

## Imaging Update: New Windows, New Views

Ronald G. Blasberg

### Introduction

The recent progress in our understanding of the molecular-genetic mechanisms active in many diseases and the application of new biologically based approaches in therapy are exciting new developments that have emerged following mapping of the human genome. Novel "molecular therapies" have been developed that target specific oncogenic mutations in chronic myelogenous leukemia (1), gastrointestinal stromal tumors (2), lung cancer (3), and renal cell carcinoma (4, 5). Concurrent with the advances in our understanding of the biological basis of disease and the development of new molecular-targeted therapies, medical imaging has also undergone a remarkable revolution and expansion in the past two decades. Imaging now provides visualization in space and time of normal as well as abnormal cellular processes at a molecular-genetic or cellular level of function, and this new focus has been described as "molecular imaging." The term molecular imaging was coined in the mid-1990s: it has its roots in both molecular biology and cell biology as well as in chemistry and imaging technology.

Recent studies have focused on noninvasive molecular imaging in live animals and in human subjects. The success of *in vivo* molecular imaging is the direct result of significant developments in imaging technology as well as new imaging paradigms (6): (a) magnetic resonance imaging (MRI; ref. 7), (b) nuclear imaging (positron emission tomography [PET] and gamma camera; ref. 8), (c) optical imaging of small animals (9–11) as well as two-photon fluorescent imaging of viable cells, small organisms, and embryos (12). Other imaging modalities, such as ultrasound, are not included in this FOCUS section because of space limitations. It should be noted that these developments occurred more or less in parallel to each other and were largely independent of simultaneous advances that were occurring in genetics and molecular and cellular biology during the 1980s and early 1990s. However, it is the convergence of the imaging and molecular/cellular biology disciplines in the mid-1990s that is at the heart of this success story and is the wellspring for further advances in this new field.

Although noninvasive molecular imaging paradigms were first applied to small animals, they are now being translated into the clinic (13, 14) and will establish new standards of medical practice. The development of versatile and sensitive noninvasive assays that do not require tissue samples will be of considerable value for monitoring molecular-genetic and cellular processes in animal models of human disease as well

as for studies in human subjects. Imaging molecular-genetic and cellular processes will complement established molecular-biological assays that are invasive and require tissue sampling. Imaging can provide a spatial as well as a temporal dimension to our understanding and monitoring of various disease states in individual subjects.

### Overview

In this issue of *Clinical Cancer Research*, the FOCUS is on noninvasive imaging: "New Windows, New Views." Five articles by experts in their field present different perspectives on the current use in clinical management and clinical research of MRI and PET as well as the use in small animal research of non-invasive optical methods (bioluminescence and fluorescence).

Jackson et al. (15) describe the advantages and limitations of imaging tumor vascular heterogeneity and angiogenesis by using dynamic contrast enhanced MRI in the assessment of antiangiogenic and vascular-targeted therapy. They review the basic technical aspects of dynamic contrast enhanced MRI to help the clinician understand the wide methodological variations seen in the literature. A review of studies where the technique has been applied to early-phase clinical trials or clinical management is also presented, and the potential for obtaining a better characterization of the heterogeneity of tumor tissue is discussed.

Mankoff et al. (16) describe the biological basis for tumor-specific PET imaging in patients with [<sup>18</sup>F]fluorodeoxyglucose (FDG) and other radiopharmaceuticals. Because FDG is by far the most widely imaged radiopharmaceutical in the assessment of cancer with PET, it is discussed in some detail. The basic principles underlying the accumulation of FDG in tumor tissue and the different analytic methods and numeric values used to express the imaging results are presented (standard uptake values; the FDG "flux constant"  $K_i$ ; and the metabolic rate for FDG, MR-FDG). The variability in FDG PET to detect different tumors and to monitor tumor response is discussed, as well as some biological insights gained from FDG PET imaging of tumors. The last section in the article, "Beyond FDG," presents a look toward the future. New radiopharmaceuticals that are being tested in both animal models and in clinical trials are briefly discussed.

