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Image Guided Tumor Resection

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Abstract

Each year, millions of individuals undergo cancer surgery that is intended to be curative or at least a necessary component of a curative regimen. Particularly for those patients whose cancer harbors cells that are resistant to chemotherapy or radiation, the extent of surgery often defines whether they will be a survivor or casualty of the disease. For many solid tumor types, the difference in survival between patients who undergo gross total resection and those who have residual bulky disease is often profound. With surgery being central to cancer survivorship, it is stunning how few resources have been invested in improving surgical outcomes, particularly in comparison to chemotherapeutic research and discovery. This article reviews recent advances related to developing targeted fluorescent agents to guide surgeons during cancer removal. The goal of these drugs and devices is to clearly distinguish cancer from normal tissue to improve surgical outcome for cancer patients.

The unmet clinical need

In certain cases, distinguishing between cancer and normal tissue affects not only survival, but quality of life for those who survive. For patients with cancer of the tongue, esophagus, or anus, preserving key normal tissues while completely resecting the cancer impacts the most basic functions of daily life.

In certain brain tumors, complete resection of non-metastatic tumors has significant clinical value resulting in improved survival and reduced requirement for radiation or chemotherapy. In pediatric ependymoma patients where it is possible to achieve gross total resection of posterior fossa lesions, patients experience an 80% event free survival (EFS), and over 90% overall survival at 5 years even without subsequent radiation or chemotherapy [1]. By contrast, children with ependymomas and residual bulky disease post-surgery surgery require chemotherapy and radiation and experience a less than 50% 5 year EFS [1]. The

situation is similar in young medulloblastoma patients where gross total resection (GTR) followed by 18 Gy craniospinal radiation experience approximately 75% 5 year EFS. By contrast, patients with incompletely resected medulloblastomas and bulky residual disease require 36 Gy craniospinal radiation and experience only approximately 60% 5 year EFS [2]. The additional radiation and chemotherapy required in these cases frequently results in severe neurocognitive damage and other toxicities [3, 4].

The potential benefit of image guided tumor resection for patients with glioblastoma multiforme (GBM), the most aggressive and common adult brain tumor, is more controversial. Skeptics correctly point out that diffuse invasion of glioma cells occur well beyond any reasonable surgical margin, and can even be found on the contralateral side of the brain at the time of diagnosis. However, it is also well known that surgical resection is the most effective therapy known to increase survival, and several studies have shown a clear improvement in survival with increased percent of resection [5, 6]. Further, fluorescent compounds that mark the tumor intraoperatively, such as 5-ALA, have been used to enhance the percent of tumor resected resulting in improved median survival, but increased deficits [7]. Likewise, multi-institutional trials of pediatric glioma patients show long-term survival of pediatric high-grade glioma patients depends on GTR [8]. Presumably radiation therapy and chemotherapy are more effective for microscopic residual disease than for bulky disease due to the mechanisms of therapy resistance that flourish within the tumor microenvironment.

Limiting the frequency of second surgeries is a key goal of many investigators. Nearly 25% of women who undergo breast conservation surgery for breast cancer [9] and 20 % of patients with prostate cancer [10] learn that the margins of their cancer were positive days to weeks *after* the surgery has been completed. Gaining insight into whether margins are free of detectable disease in real time during the initial surgery could reduce second surgeries for patients and ultimately result in significant cost savings to healthcare and insurance systems. Similarly, pathologists may be able to use benchtop imaging devices to identify the margins of a tumor that are most likely to be positive for cancer cells, thus focusing their time and effort on high payoff regions rather than potentially missing key regions because there is simply too much tissue to evaluate microscopically. Furthermore, some cancer cells may appear identical or nearly identical to non-neoplastic cells by light microscopy, even with immunohistochemical stains. While it will need to be determined for each probe and each tumor type, it is conceivable that image guided pathology will reveal microscopic disease that is undetectable by current methods.

