patients (40).

Several IMI agents target epidermal growth factor receptor (EGFR), which is expressed in over 90% of head and neck cancers. Early trials have shown strong results for IRDye800CW conjugated to either cetuximab or panitumumab. The pilot study of cetuximab-IRDye800CW in 12 patients (NCT01987375) found a tumor-to-background ratio of 5.2 and correlation of fluorescence intensity with EGFR histologic expression (41). Panitumumab-IRDye800CW had similarly strong results (NCT02415881) (42).

Equally important to tumor removal is the preservation of nerve structures and microvascular perfusion of thyroid and parathyroid. Although nerve labeling has been a challenge in IMI because of their low metabolic rate, several molecules have demonstrated potential. ALM-488, a peptide-fluorescein derivative conjugate, received fast track designation by the FDA in 2021 after strong data from a phase I/II clinical trial (NCT04420689). Since most tracers used to identify tumors have NIR emission wavelengths, ALM-488 can be used concurrently with other agents to identify the tumor and nearby nerves (9). On the other hand, efforts to create nerve NIR tracers seek to

leverage the low autofluorescence and scattering of NIR. Such experimental compounds include synthetic oxazine derivatives, which can identify nerves buried to a depth of 3 mm (43).

Sarcoma

TumorGlow (second-window ICG) has been shown to be a valuable tool for localization of pulmonary sarcoma metastasis (Videos 1-2). In a study of 30 patients undergoing IMI-guided metastasectomy, Predina et al. found that TumorGlow identified 24 occult lesions, of which 21 (88%) were malignant (25). In a long-term follow-up study, Azari et al. demonstrated that patients who underwent TumorGlow infusion had increased occult lesion detection, follow-up surveillance, use of adjuvant therapy, and survival compared to patients who received standard care (non-IMI guided metastasectomy) (26). There are plans to initiate a phase III randomized controlled trial evaluating TumorGlow for sarcoma metastasectomy.

Targeted fluorochromes have also been studied for sarcoma resection, with EGFR being a popular target given its frequent overexpression in sarcomas. ABY-029, an anti-EGFR Affibody conjugated to IRDye800CW, was found to have excellent localization to various extremity soft tissue sarcomas with an average TBR of 3.25 (NCT03154411) (9). Importantly, the fluorescence patterns were not affected by neoadjuvant chemoradiotherapy, which is significant as aggressive upfront therapy is a mainstay of treatment for high-grade disease.

Gastrointestinal Malignancies

Carcinoembryonic antigen (CEA) is expressed in over 90% of colorectal malignancies, particularly adenocarcinomas. SGM-101 (Surgimab), an anti-CEA antibody conjugated to the far-red absorbing fluorochrome BM-104, has been studied in multiple clinical trials. Schaap et al. showed in a multicenter pilot study (NCT02973672) that SGM-101 had a sensitivity of 98.5%, specificity of 62.2%, positive predictive value of 82.3%, and negative predictive value of 95.8% in detecting lesions (11). Boogerd et al. and de Valk et al. have similarly demonstrated utility of SGM-101 in CEA+ primary lesion localization and detection of occult satellite lesions in the peritoneum, liver, and omentum (44,45). There is currently a phase I trial exploring SGM-101 in the detection of CEA+ lung metastases and primary CEA+ lung tumors (NCT04315467).

Pancreatic adenocarcinoma is currently the 4th leading cause of cancer-related death in the United States and is increasing in incidence (1). The desmoplastic reaction and dense stroma present a difficulty for IMI by blocking both the tracer and excitation source. Nevertheless, some studies have demonstrated potential. Newton et al. have demonstrated that TumorGlow could identify positive microscopic margins, with fluorescence visualized in 11/12 pancreatic ductal adenocarcinoma tumors. TumorGlow was also able to detect dysplastic and pre-malignant mucinous neoplasms. Lack of fluorescence correlated with complete response to neoadjuvant chemoradiotherapy, significant because response to neoadjuvant therapy is difficult to discern due to anatomic and treatment-related changes (46).

Ovarian Cancer

Cytoreductive surgery is important in the management of ovarian cancer and improves outcomes, especially if patients have no residual disease. Given the frequent overexpression of FR α in ovarian cancer, trials have investigated the use of pafolacianine in ovarian cancer resection. A multicenter phase II clinical trial (NCT02317705) evaluated 225 lesions from 29 patients, finding that pafolacianine had a sensitivity of 86% and positive predictive value of 88%. Additional lesions not detected preoperatively were found in almost half of the patients (47). The follow-up phase III trial of 150 patients (NCT03180307) found that intraoperative imaging detected additional lesions not identified by white light in 33% of patients. Complete resection was achieved in 62.4% of patients (48). These strong results spurred the November 2021 FDA approval of pafolacianine for ovarian cancer.

