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Molecular imaging agents: impact on diagnosis and therapeutics in oncology

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Abstract

Imaging has become a crucial tool in oncology throughout the course of disease detection and management and is an integral part of clinical trials. Anatomic and functional imaging led the way, providing valuable information used in the diagnosis of disease, including data regarding the size and location of the tumor and on physiologic processes such as blood flow and perfusion. As understanding of cancer pathogenesis has advanced through the identification of genetic, biochemical, and cellular alterations in evolving tumors, emphasis has been made on developing methods to detect and serially monitor such alterations. This class of approaches is referred to as molecular imaging. Molecular imaging offers the potential for increasingly sensitive and specific visualization and quantification of biological processes at the cellular and molecular level. These approaches have become established as essential tools for cancer research, early cancer detection and staging and monitoring and predicting response to targeted therapies. Here, we will discuss recent advances in the development of molecular imaging agents and their implementation in basic cancer research as well as in more rationalized approaches to cancer care.

Keywords

Molecular Imaging; Tumor; Cancer; Targeted Molecular Imaging; Therapeutic Response; PET; MRI; SPECT

CLASSES OF IMAGING USED IN ONCOLOGY

Anatomic Imaging

Medical imaging has traditionally been used to diagnose disease using inherent differences in tissue contrast to locate and measure the extent of the pathology. In the past, most medical imaging could be divided into two classes, anatomic and functional. In oncology, anatomic images are used to locate, demarcate, and quantify the size of the tumor; important information in planning or monitoring treatment as it progresses and guiding necessary adjustments to the therapy. During the past three decades, sophisticated technologies such as Ultrasound (US), Computed Tomography (CT), and Magnetic Resonance Imaging (MRI) have entered clinical practice providing increasing anatomical definition of the disease. However, these approaches provide only limited insight into the pathophysiology of the tumor since the anatomic images contain no molecular information. Moreover, if the tumor and the tissue surrounding it share similar physical properties (e.g. the same density), the tumor may be hard to image with CT and in some cases MRI (Ref. 1).

Functional imaging

Functional imaging provides measurements of physiological processes such as alterations in blood flow or oxygenation that underlie disease at the tissue or cellular levels. Many cancer therapies involve anti-angiogenic strategies leading to loss of blood vessels, which can be detected via functional imaging and precede tumor volume decreases by days (Ref. 2). In a preclinical experimental model, injection of a high molecular weight MRI-compatible nanoparticle that remains in the vessels enables MRI-based detection of vascular impairment—resulting from genetic inactivation of an H-RAS transgene—a few days before a decrease in tumor volume is observed (Ref. 3). It follows that quantitation of vessel volume by functional imaging in a clinical setting, might provide an earlier indicator of therapeutic efficacy when compared with anatomic imaging alone. Functional imaging has also recently been utilized to determine a blood vessel normalization window, a time frame when edema is decreased and blood vessels take on a more “normal” appearance (Ref. 4). Drug delivery has been shown to increase when chemotherapeutics are given in this window (Ref. 5).

Overview of molecular imaging

Improved understanding of disease mechanisms through genomics, proteomics, and animal modeling along with advances in chemistry and molecular biology have fueled the development of a third class of imaging. This class, molecular imaging, has been formally defined as “the visualization, characterization and measurement of biological processes at the molecular and cellular levels in humans and other living systems.” (Ref. 6). Whereas anatomical, and in some cases, functional imaging provide information on gross effects, molecular imaging reports what is happening on the cellular and molecular level including information on receptor number, signal transduction aberrations and enzymatic activities. Coupled with the improved knowledge of cancer biology and genetics, and the growing number of targeted drugs entering clinical trials, it is clear that molecular imaging will continue to provide increasingly sensitive approaches for early cancer detection and staging, for prediction of therapeutic response and for monitoring therapeutic efficacy. Moreover, these approaches can enable unprecedented insights into the molecular pathogenesis of evolving cancers in the laboratory setting. Commonly used laboratory techniques rely on lysis or other means of destructive sampling of cells or tissues at defined time points (Ref. 7). This gives a static glimpse of the cell or tissue at that time point, but often a more dynamic and prolonged monitoring is desired. Investigations using animal models of disease, in particular, benefit from more temporal and dynamic observation tools such as those afforded by non-invasive molecular imaging approaches (Ref. 7).

Recently, multi-modal imaging has emerged, combining multiple classes of imaging. Fusing the molecular information from a tumor with anatomical or functional information provides an accurate understanding of the tumor while also providing greater sensitivity and specificity. Positron emission tomography (PET)/MRI (Ref. 8), PET/CT (Ref. 9), single photon emission computed tomography (SPECT)/CT (Ref. 10), MRI/Optical (fluorescence) (Refs 11,12) are all examples of this type of imaging. One retrospective study found that PET/CT imaging was significantly more accurate for staging patients' tumors using the tumor-node-metastasis system. Tumor staging with PET/CT showed an accuracy of 84% compared to 76, 63, 64% in side-by-side PET+CT, CT alone and PET alone, respectively (Ref. 9). A comparison of all three imaging classes is schematically represented in Figure 1.

DEVELOPMENT OF MOLECULAR IMAGING PROBES

The field of molecular imaging relies on the availability of 1) relevant biomarkers and the ability to produce targeted contrast agents that are specific and selective for those biomarkers (Ref. 13) and 2) improvements in detection technologies. Detailed analyses of

cancer mechanisms have identified a wealth of potential cancer markers—including altered proteins and metabolites—that are diagnostic for many aspects of the disease state (e.g. the presence of a given cancer type, the pathologic subtype and grade, and the presence of specific oncogenic alterations). The two major types of probes to these biomarkers are exogenous probes (e.g. small molecules, antibodies) and genetically encoded probes.

