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HHS Public Access

Author manuscript

Semin Radiat Oncol. Author manuscript; available in PMC 2016 October 01.

Published in final edited form as:

Semin Radiat Oncol. 2015 October ; 25(4): 313–321. doi:10.1016/j.semradonc.2015.05.005.

Tailoring Adjuvant Radiation Therapy by Intraoperative Imaging to Detect Residual Cancer

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Abstract

For many solid cancers, radiation therapy is offered as an adjuvant to surgical resection in order to lower rates of local recurrence and improve survival. However, a subset of patients treated with surgery alone will not have a local recurrence. Currently, there is no way to accurately determine which patients have microscopic residual disease in the tumor bed after surgery and therefore are most likely to benefit from adjuvant radiation therapy. To address this problem, a number of technologies have been developed to try to improve margin assessment of resected tissue and to detect residual cancer in the tumor bed. Moreover, some of these approaches have been translated from the preclinical arena into clinical trials. Here, we review different types of intraoperative molecular imaging systems for cancer. Optical imaging techniques like epi-illumination, fluorescence molecular tomography and optoacoustic imaging can be coupled with exogenous fluorescent imaging probes that accumulate in tumors passively via the enhanced permeability and retention effect or are targeted to tumor tissues based on affinity or enzyme activity. In these approaches, detection of fluorescence in the tumor bed may indicate residual disease. Protease activated probes have generated great interest because of their potential for leading to high tumor to normal contrast. Recently, the first Phase I clinical trial to assess the safety and activation of a protease activated probe was conducted. Spectroscopic methods like radiofrequency spectroscopy and Raman spectroscopy, which are based on energy absorption and scattering respectively, have also been tested in humans and are able to distinguish between normal and tumors tissues intraoperatively. Most recently, multi-modal contrast agents have been developed that target tumors and contain both fluorescent dyes and MRI contrast agents, allowing for preoperative planning and intraoperative margin assessment with a single contrast agent. Further clinical testing

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Conflict of Interest: RW and DGK are consultants to and RW is a cofounder of Lumicell, Inc., a company that is commercializing intraoperative imaging technology.

of these various intraoperative imaging approaches may lead to more accurate methods for margin assessment and the intraoperative detection of microscopic residual disease, which could guide further resection and the use of adjuvant radiation therapy.

Introduction

Radiation therapy is the backbone of curative therapy for some types of cancer. For example, for patients with locally advanced laryngeal cancer, radiation therapy alone results in long-term local control in nearly half of patients (1). Concomitant chemotherapy or laryngectomy can modestly increase local control rates to about 75% (1, 2). For some other solid tumors, like breast cancer, rectal cancer, and extremity soft tissue sarcoma, surgery serves as the primary mode to achieve local control and as a single modality can be curative for some patients, who present without metastatic disease. For example, for patients with breast cancer, who are treated with breast-conserving surgery alone, approximately 26% will develop a local recurrence and adjuvant radiation therapy decreases the rate of local recurrence to about 7% (3). Moreover, for patients with extremity soft tissue sarcoma, who are treated with limb-sparing surgery alone, approximately 25–35% will develop a local recurrence and adjuvant radiation therapy decreases the rate of local recurrence to less than 10% (4–6). Furthermore, for patients with resectable rectal cancer, who undergo total mesorectal excision alone, approximately 20% will develop a local recurrence and adjuvant radiation therapy decreases the rate of local recurrence to less than 10% (7).

Although adjuvant radiotherapy effectively kills microscopic residual disease to improve local recurrence-free survival for the entire population of patients with these cancers and for some patients may improve overall survival, a majority of patients treated with adjuvant radiation therapy for rectal cancer, extremity soft tissue sarcoma, and breast cancer would achieve local control with surgery alone (Figure 1). For these patients, radiation therapy increases short and long-term toxicity without providing any clinical benefit. To tailor adjuvant therapy to cancer patients, who have the potential to benefit from this effective therapy, new approaches are needed to predict which patients with localized cancer are at high risk for developing local recurrence after surgery alone.

