Imaging: Strategies, Controversies, and Opportunities

Ronald Blasberg[1] and David Piwnica-Worms[2]

Abstract

At a Clinical and Translational Cancer Research Think Tank meeting sponsored by the American Association for Cancer Research in 2010, one of the breakout groups focused on new technologies and imaging. The discussions emphasized new opportunities in translational imaging and its role in the future, rather than established techniques that are currently in clinical practice. New imaging methods under development are changing the approach of imaging science from a focus on the anatomic description of disease to a focus on the molecular basis of disease. Broadly referred to as molecular imaging, these new strategies directly embrace the incorporation of cell and molecular biology concepts and techniques into image generation and can involve the introduction of genes into cells with the explicit intent to image the end products of gene expression with external imaging devices. These new methods hold the promise of providing clinicians with (i) robust linkages between cell and animal models and clinical trials, (ii) *in vivo* biomarkers that can be measured repeatedly and sequentially over time to observe dynamic disease processes and responses to treatment, and (iii) tools for preselection and patient population enrichment in phase II and III trials to improve outcomes and better direct treatment. These strategies provide real-time pharmacodynamic parameters and can be powerful tools to monitor therapeutic effects in a spatially and tissue-specific manner, which may reduce cost during drug development, because pharmacodynamic studies in animals can inform clinical trials and accelerate the translation process. The Imaging Response Assessment Team (IRAT) program serves as an example of how imaging techniques can be incorporated into clinical trials. IRATs work to advance the role of imaging in assessment of response to therapy and to increase the application of quantitative anatomic, functional, and molecular imaging endpoints in clinical trials, and imaging strategies that will lead to individualized patient care. *Clin Cancer Res; 18(3); 631–7. ©2012 AACR.*

Introduction

At a Clinical and Translational Cancer Research Think Tank Meeting sponsored by the American Association for Cancer Research in 2010, one of the breakout groups focused on new technologies and imaging. These discussions emphasized translational imaging and its role in the future, rather than established techniques that are currently in clinical practice. They identified and discussed the following important categories: (i) identifying the disease phenotype, (ii) preselection of target populations, (iii) monitoring dose and dose scheduling (pharmacodynamics and pharmacokinetics), (iv) validation of drug efficacy and time course of efficacy, (v) individual patient management, and (vi) imaging to guide biopsy sites for genomic and proteomic assays. In this brief review, we focus on several of the above categories in the "Application" section. In the "Background" section, we provide a brief outline of the development and salient features of molecular imaging. We subsequently attempt to provide a glimpse into the future of imaging and its translation into modern clinical practice.

Background

Investigators have developed novel molecular therapies 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 biologic basis of disease and the development of new molecular targeted therapies, medical imaging has also undergone a remarkable revolution and expansion in the past 2 decades. Imaging now provides visualization in space and time of both normal and abnormal cellular processes at a molecular–genetic or cellular level of function. This new focus has been described as molecular imaging, a term coined in the mid-1990s that has its roots in both molecular biology and cell biology, as well as in chemistry and imaging technology.

Molecular imaging is the direct result of significant developments in several noninvasive, *in vivo* imaging

Authors' Affiliations: [1]Departments of Neurology and Radiology, Molecular Pharmacology and Chemistry Program, Memorial Sloan-Kettering Cancer Center, New York, New York; [2]Departments of Cell Biology and Physiology, Developmental Biology, and Radiology, BRIGHT Institute, and Molecular Imaging Center, Washington University Medical School, St. Louis, Missouri

Corresponding Author: Ronald G. Blasberg, Memorial Sloan-Kettering Institute, 1275 York Avenue, New York, NY 10021. Phone: 646-888-2211; Fax: 646-422-0408; E-mail: blasberg@neuro1.mskcc.org

