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Fluorescence Image-Guided Surgery – a Perspective on Contrast Agent Development

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Abstract

In the past several decades, a number of novel fluorescence image-guided surgery (FGS) contrast agents have been under development, with many in clinical translation and undergoing clinical trials. In this review, we have identified and summarized the contrast agents currently undergoing clinical translation. In total, 39 novel FGS contrast agents are being studied in 85 clinical trials. Four FGS contrast agents are currently being studied in phase III clinical trials and are poised to reach FDA approval within the next two to three years. Among all novel FGS contrast agents, a wide variety of probe types, targeting mechanisms, and fluorescence properties exists. Clinically available FGS imaging systems have been developed for FDA approved FGS contrast agents, and thus further clinical development is required to yield FGS imaging systems tuned for the variety of contrast agents in the clinical pipeline. Additionally, study of current FGS contrast agents for additional disease types and development of anatomy specific contrast agents is required to provide surgeons FGS tools for all surgical specialties and associated comorbidities. The work reviewed here represents a significant effort from many groups and further development of this promising technology will have an enormous impact on surgical outcomes across all specialties.

Keywords

fluorescence; image-guided surgery; fluorescence imaging system; clinical development; clinical trial; contrast agent; near-infrared fluorescence

1. Introduction

Fluorescence image-guided surgery (FGS) technologies have been under development for the past three decades. A movement that began with the development of clinically approved near-infrared (NIR) fluorescent agents indocyanine green (ICG) and methylene blue as vascular tracers has grown to become a multidisciplinary field studied by dozens of groups worldwide.[1–4] Through the creation of targeted contrast agents and sensitive imaging systems, FGS has the potential to revolutionize surgery, improving surgical outcomes by enhancing visualization of tissues for resection, such as tumors, or preservation, such as nerves or vasculature. Utilizing compact and relatively low-cost imaging systems, FGS can be readily implemented into many procedures to bridge the gap between preoperative

imaging, such as magnetic resonance imaging (MRI) and computed tomography (CT), and the current intraoperative reality.[5–15] FGS systems have been successfully utilized in a wide variety of clinical applications to improve outcomes, including tumor resection, sentinel lymph node mapping, angiography, lymphography, and ureter and bile duct anatomic imaging.[16–28] Additionally, a number of targeted contrast agents are under development to expand the applications of this promising technology. For example, 5-aminominolevulinic acid (5-ALA) and its fluorescent metabolite protoporphyrin IX (PpIX) has garnered clinical approval for glioma resection and has significantly enhanced complete resection rates and revolutionized neurosurgical treatment of brain tumors over the past decade.[29] Many more targeted contrast agents are currently in the clinical pipeline, representing substantial effort by the FGS community to translate this promising technology to many surgical indications.

Considered new drugs by the FDA, novel FGS contrast agents face a lengthy and expensive approval process to reach clinical use. Completion of preclinical development to enable Investigational New Drug (IND) approval for first-in-human trials represents the first major hurdle for FGS contrast agent development. Subsequently, completion of Phase I, II, and III clinical trials requires exponentially more time, effort, and money to complete successfully prior to market approval. Finding the necessary investments and strategic partners to navigate this long and costly process is difficult, especially for FGS contrast agents as non-curative diagnostic agents for single use, which provide a low financial incentive for investment from commercial sources.[30,31] Nonetheless, many startups, groups in academia, and even mid-scale biotechnology and medical device companies have worked towards clinical translation of a variety of novel FGS contrast agents. Herein, we review the current landscape of novel FGS contrast agents undergoing clinical translation and outline their integration with the current ecosystem of clinical, and pre-clinical, fluorescence imaging systems.

2. Clinically Approved Fluorescence Image-Guided Surgery Contrast Agents

A complete picture of the current clinical translation of FGS contrast agents cannot be achieved without including fluorescent agents with current clinical approval. Although few in number and most lacking specific targeting, these agents represent the majority of clinical work using FGS to date (Table 1). The majority of these contrast agents, Methylene Blue (MB), ICG, and Fluorescein, have been used as far back as 1891 for the treatment of diseases like malaria and as colorimetric reporters for tissue perfusion and angiography.[32,33] Initial use of these agents for FGS dates back to 1947, when fluorescein was used to guide brain tumor resection.[34] However, the agent used perhaps most frequently for FGS, ICG, has dominated the majority of clinical applications in recent years.

ICG is a water soluble tricarbocyanine fluorophore that possesses bright NIR fluorescence properties with excitation at 780 nm and emission at 820 nm.[35] The production and use of ICG dates back to 1955, when ICG was manufactured for use by Kodak Research Laboratories, and clinical approval dates back to 1956, when ICG was approved by the FDA for retinal angiography.[33] ICG clears rapidly from the body and possesses an excellent

safety profile, with LD_{50s} between 50–80 mg/kg in animals. ICG is currently used for sentinel lymph node mapping, angiography, reconstructive surgery, cholangiography, and tumor imaging among other uses for FGS and other clinical practices.[16,35–38]

One FGS contrast agent was only recently approved, 5-ALA, and is unique among the clinically approved agents in that it provides targeted fluorescence imaging of cancer instead of providing contrast via passive mechanisms. 5-ALA is metabolized to the fluorescent porphyrin molecule PpIX following uptake in cancer cells, possessing visible fluorescence with excitation at 380–440 nm and emission at 620–640 nm.[39,40] 5-ALA was first studied for FGS in 1998 and reached FDA approval in 2017.[41] Remarkably, 5-ALA enabled FGS has improved high grade glioma complete resection rates almost two-fold over white light imaging alone, resulting in increased overall progression free survival.[23] These FGS contrast agents, while few in number and possessing suboptimal fluorescent and/or tissue uptake properties, have demonstrated the incredible utility of FGS to improve surgical outcomes. Looking forward, many new FGS contrast agents are under development and possess advanced targeting, physiochemical, and tissue uptake characteristics.