Wester (17) then describes the process of developing and validating new radiopharmaceuticals for PET and gamma camera imaging: nuclear imaging probes (from bench to bedside). Key steps in the development of new radiopharmaceuticals are coupled to strategies for probe-target identification and selection and are presented at the beginning of the article. This is followed by a discussion of tracer concepts and methods for the optimization of imaging probes. Examples are presented for several categories, including probes that image proliferation, angiogenesis, apoptosis, receptor expression, hypoxia, and

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**Requests for reprints:** Ronald G. Blasberg, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021-6094. Phone: 212-639-7337; Fax: 212-717-3063; E-mail: blasberg@neuro.lmskcc.org.

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metastases. A brief section at the end asks a thoughtful question: "What kind of tracer, for which kind of imaging?"

It should be noted, however, that there have only been three PET radiopharmaceuticals approved by the U.S. Food and Drug Administration (FDA) for commercial distribution for human studies over the past 20 years: [ $^{18}\text{F}$ ]sodium fluoride, [ $^{18}\text{F}$ ]FDG, and [ $^{82}\text{Rb}$ ]rubidium chloride. This largely reflects the long and expensive process that is necessary to complete the required documentation of a new radiopharmaceutical for the current FDA review process as well as the necessity for a substantial commercial market to support for the production and distribution of the new radiopharmaceutical. In addition, the commercial distribution factor excludes radiopharmaceuticals labeled with short-lived positron-emitting isotopes (e.g., oxygen-15,  $t_{1/2} = 2$  min; nitrogen-13,  $t_{1/2} = 10$  min; carbon-11,  $t_{1/2} = 20$  min). Because the Center for Medicare Services required FDA approval as a precondition for reimbursement, regulatory delay at the FDA severely limited the number of new radiopharmaceuticals available for clinical PET imaging. The process for developing new radiopharmaceuticals in the United States is described in greater detail below.

A very interesting and novel combination of two modalities (MRI and ultrasound) is presented by Moonen (18). This article describes a treatment-based imaging application that is currently undergoing clinical trials in patients with solid tumors: the spatiotemporal control for cancer treatment and for gene expression using MRI-guided high-intensity focused ultrasound. High-intensity focused ultrasound is the only known technology that can be used to achieve a local temperature increase deep inside the human body in a noninvasive manner. Coupled with MRI guidance, the system provides *in situ* target definition and the means to identify and spare nearby healthy tissue. MRI is also used to provide continuous temperature mapping during high-intensity focused ultrasound and provides real-time spatial and temporal control of the heating procedure that adds another level of safety. A very interesting section at the end of the article discusses the application of local temperature elevation in targeted gene therapy protocols. The use of MRI-guided high-intensity focused ultrasound and heat-sensitive promoters to control gene expression is also discussed.

Kaijzel et al. (19) focus on optical imaging technology and the use of optical reporter genes in animal models to assess cancer development and progression. The first reporter gene approaches were based on the bacterial *chloramphenicol acetyltransferase* (CAT) gene (20) and the *lacZ* gene (21) to visualize reporter gene expression in individual cells. However, postmortem tissue sampling and processing were still required. More recent studies have focused on noninvasive imaging techniques involving live animals and human subjects, as referenced above and described in detail in this article. Different optical-based imaging methods to investigate tumor development, progression, metastasis, and treatment response in animal models are described. There is a specific emphasis on imaging metastasis to bone and bone marrow and on how optical imaging can be used to monitor angiogenesis, apoptosis, and proteolysis. The potential for translation of optical imaging methods into the clinic for the study and treatment of cancer is discussed at the end of the article.