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technologies, including nuclear imaging [e.g., quantitative autoradiography, gamma camera, single photon emission computed tomography (SPECT), and positron emission tomography (PET)] (6); MRI; and magnetic resonance spectroscopy [MRS (7)]; optical imaging of small animals (6–9); and epifluorescent and 2-photon fluorescent imaging of viable cells, small organisms, and embryos (10). It should be noted that these developments occurred more or less in parallel with each other and were largely independent of the advances that were occurring in genetics and molecular cell biology during the 1980s and early 1990s. However, each of these imaging technologies has important antecedents. For example, radionuclide-based imaging is founded on the radiotracer principle first described by George de Hevesy. In 1935 he published a letter in *Nature* in which he discussed the tracer principle using ^{32}P for the study of phosphorus metabolism (11), and in 1943 he was awarded the Nobel Prize in Chemistry. The tracer technique was later adapted for many applications in physiology, biochemistry, functional diagnosis, nuclear medicine, and, more recently, molecular imaging.

The new imaging methods that are being developed are changing the approach of imaging science from a focus on the anatomic description of disease to a focus on the molecular basis of disease. Rather than imaging macroscopic phenotypes and physiologic functions (e.g., blood flow, pH, and organ volumes), investigators are focusing on imaging molecular targets of disease and *in vivo* biochemistry. This transition requires radiologic scientists and clinical researchers to share a common conceptual framework, vocabulary, and approach to genomic and proteomic science. These new methods hold the promise of providing clinicians with *in vivo* imaging biomarkers that can be measured repeatedly and sequentially over time to observe dynamic disease processes and responses to treatment.

Application

Currently, 3 strategies for noninvasive monitoring and quantification of molecular events are being used. These can be described as direct, genetically encoded, and biomarker imaging (12–15). These imaging strategies are linked to 2 companion articles in this *CCR Focus* section. Kucherlapati provides complementary strategies for imaging in genetically altered animal models of cancer (16) to assess the effects of specific genetic alterations and targeted drug therapy on signaling pathway activity, as monitored by various molecular imaging paradigms. Similarly, Arteaga and Baselga (17) focus on human cancer genetics and drug development, in which imaging will be playing an increasingly important role.

Direct molecular imaging strategies are usually described in terms of a specific endogenous target and a target-specific probe. This strategy builds on established chemistry and radiochemistry relationships in developing novel small-molecule and macromolecular probes. 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 and now includes nanoparticles. The next generation of direct molecular imaging probes will come from enhanced interactions among pharmaceutical companies, academia, and hospital centers. Such interactions are currently being pursued with the objective of developing and evaluating new compounds and imaging paradigms, including compounds and strategies that target specific molecules [e.g., DNA, mRNA, and proteins (18)] or activated enzyme systems (19, 20), specific signal transduction pathways (21), and novel spectral analytic techniques such as *in vivo* MRS and Raman spectroscopy (22–25).

The imaging of specific antigens on the cell surface using radiolabeled antibodies or genetically engineered antibody fragments (minibodies) is an example of direct molecular imaging that has evolved over the past 30 years (26). In addition, *in vivo* visualization of receptor density/occupancy with small radiolabeled ligands, which is widely used, can also be classified as a direct imaging approach (27–29). These examples represent 2 of the first molecular imaging applications used in clinical nuclear medicine. Thousands of injectable agents have been and are continuing to be developed. They include radiopharmaceuticals, magnetic resonance contrast agents, targeted ultrasound agents, and optical probes. The development cycles have been slow, but these agents have the potential for direct translation to the clinic. However, direct imaging strategies are limited by the necessity to develop and validate a specific probe for each molecular target. Because each probe requires a detailed characterization, including sensitivity, specificity, and safety, the time and cost required to develop a new probe can be considerable. For example, it has taken more than 20 years to develop, validate, and obtain regulatory approval for [^{18}F]-fluoro-2-deoxy-D-glucose ([^{18}F]-FDG) PET imaging of glucose use in tumors. Nevertheless, once a new imaging probe has been approved, it may have wide clinical application, as is the case with [^{18}F]-FDG (30, 31).

