

## KEYNOTE LECTURE

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# Translational molecular imaging for cancer

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### Abstract

Although most clinical diagnostic imaging studies employ anatomic techniques such as computed tomography (CT) and magnetic resonance (MR) imaging, much of radiology research currently focuses on adapting these conventional methods to physiologic imaging as well as on introducing new techniques and probes for studying processes at the cellular and molecular levels *in vivo*, i.e. molecular imaging. Molecular imaging promises to provide new methods for the early detection of cancer and support for personalized cancer therapy. Although molecular imaging has been practiced in various incarnations for over 20 years in the context of nuclear medicine, other imaging modalities have only recently been applied to the noninvasive assessment of physiology and molecular events. Nevertheless, there has been sufficient experience with specifically targeted contrast agents and high-resolution techniques for MR imaging and other modalities that we must begin moving these new technologies from the laboratory to the clinic. This brief review outlines several of the more promising areas of pursuit in molecular imaging for oncology with an emphasis on those that show the most immediate likelihood for clinical translation.

**Keywords:** *Molecular imaging; clinical translation.*

### Introduction

As it relates to cancer, molecular imaging represents a group of methods to study the malignant phenotype noninvasively at high resolution with specific probes, contrast agents or MR pulse sequences with a view not only to understanding cancer biology but also to providing early diagnosis and support emerging cancer therapies. Molecular imaging is important to cancer research, diagnosis and therapy now because of the many new, specific therapies that may not be as indiscriminately cytotoxic as most have been to date. Much has been written about parallel progress in development of high-resolution, multimodality imaging devices<sup>[1–4]</sup> along with array-based techniques used to discover new targets for cancer imaging and therapy<sup>[5]</sup> and the ready availability of experimental models, many of which are genetically based and therefore provide unprecedented relevance to human cancer<sup>[6,7]</sup>. Convergence of advances in those areas has provided interesting and in some cases spectacular imaging results in experimental models with

some novel approaches beginning to find their way to the clinic.

Translational research is a somewhat nebulous term that attempts to describe the work that goes into bringing the most promising experimental therapies to the clinic after extensive testing in experimental models. In the US, the National Cancer Institute (NCI) has recognized the importance of this type of research and has invested substantial funding not only into the development of *in vivo* cellular and molecular imaging centers (ICMICs) but also into small animal imaging resource programs (SAIRPs), both of which involve translational research extensively. Because imaging agents are designed to be ‘tracers’ of physiology and therefore have no pharmacologic effect, they can be approved for human administration much more readily than most therapeutic agents. In the US, there has been a recent revision of the criteria needed to be met for an imaging agent to progress to the clinic, reflecting the general lack of toxicity of these agents, further promoting clinical translation. There are also other programs, such as the Development of

**Table 1** A sampling of recently translated and near-term clinical molecular imaging probes and methods for cancer (promising preclinical techniques are depicted in bold type)

Biology	Representative probe	Method	Reference
Angiogenesis	[ <sup>18</sup> F]Galacto-RGD	PET <sup>a</sup>	[9]
Apoptosis	[ <sup>99m</sup> Tc]Annexin-V	SPECT <sup>b</sup>	[98]
Signal transduction	[ <sup>124</sup> I]FIAU	PET	[14]
<b>Protein interaction</b>	<b>Luciferin</b>	<b>Bioluminescence</b>	<b>[16]</b>
Receptor/enzyme/transporter	[ <sup>68</sup> Ga]F(ab) <sub>2</sub> -Herceptin	PET	[72]
Metabolism	FLT	PET	[20]
<b>Cell trafficking</b>	<b>Cy5.5-CLIO</b>	<b>Fluorescence/MR</b>	<b>[55]</b>
Chemotherapy pharmacokinetics	5-[ <sup>18</sup> F]Fluorouracil	PET	[62]
Multidrug resistance	[ <sup>99m</sup> Tc]Sestamibi	SPECT	[63]
Hypoxia	[ <sup>60</sup> Cu]ATSM	PET	[82]
<b>Gene delivery/expression</b>	<b>Ormosil</b>	<b>Fluorescence</b>	<b>[32]</b>

<sup>a</sup>Positron emission tomography.<sup>b</sup>Single photon emission computed tomography.