## Imaging Paradigms

Molecular-genetic studies of cancer and our understanding of the multiple and converging pathways that are involved in oncogenesis and tumor progression have rapidly expanded. The era of molecular medicine has begun, and the benefits to individual patients with some cancers are being realized. "Biomarker imaging" that reflects endogenous molecular/genetic processes is particularly attractive for rapid translation into clinical studies in the near term. This is because existing radiopharmaceuticals and imaging paradigms may be useful for monitoring downstream changes of specific molecular/genetic pathways in diseases such as cancer. Biomarker imaging (such as FDG PET; see Fig. 1 in ref. 17) has been shown to be very useful in a clinical setting for the identification of malignant lesions and for staging the extent of disease and, in some cases, can be used as a very sensitive biomarker of treatment response (GIST-Gleevec FDG; see Fig. 3 in ref. 16). However, biomarker imaging is likely to be less specific and more limited with respect to imaging the expression of a particular protein or measuring the activity of a particular "upstream" pathway in comparison with direct-imaging paradigms, such as imaging the expression of  $\alpha_v\beta_3$  integrins that occurs during angiogenesis (see Fig. 7 in ref. 17) or the expression of the somatostatin receptor (sst2) that occurs in many (particularly, neuroendocrine) tumors (see Fig. 8 in ref. 17). Nevertheless, biomarker imaging benefits from the use of probes that have already been developed, studied, and approved for administration in human subjects. Thus, the translation of biomarker imaging paradigms into patients will be far easier than either the direct imaging or reporter transgene imaging paradigms outlined below.

The "direct" molecular imaging motif builds on established chemistry and radiochemistry relationships. Bioconjugate chemistry linking specific binding motifs and bioactive molecules to paramagnetic particles for MRI or to radionuclides for PET and gamma camera imaging is rapidly expanding. This has occurred largely through the development of new relationships and focused interactions among molecular/cellular biologists, chemists, radiochemists, imagers, and clinicians. The next generation of direct molecular imaging probes will come from better interactions among pharmaceutical companies, academia, and hospital centers. Such interactions are now being pursued, and the objective is to develop and evaluate new compounds for imaging that target specific molecules (e.g., DNA, mRNA, and proteins) or activated enzyme systems in specific signal transduction pathways. However, a constraint limiting direct imaging strategies is the necessity to develop a specific probe for each molecular target and then to validate both the sensitivity, specificity, and safety of each probe for specific applications before their introduction into the clinic. This can be very time-consuming and costly; for example, the development, validation, and regulatory approval for [ $^{18}\text{F}$ ]FDG PET imaging of glucose utilization in tumors has taken over 20 years.

Reporter gene imaging studies will be more limited in patients compared with that in animals due to the necessity of transducing target tissue with specific reporter constructs or the production of transgenic animals bearing the reporter constructs. Ideal vectors for targeting specific organs or tissue

(tumors) do not exist at this time, although this is a very active area of human gene therapy research. The clinical application of PET-based reporter gene imaging will begin to expand over the next several years. These studies will initially involve “constitutive,” “always-on” reporter systems and focus on two different applications: (a) gene therapy (viral vector tracking and monitoring) and (b) adoptive cell-based therapies. The first patient applications have already been reported in which imaging was used to track and monitor “suicide” gene therapy (13, 14). The second application of reporter gene imaging that is likely to be translated into clinical studies involves adoptive, cell-based therapies. These studies will involve autologous or donor-matched lymphocytes (T cells) or stem/progenitor cells that are genetically modified, selected, and expanded *ex vivo* in a good manufacturing practice facility and then administered to patients. Initially, such studies will focus on constitutive reporters in phase 1-like toxicity-safety studies. These initial studies will be able to track and monitor the number (expansion or contraction) of adoptively administered cells and will be similar to vector-tracking studies.