Although genetically encoded imaging strategies are more complex, they have the potential to allow highly specific and detailed analyses *in vivo* (32). They are highlighted in this overview as a look to the future of molecular imaging in the context of the Think Tank goals. In this approach, a genetically encoded reporter is introduced into cells to enable the visualization of molecular events *in vivo*. This approach is now being used extensively in small animals, as well as in a limited number of clinical studies, as reporter gene imaging. This technique requires pretargeting (delivery) of the reporter gene to the target tissue by transfection, transduction, or cell delivery. The reporter gene usually includes transcriptional control components that can function as molecular–genetic sensors to regulate or initiate reporter gene expression, as well as fusion reporters to study posttranslational modification of target proteins. These strategies have been widely applied in optical (32–35) and radionuclide-based (36–41) imaging, and to a lesser degree in MRI (42, 43).

It could be argued that the design and execution of genetically encoded imaging experiments were the

strategies that distinguished molecular imaging from previous decades of *in vivo* and *in vitro* radiotracer development and optical imaging research. The broad missions of molecular imaging directly embraced the incorporation of cell and molecular biology concepts and techniques for the introduction of genes into cells with the explicit intent to image the end products of gene expression with external imaging devices. Previously, investigators simply used what nature had provided, i.e., the endogenous receptors, transporters, and enzymes of biology, as targets for tracer development. The concept of manipulating cells and animal tissues with genetic tools to express imageable transgenes with the intent to investigate transgene biology with external macroscopic imaging techniques such as PET, SPECT, optical imaging, and MRI, represented a significant paradigm shift.

The first studies that incorporated this concept and established the new paradigm date to the early 1990s. In one early example, cells were infected with a baculovirus that had been engineered to express human *MDR1* P-glycoprotein, with the intent to image the reduced uptake of ^{99m}Tc-SESTAMIBI, a radiopharmaceutical that was found to be outwardly transported by the multidrug transporter P-glycoprotein (44). This established a new approach to noninvasive assessment of multidrug resistance in cancer. In another study, *Salmonella* bacteria were engineered to express the *lux* operon with the intent to image bacterial infection and monitor drug treatment in animals by *in vivo* bioluminescence imaging (45). Another important study showed the principles of macroscopic imaging of herpes simplex virus 1 thymidine kinase reporter transgene (HSV1-TK) using PET in the context of gene therapy (ref. 36, Fig. 1), establishing the general paradigm for noninvasive radiotracer reporter gene imaging using radiolabeled probes. All of these studies required the appropriate pairing of a genetically encoded transgene and an imageable probe that is acted on by the transgene product. The reporter transgene usually encodes for an enzyme (38), a receptor (46), or a transporter (47) that selectively interacts with a radiolabeled probe, resulting in the accumulation of the imaging agent in the transduced cell that is proportional to the expression of the reporter transgene. Recall that fluorescence microscopy of cellular GFP was first reported in 1994, changing the field of cell biology with the advent of microscopic fluorescence imaging at the cellular and subcellular scale. Molecular imaging promised to change macroscopic imaging, with the long-term intent of translating the information to live animals and patients.

Genetically encoded optical reporters, such as luciferase or fluorescent proteins, provide cost-effective strategies for preclinical small-animal studies. The tagged molecules offer a direct linkage to cancer-associated molecules and give researchers the ability to observe molecular dynamics in real time *in vivo*. This approach provides molecule-specific pharmacodynamic information noninvasively *in vivo*, allowing a time course for response to treatment to determine the specific dosage for a desired molecular action (32, 48, 49). As genetically encoded imaging reporters are engineered into mouse lines (50, 51), preclinical pharmacodynamic studies will be made more accurate due to direct observation of drug action on specific targets over time. These observations will confirm the mechanism of disease and drug action, validate that the drug is affecting the expected target, and validate the drug's efficacy (52).