Prostate Cancer

Radical prostatectomy with lymph node dissection is efficacious for prostate cancer treatment but still results in recurrence rates of 20-40%. IMI can improve lymph node dissection, especially given the atypical location of some nodes and their proximity to nerves. Several targeted tracers have been developed that take advantage of the high expression of prostate-specific membrane antigen (PSMA) on prostate cancer (49).

PSMA-targeting fluorochromes have existed since 2005, when Humblet et al. conjugated the IRDye78 to the PSMA inhibitor GPI (GPI-78) and found that it accumulated in xenograft tumors (50). Many other PSMA ligands have since been conjugated to dyes like IRDye800 and Cy5.5, although most of these molecules are still in preclinical development. However, the ProMOTe IR800 IAB2M trial in the United Kingdom is currently enrolling and evaluating the efficacy of IRDye800CW conjugated to IAB2M, a minibody engineered from an anti-PSMA antibody.

Hybrid tracers have also been developed for prostatectomy. For example, Lütje et al. conjugated the anti-PSMA antibody D2B with IRDye800CW and ^{111}In , finding that xenograft tumors could be detected using both fluorescence and SPECT/CT imaging (51). Hensbergen et al. synthesized several hybrid tracers featuring the PSMA inhibiting motif glutamate-urea-lysine (EuK), labeled them with $^{99\text{m}}\text{Tc}$, and found $^{99\text{m}}\text{Tc}$ -EuK-(SO₃)Cy5-mas₃ to have good kinetics and high tumor-to-muscle ratio (52). There are myriad combinations of fluorescent molecules with targeting molecules and radiotracers, such as ^{111}In -LICOR-800CW-Lys-DOTA-EuK and ^{68}Ga -NIR 800CW-PSMA-11, although these are all still in preclinical development.

DISCUSSION

Thanks to exponential advances in nuclear medicine, today's patients can walk into the clinic, and within a day, a surgeon will know the anatomic location and aggressiveness of the tumor, as well as the functional limitations of performing surgical intervention. However, this precision medicine often stops at the doors of the operating room. Surgeons still rely on tactile and visual feedback to perform oncologic resection, a technique that has not changed since the first days of modern surgery. Intraoperative molecular imaging promises to address the challenge of complete tumor removal with negative margins (Figure 4) and improve outcomes.