2. Novel Fluorescence Image-Guided Surgery Contrast Agents Under Clinical Development

At present, 39 novel FGS contrast agents are undergoing clinical trials in the United States. These agents account for 85 clinical trials registered in clinicaltrials.gov across a broad range of indications. These agents and their corresponding clinical trials are outlined in Table 2. These clinical trials represent a diverse field of researchers, clinicians, and industry partners undertaking the clinical translation of a variety of unique targeted FGS contrast agents. A number of probes utilize targeting moieties such as antibodies or peptides to obtain highly specific fluorescent signal.[42] Others generate specific contrast via activatable fluorescence mechanisms using fluorophore quenching groups that are cleaved via enzymatic processes.[43] Others still possess structure inherent targeting mechanisms where the fluorophore itself acts as the targeting moiety and fluorescent reporter.[44–47] This diversity in probe design is evident in the molecular type of each contrast agent (Fig. 1), where the majority of contrast agents are small molecule based, followed closely by peptide and antibody based contrast agents.

From this diverse group of contrast agents for FGS, a handful have completed significant development through phase II clinical trials on the pathway to FDA approval. The clinical trial phase of each probe is outlined in Fig. 2. Currently, SGM-101, BLZ-100, LUM015, and OTL38 have reached phase III clinical trials (NCT03659448, NCT03579602, NCT03686215, and NCT03180307, respectively). SGM-101 is a CEA targeting antibody labeled with a NIR fluorophore under phase III testing for colorectal neoplasms. SGM-101 has demonstrated a high degree of specificity in primary tumor tissue and metastases, with mean tumor to background ratios of 1.6 and 1.7, respectively.[50] BLZ-100 is composed of a chlorotoxin peptide covalently bound to ICG that demonstrates high affinity for matrix metalloprotease 2 (MMP-2) under phase III testing for pediatric central nervous system (CNS) tumors. In phase I testing, BLZ-100 demonstrated low toxicity, with no observed

adverse events, and positive fluorescence signal in tumor tissue that was positively correlated with the dose of the imaging agent and grade of the cancer.[74] OTL38 is a small molecule probe consisting of folic acid, a ligand for folate receptor alpha, conjugated to the NIR fluorophore S0456 that is in phase III clinical studies for ovarian cancer. In phase II studies OTL38 identified cancer lesions with a sensitivity of 97.97%, where 48.3% of patients had at least one additional lesion identified using FGS over white light alone.[110] These probes represent every major type of targeted FGS contrast agent and are leading the way for clinical translation of many others.

For all other FGS contrast agents under clinical translation, probes in phase I clinical trials make up the majority of clinical studies, followed closely by phase II studies (Fig. 2). One option for easing the regulatory burden for first-in-human studies of new agents is to utilize the food and drug administration's (FDA's) exploratory investigational new drug (eIND) pathway. First in human (FIH) studies conducted under an eIND require significantly less preclinical toxicology testing by allowing researchers to administer "microdoses" denoted as less than 100 μg or 30 nmol per administration for small molecule or protein products, respectively.[113] Due to the lower administered dose, substantially fewer preclinical toxicology studies are required prior to FIH clinical trials as compared to traditional translation under an IND, allowing proof of concept phase 0 studies to be performed with relative ease and significantly decreased financial burden. This alternative route to clinical use has been utilized with success in the recent phase 0 clinical trials of ABY-029, a promising tumor targeted FGS affibody probe, BBN-IRDye800CW, a dual modality PET/FGS contrast agent for brain cancer resection, fluorescein conjugated wisteria floribunda (WFA), a topically applied lectin for colon cancer resection, and EC17, a small molecule folate-FITC conjugate targeting folate receptor for renal carcinoma resection.[66,67] The eIND pathway offers a promising alternative for ease of clinical translation for new FGS imaging probes, decreasing the financial burden to obtaining FIH results.

3. Fluorescence Spectral Properties and Compatibility with Clinical Imaging Systems

Additional diversity among the novel FGS contrast agents undergoing clinical translation is found in analysis of the fluorescence reporters' spectral properties (Fig. 3). A number of fluorophores have been utilized to provide contrast for each agent and those with reported excitation and emission are graphed in Fig. 3A along with the number of probes using each fluorophore. With fluorescence centered around 800 nm, IRDye800CW is the most often utilized fluorophore, providing contrast for more than double the number of probes than any other fluorophore. Examining the distribution of all 39 probes among the respective fluorescence NIR imaging channels (700 and 800 nm) and visible channel, just over half (20 probes) utilize fluorophores with excitation and emission wavelengths centered around 800 nm, with 9 probes using fluorophores with excitation and emission wavelengths centered around 700 nm and 10 probes using fluorophores with excitation and/or emission wavelengths in the visible range (Fig. 3B).

Assessing the compatibility of these fluorophores with clinical imaging systems requires a review of the currently clinically approved fluorescence imaging systems. Table 3 outlines the FGS imaging systems that are FDA approved or under development/clinical translation for human use. Notably, all FDA approved imaging systems are developed with fluorescence imaging capabilities compatible with the current FDA approved FGS contrast agents. Thus, the majority of systems are tuned for ICG fluorescence imaging, with excitation and emission wavelengths centered around 800 nm. Few FDA approved imaging systems possess capabilities for imaging fluorescein and PpIX fluorescence, and only two approved systems, the Fluobeam and Quest Spectrum, possess capabilities for imaging MB in the 700 nm channel. Thus, there exists a gap between novel FGS contrast agents' fluorescence properties and the imaging capabilities of clinically approved FGS imaging systems, where those contrast agents outside the 800 nm channel are lacking adequate options for spectrally tuned imaging systems. This gap exists likely due to the regulatory process for FGS imaging systems, where the majority of systems have achieved 510(k) clearance via predicate devices by showing substantial equivalence with already approved systems, most often the Stryker SPY imaging system that first obtained approval in 2005. However, a number of imaging systems are under development preclinically that could provide imaging capabilities for a wide range of fluorophores and incentive for approval of these systems will grow as novel FGS contrast agents with fluorescence outside of the 800 nm channel reach FDA approval (Table 3). For example, of the four novel FGS contrast agents in phase III clinical trials, two possess fluorescence properties outside the 800 nm channel including SGM-101, which uses BM104 as its fluorescent reporter with excitation and emission in the 700 nm channel, and LUM015, which utilizes Cy5 as its fluorescent reporter with excitation and emission in visible/NIR 700 nm channel.