There are many different factors that influence the rate of local recurrence after surgery for local disease. Clinical factors such as age, tumor subtype, size, and stage can affect the risk of local recurrence (8–10). More recently, quantitative assays using multigene reverse transcriptase-polymerase chain reaction (RT-PCR) of paraffin-embedded tumor samples have been developed to estimate the recurrence risk based on gene expression profiles. These include Oncotype Dx, a 21-gene assay that can stratify breast cancer patients according to their risk of local and distant recurrence (11). Please see the article by Wolfe and Woodward in this issue of Seminars in Radiation Oncology for details of this 21-gene assay (12).

An important risk factor for local recurrence in many patients with solid tumors is margin status at the time of local excision (8–10). The margin status is determined by whether tumor cells extend to the inked surface of the resected specimen. “Tumor-on-ink” is an indirect measure of microscopic residual disease within the tumor bed, which may prompt

further resection. Intraoperatively, margins of a resected specimen can be assessed by frozen section pathology, but this approach is limited by time and sampling error leading to high false negative rates (13). Postoperative final pathology often provides a more complete assessment of the margins of the resected specimen, but positive margins may require re-excision, which is associated with increased morbidity and cost.

Although a positive margin on final pathology does increase the risk of local recurrence, margin status does not predict which individual patients with cancer will develop a local recurrence when treated with surgery alone. For example, in one study of patients with extremity soft tissue sarcomas treated with limb-sparing surgery alone, approximately 35% of patients with a positive resection margin developed a local recurrence compared to about 28% of patients with a negative final margin (5). Therefore, for individual patients, the excised margin status is not a perfect surrogate for the presence of residual disease within the tumor bed. A real-time method for quick and accurate direct examination of the entire tumor bed for the presence of microscopic residual disease could be superior to current standard histopathological methods that use the resected specimen as a surrogate. Several different intraoperative imaging modalities have been developed to detect such residual cancer, which could potentially be used to predict which patients would benefit from adjuvant radiation therapy. These methods complement traditional perioperative clinical imaging methods and are often designed to achieve the much higher spatial resolution necessary to detect and resect microscopic disease. This review summarizes intraoperative imaging technologies that are in different phases of preclinical and clinical development, which have the potential to help guide the use of adjuvant radiation therapy.

Optical Imaging

Principals of Optical Imaging

Light based imaging is used widely to guide surgical resection. Human vision, which can detect light in the visible range to resolve macroscopic structures, is used by the surgeon to visualize anatomic features as a primary guide to resection. Surgical pathologists employ a microscope to optically detect cellular features that have been removed from the patient and sectioned into thin slices. Fluorescent agents can be used to optically resolve cellular and molecular features *in vivo*, and near infrared (NIR) fluorophores are particularly attractive because they allow deeper penetration of tissues than fluorophores that emit light in the visible range and minimize interference with autofluorescence from normal tissues (14).

The development of high sensitivity charged couple device (CCD) cameras has allowed the use of simple epi-illumination, or reflectance imaging, to detect fluorescence *in vivo* with the ability to resolve features at the cellular level (15). Epi-illumination involves the use of incident light within a specific bandwidth to excite the fluorophore of interest and cause emission of light with a different wavelength that is detected on the same side of the sample as the incident light (16). Reflectance imaging can provide a qualitative representation of fluorescence activity within a region of interest, but quantification and high-resolution localization of the signal may be complicated by the light scattering properties of the tissue. Consequently, surface features can be accurately detected by two-dimensional reflectance

imaging, but detecting features deep to the surface may pose more of a challenge and require confocal or multiphoton tomographic approaches.

Fluorescence molecular tomography (FMT) provides an accurate method to measure the concentration of fluorophores below the surface of a tissue, by detecting absorbed and emitted light, and generates a three-dimensional reconstruction of fluorescent activity (16). FMT has been used *in vivo* to show spatial resolution of structures up to several mm deep (17). Another method to resolve deep fluorescent features is optoacoustic (or photoacoustic) imaging which uses short duration laser light pulses to generate acoustic waves from fluorophores. This technology can be used to measure the presence of endogenous chromophores like hemoglobin, as well as reveal information regarding oxygenation status, making optoacoustic imaging an important tool for vascular imaging (18). Optoacoustic imaging can be simultaneously used to quantify exogenous fluorophores *in vivo*, thereby enabling the reconstruction of deep targets of interest as well as providing surrounding anatomical information with the use of a single imaging modality (19).