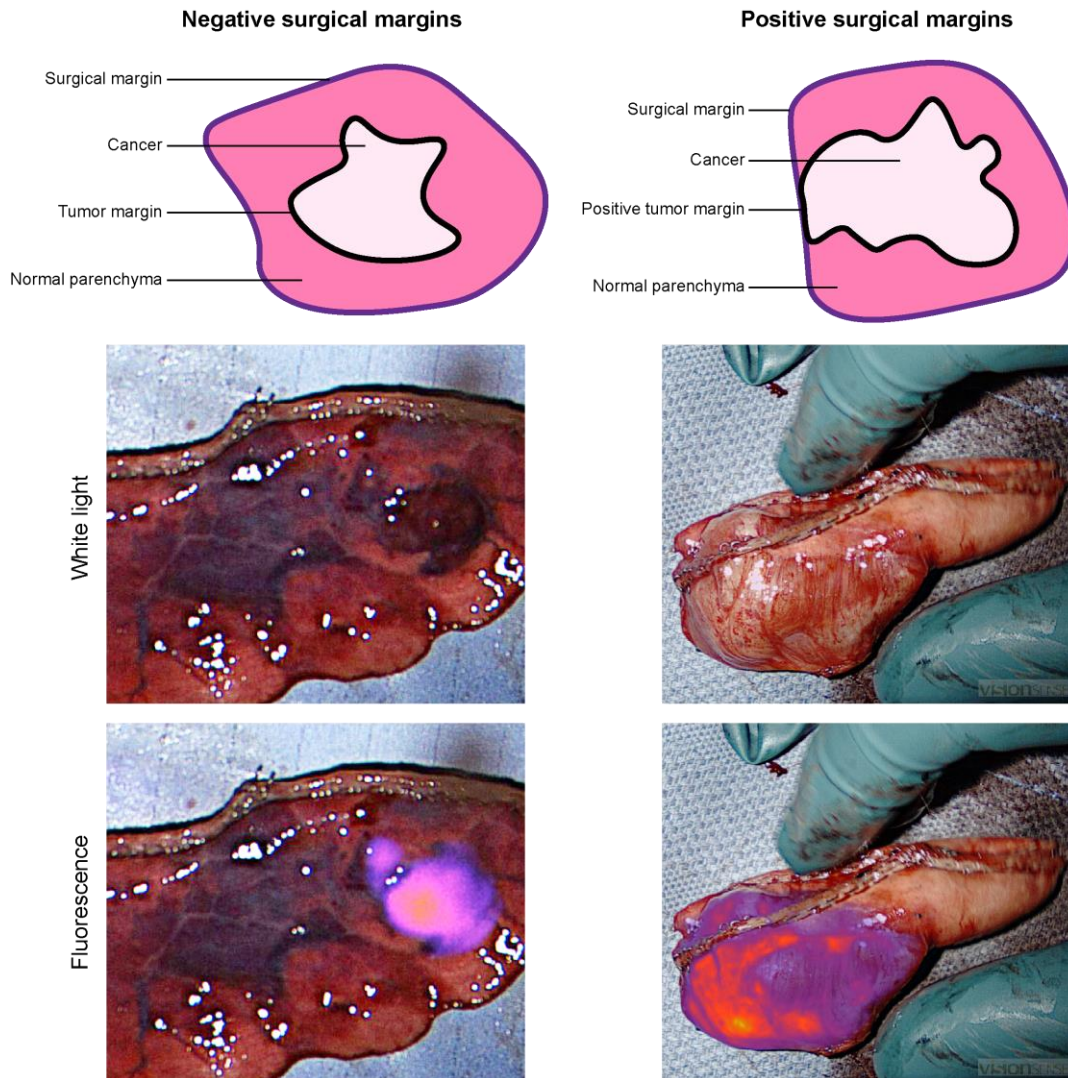


Figure 4. Oncologic resection prioritizes complete tumor removal with negative margins. Residual tumor left behind at the margin frequently necessitates reoperation and leads to poorer outcomes for patients.

The oncologic potential of IMI has been demonstrated in numerous cancers. In the lung, for example, pafolacianine can delineate primary tumors, identify occult synchronous lesions, and assess margin adequacy (20,21). Surgeons can confirm complete lesion removal within minutes and proceed with appropriate surgery, without waiting for frozen section confirmation. This saves patients time under anesthesia, reduces operating room costs, and ensures oncologically sound resection even if the lesion is not seen or felt by the surgeon (53). Furthermore, with the development of ALM-488 and oxazine derivatives, IMI can also identify neurovascular structures and prevent their damage (3,9). In our experience, IMI-guided evaluation does not add undue inconvenience and easily incorporates into routine thoracoscopic techniques and equipment.

Despite recent exponential developments in intraoperative molecular imaging, IMI is still in its technological infancy and has significant challenges to overcome. Fluorescent tracers suffer from limited tissue depth penetration, fluorescence bleaching,

and background autofluorescence, all of which can make in vivo imaging difficult (5). Autofluorescence is a particularly significant problem in lung resection, where smoking-induced anthracosis is common (19). NIR-II (>1000 nm) and hybrid radioactive-fluorescent tracers will help with these issues, as will hybrid radioactive-fluorescent tracers, which have much less tissue attenuation (7). The same radioisotope used for intraoperative guidance can also be used for preoperative imaging studies. However, hybrid tracers bring their own set of limitations, particularly with dosing—many radioisotopes are used at picomolar concentrations to minimize radiation exposure, while most fluorescent tracers are employed at micromolar concentrations (8). Microdosing techniques show promising results in combining nuclear medicine with fluorescence guidance to improve intraoperative localization.

There are already dozens of fluorochromes, radioisotopes, and targeting molecules that have been conjugated in hundreds of combinations, with possibly thousands of experimental compounds on the way (2,5,6). However, the history of IMI use in oncologic surgery is short (< 20 years), resulting in the research on these compounds being mostly preclinical. The few clinical studies that have been conducted include fewer than a hundred patients. Randomized controlled trials involving at least several hundred patients, such as those being conducted/recently concluded for pafolacianine, LUM015, and ICG-^{99m}Tc-nanocolloid, are necessary to better delineate the added value of IMI over the current standard of care (21,30,35). It will be critical to not only collect data on the number of additionally identified lesions and positive margins, but also note whether patients have a change in their staging, adjuvant treatment, and overall survival.

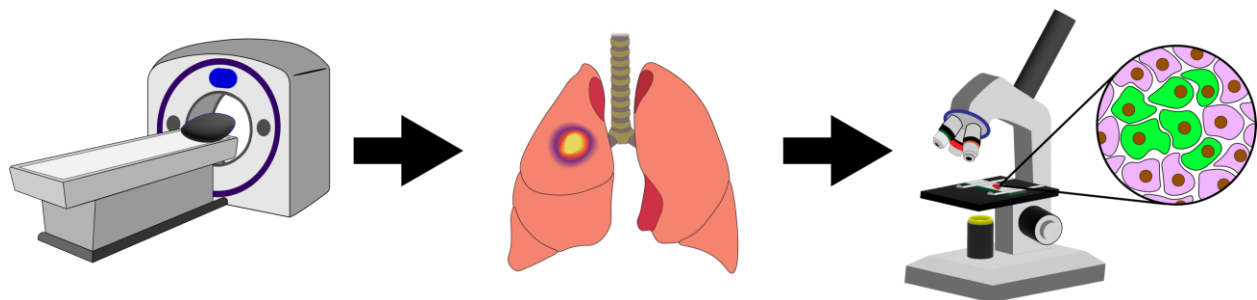


Figure 5. Intraoperative molecular imaging lies within the continuum of preoperative radiologic evaluation and intra/postoperative histologic confirmation, complementing these technologies and offering improved outcomes for patients.