4. Clinical Indications of Novel Fluorescence-Guided Surgery Contrast Agents

Analysis of the clinical indication studied in all clinical trials using novel FGS contrast agent reveals an overwhelming majority of agents targeted to cancer for enhanced detection and/or resection. 75, or 88%, of all novel FGS contrast agent clinical trials are underway for cancer related indications, while 6, or 7%, are underway for other disease related indications and only 4, or 5%, are underway for enhanced anatomical preservation. Thus, while great progress has been made in the field of FGS contrast agent development for improved cancer detection and resection, there remains a need for surgical treatment of non-cancer diseases and preservation of normal tissue function that can be enhanced using FGS. Interestingly, some cancer targeting agents have been employed in the treatment of other diseases, such as hyperparathyroidism (EC17, NCT01996072), carotid plaque instability (Bevacizumab-IRDye800CW, NCT03757507), or rheumatoid arthritis (OTL38, NCT03938701). Continued expansion of the many novel cancer targeted FGS contrast agents for other diseases could provide an excellent foundation for increasing the impact of FGS outside surgical oncology. Developing probes targeted to important anatomical structures, such as ureters or nerves, however, requires separate development efforts to identify targeting moieties for these non-diseased and intact tissues. Although, such development efforts possess a strong value and are worth undertaking, as injury to non-diseased tissues is responsible for a plethora of

surgical comorbidities that plague patient outcomes and present an enormous cost to the healthcare system. For instance, intraoperative nerve damage affects up to 63 million patients worldwide annually, causing pain or loss of function and significantly affecting quality of life.[114,115] These rates remain high despite efforts to improve nerve sparing through complex surgical techniques and nerve detection technologies in procedures that have a high incidence of injury.[115–123] Due to this need, several classes of nerve specific fluorophores have been studied for FGS preclinical.[10,124–134] Further clinical translation of these and other anatomy targeted FGS contrast agents will improve surgical outcomes overall and could be used in synergy with cancer or disease specific agents to comprehensively benefit surgical goals.

5. Conclusion

Remarkable progress has been made in the past several decades not only in the field of optical imaging and biophotonics, but in the fields of fluorophore chemistry and FGS contrast agent development. An array of novel FGS contrast agents are under clinical translation, and many more are in preclinical development, providing evidence for a rapidly expanding field that is poised to significantly affect the surgical practice. There is an incredible diversity of FGS contrast agent molecular type, targeting mechanism, and fluorescence properties, owing to the efforts of a diverse field of physicists, chemists, clinicians, biologists, and engineers. Continued development towards clinical approval of these novel FGS contrast agents and FIH clinical studies of non-cancerous disease specific and anatomy specific FGS contrast agents will enable significant advancement in the field of surgery as a whole and ultimately improve patient outcomes across many surgical specialties.

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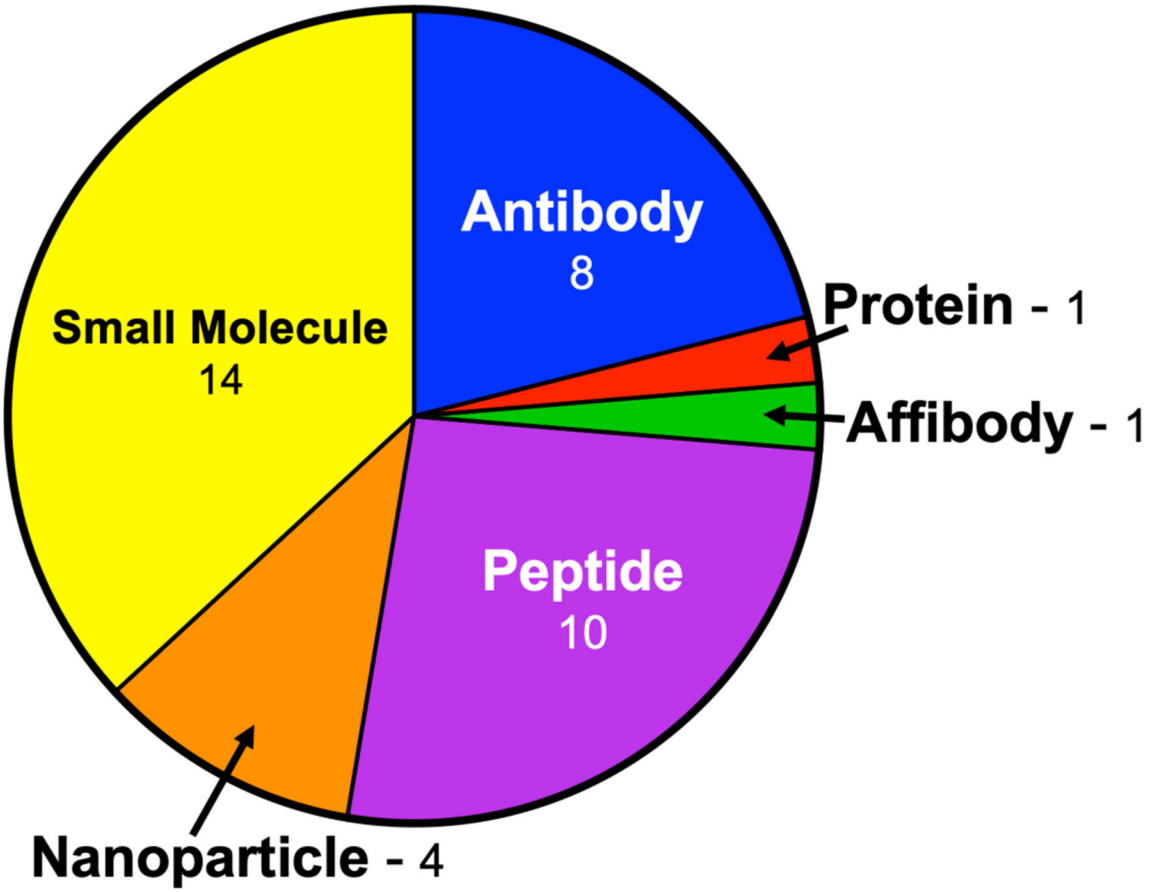

Figure 1.
A pie chart of novel FGS contrast agent currently undergoing clinical translation split by probe type. The number of each agent type is listed next to the type.