Nonspecific Fluorescent Contrast Agents

Exogenously administered fluorescent agents can be used to significantly improve contrast and thus allow more sensitive tumor detection. Nonspecific fluorescent probes, which are constitutively fluorescent, work by preferential distribution to different tissues, accumulate in some tumors via the enhanced permeability and retention (EPR) effect, and result in modest tumor to normal contrast. EPR occurs when growing tumors develop irregular vasculature with a discontinuous endothelium, resulting in “leaky vessels” that allow the passive movement of macromolecules and nanoparticles into the tumor tissue (20). Furthermore, tumors often have defective lymphatic drainage of extracellular fluids, causing extended retention of some fluorochromes (20, 21). After administration of a nonspecific fluorescent probe, the concentration of the probe will increase more quickly in tumors when compared to normal tissues, creating tumor to normal fluorescence contrast.

Many of the nonspecific fluorescence contrast agents currently being studied for intraoperative imaging have been historically used for other purposes. Indocyanine Green (ICG), for example, is a NIR fluorophore that has been used extensively for visualization of vascular and lymphatic structures by virtue of its dark green color (22). The majority of human studies with ICG have shown its utility as an interstitially administered sentinel lymph node marker for patients with breast cancer (22–24). Kosaka et al used ICG to label ovarian cancer metastatic nodules using a microendoscope in a mouse model and recorded tumor to background ratios ranging from 1.2–1.7 (25). Another nonspecific fluorescent agent currently being investigated for its ability to label tumor cells is fluorescein sodium, commonly used for the diagnosis of vascular disorders of the eye. Intravenously administered fluorescein sodium has been shown to localize to brain tumors in humans and mice via disruption of the blood brain barrier in affected regions, acting as a marker for intraoperative confocal microscopy during resection (26, 27). Methylene blue is a third clinically available imaging agent that has been tested for its ability to label tumor tissues. Tummers et al reported a study in which patients with breast cancer patients were administered intravenous methylene blue prior to tumor resection, and subsequent

fluorescence imaging of the resected tissue showed a tumor to background fluorescence ratio of 2.4 (28). Nonspecific fluorescent contrast agents provide one reasonable option for intraoperative imaging as they are clinically available. They also have disadvantages: 1) relatively low tumor to background ratio, 2) lack of cellular accumulation, and 3) extravasation during surgery. For these reasons, targeted agents are being developed.

Targeted Contrast Agents

In order to improve the tumor-specificity of fluorescence imaging agents and further increase the tumor to background ratio, a number of investigators have developed tumor-targeted contrast agents. Several of these targeted agents have progressed to clinical testing (Table 1). An ideal targeted contrast agent has high affinity for the molecular target within the tumor with minimal nonspecific uptake into normal tissues (29). One way of targeting fluorescent imaging agents is by fluorescently labeling antibodies that recognize tumor antigens. For example, bevacizumab and trastuzumab, clinically effective inhibitor antibodies of the human vascular endothelial growth factor (VEGF) and human epidermal growth factor (HER2), respectively, were labeled with an infrared dye and resulted in tumor to background ratios of 1.9 and 2.9 when injected into human xenograft-bearing mice (30). Furthermore, it has been shown that such probes can even quantify the *in vivo* expression of their target, which may allow the surgeon to gain such information intraoperatively, whereas this is information is currently obtained only postoperatively on fixed tissues (31).

Alternatively, fluorescent moieties can be conjugated to peptides or other molecules that have an affinity for cancer cells. For example, to target the overexpression of folate receptor-α in ovarian cancer, folate was conjugated to fluorescein isothiocyanate and intravenously administered to patients undergoing surgery for ovarian cancer. In this clinical trial, fluorescence was detected intraoperatively in all patients with malignant tumors with a tumor to background ratio of about 3 (32). Furthermore, the surgeons were able to detect more intraperitoneal deposits of ovarian cancer when guided by fluorescence imaging (32). Other investigators have used a similar approach with quantum dots, which may be superior to organic fluorophores because of their increased photostability and highly tunable excitation and emission spectra (33). By labeling quantum dots with an RGD (arginylglycylaspartic acid) peptide which targets the $\alpha_v\beta_3$ integrin receptor(34), Li et al observed a 5-fold increase in mouse tumor fluorescence compared to mice injected with untargeted quantum dots (33).