Exogenous probes

One of the earliest molecular imaging methods was provided by the creation of ^{18}F -labeled fluorodeoxyglucose (FDG), thus enabling PET-based monitoring of glucose uptake as a measure of the tumor's metabolic activity. Increased glucose metabolism in cancer cells was first observed by Otto Warburg in the 1950's (Ref. 14) and is now referred to as the “Warburg Effect.” However, any cells that have high glucose metabolism are labeled by ^{18}F -FDG, including cardiac myocytes (Ref. 15) and inflammatory cells (Ref. 16); therefore, in the background of inflammation such as colitis (Ref. 17) for colon cancer or pancreatitis (Ref. 18) for pancreatic cancer, ^{18}F -FDG mediated PET imaging will not be specific.

In addition to the use of labeled metabolites, biomarkers can be targeted with various molecules such as antibodies, proteins, peptides, aptamers, or small molecules, which are conjugated to imaging agents suitable for detection with imaging modalities (optical, Ultrasound, MRI, PET, SPECT and CT approaches). Alternative approaches include various types of probes that can be activated by specific enzymes and therefore gauge their catalytic activity. The application of these probes will be discussed in detail below.

Genetic Probes

Advances in transgenic technology and bioluminescent/chemiluminescent reporters have fueled the development of powerful genetic tools for imaging in the laboratory setting. Genetically encoded reporters allow spatial and temporal visualization of molecular events in vivo via expression of transgenes whose expression level correlates with specific molecular changes, or that encode modified proteins whose expression level or biochemical activity tracks with such changes.

Luciferase Reporters—Genetic reporters encoding chemiluminescence proteins related to the firefly luciferase enzyme have found great utility for in vivo imaging. The activity of such reporters can be readily detected using a CCD camera following injection of the appropriate chemiluminescent substrate. This type of approach has been used to generate mouse models that enable monitoring of tumor microenvironments. For example, regional hypoxia has been studied using a transgene that drives the expression of a chimeric protein consisting of the oxygen-dependent degradation domain (ODD) of the Hypoxia-inducible factor (HIF1A) fused to firefly luciferase (Ref. 19). HIF1A is constitutively expressed, but is degraded by the ubiquitin-proteasome degradation pathway under normoxic conditions due to the presence of the ODD. Under hypoxic conditions, HIF1A becomes stabilized. Therefore, ODD-luciferase reporters, whose stability is also regulated by tissue oxygen levels, serve as a gauge of both the degree of hypoxia and the relative stability of HIF1A in vivo (Ref. 19).

A different type of genetic reporter system has been used to monitor the activity of the nuclear factor kappa-light-chain-enhancer of activated B cells (NFkB) pathway in vivo. Defects in this pathway have been implicated in a myriad of diseases including cancer (Refs 20,21,22). In resting cells, the heterodimeric NFkB transcription factor is sequestered in the cytoplasm through dimerization with inhibitor of kappa B (NFkB I) family proteins (Refs 20,21,23,24,25). Inhibitor of kappa B kinase (IKBK) activates the transcriptional function of

NFKB by phosphorylating specific serine residues on NFKBI resulting in its degradation (Ref. 24). An NFKBI- α (NFKBIA)-firefly luciferase fusion reporter has been engineered to study IKBK activity (Ref. 26). Alterations in the bioluminescence signal can be correlated with IKBK activity and thus, this presents a method to monitor the activity of IKBK in vivo in relation to pathophysiological stimuli or to drugs that are designed to manipulate this pathway.

FRET signal reporters—Fluorescence resonance energy transfer (FRET) signal reporters, designed to lose signal intensity upon proteolytic cleavage, have been used to track protease activity in vivo. Recently, fluorescent proteins were shown to be suitable for FRET and to be useful in a wide range of applications including studying protein binding interactions and conformational changes, GTPase, protease and kinase activity, and metabolism (Refs 27,28). FRET based approaches have been used to track in vivo caspase 3 activity, an important measure of apoptosis that can be utilized to determine extent of therapeutic response. Tumor cells have been engineered to express SCAT3, a protein with two fluorescent proteins, cyan fluorescent protein (CFP) and yellow fluorescent protein (YFP) linked through a DEVD caspase 3 cleavage site (Ref. 29). Caspase 3 activation leads to cleavage at the DEVD site, resulting in separation of the fluorescent proteins and a decreasing FRET signal (Ref. 29). Therefore, activation of caspase 3 can be correlated directly with a decrease in FRET signal (Ref. 30). This system was effectively utilized by Zhou et al. where they subcutaneously injected lung cancer cells expressing SCAT3. Monitoring FRET signal served as a system for documenting caspase 3 activity after photodynamic therapy (PDT) or treatment with the chemotherapeutic cisplatin (Ref. 30).

Detection of genetic reporters with PET and MRI—PET reporter transgene systems have been used both in vitro and in vivo (Ref. 31). One of the most common reporter genes is herpes simplex virus type 1 thymidine kinase (HSV1-tk) (Ref. 32). The HSV1-tk gene encoded enzyme phosphorylates a wider range of substrates than mammalian thymidine kinase (TK1). The difference in phosphorylation between mammalian and virus thymidine kinase permits imaging of thymidine analog substrates such as 5-iodo-2'-fluoro-2'-deoxy-1- β -D-arabino-furanosyl-uracil (FIAU) which are preferentially phosphorylated and accumulated in transduced cells (Ref. 31). HSV1-tk has also been used as a suicide gene in the treatment of cancer. Animal models in which HSV1-tk transduced tumor cells were treated with ganciclovir have shown success (Refs 33,34). However, clinical results have shown limited gene transfer to tumor cells, ultimately limiting therapeutic efficacy (Ref. 35). Therefore, a non-invasive system to monitor gene transfer effectiveness may be valuable. An in vivo experiment used radiolabeled ^{18}F -FIAU to image HSV1-tk suicide gene expression in transduced tumors. Researchers found that the uptake of ^{18}F -FIAU in HSV1-tk transduced HT-29 tumors was much greater than wild-type HT-29 tumors (Ref. 36). Molecular-genetic PET imaging provides exquisite sensitivity that can be exploited to non-invasively detect small changes in induced, normal, and aberrant gene expression.