Informing adjuvant therapy

In some instances, fluorescence imaging for cancer can guide more than surgery. In cases where cancer is thought to be localized to a single location based on CT or MRI scans, fluorescence imaging can illuminate microscopic tumor studding that is below the limit of detection for other modalities. A recent study with fluorescein-labeled folate revealed that surgeons observed an average of seven tumor loci per ovarian cancer patient without the imaging agent and observed an average of 34 tumor loci per patient with fluorescence guidance [11]. Clearly it would be futile to surgically remove dozens of microscopic cancer

foci and clearly these patients require adjuvant therapy even if CT scans reveal a single site of disease and surgeons achieve a complete resection of that locus with clean surgical margins.

The National Cancer Institute created a devoted community of investigators that are advancing image guided surgery strategies. The US Food and Drug Administration (FDA) and its European counterpart have issued clear and helpful guidance documents to assist those who intend to create clinical candidates [12–16]. While reporting all progress in this rapidly evolving field is beyond the scope of a single review article, the following sections attempt to provide a high-level overview of progress and considerations related to ligands and devices for image guided resection.

Emerging image guidance modalities

The use of imaging to guide tumor resection encompasses everything from simple white light illumination of a surgical field to the sophisticated neuronavigation systems that enable neurosurgeons to orient the surgical field to a pre-operative MRI scan. Recent innovation in this field, driven by the need for better real-time intraoperative tools, has taken the form of optical, primarily fluorescence, imaging in real or near-real time. Approaches across all surgical oncology indications include systems that measure differences in endogenous tissue properties, and systems that depend on an exogenous imaging agent.

Systems that measure changes in endogenous tissue properties, including autofluorescence, exploit cellular characteristics that may be altered in neoplastic tissue. These include differences in DNA, amino acid, collagen, and glycolipid composition, porphyrin levels, hemoglobin content and saturation, oxidation, cellular architecture, or angiogenesis (for reviews, see [17], [18], and [19]). These changes are inherently subtle, but consistent enough to offer additional information that supports lesion localization and margin assessment. Clinically they have been applied, for example, in bedside margin assessment for breast cancer [18], and in indications such as head & neck cancer [17], esophageal cancer [20], and lung cancer [19]. More recently, mass spectrometry has been applied to analyze lipid profiles in aerosolized samples [21, 22].

Non-targeted imaging agents

The use of an exogenous contrast agent may enhance differences between tumor and non-tumor tissue, and thereby simplify the distinction. Exogenous agents can be either non-targeted or targeted to antigens that are preferentially expressed in tumor, tumor vasculature, or microenvironment. Non-targeted agents provide contrast due general properties of tumors, rather than presence of a specific antigen, and they include unconjugated fluorescent dyes and a subset of nanoparticles. Unconjugated fluorescent dyes such as fluorescein [23], methylene blue [24], or indocyanine green [23, 25] can be delivered locally or systemically, and accumulate in areas of tumor tissue that have disrupted or altered tumor vasculature and drainage.

The metabolic agent 5-aminolevulinic acid (5-ALA) induces production of fluorescent protoporphyrin IX (PpIX) in tumor cells. 5-ALA has been used in a variety of clinical

settings, including photochemotherapy in the dermatology setting, detection of bladder tumors following interstitial delivery, and detection of glioma following systemic delivery. A randomized Phase 3 clinical trial in adult glioma showed a significant increase in “complete” resections compared to conventional surgery (65% vs. 36%), with complete resection significantly influencing progression free survival [26]. A prospective comparison with intraoperative MRI has shown that the use of 5-ALA can improve detection of tumor, particularly in infiltrative zones [27]. Unfortunately, many low-grade and pediatric brain tumors do not produce clinically useful fluorescence following 5-ALA administration [28, 29]. Due to its excitation in the ultraviolet and emission in the visible spectrum, visualization of PpIX fluorescence is restricted to within a few millimeters of the tissue surface. Normal brain tissue can obscure 5-ALA – induced fluorescence, in some cases resulting in residual tumor being left behind and repeat surgery [30].