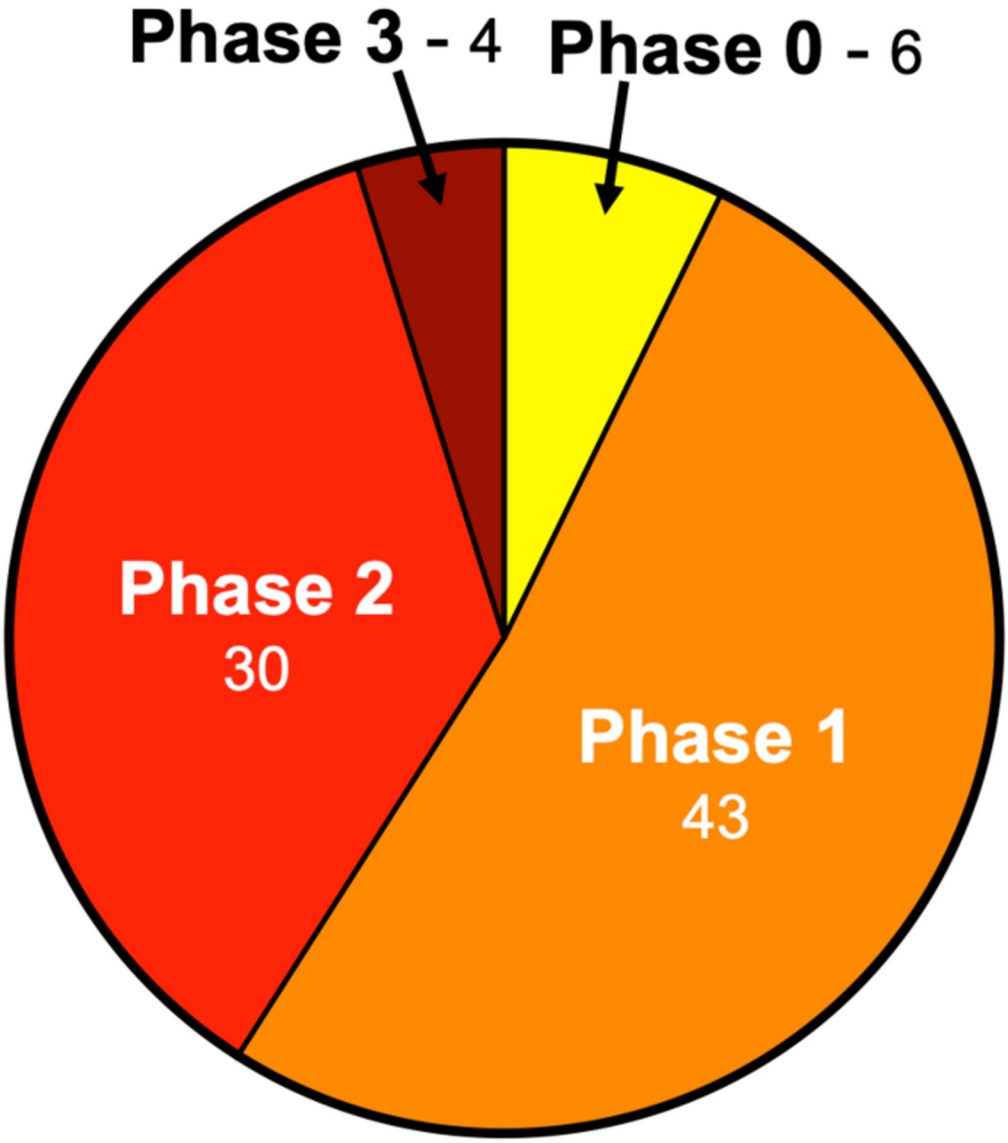

Figure 2.
A pie chart highlighting the clinical trial phase of the novel FGS contrast agents currently undergoing clinical translation. The number of agents in each phase is listed.

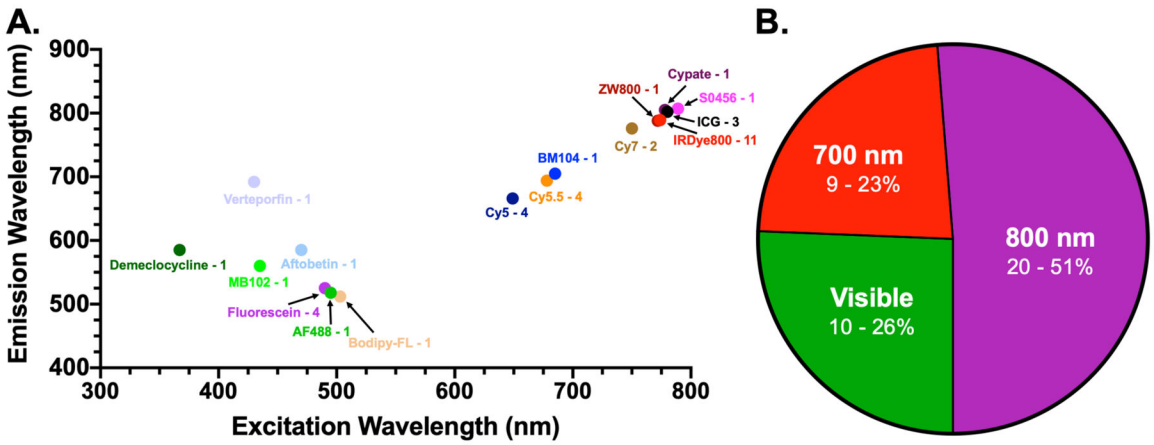

Figure 3. A.