CONCLUSION

IMI guided surgical resections are becoming commonplace in the management of oncologic diseases, with dozens of fluorescent molecules being investigated. The technology lies in the continuum of radiologic diagnostic evaluation and histologic confirmation, drawing upon significant advances developed by nuclear medicine (Figure 5). Although preclinical and early clinical trials have already shown the ability of IMI to refine oncologic resections, further studies are needed to elaborate on the optimal patient characteristics that stand to benefit from IMI. Continued innovation in combining radiotracers with fluorescent molecules, together with development of novel NIR-II fluorophores, will alleviate some of the current challenges of IMI implementation. Clinicians should be aware of the developments in this field, as IMI will likely be added to the mainstay arsenal of tools in surgical oncology.

NOTEWORTHY

- Intraoperative molecular imaging uses fluorescent tracers to intraoperatively locate tumors, facilitating their complete removal.
- Intraoperative molecular imaging has repeatedly been proven to improve surgical outcomes for patients.
- Hybrid tracers incorporating both a radioactive and fluorescent molecule mitigate problems of autofluorescence and lack of depth penetration seen with fluorescence alone.

REFERENCES

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. *CA: A Cancer Journal for Clinicians*. 2022;72:7-33.
2. Joshi BP, Wang TD. Targeted optical imaging agents in cancer: focus on clinical applications. *Contrast Media & Molecular Imaging*. 2018;2018:1-20.
3. Wang LG, Barth CW, Combs JR, Montaña AR, Gibbs SL. Investigation of oxazine and rhodamine derivatives as peripheral nerve tissue targeting contrast agent for in vivo fluorescence imaging. *Proceedings of SPIE--the International Society for Optical Engineering*. 2019;10862:1-13.
4. Stummer W, Stocker S, Novotny A, et al. In vitro and in vivo porphyrin accumulation by C6 glioma cells after exposure to 5-aminolevulinic acid. *Journal of photochemistry and photobiology*. 1998;45:160-169.
5. Nagaya T, Nakamura YA, Choyke PL, Kobayashi H. Fluorescence-guided surgery. *Frontiers in Oncology*. 2017;7:1-16.
6. Hernot S, van Manen L, Debie P, Mieog JSD, Vahrmeijer AL. Latest developments in molecular tracers for fluorescence image-guided cancer surgery. *Lancet Oncol*. 2019;20:e354-e367.
7. KleinJan GH, van Werkhoven E, van den Berg NS, et al. The best of both worlds: a hybrid approach for optimal pre- and intraoperative identification of sentinel lymph nodes. *European journal of nuclear medicine and molecular imaging*. 2018;45:1915-1925.
8. van Leeuwen FWB, Schottelius M, Brouwer OR, et al. Trending: radioactive and fluorescent bimodal/hybrid tracers as multiplexing solutions for surgical guidance. *Journal of Nuclear Medicine*. 2020;61:13-19.
9. Azari F, Kennedy G, Bernstein E, et al. Intraoperative molecular imaging clinical trials: a review of 2020 conference proceedings. *Journal of biomedical optics*. 2021;26:1-22.
10. Predina JD, Newton AD, Keating J, et al. Intraoperative molecular imaging combined with positron emission tomography improves surgical management of peripheral malignant pulmonary nodules. *Annals of surgery*. 2017;266:479-488.
11. Schaap DP, de Valk KS, Deken MM, et al. Carcinoembryonic antigen-specific, fluorescent image-guided cytoreductive surgery with hyperthermic intraperitoneal chemotherapy for metastatic colorectal cancer. *The British Journal of Surgery*. 2020;107:334-337.