Until recently, there was a lack of genetic reporters for molecular MRI. Bengtsson et al. have described an MRI-compatible lacZ genetic reporter system (Ref. 37). The lacZ gene encodes β -galactosidase (β -gal) which can be detected by cleavage of the commercially available substrate S-Gal, both in vitro and in vivo. Cleaved S-Gal chelates ferric iron (derived from ferric ammonium citrate, FAC) and creates a dark colored reaction product that can be used to identify cells expressing lacZ (Ref. 38). The authors reasoned that accumulation of iron in lacZ⁺ cells could be detected by MRI. Injections of S-Gal/FAC into the muscles of lacZ⁺ animals and lacZ⁺ tissue implanted animals allowed visualization of labeled cells by MRI (Ref. 37). This finding, which utilized an already commercially available reagent, provided a new application of the lacZ genetic reporter system allowing non-invasive MR imaging in vivo.

CLINICAL APPLICATIONS OF MOLECULAR IMAGING

Beyond its use in the laboratory setting, molecular imaging has also made considerable contributions to oncologic practice in the clinic. Moreover, evolving understanding of the molecular signatures associated with different aspects of tumor biology or drug responsiveness will ensure that molecular imaging has an increasingly important role in future cancer care.

Early detection, diagnosis, and prognosis

The 5-year survival rate is greatly increased when cancer is detected and diagnosed in its earliest stages (when it is confined locally or regionally) (Ref. 39). Extensive progress has been made in early detection of some cancers including colon, breast and bladder, and correspondingly, screening programs have been developed with the aim of reducing mortality (Ref. 39). Significantly, these cancers are readily accessible to non-invasive or minimally invasive techniques. Since molecular imaging can provide reliable information about the biology and natural history of the detected lesions, it still has considerable value as a prognostic tool for cancers arising at these physically accessible sites. Molecular imaging can enhance traditional imaging because of its ability to highlight small abnormalities and allow the distinction between normal or inflammatory tissues and cancer tissue based on early molecular changes and not gross anatomic difference. High sensitivity and specificity of molecular biomarkers is a requirement that, when fulfilled, offers the ability to acquire quantitative data from repeat studies (e.g. pre and post treatment). It also serves as an important adjunct to traditional imaging. For example, molecular imaging via colonoscopy adds molecular information, which enhances the sensitivity and diagnostic power of the original technique. Molecular imaging has the potential to allow accurate staging, prediction of prognosis and the stratification of patients into appropriate treatments based on staging and the pathways that are aberrant for each patient.

Endoscopic molecular imaging in colorectal cancer diagnosis—As mentioned above, existing noninvasive and minimally invasive imaging methods are currently available for early detection of colorectal cancer (CRC). Endoscopic colonoscopy has been widely used as a first or second level exam for specific ages among the general population and high-risk group with familial aggregation for colorectal cancer (Ref. 40). This exam allows the identification of precancerous lesions, commonly polyps, and their concurrent sampling or resection and has been shown to reduce incidence of colorectal cancer and improve cancer outcomes. However, improvement is still needed, as routine endoscopy is reported to have miss rates of early adenomas of up to 20% (Ref. 41), especially in flat polyps (Ref. 42) or in polyps that are present in inflammatory environments i.e. colitis (Ref. 43). Therefore, an approach of emerging importance is the implementation of fibre-optic endoscopy with high affinity optical molecular imaging agents that aid in the identification of difficult-to-detect early malignant adenomas (and to reduce the overtreatment of benign lesions).

One example of such an application has been the development of methods for monitoring cathepsin B activity in vivo. Cathepsin B proteolytic activity is commonly active in the tumor microenvironment and contributes to the regulation of angiogenesis and invasion during cancer progression (Refs 44,45). Using a probe that fluoresces in response to cathepsin activity (ProSense 680) Gounaris et al. were able to image cathepsin activity in vivo by intravital endoscopic microscopy in a mouse model of intestinal polyposis (Ref. 46). Notably, cathepsin B is a biomarker of cancer-associated inflammation in tumor infiltrating myeloid cells, but it can be used as a surrogate marker for demarcation of dysplasia (Ref. 46). Indeed, the fact that more than 3/4 of the probe signal was localized in CD11b+Gr1+ myeloid derived suppressor cells (MDSC) and CD11b+F4/80+ macrophages infiltrating the

lesions in the mouse polyposis model also suggested a pathogenic role of MDSC in polyp development and/or maintenance. As a consequence, the authors were able to show that upon treatment of mice with the anti-inflammatory anti-TNF α , cathepsin activity was suppressed. This cathepsin sensing system is therefore also a suitable approach to monitoring response to therapy (Ref. 46).

Additional approaches have used antibodies and peptides to target imaging agents to emerging colorectal cancer. An example of this is the use of fluorescently labeled anti-epidermal growth factor receptor (EGFR) antibodies for in vivo endomicroscopic molecular imaging. This approach successfully visualized and differentiated human CRC tumor types based on their EGFR expression in a rodent xenograft model (Ref. 47). Intravenous application of labeled anti-EGFR in this same study resulted in a quick delivery to the tumor without the necessity to clear the tissue from adherent tissue or mucus. Topical application may prove more practical for clinical use during colonoscopy and may be associated with less immunogenicity and other adverse events.