Fluorescence excitation and emission wavelengths and **B.** imaging channel distribution of all fluorophores utilized by all novel FGS contrast agents undergoing clinical translation. The number of probes using each fluorophore are listed next to their names and the number of fluorophores in each channel are listed as well as the percentage of the total that number represents.

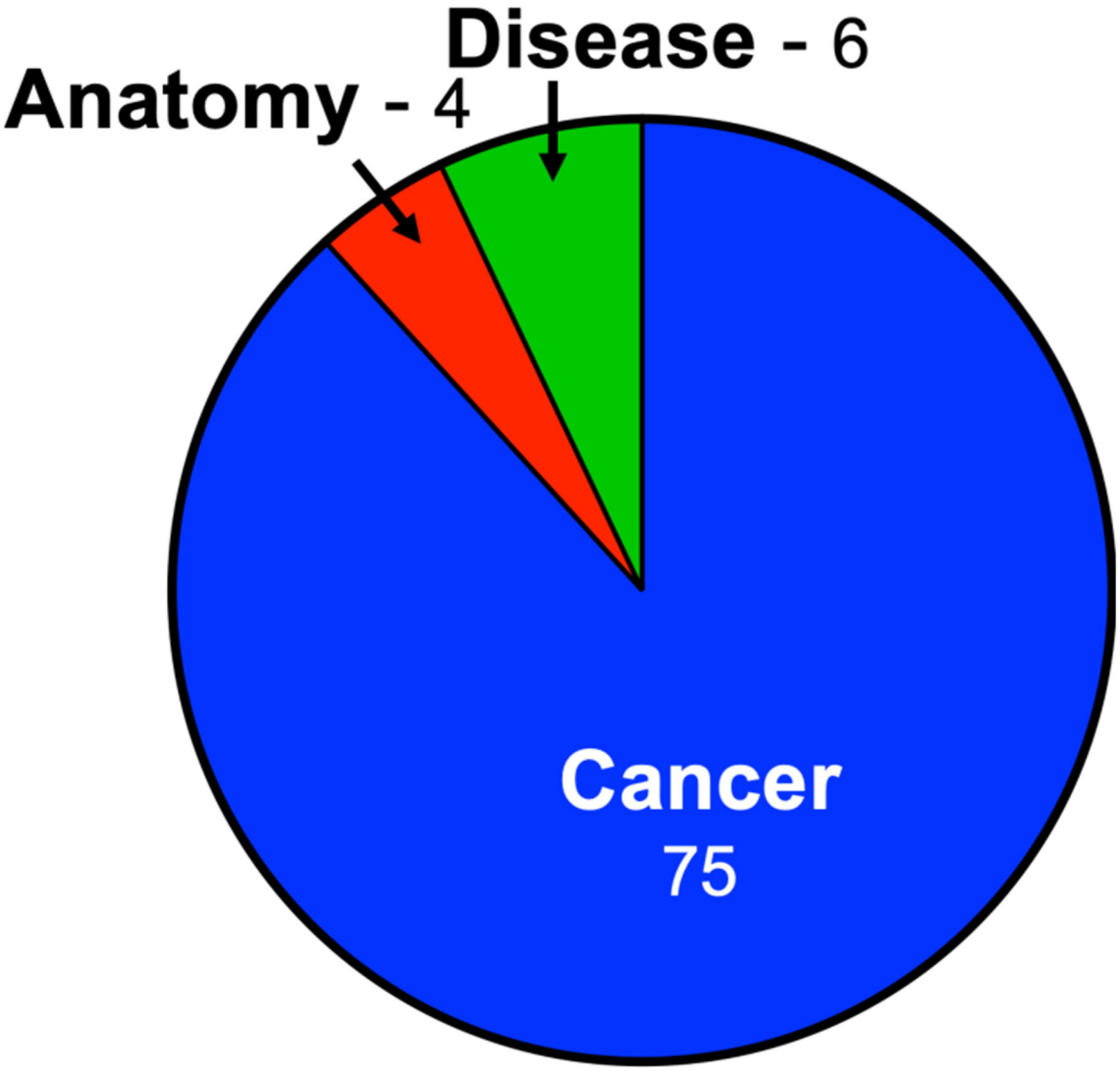

Figure 4.
A pie chart highlighting the broad clinical indication of the novel FGS contrast agents currently undergoing clinical translation. The number of agents targeted for each indication is listed next to it.

Table 1.

FDA-approved FGS contrast agents.

Name	Description	Chemical Class	Excitation Wavelength	Emission Wavelength
Fluorescein	Visible fluorophore often used for angiography and glioma resection. No specific targeting mechanism	Xanthene	494 nm	521 nm
Methylene Blue (MB)	Near-infrared fluorophore used in many FGS applications. No specific targeting mechanism.	Thiazine	668 nm	688 nm
Indocyanine Green (ICG)	Near-infrared fluorophore with the broadest clinical adoption and use. No specific targeting mechanism	Cyanine	780 nm	820 nm
5-aminominolevulinic acid (5-ALA)	Pro-drug that is metabolized to the visible fluorophore protoporphyrin IX (PplX) in cancer cells. Specifically targeted to cancer tissue.	Porphyrin	380–440 nm	620–640 nm

Table 2.

Novel FGS contrast agents under clinical development.