Clinical Imaging Drug Enhancers (DCIDE) program at the NCI, that are beginning to hasten translation of new molecular imaging agents. The questions become: what are the promising areas in molecular imaging research on which to focus for near-term clinical translation? In light of the abundant, new targets and technologies, how do we know where to place our efforts? Small animal imaging can certainly help in validating or eliminating potential molecular imaging probes from the pool of available materials, however, small animal imaging itself is a time- and labor-intensive process such that only the most promising targets for the most useful indications should be pursued.

### *Cellular events and molecular pathways as imaging targets for cancer*

Among the many possible targets for imaging cancer, those that have been the focus of the most intense research include angiogenesis<sup>[8,9]</sup>, apoptosis<sup>[10,11]</sup>, signal transduction<sup>[12–15]</sup>, and study of protein interaction networks<sup>[16]</sup> as well as more conventional approaches to receptor-<sup>[17]</sup> or enzyme-based<sup>[18]</sup> and metabolic imaging<sup>[19–23]</sup>. Methods for imaging cellular trafficking are no longer experimental curiosity with the use of functionalized nanoparticles providing among the most compelling evidence for the use of MR in molecular imaging for clinical applications to cancer, arguably at sensitivities superior to those demonstrated for the radionuclide-based techniques<sup>[24–26]</sup>.

Other targets that have been the subject of significant imaging inquiry include probing multidrug resistance<sup>[27–30]</sup> and gene delivery and expression<sup>[15,31–33]</sup>. Among the most promising receptor-based targets are the prostate specific membrane antigen (PSMA)<sup>[18,34]</sup>, HER-2/neu<sup>[35]</sup>, the vascular endothelium through the  $\alpha_v\beta_3$  receptor using RGD peptides<sup>[8,9,36,37]</sup> and steroid receptor proteins including the estrogen<sup>[38–40]</sup>, progesterin<sup>[41,42]</sup>

and androgen<sup>[43,44]</sup> receptors. Promising metabolic techniques involve the use of [<sup>18</sup>F]fluorodeoxyglucose (FDG) for studying tumor glycolysis<sup>[45,46]</sup> and therapeutic monitoring in clinical<sup>[47]</sup> and preclinical studies<sup>[48]</sup>, radiolabeled choline analogs<sup>[49]</sup> for prostate cancer, and [<sup>18</sup>F]fluorothymidine (FLT) as well as other thymidine analogs as tumor proliferation agents, the uptake of which are dependent upon activity of the cell cycle<sup>[20]</sup>. Although an incomplete list, those entities were chosen because they have all been imaged clinically, have all proved useful and provide models for the translation of experimental molecular imaging agents (Table 1). Several illustrative examples are discussed in greater detail below.

### *A word on instrumentation and modality choice*

Because molecular imaging is a biology-driven enterprise, workers in this field are generally less interested in applying a specific imaging modality than at uncovering a particular biological process, which may require complementary modalities. Fig. 1 summarizes the most commonly used molecular imaging modalities with respect to their relative sensitivities. The imaging modalities must be thought of as complementary in that while some, such as MR spectroscopy, may have a built-in correlative anatomic mode, i.e. MR imaging, and therefore provide high spatial resolution, that technique is of considerably less sensitivity than, for example, positron emission tomography (PET). On the other hand, the use of superparamagnetic iron oxide (SPIO) particles for cellular trafficking has enabled the visualization of a single cell using a clinical magnet<sup>[26,50]</sup>. That fact appears to contradict the sensitivity scale shown in Fig. 1. Nevertheless, the radionuclide-based techniques have been used for many years to study specific molecular species, and remain supremely translatable. As we gain more experience with experimental models, we continue to learn the strengths and weaknesses of the modalities for particular