Importantly, all of the aforementioned targeted probes are constitutively fluorescent, and therefore, result in detectable fluorescence when passively distributed to non-tumor tissues, which can decrease tumor to background fluorescence ratios. To limit background fluorescence, investigators have developed “activatable” imaging probes, which can be specifically turned by tumors. Often, these probes consist of a quenched fluorophore, a protease peptide substrate, and a polymer like polyethylene glycol (PEG) to promote accumulation in tumors (35, 36). Because the fluorophore is quenched, the probe is optically inactive in the native state but emits fluorescence after cleavage of the peptide by a proteolytic enzyme (Figure 2). Many enzymes are upregulated intracellularly in cancer cells and extracellularly in the tumor microenvironment where they may play a role in tumor

progression and spread (37). Cathepsin proteases are a specific family of proteases that are highly upregulated in a variety of cancers when compared to normal tissues (38) and therefore, have been used to target activatable probes. Furthermore, Cuneo et al showed that preoperative radiation therapy to the tumor does not decrease the expression of cathepsin proteases in a mouse model of soft tissue sarcoma (39). Therefore, protease activatable probes are likely to retain tumor-specificity in patients who have received radiation therapy prior to cancer surgery.

In 1999, Weissleder et al reported the use of a protease activatable fluorescent probe that resulted in tumor to normal muscle fluorescence ratio of about 7 when injected into mice bearing a human breast cancer xenograft (35). They found that the probe was intracellularly activated by lysosomal cysteine and serine proteases and that extracellular activation was minimal (35). The same probe was then tested in a spontaneous mouse model of breast cancer, showing clear delineation of the tumor via whole animal epi-illumination and FMT imaging methods (40). Interestingly, the tumor to normal muscle fluorescence ratio in the primary breast cancer model was lower at approximately 2.1 (40). Activatable probes have also been developed that target matrix metalloproteinases (MMPs), resulting in significantly higher *in vivo* tumor fluorescence when injected into mice bearing MMP-expressing human xenograft tumors compared to mice with tumors expressing low levels of MMPs (36). One study compared the tumor-labeling kinetics of two highly similar probes that differed only in their protease-specificity for either cathepsin B or MMPs. Although both resulted in tumor specific fluorescence of transplanted subcutaneous tumor nodules, signal from the MMP-sensitive probe peaked about 3 hours after injection and decayed more quickly than signal from the cathepsin B-sensitive probe which peaked at 6 hours after injection. Differences of background fluorescence detected in normal organs was also noted (41).

Kirsch et al and Mito et al intravenously administered commercially available cathepsin and MMP-specific activatable probes to label tumor cells in a primary mouse model of soft tissue sarcoma (42, 43). Tumor-specific fluorescence detected using FMT yielded a tumor to normal tissue fluorochrome ratio of 6.7 (42) while the tumor to normal tissue fluorescence ratio using a handheld epi-illumination device ranged from 5 to over 20 (43). In a mouse model of sarcoma surgery with intraoperative fluorescent imaging, residual fluorescence in the tumor bed after gross tumor resection correlated with the presence of residual sarcoma cells and the risk of local recurrence (42, 43). Furthermore, when residual fluorescence was used to guide additional resection of tissues, local recurrence rates significantly decreased (43). Recently, a Phase I clinical trial was conducted to test the safety and activation of a protease activatable probe, LUM015, in humans with soft tissue sarcoma and breast cancer (NCT01626066). LUM015 consists of a PEGylated fluorophore linked to a quencher molecule via a protease-sensitive polypeptide linker and results in a tumor to normal muscle fluorescence ratio of 4.8 when administered to primary sarcoma-bearing mice via tail vein injection (44). The results of this Phase I trial are eagerly anticipated.