Probe Name	Description	Fluorophore	Company/ Group	Indications	Clinical Trial Phase	NCT#	Status
Antibody							
SGM-101[48–50]	Monoclonal antibody to carcinoembryonic antigen (CEA) labelled with 700nm BM104fluorophore. IV administration 4 days before surgery	BM104	Surgimab	Colon Cancer	Phase 2	NCT02973672	completed
				Rectum Cancer	Phase 2		
				Pancreatic Cancer	Phase 2		
				Metastatic Colorectal Cancer	Phase 2		
				Recurrent Colorectal Carcinoma	Phase 2		
				Colorectal Neoplasms	Phase 3	NCT03659448	recruiting
				Peritoneal Carcinomatosis	Phase 1	NCT02784028	recruiting
Panitumumab-IRDye800CW[51–54]	EGFR targeting antibody conjugated with IRDye800. IV administered over60min, 1–5 days prior to surgery	IRDye-800	Rosenthal-Stanford	Pediatric Neoplasms	Phase 2	NCT04085887	not yet recruiting
				Malignant Glioma	Phase 2	NCT03510208	recruiting
				Head and Neck Cancer	Phase 2	NCT03405142	recruiting
				Lung Cancer	Phase 2	NCT03582124	suspended (business decision)
				Pancreatic Cancer	Phase 2	NCT03384238	recruiting
Cetuximab-IRDye800CW[52,53,55–59]	EGFR targeting antibody conjugated with IRDye800. IV administered 4 days prior to surgery	IRDye-800	Rosenthal-Stanford	Head and Neck Cancer	Phase 2	NCT03134846	recruiting
				Pancreatic Cancer	Phase 2	NCT02736578	terminated (logistics)
				Esophageal Cancer	Phase 1	NCT04161560	recruiting
				Brain Cancer	Phase 2	NCT02855086	terminated (logistics)
Bevacizumab-IRDye800CW[52,60–62]	VEGF targeting antibody conjugated to IRDye800. IV administration 3 days prior to surgery at doses of 10, 25, or 50 mg	IRDye-800	van Dam-Groningen	Adenomatous Polyposis	Phase 1	NCT02113202	completed
				Rectal Cancer	Phase 1	NCT01972373	completed
				Breast Cancer	Phase 2	NCT02583568	completed
				Esophageal Cancer	Phase 2	NCT03877601	recruiting
				Hilar Cholangiocarcinoma	Phase 2	NCT03620292	recruiting
				Soft Tissue Sarcoma	Phase 2	NCT03913806	recruiting
				Pancreatic Cancer	Phase 2	NCT02743975	recruiting
				Endometriosis	Phase 1	NCT02975219	recruiting
				Inverted Papilloma	Phase 1	NCT03925285	recruiting
				Pituitary Adenoma	Phase 1	NCT04212793	not yet recruiting
				Carotid plaque instability	N/A	NCT03757507	not yet recruiting

Probe Name	Description	Fluorophore	Company/ Group	Indications	Clinical Trial Phase	NCT#	Status
lndium-111-DOTA-Labetuzumab-IRDye800CW[63]	CEA targeting antibody conjugated with dual modality (SPECT/CT and fluorescence) tracers. Administered 6–7 days before surgery.	IRDye-800	Boerman-Radbound University	Colorectal Cancer	Phase 2	NCT03699332	recruiting
lndium-111-DOTA-Girentuximab-IRDye800CW[64]	Carbonic anhydrase IX (CAIX) targeting antibody conjugated with dual modality (SPECT/CT and fluorescence) tracers. Administered 7 days before surgery.	IRDye-800	Boerman-Radbound University	Renal Carcinoma	Phase 1	NCT02497599	active, not recruiting
MDX1201-A488	PSMA targeting antibody conjugated with Alexa Fluor 488. IV administration 4 days prior to surgery	AF488	Zhumkhawala - City of Hope Medical	Prostate Cancer	Phase 1	NCT02048150	active, not recruiting
ProstaFluor	PSMA targeting antibody huJ-591 conjugated to IR-800CW	IRDye-800	Spectros	Prostate Cancer	Phase 1	NCT01173146	withdrawn - cost of antibody production increased, no supplemental funding obtained
Protein							
Fluorescein conjugated wisteria floribunda	Fluorescein conjugated lectin. Sprayed onto colonic surface during surgery	Fluorescein	Yeung - Oxford	Colorectal Cancer	Phase 0	NCT03070613	enrolling by invitation
Affibody							
ABY-029[28,65–67]	EGFR targeting affibody labeled with IRDye800CW. Microdose injection 1–3 hours prior to surgery	IRDye-800	Pogue - Dartmouth	Glioma	Phase 0	NCT02901925	suspended - awaiting FDA approval of eIND amendment
				Primary soft tissue Sarcoma	Phase 0	NCT03154411	suspended - awaiting FDA approval of eIND amendment
				Head and Neck Cancer	Phase 0	NCT03282461	suspended - awaiting FDA approval of eIND amendment
Peptide							

Probe Name	Description	Fluorophore	Company/ Group	Indications	Clinical Trial Phase	NCT#	Status
BLZ-100 (tozuleristide)[68–74]	Chlorotoxin (scorpion venom) with high affinity to matrix metalloprotease 2 (MMP-2) conjugated with NIR fluorophore. IV administration at least 1 hour before surgery	ICG	Blaze Bioscience	Soft Tissue Sarcoma	Phase 1	NCT02464332	withdrawn - not enough subjects enrolled
				CNS Tumors	Phase 3	NCT03579602	recruiting
				Glioma	Phase 1	NCT02234297	completed
				Breast Cancer	Phase 1	NCT02496065	completed
				Skin Neoplasms	Phase 1	NCT02097875	completed
AVB-620[75,76]	Ratiometric MMP activatable peptide labeled with cy5 and cy7. Upon activation, cy5 is cleaved, and cy5 fluorescence increases. IV administration up to 24 hr before	Cy5& Cy7 (cy5 detected)	Avelas Biosciences	Breast Cancer	Phase 2	NCT03113825	recruiting
BBN-IRDye800CW[76]	Peptide targeting gastrin-releasing peptide receptor (GRPR) labeled with IRDye 800 and radiotracer. IV administration 2 hr before surgery at 1 mg dose	IRDye-800	Chen-NIH	Brain Cancer	Phase 0	NCT02910804	recruiting
EMI-137[77]	Human hepatocyte growth factor receptor (c-MET) targeting peptide conjugated to cyanine based fluorophore (Cy5?). IV administration 1–3 hours before surgery	Cy5	Edinburgh Molecular Imaging	Colon Cancer	Phase 2	NCT03360461	recruiting
				Thyroid Cancer	Phase 1	NCT03470259	completed
				Barret Esophagus	Phase 1	NCT03205501	recruiting
				Lung Cancer	Phase 1	NCT02676050	not yet recruiting
QRH-882260 Heptapeptide[78]	Seven amino acid long peptide that binds to EGFR labeled with cy5. Orally administered and binds to tumor cells in Gl tract.	Cy5	Wang-University of Michigan	Colon Cancer	Phase 1	NCT03148119	terminated (QRH to be used for other indications)
				Cholangiocarcinoma	Phase 1	NCT03438435	recruiting
				Barret Esophagus	Phase 1	NCT03589443	completed
				Safety study	Phase 1	NCT02574858	completed
KSP/QRH peptide dimer[79]	EGFR and HER2 targeting peptide labelled with IRDye800. Orally administered or sprayed onto area of interest	IRDye-800	Wang-University of Michigan	Barret Esophagus	Phase 1	NCT03852576	recruiting
Gl heptapeptide[80]	Heptapeptide labeled with FITC. Orally administered or sprayed onto area of interest	FITC	Wang-University of Michigan	Barret Esophagus	Phase 1	NCT01630798	completed