Nanoparticles

A variety of nanoparticle formats are being investigated for fluorescence-guided resection as well as diagnostic imaging. Non-targeted nanoparticles, like unconjugated dyes, can enter and be retained within tumors due to EPR (enhanced permeability and retention), an effect of tumor vasculature that is non-specific and varies with the size and type of lesion. Because they have large surface areas and can be functionalized, nanoparticles can be targeted to antigens as well as carry multiple “payloads,” including fluorescent dyes, radioactive tags for imaging or therapeutic utility, and agents for photodynamic or photothermal therapy. The utility of near-infrared (NIR) fluorescent dyes is well-recognized in the field [31], and many nanoparticle-NIR dye conjugates are being developed. While these conjugates can provide very intense labeling and good contrast, they face significant technical and regulatory hurdles for clinical applications. Nanoparticles intended solely for imaging will have a different risk-benefit profile as compared to those used for therapeutic purposes [32], requiring them to be safer. Early clinical development has been initiated with C-dots, an integrin-targeting nanoparticle that is used for pre-operative PET imaging as well as intraoperative fluorescence imaging [33].

Targeted imaging agents

Other targeted imaging agents have been constructed using dye conjugates with antibodies, peptides, or natural ligands to tumor targets. A number of RGD-containing peptides are being developed to target integrins on tumor vasculature [34]. These probes will highlight tumor vasculature and in some cases tumor cells themselves. Another approach that focuses on the tumor microenvironment uses tumor associated enzymes (e.g., caspase) to cleave imaging pro-drugs into fluorescing metabolites [35, 36]. There have been several preclinical studies and a few clinical trials of approved therapeutic antibodies conjugated to NIR fluorophores; these include cetuximab and panitumumab (EGFR), trastuzumab (HER-2), and bevacizumab (VEGF) [37–39]. Due to their large size, antibody conjugates may penetrate less effectively into tumor tissue compared with small molecule, aptamer, or peptide conjugates. Small molecule conjugates are being developed that target folate receptor alpha and PSMA [40]. As noted above, a folate-FITC conjugate was evaluated for intraoperative imaging during ovarian cancer resection, where fluorescence imaging resulted in

identification of peritoneal metastases that were not visible to surgeons using white light alone [11]. While offering high specificity for tumors expressing the targeted antigen, these conjugates are not likely to be broadly applicable across multiple tumor types.

The cationic peptide chlorotoxin is internalized by cancer cells in a wide variety of solid tumor indications [41, 42]. A radiolabeled chlorotoxin peptide validated these findings in humans [43–45]. Furthermore, BLZ-100, a variant of chlorotoxin conjugated to a form of indocyanine green, is able to distinguish very small foci of brain tumor cells from normal brain (Fig 1). Compared to non-targeted agents that rely on disrupted blood brain barrier and EPR, molecules that specifically target cells and are internalized have the potential to distinguish cancer from normal tissue on a near cell by cell basis provided that high resolution detection devices are used.

Exploiting more general properties of cancer cells is a strategy that may lead to other imaging agents that can be applied to multiple tumor types. Examples of such approaches include a phospholipid ether analog that targets lipid raft structures [46], and probes that are cleaved by extracellular matrix enzymes overexpressed at tumor borders, such as cathepsins and matrix metalloproteinase [47]. One example of this technology is a cell-penetrating peptide that is protected until the probe is cleaved or processed by the matrix metalloproteinase enzymes, after which the peptide penetrates nearby cells [48]. This approach ensures that the activated probe is retained in the tissue surrounding the site of activation, which is important for maintenance of distinct tumor boundary imaging throughout the time frame of a surgery.

Targeting agent considerations

The observation that some fluorescent molecules preferentially distribute to some cancers compared to adjacent tissue was first made over 50 years ago. These agents have not advanced to human clinical use primarily because the disrupted barriers in tumors are heterogeneous, so some regions of cancer are not detected. Also, some non-neoplastic tissues surrounding the tumor non-specifically take up the agents as well. Hence both sensitivity and specificity are problems and these are the two parameters that are critical for providing useful information to surgeons. For this reason, many investigators and clinicians favor targeted agents, particularly those that bind directly to tumor cells with sufficient affinity or avidity that tumor cell rather than interstitial or peri-tumoral fluid is marked by fluorescent signal.