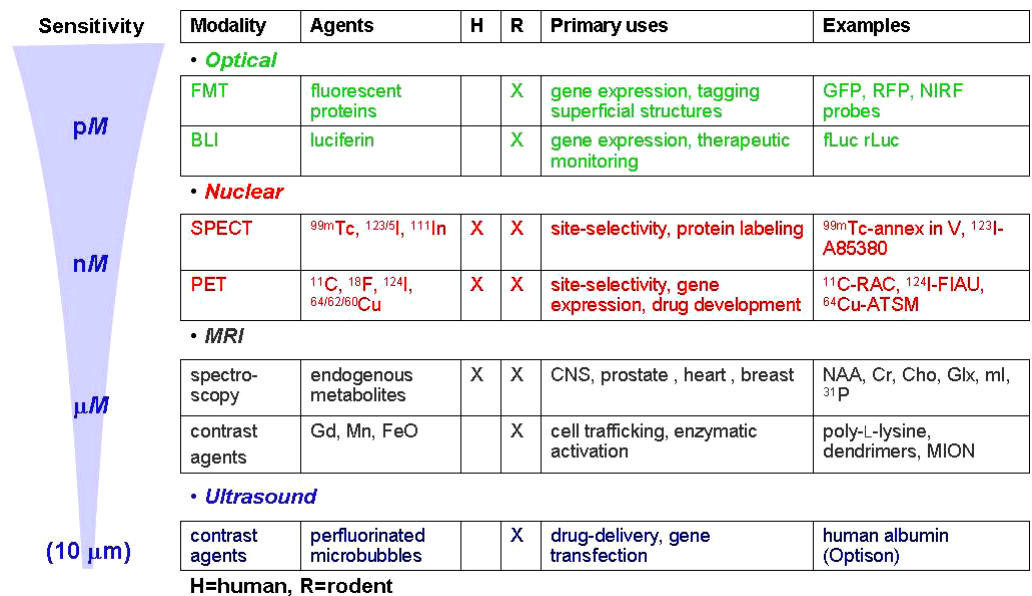


Figure 1 Modalities for molecular imaging.

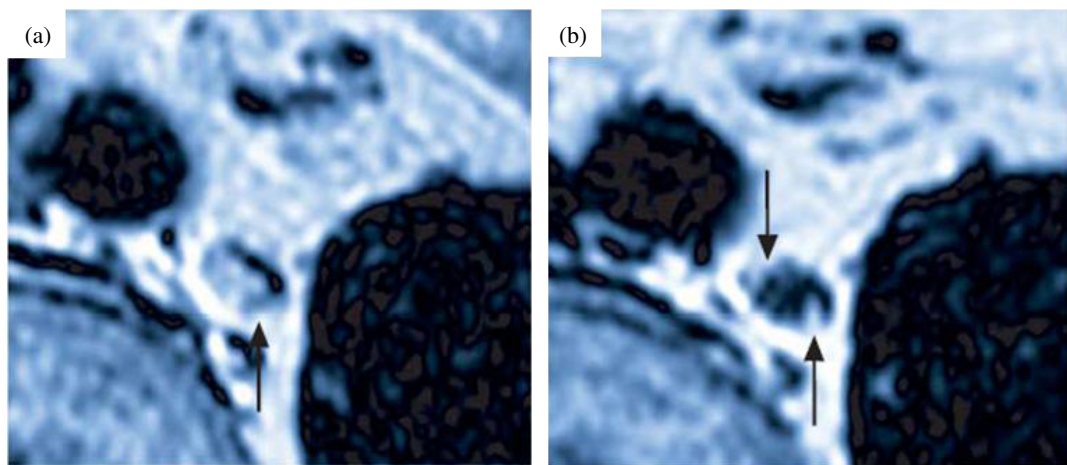


Figure 2 Use of a lymphotropic MR contrast agent (iron oxide-containing nanoparticle) in a patient with prostate cancer. Arrows indicate micrometastases, i.e. where nanoparticles are excluded from lymph node uptake (from reference [25]).