12. De Vries HM, Bekers E, Van Oosterom MN, et al. c-MET receptor–targeted fluorescence on the road to image-guided surgery in penile squamous cell carcinoma patients. *Journal of Nuclear Medicine*. 2022;63:51-56.
13. Smith BL, Gadd MA, Lanahan CR, et al. Real-time, intraoperative detection of residual breast cancer in lumpectomy cavity walls using a novel cathepsin-activated fluorescent imaging system. *Breast cancer research and treatment*. 2018;171:413-420.
14. Voskuil FJ, Steinkamp PJ, Zhao T, et al. Exploiting metabolic acidosis in solid cancers using a tumor-agnostic pH-activatable nanoprobe for fluorescence-guided surgery. *Nature Communications*. 2020;11:1-10.
15. Hadjipanayis CG, Stummer W. 5-ALA and FDA approval for glioma surgery. *Journal of neuro-oncology*. 2019;141:479-486.
16. Zeh R, Sheikh S, Xia L, et al. The second window ICG technique demonstrates a broad plateau period for near infrared fluorescence tumor contrast in glioblastoma. *PloS one*. 2017;12:1-16.
17. Lee JYK, Thawani JP, Pierce J, et al. Intraoperative near-infrared optical imaging can localize gadolinium-enhancing gliomas during surgery. *Neurosurgery*. 2016;79:856-871.
18. Leary S, Blatt JE, Cohen AR, et al. A phase II/III randomized, blinded study of tozuleristide for fluorescence imaging detection during neurosurgical resection of pediatric primary central nervous system (CNS) tumors: PNOC012 (Pacific Pediatric Neuro-oncology Consortium). *Journal of Clinical Oncology*. 2020;38:TPS2575-TPS2575.
19. Azari F, Kennedy G, Zhang K, et al. Effects of light-absorbing carbons in intraoperative molecular imaging-guided lung cancer resections. *Molecular imaging and biology*. Epub ahead of print.
20. Predina JD, Newton AD, Keating J, et al. A phase I clinical trial of targeted intraoperative molecular imaging for pulmonary adenocarcinomas. *Annals of Thoracic Surgery*. 2018;105:901-908.
21. Gangadharan S, Sarkaria IN, Rice D, et al. Multiinstitutional phase 2 clinical trial of intraoperative molecular imaging of lung cancer. *The Annals of Thoracic Surgery*. 2021;112:1150-1159.
22. Azari F, Kennedy G, Bernstein E, et al. Evaluation of OTL38-generated tumor-to-background ratio in intraoperative molecular imaging-guided lung cancer resections. *Molecular Imaging and Biology*. June 2021:1-12.
23. Okusanya OT, Holt D, Heitjan D, et al. Intraoperative near-infrared imaging can identify pulmonary nodules. *The Annals of thoracic surgery*. 2014;98:1223-1230.

24. Holt D, Okusanya O, Judy R, et al. Intraoperative near-infrared imaging can distinguish cancer from normal tissue but not inflammation. *PloS one*. 2014;9:1-11.
25. Predina JD, Newton AD, Corbett C, et al. Near-infrared intraoperative imaging for minimally invasive pulmonary metastasectomy for sarcomas. *The Journal of thoracic and cardiovascular surgery*. 2019;157:2061-2069.
26. Azari F, Kennedy GT, Zhang K, et al. Impact of intraoperative molecular imaging after fluorescent-guided pulmonary metastasectomy for sarcoma. *Journal of the American College of Surgeons*. 2022;234:748-758.
27. Arlauckas SP, Kumar M, Popov AV, Poptani H, Delikatny EJ. Near infrared fluorescent imaging of choline kinase alpha expression and inhibition in breast tumors. *Oncotarget*. 2017;8:16518-16530.
28. Chiorazzo MG, Tunset HM, Popov AV, Johansen B, Moestue S, Delikatny EJ. Detection and differentiation of breast cancer sub-types using a cPLA2 α activatable fluorophore. *Scientific reports*. 2019;9:1-11.
29. Berger DMS, van den Berg NS, van der Noort V, et al. Technologic (r)evolution leads to detection of more sentinel nodes in patients with melanoma in the head and neck region. *Journal of Nuclear Medicine*. 2021;62:1357-1362.
30. Dell'Oglio P, de Vries HM, Mazzone E, et al. Hybrid indocyanine green–99mTc-nanocolloid for single-photon emission computed tomography and combined radio- and fluorescence-guided sentinel node biopsy in penile cancer: Results of 740 inguinal basins assessed at a single institution. *European Urology*. 2020;78:865-872.
31. Kim H, Chang JH. Multimodal photoacoustic imaging as a tool for sentinel lymph node identification and biopsy guidance. *Biomedical Engineering Letters*. 2018;8:183-191.
32. Kang J, Chang JH, Kim SM, et al. Real-time sentinel lymph node biopsy guidance using combined ultrasound, photoacoustic, fluorescence imaging: in vivo proof-of-principle and validation with nodal obstruction. *Scientific Reports*. 2017;7:1-9.
33. Nishio N, van den Berg NS, Martin BA, et al. Photoacoustic molecular imaging for the identification of lymph node metastasis in head and neck cancer using an anti-EGFR antibody-dye conjugate. *Journal of Nuclear Medicine*. 2020;62:648-655.
34. Smith BL, Lanahan CR, Specht MC, et al. Feasibility study of a novel protease-activated fluorescent imaging system for real-time, intraoperative detection of residual breast cancer in breast conserving surgery. *Annals of Surgical Oncology*. 2020;27:1854-1861.
35. Ferrer J, Carr D, Blumencranz P, et al. Abstract P1-20-06: results from the expansion into multiple institutions for training in the use of the LUM imaging

system for intraoperative detection of residual cancer in the tumor bed of female subjects with breast cancer clinical trial. *Cancer Research*. 2020;80:P1-20-06.