The aforementioned protease activatable probes target different proteases based on substrate specificity. Other investigators have developed activity-based probes (ABPs) which form covalent bonds with the protease of interest while simultaneously releasing the fluorescence quencher, which may allow the quantification of tissue protease activity (45). Verdoes et al

developed a series of pan-cathepsin ABPs that are quickly internalized by cells and result in tumor-specific fluorescence in a mouse model of breast cancer as soon as one hour after injection (46). One of these ABPs was then shown to generate tumor-specific fluorescence when administered either intravenously or intrarectally in a mouse model of intestinal adenoma and adenocarcinoma with a polyp to normal intestine fluorescence ratios of greater than 3. Furthermore, this ABP was able to specifically label human polyps when applied topically to fresh frozen tissue sections (47)

In addition to targeting protease overexpression in tumors, there are several other pathways that can be used to fluorescently label cancer during surgery. Hyun et al have developed halogenated fluorophores that target thyroid and parathyroid tissues simply due to their structure and the presence of halogen side chains. Identification of parathyroid tissues during thyroid surgery is important because inadvertent removal of the parathyroid glands can cause significant morbidity. These halogenated fluorophores were administered intravenously to mice, rats, and pigs and allowed for the simultaneous visualization of thyroid and parathyroid tissues (48).

Another way to differentiate between tumor and normal tissues is by analyzing the pattern of staining of nuclei-specific fluorescence contrast agents. Because tumors can have a higher nuclear density and increased nuclear size compared to normal tissues, staining tissues with fluorescent agents like acriflavine or acridine orange, which bind nucleic acids, can result in tumor-specific patterns of fluorescence that can be detected by microscopy (49). Acriflavine is used clinically as a topical antiseptic agent. Fu et al used a mouse model of soft tissue sarcoma surgery to show that when acridine orange was applied to a resected specimen, the pattern of fluorescence detected using structured illumination microscopy allowed differentiation between cancerous and normal tissues (49). Therefore, this system could potentially be used for margin assessment, either on the resected specimen or within the tumor bed itself.

5- aminolevulinic acid (5-ALA) is one example of a targeted fluorescent imaging agent that has been successfully introduced into clinical use for tumor margin assessment. 5-ALA is a natural precursor of hemoglobin synthesis, and upon exogenous administration of the probe, the penultimate heme synthesis pathway product, protoporphyrin IX, accumulates (50, 51). 5-ALA is preferentially taken up into tumors because of increased vascular permeability and fluorescent protoporphyrin IX accumulates preferentially in epithelial tissues as well as tumor tissues because of slower heme production (50, 51). Stummer at al showed that 5-ALA administered orally to patients with malignant glioma before surgery, resulted in tumor-specific fluorescence that could be detected intraoperatively(52). In a subsequent randomized phase III clinical trial of patients with malignant glioma undergoing complete surgical resection, use of 5-ALA fluorescence to guide surgery was associated with a 20% increase in 6-month progression free survival (53). As the other probes discussed in this section undergo further preclinical and clinical testing, the use of 5-ALA to guide surgery in patients with brain cancer serves as an example of the ability of intraoperative fluorescence imaging to detect residual disease for further resection or potentially to stratify patients for adjuvant therapies, like radiotherapy.

Spectroscopy

While significant advances are being made in the use of optical fluorescence imaging for intraoperative imaging of tumor resection margins (Table 1), one potential limitation of this approach is the need for exogenous contrast agents. This presents an additional regulatory challenge as novel agents must be approved for use in humans, requiring extensive safety testing. Therefore, imaging approaches that do not require exogenous contrast agents, and instead, create tumor to normal contrast based on the intrinsic properties of the tissue are being explored within the field of spectroscopy. The two main spectroscopy approaches that have been developed for intraoperative imaging of cancer are radiofrequency spectroscopy and Raman spectroscopy.

Radiofrequency Spectroscopy

Radiofrequency spectroscopy measures the absorption of radiowaves by the sample to differentiate between two different types of tissues, like tumor and normal muscle, because of varying electromagnetic properties. A handheld radiofrequency spectroscopy device has been developed for the assessment of margins on resected breast cancer specimens. In a study of resected breast cancers from 57 patients, this device was found to detect positive margins with 71% sensitivity and 68% specificity (54). A prospective clinical trial of a radiofrequency spectroscopy device to guide surgical resection in patients undergoing breast-conserving surgery reported that spectroscopy guidance decreased the rate of surgical re-excision (55).