Molecular endoscopy has started to be applied in humans. A pilot colonoscopy trial used labeled monoclonal antibodies against carcinoembryonic antigen with a prototype fluorescence endoscope for macroscopic tumor detection. Specific fluorescence after topical administration was seen in 19 of 25 carcinomas (Ref. 48). Other investigators have sought to identify new markers for colonic dysplasia. Peptide probes against CRC have been developed using a cell based phage display approach similar to that described above (Ref. 49). I.V. injection of fluorescein isothiocyanate (FITC)-conjugated peptides into mice harboring orthotopically implanted colon tumors resulted in enhanced tumor detection upon fluorescence endoscopy (Fig. 2A) (Ref. 50). In an extension of this work, screens were performed to isolate a heptapeptide with preferential binding to premalignant lesions in ex vivo human colonic biopsies. The FITC-labeled peptide imaging agent was topically administered through the instrument channel of a standard colonoscope in patients undergoing colonoscopy and enabled effective detection of dysplastic regions by an endoscopic fluorescence confocal imaging system (Fig 2B). The agent is bound more strongly to dysplastic colonocytes than to adjacent normal cells and allows imaging of dysplastic crypts with a reported 81% sensitivity and 82% specificity (Ref. 51). The combination of endoscopy with intravital microscopy appears likely to translate in a significant shift in the approach to treatment of many premalignant lesions such as colorectal polyps.

Detection of breast cancer with antibodies and peptides—Similarly, molecular imaging probes have also been developed for the early detection of breast cancer. The receptor tyrosine kinase, ERBB2 is overexpressed in a subset of breast cancers and is a marker of poor clinical prognosis (Ref. 52). A variety of molecules and modalities have been used to target the ERBB2 receptor as a therapeutic approach. These include monoclonal antibodies (Ref. 53), vaccines (Ref. 54) and gene therapy (Ref. 55). The humanized monoclonal antibody against ERBB2 Herceptin (trastuzumab, Genentech) was the first agent to be widely used in the clinic; in fact, it is still widely used. However, patients treated with Herceptin are found to be at a higher risk for cardiac dysfunction (Refs 56,57,58). Because they are less immunogenic, peptides such as those developed against colorectal cancer biomarkers have shown lower toxicity and exhibit desirable pharmacokinetic properties combined with high affinity and selectivity (Ref. 49). Therefore, a targeted peptide conjugated with a contrast agent of any relevant modality provides a minimally toxic, noninvasive imaging tool for the detection of ERBB2 positive breast cancer. Such a peptide was identified using a phage display peptide screen directed against the extracellular domain of ERBB2. This peptide was conjugated to ^{111}In -1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (^{111}In -DOTA) and injected i.v. into mice

bearing human breast cancer xenografts. SPECT imaging revealed that the tumor was clearly demarcated by the labeled peptide (Fig. 3) (Ref. 59). This strategy could potentially be extended to the clinic to noninvasively assess the molecular status of the developing tumor. Combined with the white light imaging, molecular imaging was able to provide more information than a purely anatomical imaging scan would reveal.

Peptide probes for early detection of pancreatic cancer—In comparison, a number of other cancers have been more difficult to detect due to their propensity to metastasize when relatively small (<10 mm) or to their location within the body. Additionally, the vagueness of symptoms associated with the tumor's location in the body (usually visceral) causes patients to present with advanced disease. Examples of these types of cancers include pancreatic ductal adenocarcinoma (PDAC) and ovarian cancers, which elude today's tests.

PDAC is among the most lethal types of cancer, with median survival only around 6 months and a 5-year survival rate of only 4% (Refs 60,61). Currently the only potential cure is surgical resection; however, 80-90% of patients with PDAC present with surgically unresectable disease (Ref. 62). These statistics highlight the need for identification of biomarkers and subsequent development of noninvasive molecular imaging probes for early detection and diagnosis of PDAC. Although the incidence of PDAC is relatively low and therefore population-based screening using imaging would not be cost-effective, subjects with increased risk such as those with hereditary disease or patients with new onset diabetes would benefit from such agents. Further, clinical benefits would come from monitoring cancer spread before surgery and recurrence after surgery. Recent efforts have been made to develop noninvasive molecular imaging probes capable of detecting early stage PDAC and identifying extent of metastases. Toward this end, peptides that distinguish between PDAC and normal pancreatic ductal cells in humans and mice were identified through a cell based phage display screen against cells isolated from murine models of PDAC (Ref. 63). Further proteomic studies identified the target of one of the isolated peptides, Plectin-1 (PLEC1), as a potential novel biomarker of PDAC (Ref. 63). The peptide identified by the phage screen was conjugated to magnetic and fluorescent nanoparticles (MFNP) and injected into genetically engineered mice, which develop PDAC that is histopathologically similar to human cancer (Refs 63,64). Molecular imaging through targeting plectin-1 allowed the detection of tumors via both MR and optical methods (Fig. 4). The immunohistochemical data combined with the imaging data suggest that this molecularly targeted imaging agent could identify PDAC at a stage in which it is still susceptible to potentially curative surgery. In addition this has the potential to non-invasively detect metastasis as well as differentiate between benign and malignant lesions. Since only patients with locally invasive disease confined to the pancreas benefit from surgical resection, this approach could aid in clinical decision-making and potentially help avoid the high mortality and morbidity commonly associated with major pancreatic interventions.

Practical considerations regarding the implementation of cancer screening programs—While the discovery of cancer biomarkers and molecular probes that target them holds considerable promise for improvement in cancer detection, it should be noted that the availability of such tools does not necessarily justify the development of a pan-screening program. In 1968, the World Health Organization (WHO) commissioned Wilson and Jungner to write a report on screening (Ref. 65). The original criteria they set forth remains “classic” (Ref. 66); however, these criteria have changed slightly to reflect the changing times. Considerations such as the frequency of the cancer type, the amount of knowledge of the cancer, the availability of treatments should a tumor be discovered, and the cost of the test need to be taken into account in order to select the population most likely to benefit from such a test.