Probe Name	Description	Fluorophore	Company/ Group	Indications	Clinical Trial Phase	NCT#	Status
KCC heptapeptide[81]	Heptapeptide labeled with FITC. Orally administered or sprayed onto area of interest	FITC	Wang- University of Michigan	Colorectal Cancer	Phase 1	NCT02156557	completed
LS301[82,83]	integrin receptor targeting octapeptide conjugated to NIR fluorophore cypate. IV administration 1 day prior to surgery.	Cypate	Achilefu - Washington University	Breast Cancer	Phase 2	NCT02807597	not yet recruiting
				Pancreatic Cancer	Phase 2	NCT04105062	not yet recruiting
				Liver Cancer	Phase 2		
				Gastric Cancer	Phase 2		
				Gastrointestinal Stromal Cancer	Phase 2		
				Metastatic Cancer	Phase 2		
RGD peptide -cy7 (ORL-1)[84–88]	Alpha(v) beta(3) integrin targeting peptide labeled with cy7. Topically applied to skin surface.	Cy7	Orlucent	Melanoma		NCT03535077	recruiting
Nanoparticle							
cRGDY-PEG-Cy5.5-C*[89]	Integrin-targeting, dual modality (PET & fluorescent) silica nanoparticle labeled with cy5.5. Injected at site of tumor before or during surgery	Cy5.5	Patel - Sloan Kettering	Head and Neck Cancer	Phase 2	NCT02106598	recruiting
				Breast Cancer	Phase 2		
				Colorectal Cancer	Phase 2		
cRGD-ZW800–1[87,90]	Integrin-targeting silica nanoparticle labelled with ZW800. Injected 4–24 hours prior to surgery.	ZW-800	Keereweer - Erasmus Medical Center	Head and Neck Cancer	Phase 2	NCT04191460	not yet recruiting
64Cu-NOTA-PSMAi-PEG-Cy5.5-C*[91]	PSMA targeting nanoparticle, multi-modality (**PET**, MRI, fluorescent) labelled with Cy5.5.	Cy5.5	Memorial Sloan Kettering	Prostate Cancer	Phase 1	NCT04167969	recruiting
ONM-100[92]	pH-sensitive micelles conjugated with ICG. IV administration on day of surgery.	ICG	OncoNano Medicine	Breast Cancer	Phase 2	NCT03735680	recruiting
				Head and Neck Cancer	Phase 2		
				Colorectal Cancer	Phase 2		
				Bladder Cancer	Phase 2		
				Prostate Cancer	Phase 2		
				Ovarian Cancer	Phase 2		
Small Molecule							
Demeclocycline[93]	Antibiotic with UV abs/yellow fl. Administered orally for 2 day	Demeclocycline	Curry - Massachusetts General	Brain Tumor	Phase 1	NCT02740933	not yet recruiting

Probe Name	Description	Fluorophore	Company/ Group	Indications	Clinical Trial Phase	NCT#	Status
IRDye-800BK[94]	injected into urethra and imaged immediately	IRDye-800BK	Barnes - Oxford	Ureter Injury	Phase 2	NCT03387410	completed
LUM015[95–97]	Cathepsin-activatable labeled with cy5 and fluorescent quencher linked by a pan-cathepsin protease cleavable peptide. IV injection 2–6 hrs prior to surgery at 1 mg/kg dose.	Cy5	Lumicell	Breast Cancer	Phase 3	NCT03686215	recruiting
				Colorectal Cancer	Phase 2	NCT02584244	recruiting
				Barret Esophagus	Phase 2		
				Pancreatic Cancer	Phase 2		
				Brain Cancer	Phase 1	NCT03717142	recruiting
				Prostate Cancer	Phase 1	NCT03441464	recruiting
IS-001[44]	Agent for use with daVinci robot	IS-001	Intuitive Surgical	Hysterectomy - Ureter Injury	Phase 2	NCT03937505	recruiting
PARPi-FL[98–100]	PARP1 inhibitor (olaparib) labeled with BODIPY-FL. Applied topically for basal cell carcinoma	BODIPY-FL	Reiner - Sloan Kettering	Oral Squamous Cell Carcinoma	Phase 2	NCT03085147	recruiting
HS-196[101,102]	Heat shock protein 90 (HSP90) inhibitor labelled with NIRdye. IV administration	N/A	Lyerly - Duke	Solid Tumor	Phase 1	NCT03333031	recruiting
TMVP1-ICG	Used for SLN mapping, injected into the cervix	ICG	Ding Ma - Huazhong University	Cervical Cancer	Phase 1	NCT03320772	recruiting
EC17[103–105]	Folate-FITC conjugate targeting folate receptor. IV administration 2–4 hours before surgery at 0.1 mg/kg dose	FITC	Singhal - University of Pennsylvania	Hyperparathyroidism	Phase 1	NCT01996072	completed
				Breast Cancer	Phase 1	NCT01994369	completed
				Ovarian Cancer	Phase 1	NCT02000778	completed
				Renal Carcinoma	Phase 0	NCT01778933	completed
				Lung Cancer	Phase 1	NCT01778920	completed
OTL38[106–110]	Folate receptor alpha (Fra) targeting ligand (folic acid) conjugated to NIR fluorophore S0456. IV administration 2–3 hrs before surgery	S0456	On Target Laboratories	Ovarian Cancer	Phase 3	NCT03180307	recruiting
				Lung Cancer	Phase 2	NCT02872701	completed
				Lung Cancer	Phase 1	NCT02769156	recruiting
				Lung Cancer	Phase 1	NCT02602119	recruiting
				pituitary adenoma	Phase 1	NCT02629549	terminated (recruitment fulfilled)
				Malignancies in pituitary gland	Phase 1	NCT02769533	completed
				Bladder Cancer	Phase 1	NCT02852252	recruiting
				Rheumatoid Arthritis	Phase 1	NCT03938701	not yet recruiting
				Renal Carcinoma	Phase 1	NCT02645409	completed
Li-COR Ureter Agent	IV administration during surgery at 0.06 mg/kg	IRDye-800BK	Li-COR	Ureter Injury	Phase 2	NCT03106038	completed
MB-102[46,47]	Human plasma fluorescence tracer.	MB-102	MediBeacon	Acute Kidney Injury	Phase 2	NCT02772276	recruiting