Reporter gene imaging studies will be more limited in patients than in animals because of 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. Studies of this approach will initially involve constitutive (always-on) reporter systems and focus on 2 different applications: (i) gene therapy (viral vector tracking and monitoring), and (ii) adoptive cell-based therapies. The first patient applications, in which imaging was used to track and monitor suicide gene therapy, have already been reported (53, 54). 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 Processes facility, and then administered to patients. Initially, such studies will focus on constitutive reporters in phase I–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. A subsequent phase of reporter gene imaging studies in patients may involve combinations of reporter systems, involving both constitutive reporters for tracking and inducible reporters that are 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.

Although each new vector requires extensive and time-consuming safety testing before regulatory approval for administration in humans can be obtained, reporter gene imaging has several advantages. It is possible to develop and validate reporter imaging strategies more rapidly and at considerably lower cost compared with direct imaging strategies. This is because only a small number of well-characterized and validated reporter gene–reporter probe pairs have to be established. 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 biologic 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

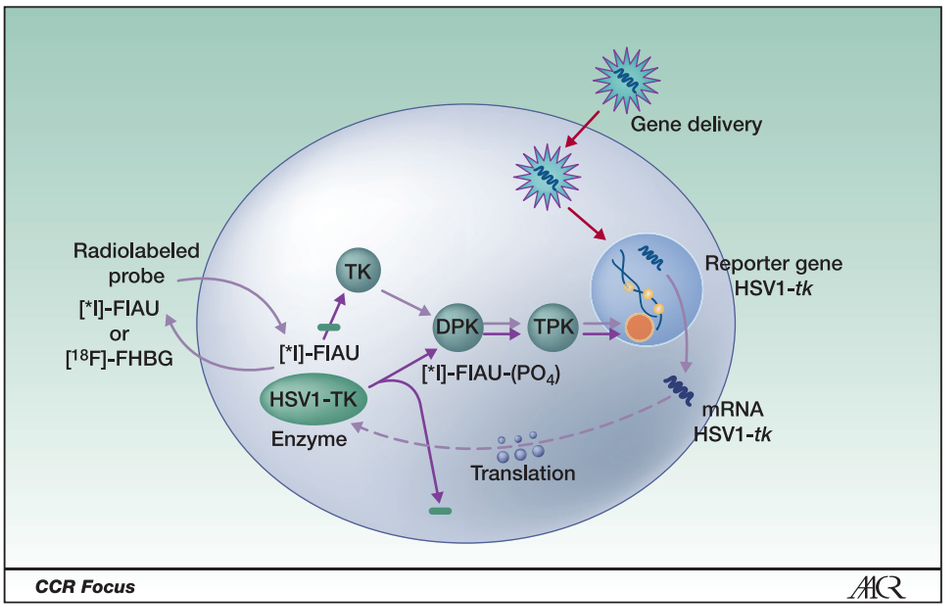

Figure 1. Schematic for imaging herpes simplex virus 1 thymidine kinase reporter gene (HSV1-*tk*) expression with reporter probes ^{124}I-, ^{131}I-, or ^{18}F-labeled FIAU and ^{18}F-labeled FHBG. The HSV1-*tk* gene complex is transfected into target cells by a vector. Inside the transfected cell, the HSV1-*tk* gene is transcribed to HSV1-*tk* mRNA in response to activation by either constitutive or inducible promoters, and then translated on the ribosomes to a protein (enzyme), HSV1-TK. After a radiolabeled probe is administered and then transported into the cell, the probe is phosphorylated by HSV1-TK (the gene product). The phosphorylated radiolabeled probe does not readily cross the cell membrane and is trapped within the cell. Thus, the magnitude of probe accumulation in the cell (i.e., the level of radioactivity) reflects the levels of HSV1-TK enzyme activity and HSV1-*tk* gene expression. Reprinted with permission from Blasberg and Tjuvajev (12).

have potential for implementation in future clinical studies using human reporter genes (55).