36. Lamberts LE, Koch M, De Jong JS, et al. Tumor-specific uptake of fluorescent bevacizumab-IRDye800CW microdosing in patients with primary breast cancer: A phase I feasibility study. *Clinical Cancer Research*. 2017;23:2730-2741.
37. Sampath L, Kwon S, Hall MA, Price RE, Sevick-Muraca EM. Detection of cancer metastases with a dual-labeled near-infrared/positron emission tomography imaging agent. *Translational Oncology*. 2010;3:307-217.
38. Schaafsma BE, Verbeek FPR, Rietbergen DDD, et al. Clinical trial of combined radio- and fluorescence-guided sentinel lymph node biopsy in breast cancer. *The British journal of surgery*. 2013;100:1037-1044.
39. Manca G, Garau LM, Mazzarri S, et al. Novel experience in hybrid tracers: clinical evaluation of feasibility and efficacy in using ICG-99mTc nanotop for sentinel node procedure in breast cancer patients. *Clinical nuclear medicine*. 2021;46:e181-e187.
40. Stoffels I, Leyh J, Pöppel T, Schadendorf D, Klode J. Evaluation of a radioactive and fluorescent hybrid tracer for sentinel lymph node biopsy in head and neck malignancies: prospective randomized clinical trial to compare ICG-99mTc-nanocolloid hybrid tracer versus 99mTc-nanocolloid. *European Journal of Nuclear Medicine and Molecular Imaging*. 2015;42:1631-1638.
41. Rosenthal EL, Warram JM, De Boer E, et al. Safety and tumor specificity of cetuximab-IRDye800 for surgical navigation in head and neck cancer. *Clinical Cancer Research*. 2015;21:3658-3666.
42. Nishio N, van den Berg NS, van Keulen S, et al. Optical molecular imaging can differentiate metastatic from benign lymph nodes in head and neck cancer. *Nature Communications*. 2019;10:1-10.
43. Wang LG, Barth CW, Kitts CH, et al. Near-infrared nerve-binding fluorophores for buried nerve tissue imaging. *Science Translational Medicine*. 2020;12:1-13.
44. Boogerd LSF, Hoogstins CES, Schaap DP, et al. Safety and effectiveness of SGM-101, a fluorescent antibody targeting carcinoembryonic antigen, for intraoperative detection of colorectal cancer: a dose-escalation pilot study. *The Lancet Gastroenterology & hepatology*. 2018;3:181-191.
45. de Valk KS, Deken MM, Schaap DP, et al. Dose-finding study of a CEA-targeting agent, SGM-101, for intraoperative fluorescence imaging of colorectal cancer. *Annals of surgical oncology*. 2021;28:1832-1844.
46. Newton AD, Predina JD, Shin MH, et al. Intraoperative near-infrared imaging can identify neoplasms and aid in real-time margin assessment during pancreatic resection. *Annals of Surgery*. 2019;270:12-20.

47. Randall LM, Wenham RM, Low PS, Dowdy SC, Tanyi JL. A phase II, multicenter, open-label trial of OTL38 injection for the intra-operative imaging of folate receptor-alpha positive ovarian cancer. *Gynecologic oncology*. 2019;155:63-68.
48. Tanyi JL, Chon HS, Morgan MA, et al. Phase 3, randomized, single-dose, open-label study to investigate the safety and efficacy of pafolacianine sodium injection (OTL38) for intraoperative imaging of folate receptor positive ovarian cancer. *Journal of Clinical Oncology*. 2021;39:5503-5503.
49. Maurer T, Graefen M, van der Poel H, et al. Prostate-specific membrane antigen-guided surgery. *Journal of Nuclear Medicine*. 2020;61:6-12.
50. Humblet V, Lapidus R, Williams LR, et al. High-affinity near-infrared fluorescent small-molecule contrast agents for in vivo imaging of prostate-specific membrane antigen. *Molecular Imaging*. 2005;4:448-462.
51. Lütje S, Rijpkema M, Franssen GM, et al. Dual-modality image-guided surgery of prostate cancer with a radiolabeled fluorescent anti-PSMA monoclonal antibody. *Journal of Nuclear Medicine*. 2014;55:995-1001.
52. Hensbergen AW, Buckle T, van Willigen DM, et al. Hybrid tracers based on cyanine backbones targeting prostate-specific membrane antigen: Tuning pharmacokinetic properties and exploring dye-protein interaction. *Journal of Nuclear Medicine*. 2020;61:234-241.
53. Kennedy GT, Azari FS, Bernstein E, et al. Targeted intraoperative molecular imaging for localizing nonpalpable tumors and quantifying resection margin distances. *JAMA Surgery*. 2021;156:1043-1050.
54. Gutowski M, Framery B, Boonstra MC, et al. SGM-101: An innovative near-infrared dye-antibody conjugate that targets CEA for fluorescence-guided surgery. *Surgical oncology*. 2017;26:153-162.
55. Miampamba M, Liu J, Harootunian A, et al. Sensitive in vivo visualization of breast cancer using ratiometric protease-activatable fluorescent imaging agent, AVB-620. *Theranostics*. 2017;7:3369-3386.
56. Kennedy GT, Okusanya OT, Keating JJ, et al. The optical biopsy: A novel technique for rapid intraoperative diagnosis of primary pulmonary adenocarcinomas. *Annals of surgery*. 2015;262:602-609.
57. Nakamura Y, Harada T, Nagaya T, et al. Dynamic fluorescent imaging with the activatable probe, γ -glutamyl hydroxymethyl rhodamine green in the detection of peritoneal cancer metastases: Overcoming the problem of dilution when using a sprayable optical probe. *Oncotarget*. 2016;7:51124-51137.
58. Azari F, Kennedy G, Singhal S. Intraoperative detection and assessment of lung nodules. *Surgical oncology clinics of North America*. 2020;29:525-541.