Device considerations

Most new fluorescent imaging agents are being developed with probes that emit in the near infrared (NIR) range because normal tissues have little autofluorescence at these wavelengths and because the light passes through small amounts of tissue that might lie between the camera and the fluorescent tissue (Fig. 2). The devices for detecting NIR fluorescence are evolving rapidly and include endoscopes or exoscopes, surgical microscopes, and robotic systems, most incorporating CCD camera technology [49, 50]. Devices that collect and integrate images over short periods of time may have superior sensitivity compared to microscopes, but devices that can display high-definition images in

real time will likely be preferred. For surgical margin evaluation, particularly in deep pockets during tumor debulking, flexible devices that can “see” around corners will provide an advantage over cameras or microscopes that are suspended over the patient.

Economic considerations of developing molecular imaging agents

The FDA regulates imaging agents as drugs. Published guidance documents and regulatory precedent focus on safety and the predictive value of the agents for accurately distinguishing cancer from normal tissue. As such, the cost of non-clinical studies, manufacturing, early stage clinical and registration trials will often exceed \$100M US dollars regardless of the market potential. Unlike chemotherapeutic agents that are prescribed for months or years, imaging agents are often used only once per patient so the potential to recover research and development costs is challenging.

Targeting molecules that bind to many types of cancer have potential to help more patients than those developed for a narrow subclass of cancer patients and may be more economically viable to develop. That said, there are certain instances in which a target is very highly expressed on a specific type of cancer cell and in which residual microscopic disease cannot be adequately addressed by adjuvant therapy due to inherent resistance to chemotherapy or radiation therapy. In these situations, agents with exceptional tumor to background ratio coupled with exquisitely sensitive devices could benefit patients and still be economically viable.

Conclusion

It seems to be only a matter of time before image guided surgery is commonplace and surgeons will reminisce about trying to distinguish cancer from normal tissue using their eyes, fingers and thumbs. In the meantime, the race is on among device designers and manufacturers. Clinical proof of concept experiments with intriguing molecular imaging agents reveal the potential power of this approach and increasingly candidate agents are being tested in larger numbers of cancer patients (Table 1). The evolution of image guidance will likely be to surgeons and pathologists what the CT or MRI scan was to radiologists. Importantly, these imaging modalities provide new information to clinicians rather than absolute diagnostics. Thus, medical judgment, experience, and expertise will continue to play a key role to minimize both undertreatment and overtreatment of cancer patients following image guided tumor resection.

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Figure 1. Detection of tumors in a mouse medulloblastoma model using BLZ-100 fluorescence (A) Odyssey image of whole brain. Fluorescence from the gross tumor is evident in the lower left quadrant. Signal in tumor was about 20-fold higher than in normal brain. A fluorescent spot that was well above background was noted (arrow). (B) H&E stained sections showed a small tumor lesion in this area of the brain. (C) High resolution image of the area delineated by the blue box in panel B. Scale bar = 138 microns.

Figure 2. Imaging BLZ-100 fluorescence in a mouse orthotopic glioma xenograft model
 (A, B) White light and NIR images prior to exposure of tumor. Lesion is visible through skin and skull. (C, D) White light and NIR images following removal of overlying skin. Note the increase in brightness, contrast, and sharpness. (E, F) White light and NIR images of fully exposed tumor (which had adhered to the inside of the skull) and normal brain. Fluorescence scale bars are shown to the right of each NIR image. NIR images were captured using a prototype open imaging system [51] by PerkinElmer.

Table 1

Selected current clinical trials of fluorescent imaging agents for tumor resection