Raman Spectroscopy

A different spectroscopic technique, Raman spectroscopy, measures the scattering properties of tissues. Therefore, Raman spectroscopy can reveal information about the chemical composition of tissue, and thereby distinguish between tumor and normal tissues (56) (Figure 3). In a clinical trial of patients undergoing partial mastectomy, a Raman probe was placed in contact with the tumor bed to measure Raman spectra *in vivo*. The measured tissue was then excised and examined for the presence of tumor or fibrocystic change, which the system was able to detect with 93% accuracy (57). Intraoperative Raman spectroscopy has also been used to detect residual cancer cells in the resection cavity during glioma surgery with sensitivity and specificity of greater than 90% (58). In this study, the investigators reported the ability to detect a cluster as small as 17 human cancer cells. Although the use of exogenous contrast agents is not necessary to differentiate tumor and normal tissues via Raman spectra, tumor-targeted Raman reporters can be used to further improve tumor detection. For example, a PEGylated gold nanoparticle with attached Raman reporter molecules was used to delineate tumors in primary mouse models of pancreatic cancer, prostate cancer, sarcoma, liposarcoma, and breast cancer (59). Furthermore, Raman spectra of the resection cavity of the mouse models allowed detection of residual disease and infiltration into neighboring tissues (59). Together, these studies suggest that Raman spectroscopy may be a promising approach for intraoperative margin assessment, with or without an exogenous contrast agent.

Multi-modal Imaging

Currently, most preoperative planning for surgical resection is done using noninvasive imaging like computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET). Tumor to normal tissue contrast achieved with each of these modalities can be improved by using the appropriate exogenous contrast agents. Recently, investigators have developed multi-modal contrast agents that provide contrast for preoperative imaging studies as well as intraoperative fluorescent optical imaging. For example, fluorescent dyes can be conjugated to Gadolinium-DTPA-polylysine, a polymeric paramagnetic contrast agent for MRI, to create a non-specific contrast agent for MRI and fluorescence imaging. Using molecules with a low dye to gadolinium ratio, tumor enhancement could be visualized with MRI and tumor-specific fluorescence could be achieved with a signal to background ratio of greater than 4 (60).

As with the fluorescent contrast agents described above, the specificity of multi-modal contrast agents can be improved by targeting the molecule to tumor tissues. Satpathy et al describe a method in which a Her2 affibody is labeled with a fluorescent dye and then conjugated to a class of supermagnetic MRI contrast agent, iron oxide nanoparticles, for the labeling of tumor cells in a mouse model of ovarian cancer (61). After administration of this probe, MR imaging allowed detection of tumor foci as small as 1 mm. Furthermore, tumors-specific fluorescence signal could be measured with noninvasive and invasive imaging techniques with a signal to background ratio of nearly 2 (62). Similarly, multi-modal contrast agents can be targeted to tumor tissues by using MMP-activatable cell penetrating peptides that are labeled with Cy5 and gadolinium (63). This contrast agent resulted in tumor-specific fluorescence in a transplanted mouse model of sarcoma and a primary mouse model of breast cancer with a tumor to muscle fluorescence ratio of about 4. Furthermore, the contrast agent allowed visualization of protease activity via MRI, with the gadolinium enhancement ratio of tumor to muscle being similar to that found by fluorescence imaging (63). These preclinical studies suggest the feasibility of using a single contrast agent to image cancer for preoperative planning of the surgery as well as for intraoperative surgical guidance.