59. Xu X, Samkoe KS, Henderson ER. Effect of preoperative cancer treatment on epidermal growth factor receptor (EGFR) receptor expression level in ABY-029 guided sarcoma surgery. *Proceedings of SPIE--the International Society for Optical Engineering*. 2020;11222:33.
60. Kennedy GT, Azari FS, Bernstein E, et al. A prostate specific membrane antigen-targeted near-infrared conjugate for identifying pulmonary squamous cell carcinoma during resection. *Molecular Cancer Therapeutics*. 2022;21:546-554.

Table 1. List of imaging agents that are FDA approved or currently in clinical trials in IMI.

Imaging Agent	Fluorochrome	Excitation (nm)	Emission (nm)	Molecular Target	Mechanism
SGM-101	BM-104 (6,54)	685	705	Carcinoembryonic antigen (CEA)	Active
LUM015	Cy5 (13)	650	675	Cathepsins, MMP	Activatable
Pegloprastide (AVB-620)	Cy5 and Cy7 (55)	630	620-850	MMP	Activatable
Fluorescein	Fluorescein	494	521	N/A	Passive
EC17	Fluorescein derivative (9,56)	470	520	Folate-receptor alpha	Active
ALM-488	Fluorescein derivative (9)	488	530	Nerve cell membrane	Active
gGlu-HMRG	Hydroxymethyl rhodamine green (HMRG) (57)	500	524	γ -glutamyl transpeptidase	Activatable
Indocyanine Green (ICG)	ICG (58)	780	805	N/A	Passive
TumorGlow	ICG (9)	780	805	N/A	Passive
BLZ-100 (Tozuleristide; TumorPaint)	ICG (18)	780	805	Annexin A2, MMP2	Active
Pegsitacianine (ONM 100)	ICG (14)	780	805	N/A	Activatable
Bevacizumab-IRDye800CW	IRDye800CW (36)	775	805	VEGF-A	Active
Cetuximab-IRDye800CW	IRDye800CW (41)	775	805	EGFR	Active
Panitumumab-IRDye800CW	IRDye800CW (42)	775	805	EGFR	Active
ABY-029	IRDye800CW (59)	775	805	EGFR	Active
Methylene Blue	Methylene Blue	668	688	N/A	Passive
5-Aminolevulinic Acid (5-ALA; Gleolan)	Protoporphyrin IX (15)	405	635	N/A	Passive
Pafolacianine (OTL38)	S0456 (10)	774	794	Folate-receptor alpha	Active
OTL78	S0456 (60)	774	794	Prostate specific membrane antigen (PSMA)	Active