Probe Name	Description	Fluorophore	Company/ Group	Indications	Clinical Trial Phase	NCT#	Status
Aftobetin - HCI	Amyloid beta binding ligand. Administered via opthalmic ointment, eye lens imaged.	Aftobetin	Cognoptix	Alzheimer's Disease	Phase 1	NCT02928211	recruiting
HS201	HSP90 inhibitor connected by linker to verteporfin (imaging agent and photosensitizing agent).	Verteporfin	Lyerly - Duke	Solid Tumor	Phase 1	NCT03906643	not yet recruiting
LuminoMark	Fluorescence localization in patients with nonpalpable breast lesions - not clear if this is a functionalized version of ICG or tagged molecule	ICG	Hanlim Pharm. Co. Ltd.	Breast Cancer	Phase 2	NCT03743259	completed
Prosense/VM110[111,112]	Near infrared fluorophore self-quenched and activated when cleaved by proteases, cathepsin B,L, and S and plasmin in cancer cells, fluorescent cleavage product detected. Combination product.	Cy5.5	Weissleder - Harvard	Ovarian Cancer	Phase 1	NCT03286062	active, not recruiting
				Pancreatic Cancer	Phase 1		

Table 3.

Current FGS imaging systems.

System Name	Description	Company/Group	Excitation (nm)	Emission (nm)	Approved?
Fluobeam	Hand held NIR imaging system (800nm channel approved, 700nm channel (680nm ex - 700 nm em developed)	Fluoptics Imaging	750	800 longpass	Yes
PDE/Photodynamic EyeNeo(ll)	Hand held NIR imaging, uses LEDs	Hammamatsu/ Mitaka	760	820 longpass	Yes
Firefly	Endoscopic fluorescence imaging system for robotic surgery	Intuitive Surgical	805	805 blocking	Yes
Image 1S Camera System	Endoscopic fluorescence imaging system	Karl Storz	N/A	N/A	Yes
Vitom II	Karl Storz Image 1s camera system, but used in open surgery as a microscope	Karl Storz	N/A	N/A	Yes
GLOW800	Leica Surgical Microscope accessory (M530)	Leica	790	835	Yes
FL560	Leica Surgical Microscope accessory (M530)	Leica	460–500	510 longpass	Yes
FL800	Leica Surgical Microscope accessory (M530)	Leica	700–800	820–860	Yes
FL400	Leica Surgical Microscope accessory (M530)	Leica	380–430	444 longpass	Yes
Pinpoint	Endoscopic fluorescence imaging system (“deep red” [for MB] system used in clinical trials)	Novadaq/Stryker	805	825–850	Yes
SPY PHI	Hand held fluorescence imaging system	Novadaq/Stryker	805	825–850	Yes
SPY Elite	On cart for open surgery fluorescence imaging system	Novadaq/Stryker	805	825–850	Yes
AIM (Advanced Imaging Modality)	Stryker’s AIM camera combined with SPY, SPY Elite, Pinpoint	Stryker	805	825–850	Yes
Visera Elite II	NIR and narrow band imaging in endoscope (Approved in Europe but not US?)	Olympus	N/A	N/A	Yes
Artemis	Hand held NIR fluorescence imaging system	Quest Medical Imaging	N/A	N/A	Yes
Spectrum	Replaced Artemis, hand held with 700 and 800 nm imaging channel, uses LEDs	Quest Medical Imaging	N/A	700–830, 830–1000	Yes
VS3 IR/Iridium System	3D endoscope or open surgical system with NIR fluorescence channel	VisionSense/ Medtronic	805	825–850	Yes
Yellow 560	Zeiss Surgical Microscope accessory (Kinevo 900)	Zeiss	460–500	550–700	Yes
Infrared 800	Zeiss Surgical Microscope accessory (Kinevo 900)	Zeiss	700–780	820–900	Yes
Blue 400	Zeiss Surgical Microscope accessory (Kinevo 900)	Zeiss	400–410	620–710	Yes
Fluorescence Goggle System	Augmented reality goggle system for FGS	Achilefu (Wash U)	780	N/A	No

System Name	Description	Company/Group	Excitation (nm)	Emission (nm)	Approved?
OPAL	Light projection system for visualization on tissue surface in open surgery	Akers (Wash U)	780	785 longpass	No
GXMI Navigator	Cart based system	Chinese Academy of Sciences	760	810–870	No
FLARE	Cart based system for open surgery, 700 & 800 nm channel (mini-FLARE latest iteration)	Curadel	656–678, 745–779	689–725, 803–853	No
IC-flow	Handheld imaging system	Diagnostic Green	780	N/A	No
HyperEye Medical System	Hand held fluorescence imaging system	Mizuho Medical Company	760–780	800–850	No
Solaris	Filtered LED light source, on cart	PerkinElmer	488, 667, 743, 757	516–523, 692–742, 770–809, 784 LP	No
Visual Navigator	Hand held system	SH System	740	820	No
Explorer Air	Multispectral imaging platform (currently in clinical trials in EU, formerly SurgOptix T-3)	Surgvision	520, 800	N/A	No