The subsequent, more exciting next phase of reporter gene imaging studies in patients will involve a combination of reporter systems. Transduction vectors will contain at least two different reporter constructs. One will be a “constitutive” reporter that will be used to identify the site, extent, and duration of vector delivery and can be used to monitor the efficiency of tissue transduction (the normalizing term) for subsequent image and data analysis. A second reporter will be “inducible” and sensitive to endogenous transcription factors as well as to posttranscriptional processing, modulation of reporter protein translation, protein-protein interactions, and reporter protein ubiquitination. The “inducible” reporter constructs are “sensors” and will be used to monitor the functional status and characteristics of the transduced cells. It is now recognized that the readout from “inducible” reporters requires coupling with the readout from a “constitutive” reporter to appropriately interpret the data. That is, the data obtained from the “inducible” reporter must be normalized to reflect the efficacy (extent) of target tissue transduction or the number of transduced cells that traffic to the target tissue or organ. The initial application of such double-reporter systems in patients will most likely be done as part of a gene therapy protocol involving viral vectors, or part of an adoptive therapy protocol involving T cells or stem/progenitor cells that couple reporter gene imaging with existing adoptive therapies.

Although each new vector requires extensive and time-consuming safety testing before regulatory approval for human administration, reporter gene imaging has several advantages. For example, it is possible to develop and validate reporter imaging strategies more rapidly and at considerably lower cost than “direct” imaging strategies. This is because only a small number of well-characterized and validated reporter gene/reporter probe pairs needs to be established. For example, a single reporter gene/reporter probe pair combination can be used in many different reporter constructs, and reporter expression can be controlled by different promoter/enhancer elements to image many different biological and molecular-genetic processes. Reporter gene imaging strategies are already

providing the opportunity for a wider application of imaging in the study of experimental animal models of human disease, and they have potential for implementation in future clinical studies using human reporter genes (22).

## Developing New Radiopharmaceuticals in the United States

Because of the regulatory and commercial impediments to the development of new radiopharmaceuticals, new applications for PET imaging in clinical medicine developed very slowly until 1997. In that year, the situation both for FDA approval and Medicare reimbursement was drastically improved by a legislative remedy, the 1997 FDA modernization act. The FDA modernization act required FDA to develop modernized rules for regulation and approval of new radiopharmaceuticals such as FDG within a reasonable timeframe (2 years was suggested). The Act went on to propose interim methods of regulatory approval that focused on the practice of medicine and pharmacy. In essence, radiopharmaceuticals for PET imaging could be formulated in radiopharmacies by licensed radiopharmacists and dispensed based on a prescription to patients for PET imaging, as long as the particular radiopharmaceutical, such as FDG, had a monograph in the U.S. pharmacopeia. In parallel, the Center for Medicare Services agreed to accept this legislative authority and began reimbursing for oncology examinations in major human cancers such as lung, melanoma, head and neck, colorectal, and lymphoma.

With better clinical reimbursement, there has been a surge in clinical applications, and the demand for PET has increased so that there are more than a thousand PET units, scanning more than 1,000,000 patients per year, in the United States alone. Particularly in the area of oncology, there has been a major effect on clinical management based on improved imaging for staging of common human tumors. In parallel with this increasing clinical demand, there has been a corresponding investment in the development of new PET instruments as well as new radiopharmaceuticals. An important outcome of this investment is the novel combination of tomographs (i.e., the combination of PET and CT into one instrument, the PET/CT hybrid). These PET/CT units have rapidly become the norm for PET imaging throughout the world, combining functional/metabolic and anatomical imaging. It should also be noted that PET/MRI hybrids are currently in the developmental stage.

Although FDG is by far the most commonly used radiopharmaceutical, the three major instrument manufacturers (Siemens, General Electric, and Philips) have joined forces with radiopharmaceutical manufacturers to promote development of additional radiopharmaceuticals suitable for PET imaging. In the next 5 to 10 years, we can anticipate a growing pharmacopeia for PET imaging that will go well beyond FDG into the imaging of the key molecules underlying pathogenesis of major human disease, as described by Wester (17) and Mankoff et al. (16).