Agent	Imaging system	Phase	Indication	# of subjects	Design	Primary endpoints	NCT number (clinicaltrials.gov)
Targeted Agents							
Bevacizumab-IRDye800	MSOT FDOT	1	Breast cancer	30	Single arm	Uptake in cancer, LN, surrounding tissue	NCT01508572
Bevacizumab-IRDye800	NIR endoscope	1	Esophageal cancer Dysplasia	10	Single arm	Fluorescence intensity	NCT02129933
Bevacizumab-IRDye800	NIR endoscope	1	Familial adenomatous polyposis	15	Non-randomized (time point groups)	Number of fluorescent adenomatous polyps	NCT02113202
BLZ-100	Fluobeam 800	1	Glioma	21	Single arm	Safety	NCT02234297
BLZ-100	Fluobeam 800	1	Skin neoplasms	30	Single arm	Safety	NCT02097875
Cetuximab-IRDye800		1	Head and neck cancer	15	Single arm	Safety	NCT01987375
cRGDY-PEG-Cy5.5-C dots	Artemis	0	Multiple solid tumor types	30	Single arm	Feasibility for SLN mapping	NCT02106598
Folate-FTTC		1	Breast cancer	10	Single arm	Feasibility	NCT01994369
Folate-FTTC		1	Lung cancer	48	Single arm	Feasibility	NCT01778920
Folate-FTTC		1	Ovarian cancer	10	Single arm	Feasibility	NCT02000778
Folate-FTTC		0	Renal cell carcinoma	10	Single arm	Feasibility	NCT01778933
LUM015		1	Sarcoma Breast cancer	15	Single arm	Safety, dose	NCT01626066
MDX1201-A488			Prostate cancer	30	Single arm	Fluorescence intensity	NCT02048150
OTL38		2	Ovarian cancer	45	Single arm	Safety Tumor to background ratio	NCT02317705
Non-Targeted Agents							
5-ALA		2	Brain cancer	120	Randomized (dose groups)	Fluorescence intensity	NCT00752323
5-ALA		1/2	Brain cancer	15	Single arm	Safety	NCT01128218
5-ALA		2	Brain cancer	108	Randomized (5-ALA vs 5-ALA + iMRI)	Extent of resection	NCT01798771
5-ALA		1	Brain cancer	540	Single arm	Fluorescence correlation with histopathology	NCT02191488
5-ALA		1	Brain tumors	234	Single arm	Correlation of fluorescence with conventional imaging data	NCT00870779
5-ALA	PRODIGI		Breast cancer	45	Controlled (No agent vs 5-ALA dose groups)	Sensitivity, specificity	NCT01837225
5-ALA		2	Glioblastoma	60	Single arm	Safety in combination with Gliadel wafers	NCT01310868
5-ALA		3	Glioblastoma	204	Randomized (5-ALA vs placebo)	Rate of complete resections	NCT01811121

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Agent	Imaging system	Phase	Indication	# of subjects	Design	Primary endpoints	NCT number (clinicaltrials.gov)
5-ALA	Modified neurosurgical microscope	2	Glioma	300	Single arm	Efficacy	NCT011116661
5-ALA		2	Glioma	83	Single arm	Extent of resection	NCT014445691
5-ALA	Confocal microscopy	3	Glioma	192	Randomized (5-ALA vs placebo)	Extent of resection	NCT01502280
5-ALA		1/2	Pediatric CNS tumors	100	Single arm	Safety Sensitivity	NCT02050243
5-ALA			Recurrent glioma	60	Single arm	Fluorescence in tumor Comparison with pathology	NCT02119338
Fluorescein	CellVizio		Rectal cancer	70	Single arm	Margin identification Concordance with histopathology	NCT01887509
ICG		0	Breast cancer	10	Single arm	Proportion of tumors identified by ICG uptake	NCT01796041
ICG	PDE	2	Breast cancer	20	Single arm	Fluorescence in tumor	NCT02027818
ICG	PDE	2	Breast cancer	20	Single arm	Fluorescence intensity following neoadjuvant therapy	NCT02032563
ICG		1/2	Breast cancer	20	Single arm	Non-palpable lesion localization rate	NCT02172989
ICG	PDE	2	Colorectal cancer peritoneal carcinomatosis	10	Single arm	Fluorescence contrast	NCT02032485
ICG		2	Head and neck cancer	10	Single arm	Fluorescence in tumor and cervical LN	NCT02027831
ICG	Fluobeam 800	1/2	Liver cancer	46	Single arm	Feasibility Rate of lesion detection	NCT01738217
ICG		1	Lung cancer	10	Single arm	Feasibility	NCT01335893
ICG	SPY thoracoscope	2	Lung cancer	20	Single arm	Safety and feasibility	NCT02090660
ICG		3	Prostate cancer	37	Single arm	Sensitivity compared to histopathology	NCT02260349
ICG	da Vinci	1	Renal cell carcinoma	50	Single arm	Dose finding	NCT01281488
ICG			Solid tumor	50	Single arm	Contrast ratio	NCT01884584
ICG		1	Solid tumor	48	Single arm	Detection by imaging system	NCT02280954