Molecular imaging and cancer therapy

The emergence of molecularly targeted therapies and personalized medicine in oncology has the potential to revolutionize patient care. Compounds such as imatinib in chronic myelogenous leukemia and gastrointestinal stromal tumors, or trastuzumab, in breast cancer, are integral parts of established therapeutic protocols, and an increasing number of molecules see their indications expanding on the basis of the results of clinical trials or on earlier phases of development. Despite the wide diffusion of targeted therapies, there has yet to be a commensurate cultural and technological shift able to hold to the promise of a personalized medicine based on the patient's and disease biological characteristics. To date, not only are we not able to predict the response to most of available cancer therapies, but the imaging criteria we use to define response to therapy, namely Response Evaluation Criteria in Solid Tumors (RECIST), is not informative with respect to important biological endpoints (Ref. 67). RECIST is a set of criteria that defines if a patient responds, stabilizes, or progresses and is used to objectively evaluate treatments in the majority of solid tumor clinical trials today (see www.RECIST.com). Molecular imaging is a natural counterpart of targeted therapies in that it has the potential to help clinicians match specific therapies to patient populations most likely to respond, to more accurately track individual response, and to allow monitoring of therapeutic resistance (Ref. 68).

Assessment of drug distribution—An important consideration in the case of therapeutic approaches to most cancer types is that the majority of patients have tumors that are refractory to current therapies or develop resistance over the course of treatment. This may relate to the intrinsic molecular features of the tumor. Alternatively, although usually ignored, another cause might be poor perfusion and consequently reduced drug distribution (Ref. 69). So, which is it? Molecular imaging can answer this question, as a wide variety of targeted molecules can be modified to include imaging and/or therapeutic agents. Aptamers, a class of molecularly targeted molecules, are short DNA or RNA oligonucleotides which bind specific targets and can be used in both imaging and therapeutic applications. An example of this involves the use of two aptamers that bind the extracellular portion of prostate specific membrane antigen (PSMA)—a prostate cancer marker—that were identified through high throughput screening techniques. These aptamers, short 40mer synthetic oligonucleotides which bind with low nanomolar affinity, could be used to carry a drug (e.g. N-acetylated alpha-linked acidic dipeptidase (NAALADA) inhibitor) or modified to carry both imaging and therapeutic agents (Ref. 70). Although this approach has yet to be widely adopted it may be of particular value in the study of tumors types in which there is speculated to be a stromal barrier to drug delivery.

Overview of use of molecular imaging in prediction of response to therapy and monitoring therapeutic efficacy—Independent of perfusion and drug distribution, many tumors are initially resistant or later acquire resistance to therapies. The question of identifying which patients will respond to particular drugs is an important one as nonresponders in a drug study decrease the apparent effectiveness of the drug. In turn, more patients will need to be enrolled to achieve statistical significance, driving up the costs of the trial. Further, appropriate patient selection increases the likelihood of a robust clinical response, whereas including nonresponders could potentially stall or halt the trial of a drug. Additionally, a nonresponder who receives therapy may unnecessarily suffer from drug side effects while also not receiving the appropriate treatment regimen. Therefore, it is important to identify patients that will respond favorably to treatment. (Ref. 68).

Another critical element in the implementation of targeted therapeutics is the development of pharmacodynamic markers of drug effects (i.e. biomarkers that document that the drug is able to hit its specific target) (Ref. 71). Such markers are essential in the validation of

appropriate targets by enabling correlation between effective modulation of the molecular target and whether the desired biologic effect is being obtained. In addition, during preclinical and clinical drug development, measurement of biomarkers via non-invasive molecular imaging is important to aid in the selection of the appropriate patients, to provide demonstration of proof of concept for target modulation, to help the interpretation of clinical trial data, and to inform the identification of the optimal dose and schedule. Most current approaches to assess the pharmacodynamic effects of a drug rely on measuring target inhibition by analysis of tumor biopsies. However, this approach suffers from a number of drawbacks including invasiveness, sampling error, and lack of broad applicability because many patients will not be suitable for biopsy. In some cases, measurement of target inhibition by sampling of normal tissues (e.g. in the blood) may have utility, but this approach requires demonstration that target level in the surrogate tissue correlates with that in the tumor and suffers from the same disadvantages of tumor biopsies. Recently, molecular imaging of glucose metabolism via FDG-PET has been used as an assay for mechanistic target of rapamycin (MTOR) inhibition, providing a proof of concept for the use of non-invasive approaches to measure target inhibition (Ref. 72).

Because molecular imaging allows monitoring of molecular processes, it lends itself to responsiveness prediction and pharmacodynamic monitoring of therapeutic efficacy. Identifying biomarkers and designing molecular imaging probes that can be used to predict whether a patient is likely to be a responder or develop resistance could greatly drive down drug development time and cost, while prolonging patient life as patients that will not respond or develop resistance will be switched to a more effective course of treatment.

Development of probes predictive of therapeutic response—Using phage display, systematic evolution of ligands by exponential enrichment (SELEX), or other high throughput screening techniques, fundamental molecular differences between responders and nonresponders can be ascertained. The molecular differences between responders and nonresponders can direct the creation of molecular imaging probes to quickly and non-invasively determine if the patient is likely to respond. A rapid in vitro screen might involve collecting cells from patients in the responder and nonresponder group, then screening for molecular differences between the two populations of cells. The molecular differences can be used to develop an imaging probe, appropriately validated in vivo, thus allowing prediction of the therapeutic outcome before initiation of therapy. In a similar situation, treating cells with a drug of interest, then screening for molecular differences between the treated and vehicle treated cells could provide useful information for the design of an imaging probe to monitor therapeutic progress. After initiation of therapy, a molecular imaging probe could be used to determine if the cells have changed their molecular phenotype to one that is indicative of drug response. Alternatively, transgenic mouse models of human cancers can be used for in vivo screening or mouse models where human biopsy specimens are orthotopically implanted can be used.