The FDA has recognized that radiopharmaceuticals, when used as radiotracers, have a strong safety record and play a key role in research. For this reason, special regulatory pathways have been developed through the mechanism of radioactive

drug research committees and exploratory investigational new drug applications (eIND), which are intended to foster the development of research applications for radiotracers.

**Radioactive drug research committee.** Under 21 CFR 361.1, the FDA describes regulations that in effect delegate the oversight for radiopharmaceutical research to specially constituted institutional committees: the radioactive drug research committee. The radioactive drug research committee should include nuclear medicine physicians, pharmacologists, dosimetrists, radiopharmacists, and other scientists who may be trained in the preparation, formulation, and use of free pharmaceuticals in clinical research. Radiopharmaceuticals can be used in individual protocols when they have been shown to have no pharmacologic effect in humans (i.e., used in tracer quantities) at radiation doses to organs and tissues that are less than what an occupational worker would receive in 1 year and when the research is for the purpose of dosimetry, metabolism, or biochemistry (i.e., to obtain fundamental clinical research knowledge of a general nature and that such information would not be used for the management of an individual patient). The FDA requires appropriate record keeping, safe facilities for the production of radiopharmaceuticals that meet current good manufacturing practices (as defined by 21 CFR 210 and 211), and accurate reports on an annual basis regarding outcome of the research. Normally, not more than 30 patients are permitted for study, although, if required to obtain adequate research data, the FDA will approve additional study subjects based on petition. The interested reader is referred to the regulation 21 CFR 361.1 that details how the committee may be set up and operate.

**eIND.** The radioactive drug research committee mechanism is not appropriate for “first in humans studies,” and an alternative has recently been developed. The FDA has proposed the eIND. Under the eIND, an investigator may combine early human studies at a phase 0 or “micro dose” level as a basis for getting important information that may provide drug developers with an early indication of whether drugs are promising (or are lacking in promise) for further development. Drugs may be used at a dose not to exceed 1/100 of the dose calculated to cause a pharmacologic effect and, in any event, not to exceed

100 µg. The concept is for study of a limited number of subjects, with a limited dose range for a limited period of time as a basis for better understanding a drug pharmacokinetics; for selecting the most important lead candidate from a group of candidates; and for exploring a drug distribution by using radiotracer imaging. As a rule, toxicity testing will be much less demanding than for a full-fledged IND because very small amounts of drug are given in radiotracer imaging studies. For example, the FDA will usually accept toxicity in one mammalian species treated via the same route with 100 times the proposed eIND study dose. Typically, observation would proceed in the animals for 14 days with an interim sacrifice day, typically on day 2 after administration, with observations including body weight, clinical signs, blood chemistries, and histopathologic analyses. In its current form, the eIND would appear to have excellent advantages for early drug discovery, including the early development of radiopharmaceuticals. This is particularly important for radiopharmaceuticals that are intended for diagnostic and therapeutic purposes. The brochure, “Guidance for Industry, Investigators, and Reviewers: Exploratory IND Studies,” is available for all drugs, including radiopharmaceuticals, and for therapeutic biological products online.<sup>1</sup>

## Conclusions

I remain optimistic; the imaging tools and resources largely exist and they will continue to be improved. We are now viewing the different imaging modalities in a new, complimentary light. By combining different imaging modalities, we are better able to image the malignant phenotype of an individual patient's tumor at a molecular level and to monitor changes in that phenotype over time. The potential to image a drug effect on the expression of specific endogenous proteins or the activity of specific signal transduction pathways in an individual patient's tumor exists and provides the opportunity for monitoring treatment response at the molecular level. At the moment, this requires the development of specific-targeting probes and the use of “diagnostic” reporter gene transduction vectors. These issues are addressed in greater detail in this FOCUS.

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<sup>1</sup> <http://www.fda.gov/CDER/guidance/7086fnl.htm>

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