Conclusion

Surgical resection is the primary treatment for many patients with solid cancers, but must often be supplemented with radiation therapy or other adjuvant therapies to achieve local control. Local recurrence is most likely due to residual disease that is not readily apparent and thus not removed at the time of surgery. Although histological examination of the margins of resected specimen provides an indirect measure of whether cancer remains in the tumor bed, clinical trials of surgery alone demonstrate that margin status is an imperfect method to predict local recurrence in an individual patient. Therefore, adjuvant radiation therapy is often delivered to a population of cancer patients to improve local control for the entire group even though only a minority of patients would develop a local recurrence if treated with surgery alone. Consequently, the majority of patients receive side effects of radiation therapy without deriving any benefit. Intraoperative imaging is a clinically feasible method to identify residual disease within the tumor bed of patients at the time of surgery

and has the potential to identify patients, who will benefit from adjuvant radiation therapy. Ongoing and future clinical trials will define the sensitivity and specificity of the different types of intraoperative imaging approaches in patients and determine how each can best be applied to different types of cancer. An important long-term goal of these clinical trials should be to determine whether the imaging technologies can accurately predict the risk of local recurrence in individual patients treated with surgery alone. If intraoperative imaging of tumor beds can stratify patients at low or high risk of a local recurrence, then this technology may allow radiation oncologists to tailor adjuvant radiation therapy specifically to those patients who are at high risk for local recurrence and therefore most likely to benefit from adjuvant radiation therapy.

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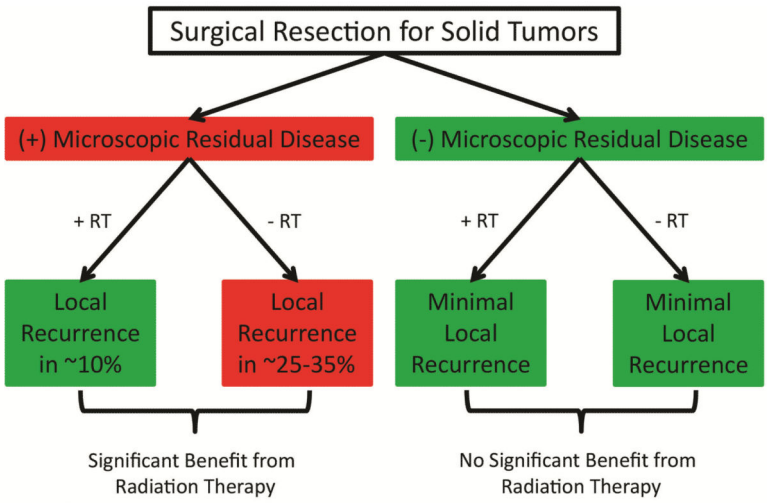

Figure 1.

Only patients with microscopic residual disease benefit from adjuvant radiation therapy. The use of adjuvant radiation therapy (RT) for all patients can decrease local recurrence by more than 50% across all patients, however, this benefit is only significant in the subset of patients with microscopic residual disease after surgical resection. A method to accurately identify these patients intraoperatively will diminish the need to administer adjuvant radiation therapy to all patients, many of whom do not benefit.

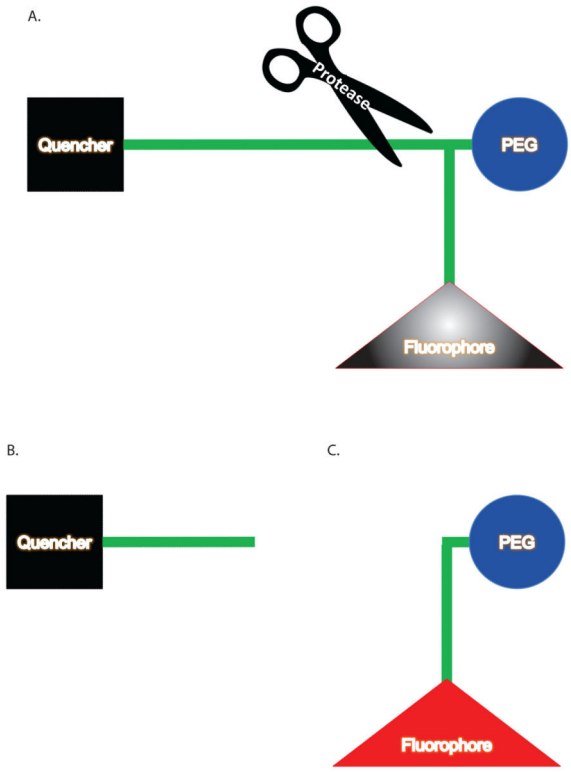

Figure 2.