Prescreening patients to predict therapeutic outcome has shown some success. One study divided osteosarcoma patients into two groups, those with $\geq 90\%$ necrosis (good responders) and those that exhibit $< 90\%$ necrosis (poor responders) after preoperative chemotherapy. Despite efforts to modify post-operative chemotherapy, the poor responders still suffered from a poor prognosis (Ref. 73). Therefore, therapy for poor responders is essentially not helpful, despite the cost and use of clinical resources. A noninvasive screen to determine into which group (good responder or poor responder) the patient should be placed is needed at the time of diagnosis so that the most effective treatment regimen can be established. In the same study, analysis of 34 pediatric osteosarcoma samples revealed 45 genes that are predictors of responsiveness to therapy (Ref. 73). In another study, researchers collected tumor interstitial fluid (TIF) and normal interstitial fluid (NIF) of breast cancer and

compared the proteins through 2-dimensional electrophoresis and mass spectrometry. Analysis revealed significant differences in protein expression between TIF and NIF. This group also compared protein expression patterns of interstitial fluid between responders and nonresponders to chemotherapy. Protein expression differences were found between the two groups (Ref. 74). Using this information, imaging probes can be designed and a non-invasive early detection and responsive predictor test can be created.

In breast cancer, there are a number of molecular features predicting drug responsiveness. Specifically, the status of estrogen receptor (ER) and progesterone receptor (PR) or the overexpression/amplification of the receptor tyrosine kinase, ERBB2 (alternatively named HER2), are useful predictors of the response to endocrine and anti-HER2 (e.g. trastuzumab and lapatinib) therapies, respectively. Therefore, knowing the status of these biomarkers is the milestone for any therapeutic planning including neoadjuvant therapies. Furthermore, it has been shown that the status of ER and PR expression changes between primary and recurrent tumors (Ref. 75), but this cannot be easily taken into account in most cases, as it requires additional invasive procedures. Non-invasively measuring ER expression with ^{18}F -16 α -17- β -fluoroestradiol (FES) PET has shown promise (Ref. 76). In another application of Susan Deutchers's work, ERBB2 status could be non-invasively obtained with the ^{111}In -DOTA peptide discussed previously in this review (Ref. 59). Continuously monitoring the ER, PR and/or ERBB2 status of the patient might provide insightful correlations in clinical trial and help to refine indications for breast cancer therapies.

Preclinical animal screening for drug therapies can be used to predict patient responsiveness in the clinic. Engelman et al. created a novel phosphoinositide 3-kinase (PIK3) mutant mouse model of lung cancer and then treated the resulting tumor with a dual pan-PIK3 and mTOR inhibitor (NVP-BEZ235) already in clinical development. Treatment with NVP-BEZ235 led to a marked decrease in tumor avidity detected by FDG-PET (Fig. 5A), MRI and microscopic techniques. They also showed that mouse cancers initiated by v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS) mutation did not respond well to treatment with NVP-BEZ235 (Fig. 5B), but combination therapy with a mitogen-activated protein kinase kinase (MEK) inhibitor (ARY-142886) produced marked tumor reduction (Ref. 77). These in vivo experiments suggest that knowing the mutation status of a patient's lung cancer may predict which drug(s) should be used to obtain the most efficacious therapeutic response.

Pharmacodynamic markers—Without molecular imaging, tumor treatment efficacy can only be assessed by anatomic or structural measurements, namely size of the tumor. In fact, the evaluation of treatment still lags behind the advances in molecular imaging and primarily consists of determination the size of the tumor before and after treatment (Ref. 78). The inadequacy of size measurements is illustrated by a study of two EGFR inhibitors erlotinib and gefitinib. These two inhibitors prolonged median survival of chemotherapy-refractory patients by 50%. Yet, CT acquired size measurements only showed a 9% tumor response rate (Ref. 78). Responses to targeted therapies might be cytostatic or cause fibrosis, meaning the tumor doesn't decrease in size. Therefore, in this instance, tumor size measurements are a rather poor indicator of therapeutic outcome and success (Ref. 78). PET allows quantification of ^{18}F -FDG uptake in tissues and previous studies have shown that standardized uptake values (SUV) are accurate indicators of tumor glucose metabolism. Following therapy, the reduction of glucose metabolism is correlated to therapeutic efficacy. After the first round of chemotherapy, FDG-PET can be used to determine the progress of the chemotherapy and effectiveness can be assessed before future surgical procedures (Ref. 79). Quantification of 3'-deoxy-3'-[^{18}F]fluorothymidine (FLT) uptake can also be used to quickly predict response to therapy. In one study, response to gefitinib in lung cancer patients as measured by chest CT (after 6 weeks of therapy) could be predicted with positive

and negative predictive values of 92.9% by FLT-PET just 7 days after initiation of treatment (Fig. 6) (Ref. 80). Also noteworthy, the time to progression was significantly increased in the patients who responded to FLT-PET (median 7.9 months) compared to those patients who didn't respond (median 1.2) (Ref. 80). Similarly, molecularly targeted agents to integrin $\alpha V\beta 3$ (ITGAVB3) and vascular endothelial growth factor (VEGF) have shown promise in predicting therapeutic response. VEGF is the key mediator of angiogenesis and has become one of the most targeted molecules in antiangiogenic and anticancer therapies (Ref. 81). Likewise, ITGAVB3 has been identified as a marker of angiogenic tissue (Ref. 82) and molecular PET (Ref. 83) and SPECT (Ref. 84) based probes have been developed. Using molecular imaging agents targeted to these molecules, tumor angiogenesis and vascular response to antitumor therapies can be assessed noninvasively (Ref. 85).