Biomarker or surrogate-marker imaging can be used to assess the downstream effects of single or multiple endogenous molecular–genetic processes. This approach is particularly attractive for potential translation into clinical studies in the near term because existing radiopharmaceuticals and imaging models may be useful for monitoring the downstream effects of alterations in specific cellular pathways that occur in various tumors. For example, [^{18}F]-FDG PET has been used successfully as an imaging biomarker in early treatment response assessments of many solid tumors. This was most dramatically shown with [^{18}F]-FDG PET imaging of gastrointestinal stromal tumors before and after imatinib treatment (21). However, it remains to be determined whether there is a sufficiently high correlation between biomarker imaging and direct molecular assays that reflect the activity of a particular molecular–genetic pathway of interest.

Imaging and Individual Patient Management

Preselection of target populations

Clinical imaging could be a valuable tool for preselection and patient population enrichment in phase II and III trials to improve outcomes and better direct treatment. This theme is developed more fully in 2 companion articles (56, 57) in this *CCR Focus* section. Imaging can serve as an *in vivo* biomarker for drug efficacy and can be used after treatment to assess a treatment's long-term efficacy. Eventually, imaging techniques may be used to guide treatment in the clinic and guide biopsies to allow for standardization of genomic and proteomic correlation studies. Although clinical imaging techniques may be expensive, potentially inexpensive and easily obtained biomarkers could be used to define optimal imaging approaches.

Monitoring dose and dose scheduling (pharmacodynamics and pharmacokinetics)

Recent advances in molecular imaging combined with the use of reporter animals can recapitulate genomic alterations in human diseases and are reshaping the process of new drug development at many levels (52). First, an effective readout reflecting real-time pharmacodynamic changes can be a powerful tool to monitor therapeutic effects in a spatially and tissue-specific manner (58). Second, molecular imaging may reduce costs during drug development, because pharmacodynamic studies in animals can inform clinical trials and accelerate the translation process. Third, noninvasive imaging allows chronologic monitoring for delayed activities and toxicity of drugs in living animals, which may translate into clinical trials.

Treatment efficacy and time course of efficacy

The Imaging Response Assessment Team (IRAT) project is an example of how imaging techniques can be incorporated into clinical trials (Box 1). IRATs work to advance the role of imaging in assessment of response to therapy, and to increase the application of quantitative anatomic,

Box 1. Recommendations

- Preselect and enrich patient populations for entry into trials, or focus clinical practice on prevention.
- Monitor neoplastic progression across all stages of disease, absent any intervention.
- Guide tissue sampling to try to standardize approaches and improve the signal:noise ratio that might be evident in some biospecimen-based markers, which could be critical for genomic expression.
- Perform metabolic and proteomic assessments.
- Assess the pharmacokinetic parameters of chemopreventive agents or other interventions that could be proposed.
- Validate the mechanisms of action of drugs and drug combinations.
- Provide real-time and recurrent *in vivo* pharmacodynamic assessments focused on either intended (efficacy) or unintended (so-called safety parameter) targets throughout intervention.

functional, and molecular imaging endpoints in clinical trials. The IRAT Network, supported by the National Cancer Institute, aims to strengthen clinical collaboration between imaging scientists and oncologic investigators, integrate IRAT teams into the protocol planning process of clinical trial design, and facilitate an ongoing exchange of ideas that will serve to advance the role of imaging as a biomarker in the oncology community. This kind of imaging could guide clinical trial design and patient management and result in faster and more efficient analyses.