Protease activated fluorescent imaging probe. (A) In the native state, a protease activated fluorescent probe consists of the fluorophore which is attached to a dark quencher molecular via a polypeptide linker (green). When the quencher is held in close proximity to the fluorophore, minimal fluorescence is detected. Often the fluorophore will also be conjugated to polymer like polyethylene glycol (PEG) to improve delivery to tumors. Upon cleavage of the polypeptide linker by proteases that are overexpressed in tumors, the quencher is released (B) and fluorescence can be detected from the liberated fluorophore (C).

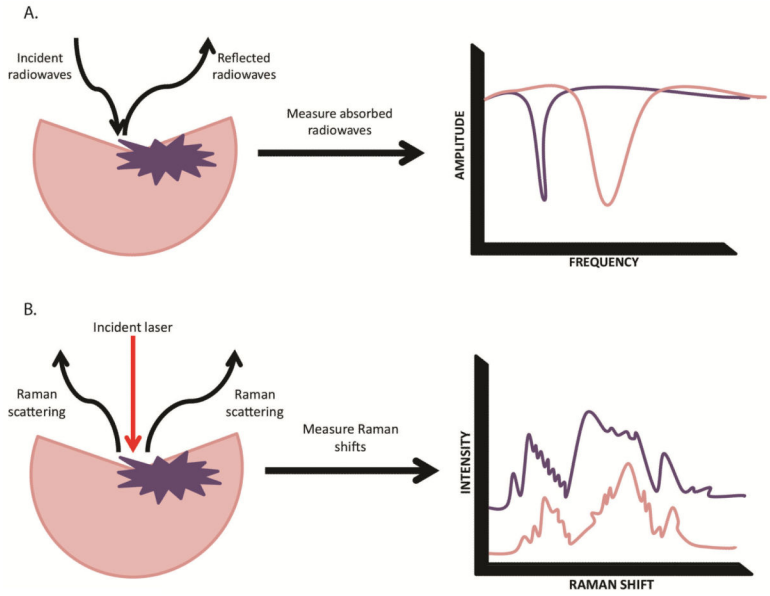

Figure 3.

Spectroscopic analysis of the tumor bed. (A) Radiofrequency spectroscopy measures the frequency of radiowaves that are absorbed by the tissue, creating distinct spectra for tumor (purple) and normal (pink) tissue. (B) Raman spectroscopy measures the scattering of incident light by tissue, resulting in a tissue-specific Raman spectra that can distinguish between tumor (purple) and normal tissue (pink).

Table 1

Targeted contrast agents that are undergoing or have completed clinical testing in human patients with cancer.

Imaging Agent	Mechanism of Action	Phase of Clinical Research	Tumor Type	Tumor to Background Ratio	Sensitivity/Specificity (%)	Re
Bevacizumab-IRDye800CW	Antibody-conjugated	Preclinical	Breast	1.9	NR	
		Preclinical	Melanoma	3, 5.8	NR	
		Phase I	Colorectal	NYR NYR	NYR NYR	NCT NCT
		Phase I	Breast	NYR	NYR	NCT
		Phase I	Esophageal	NYR	NYR	NCT
Folate-FITC (EC17)	Receptor-Targeted	Preclinical	Head & Neck	12	NR	
		Phase 0	Kidney	NYR	NR	NCT
		Phase I	Ovarian	3 NYR	NR NYR	NCT
		Phase I	Breast	NYR	NYR	NCT
		Phase I	Lung	NYR	NYR	NCT
LUM015	Protease-Activated	Preclinical	Sarcoma/Breast	4.8	NR	
		Phase I	Sarcoma	NYR	NYR	NCT
5-ALA	Fluorescent metabolite production	Phase 0	Non-melanoma Skin Cancer	NYR	NYR	NCT
		Phase I	Brain	NYR	NYR	NCT
		Phase I/II	Breast	NYR	NYR	NCT
		Phase I/II	Brain	NYR	NYR	NCT
		Phase II	Brain	NR NYR NYR	85/100 NYR NYR	NCT NCT
		Phase III	Brain	NR	NR	

NR= Not Reported; NYR= Not Yet Reported