In order to maintain the most cost and time effective therapy, imaging screens for responsive status as well as real-time monitoring of therapeutic progress are required. Because molecular imaging can non-invasively examine the tumor and its microenvironment, it is a natural fit for this type of predictive information. A series of imaging probes could be used to predict the outcome and monitor the progress of chemotherapy. Knowing how the therapy is progressing and if tumors are acquiring resistance would allow physicians to make more informed adjustments to the treatment. The limitations of anatomic tumor response measurements coupled with the widespread use of FDG-PET to assess early treatment response have led to the creation of new criteria appropriately called PET Response Criteria in Solid Tumors (PERCIST). In PERCIST, response to therapy is continuously assessed and expressed as a percentage change between pre and post-treatment maximum SUL (SUV corrected for lean body mass) (86). Establishing endpoints through molecular imaging would allow the physicians to know when the chemotherapy has successfully run its course and that the next procedure can be initiated.

RESEARCH IN PROGRESS

There is a clear need to improve the drug development pipeline. The reality is that the vast majority of new therapeutic approaches fail somewhere in the bench to bedside transition. As a result, considerable amounts of time and resources are expended without any improvement in detection and diagnosis or treatment. In fact, in 2004, the Food and Drug Administration (FDA) released its "Critical Path Report" explaining, to reduce the expenditures on candidate products that are unlikely to succeed, new tools are needed to distinguish those that are likely to succeed from those that show very little promise (Challenges and Opportunities Report – March 2004, FDA). Molecular imaging can certainly provide the tools that are needed and called for by the FDA, however there is a paucity of approved molecular imaging agents. In a report titled "Guidance for Industry, Investigators, and Reviewers: Exploratory IND Studies" (2006), the FDA encourages researchers to take advantage of "Exploratory IND (eIND) Studies" which it feels are being underutilized (see <http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM078933.pdf>). An eIND Study involves very limited human exposure but allows a quick and more cost effective mechanism to determine whether lead agents may have clinical relevance. Combined information including pharmacokinetics and toxicity obtained from eIND studies can allow the researchers to select the most promising product from a group of candidates.

Additional challenges in molecular imaging agent development are discussed in an excellent review written by John Frangioni (Ref. 87). Frangioni describes five basic roadblocks that stand in the way of successful image probe development – inadequate preclinical critique and acceptance of small effect sizes, dollar and time costs of traveling through the regulatory

system, intellectual property issues, fear of litigation, and slow and unpredictable industrial timeframes. It is estimated that development (phase 1, 2, and 3) of a novel in vivo imaging agent ranges from \$35-61 million (Ref. 87). As such, industrial investment follows a “\$100 million market” rule, meaning a company will not invest unless sales of the novel agent will exceed \$100 million per year. Most diseases do not affect enough people to bring sales above that threshold. By their very nature, molecular imaging and personalized medicine decrease the size of the possible patient set, further ensuring that novel probes and therapies designed with these characteristics in mind will not reach the industry-prescribed threshold. The result – the majority of new agents are ignored. Frangioni proposes a paradigm shift, one in which academic institutions take a more involved role, utilizing the eIND approach to further their novel agents. Academic institutions can accomplish most eIND studies using smaller amounts of money, approximately \$20,000-100,000 (Ref. 87). This amount of money can be obtained from institutional funds, National Institutes of Health (NIH) grants, or philanthropic/interest groups. Not only can molecular imaging probes aid in the eIND process, but their development will increasingly depend on eIND studies, further cementing the relationship between molecular imaging probes and eIND studies.

The FDA, National Cancer Institute (NCI), and Centers for Medicare & Medicaid Services (CMS) have proposed a new initiative called “Oncology Biomarker Qualification program” (OBQI) (see <http://www.cancer.gov/newscenter/pressreleases/OBQI>). In this program, biomarkers are defined as “indicators of disease or therapeutic effects, which can be measured through dynamic imaging tests, as well as tests on blood tissue, and other biologic samples”. Biomarkers are needed to help reduce time and cost of drug trials and provide better way to monitor therapy. A more fulsome assessment of clinical trials is needed and can easily be accomplished with molecular imaging of biomarkers. Biomarker assessment of progression and outcome is desired due to the wealth of information that is provided. Current methods to assess the response to treatment rely primarily on structural and anatomic methods like RECIST, therefore, expansion of biomarkers is greatly needed. The search for novel biomarkers is on and many researchers from all corners of the world are discovering biomarkers at a rapid pace. In an effort to reduce time wasted on redundant findings, the NIH has developed a database called the Molecular Imaging and Contrast Agent Database (MICAD, <http://micad.nih.gov>). MICAD is a freely accessible database that catalogs key research information on in vivo molecular imaging and contrast agents that was created as a key component of the “Molecular Libraries and Imaging” program of the NIH’s “Roadmap for Medical Research”. Information in the MICAD database includes information on agents developed for PET, SPECT, MRI, US, CT, optical imaging, planar radiography, and planar gamma imaging. Information is in the form of text, references, links to MEDLINE and other resources.