An example of recent advances: *in vivo* Raman spectroscopy

Raman spectroscopy is based on the inelastic scattering of a photon and has been widely used as an analytical tool in many research and industrial fields. More recently, Raman spectroscopy has also been explored for biomedical applications, including cancer diagnosis, because it can provide detailed, specific information about the chemical composition of cells and tissues (22–25). For imaging applications, investigators have developed several variations of Raman spectroscopy to enhance imaging sensitivity and take advantage of its exquisite specificity, nonexistent background noise compared with fluorescence imaging, and superb multiplexing capability. Surface-enhanced Raman spectroscopy (SERS) uses a variety of nanoparticles as contrast agents. SERS-based nanoparticle Raman imaging will be extremely useful in future research because different agents can be attached to different Raman tags to enable the interrogation of multiple biologic events simultaneously in living subjects. Raman imaging and other optical techniques have similar limitations in humans; however, whereas monochromatic laser activation occurs in the infrared, unique Raman spectral emissions occur in the far-infrared regions of the spectrum, where tissue absorption and scatter of photons are low.

Challenges for future research and implementation

MRI and PET technologies are expensive and can be labor intensive. Optical imaging in small animals is much less expensive. The sensitivity of these techniques for detecting intraepithelial neoplasia is almost totally unexplored, but it is expected to be lower than their sensitivity for advanced lesions, and researchers have not had a great deal of experience in applying them to patients at risk who are without detectable neoplasia. Optical imaging of malignant and premalignant surface lesions (such as basal cell cancer and melanoma) is now beginning to be investigated. Many of these tools have the potential for broad applicability, but, as with mouse models, their power can generate considerable noise in addition to the sought-after signal. This is particularly true for fluorescence imaging at lower wavelengths. The path to commercialization is unclear, raising more questions about how they might be developed in the prevention domain. This challenge is not unique to imaging technologies and applies as well to pharmacologic and biospecimen-based risk assessment tools used in a preventive context. To succeed, most of the imaging tools will have to be developed with the support of the bioinformatics and systems biology communities.

Several important activities could enhance the value of imaging for cancer prevention. First, we need to support applications of current and emerging imaging technologies directed specifically toward at-risk populations and patients with intraepithelial neoplastic lesions. This would encourage their development for preventive applications and allow their performance characteristics to be evaluated in relevant settings. Second, we need to work with the U.S. Food and Drug Administration, Centers for Medicare and Medicaid

Box 2. Imaging Response Assessment Team

An Imaging Response Assessment Team (IRAT) provides analysis and quality control for conventional and molecular imaging studies used in assessment of response to therapy in cancer clinical trials. Cancer centers of excellence and scientifically sophisticated radiology departments are the hub of National Cancer Institute (NCI)– IRAT network. The NCI's IRAT initiative is a collaborative effort to foster the growth of anatomic, functional, and molecular imaging techniques and their application to cancer research and treatment. Its goal is to advance the role of imaging in assessment of response to therapy; increase the application of quantitative anatomic, functional, and molecular imaging endpoints in clinical therapeutic trials; and establish oncologic IRATs as formal shared resources in cancer centers.

Services, and other agencies to establish the performance, practice, and reimbursement standards for imaging technologies applied in a preventive context as they appear. Finally, we need to support research focused on correlations between biospecimen- and image-based biomarkers of risk and chemopreventive response.

Challenges and recommendations

Several new directions and opportunities for progress were discussed at the Think Tank meeting (Box 2). These included better integration of genomics/proteomics with imaging, linking therapeutic targets to imaging targets to improve outcome measures, imaging therapeutic targets in clinical trials, linking susceptibility genes to guide image screening (e.g., mammography, colonoscopy, and bronchoscopy), and a new imaging emphasis on tumor metabolism and the tumor microenvironment. The participants also discussed the use of novel imaging technologies such as combined MRI/MRSI/PET and hyperpolarized molecules, and new optical imaging technologies involving both near- and far-infrared emitting probes and Raman-emitting nanoparticles and spectroscopy.

Disclosure of Potential Conflicts of Interest

D. Piwnica-Worms is a consultant to Carestream. R. Blasberg disclosed no potential conflicts of interest.

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