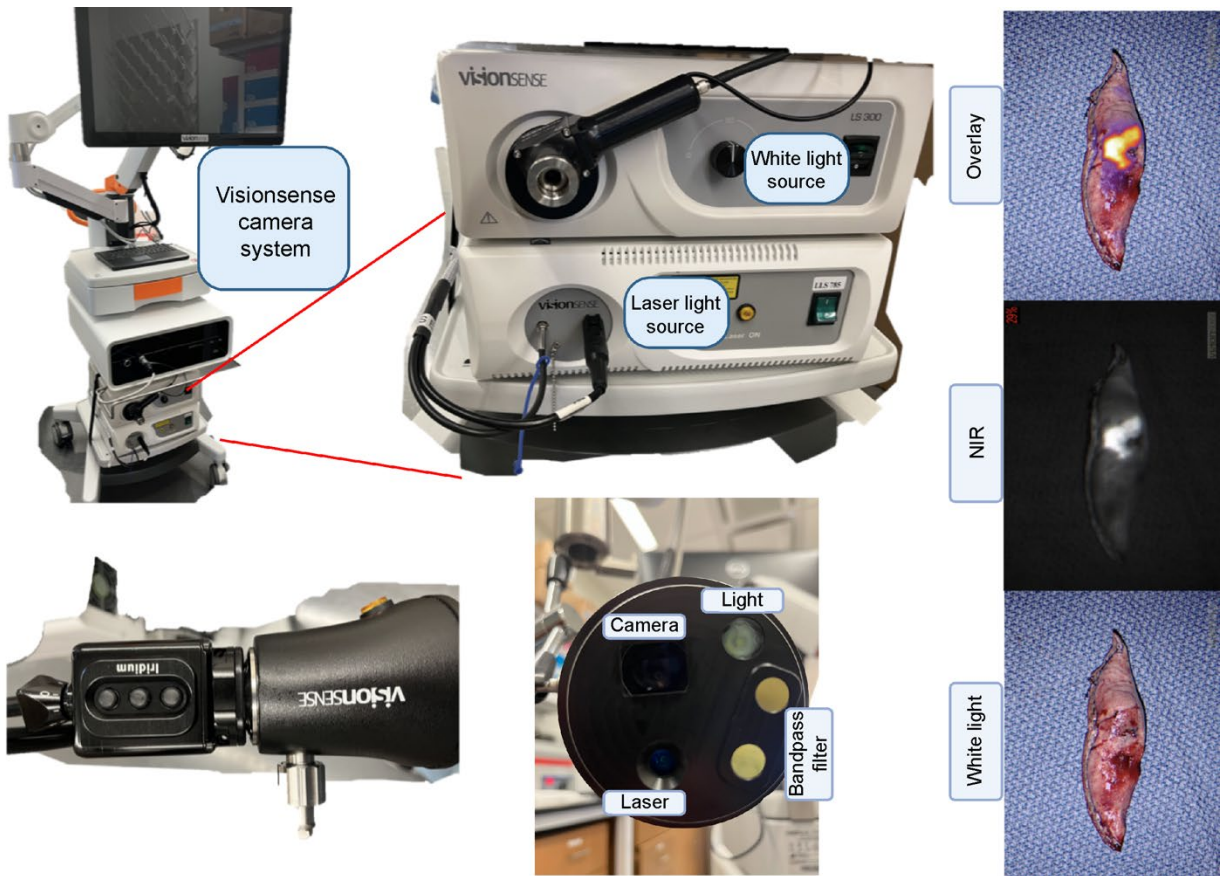
SUPPLEMENTAL DATA

Video 1: Demonstration of IMI guided localization of a chondrosarcoma metastatic lesion with ICG. The lesion was followed with serial cross-sectional imaging in a patient with high grade chondrosarcoma that was resected 11 months prior. Standard white light (left), NIR (middle), and overlay (right) video sequences are demonstrated in the segment in a similar manner that the thoracic surgeon would view during the operation.

Video 2: IMI guided detection of an occult lesion that was discovered in the same patient as Video 1. This nodule was not noted on cross sectional surveillance imaging. Standard white light (left), NIR (middle), and overlay (right) video sequences are demonstrated in the segment in a similar manner that the thoracic surgeon would view during the operation.

Supplemental Table 1. Imaging systems commonly used in IMI (60).

Imaging System	Company	Excitation Wavelength	Light Source	Real-Time Overlay	Clinical Applications
SPY	Novadaq Technologies	805	Laser	No	Perfusion, Tissue Reconstruction, Lymphatic Mapping, Cerebral Angiography
FL800/400	Leica	750-850	Xenon Light	No	Cerebral Angiography
PDE NEO II	Hamamatsu Photonics	760	LED	Yes	Lymphatic Mapping, Perfusion
Infrared 800	Zeiss	700-780	Laser	No	Neuro-oncology
Fluobeam LX	Fluoptics	680-750	Laser	No	Endocrine surgery
Quest Spectrum	Olympus	400-1000	Laser	Yes	Colorectal Surgery, hepaticopancreaticobiliary surgery, breast cancer, lymphatic mapping, lung cancer
Iridium	EleVision (Medtronic)	805	Laser	Yes	Pancreatic cancer, ovarian cancer, lung cancer, neurosurgery, breast cancer, endocrine surgery.
Onlume	OnLume Surgical	778	Laser	Yes	Neurosurgery, Tissue Perfusion, Vascular and Reconstructive Surgery, Head and Neck Surgery



Supplemental Figure 1. The Visionsense camera system features many of the components common to systems used in intraoperative molecular imaging.