CONCLUSIONS

As more information about diseases and their genetic, biochemical, and cellular aberrations becomes available, tools such as molecular imaging are needed to diagnose and monitor treatment. Although highly sought after as a means to detect and diagnose disease, molecular imaging finds important roles in basic science as well. The study of protein-protein interactions, gene regulation, and metabolism are areas of research that have benefited from advances in molecular imaging. Key areas of future development include the need for improved methodologies to serially monitor changes in activity state of intracellular signal transduction pathways as well as metabolic function. Applications in the clinic include early detection and diagnosis, prediction of response to therapy, and monitoring therapeutic efficacy once treatment has commenced. Using molecular imaging, two potential causes of unresponsiveness to treatment can be studied with the goal of eliminating resistance (inherent and physical) and providing the best treatment possible. Molecular

imaging is an emerging field with important implications to the clinic. Institutions such as the NIH, NCI, CMS and FDA realize the importance of biomarkers and developing molecular imaging technologies. As such, infrastructure and databases are already being put in place to handle the rapid expansion of biomarkers and molecular imaging technologies.

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FURTHER READING

1. Informa Healthcare USA, Inc. *Molecular Imaging in Oncology*. New York, NY, USA: 2008. This textbook describes a multitude of applications and modalities spanning all areas of molecular imaging in oncology.
2. Frangioni JV. Translating in vivo diagnostics into clinical reality. *Nat Biotechnol* 2006;24:909–913. This is an excellent commentary about the current status of molecular imaging agents and how their future depends on the ability to alter the way agent development has traditionally been accomplished. [PubMed: 16900127]

Figure 1. Schematic representation of the three classes of imaging. Broad categories of applications are shown with a few examples from each category. Many applications such as structures and flow span more than one class of imaging.

Figure 2.

Colonic tumor visualization via fluorescence endoscopy. A. Endoscopy of mice with orthotopically implanted colon tumors. After one hour, the targeted peptide near-infrared signal (A, top right) is much higher than control near-infrared signal (A, bottom right). A, top and bottom right. Light endoscopy images. Notice that polyps are not as easily detected via light endoscopy compared to fluorescence endoscopy. B. In vivo confocal fluorescence endoscopy in human patients showing targeted peptide binding to dysplastic colonocytes, but weakly binding to adjacent normal mucosa.

Figure 3.

In vivo detection of breast tumors. Left. ^{111}In -labeled scrambled peptide against MDA-MB-435 breast tumor. Right. ^{111}In -labeled targeted peptide. SPECT images were fused with conventional microCT images for spatial validation. Note the effective labeling of the tumor with targeted peptide, but absence of accumulation when scrambled peptide is injected. Imaging was performed 2 hours after injection of labeled probes.

Figure 4.

Visualization of pancreatic lesions via targeted nanoparticles. Top left. Intravital confocal microscopy of targeted nanoparticles (Cy5.5) to pancreatic lesions. Bottom left. Merge of targeted nanoparticles (Cy5.5, red) and blood pool agent (AF-750, blue). Right. Intravital low magnification view of targeted nanoparticle (FITC, green) on a larger section of the pancreas. All images were acquired on an Olympus IV 100 system.

Figure 5. Monitoring therapeutic efficacy with ^{18}F -FDG-PET/CT. A. CT (top) and PET (bottom) scans of PIK3CAH1047R-CCSP-rtTA double transgenic mice treated with doxycyclin until tumors developed (before) then treated with NVP-BEZ235 for 4 days (after). Red arrows indicate the tumor and the heart is indicated with “H”. A marked reduction in tumor size is seen after just 4 days of NVP-BEZ235 treatment. B. Tet-op-Kras mice developed tumors (before). Treatment with NVP-BEZ235 for 2 weeks produced no substantial shrinkage (after).

Figure 6.

^{18}F -FLT-PET uptake is substantially decreased 7 days after initiation of gefitinib treatment in responders compared to nonresponders. A, By day 7, marked decreases in ^{18}F -FLT uptake are seen in responding patients. Subsequent CT image taken 6 weeks after initiation gefitinib treatment shows tumor size markedly decreased. B, Nonresponder shows no visible change in ^{18}F -FLT uptake and CT images taken 6 weeks after treatment initiation confirm nonresponder status.

Table 1

Functional and molecular imaging probes discussed in this review organized by imaging modality.

Imaging Agent	Class	Modality	Preclinical (P)/Clinical (C)	Reference
Magnetic nanoparticles	Functional	MRI	P	3
Gadolinium-DTPA	Functional	MRI	C	4
lacZ/S-Gal	Molecular	MRI	P	37
PTP-magnetofluorescent nanoparticle *	Molecular	MRI	P	63
HSV1-tk/18F-FIAU	Molecular	PET	P	36
18F-FDG	Molecular	PET	P,C	72, 77, 79
¹⁸ F-16a-17-b-fluoroestradiol (FES)	Molecular	PET	C	76
3'-deoxy-3'-[¹⁸ F]fluorothymidine (FLT)	Molecular	PET	C	80
¹⁸ F-Galacto-RGD *	Molecular	PET	C	83
¹¹¹ In-DOTA-KCCYSL *	Molecular	SPECT	P	59
^{99m} Tc-NC100692	Molecular	SPECT	C	84
HIF1A-luciferase Fusion	Molecular	Optical (BL)#	P	19
NFKBIA-luciferase Fusion	Molecular	Optical (BL)#	P	26
SCAT3	Molecular	Optical (FR)#	P	29
PTP-magnetofluorescent nanoparticle *	Molecular	Optical (FL)#	P	63
Prosense 680	Molecular	Optical (FL)#	P	46
EGFR antibody-FITC	Molecular	Optical (FL)#	P	47
carcinoembryonic antigen antibody-FITC	Molecular	Optical (FL)#	C	48
RPMC-FITC *	Molecular	Optical (FL)#	P	50
VRPMPLQ-FITC *	Molecular	Optical (FL)#	C	51
Rhodamine-labeled aptamer against PSMA	Molecular	Optical (FL)#	P	70

* PTP, RGD, KCCYSL, RPMC, VRPMPLQ – peptide sequences