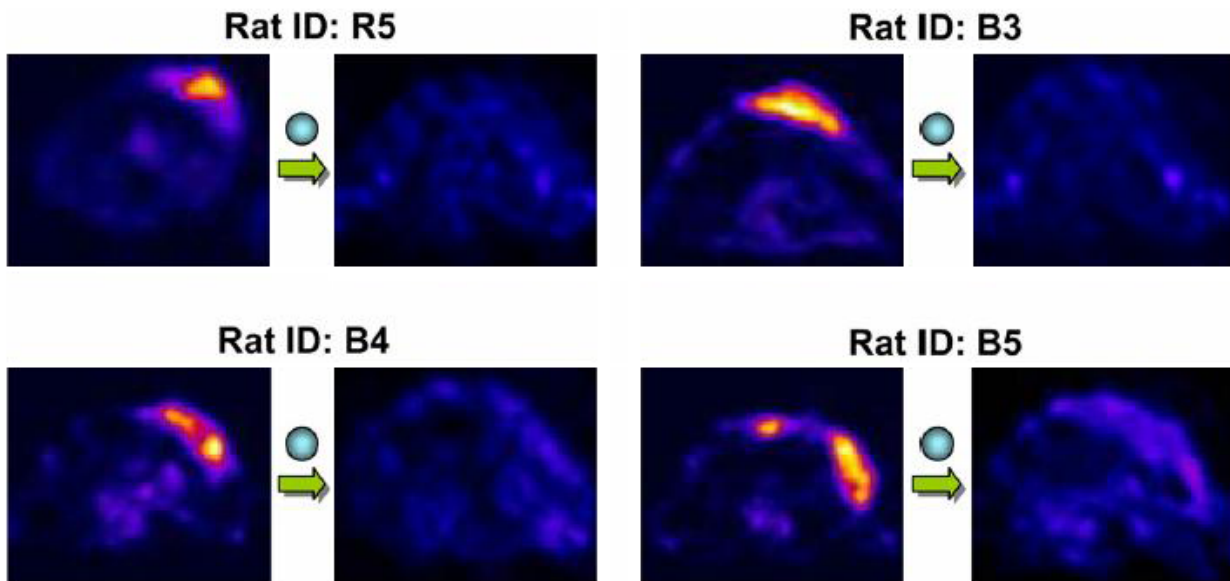
oncologic indications, leading to certain generalizations. For example, MR-based nanoparticles are proving useful for cell trafficking studies, both clinically<sup>[25]</sup> (Fig. 2) and preclinically<sup>[51,52]</sup>, while radionuclide- and optically-based molecular-genetic reporter systems, although useful experimentally, have not yet enjoyed much clinical exposure. On the other hand, for receptor- or enzyme-based imaging or for studying the pharmacokinetic disposition of chemotherapeutic agents, the radionuclide-based techniques predominate. MR- and optically-based, activatable probes have proved useful experimentally<sup>[53–56]</sup> but have not yet achieved clinical translation. Of course the development of nanodiagnostics, which are often engineered to provide multimodality imaging, will soon add a new dimension to clinical molecular imaging

as the translation of such agents is being vigorously pursued<sup>[57,58]</sup>.

### Translational molecular imaging: examples

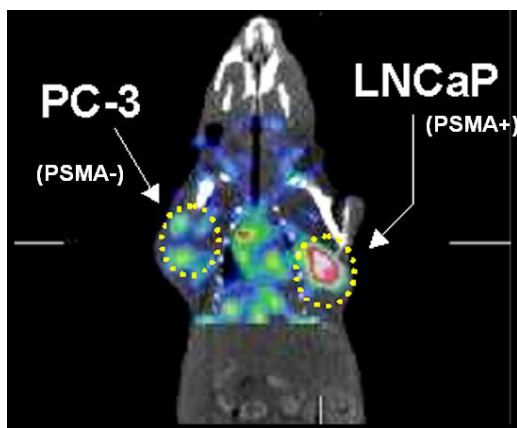
#### Radiolabeled chemotherapeutic agents and monitoring chemotherapy

A number of groups, most notably that of Aboagye *et al.* at the Hammersmith Hospital, have worked to provide positron-emitting analogs of chemotherapeutic agents such as paclitaxel<sup>[59,60]</sup>, fluorouracil<sup>[61]</sup> and others, in an effort to assess the pharmacokinetic profile of these agents in specific patients as well as studies in modulation



**Figure 3** FDG-PET images of tumor-bearing rats before and after 12 days of therapy with 3-bromopyruvate (from reference [46]).

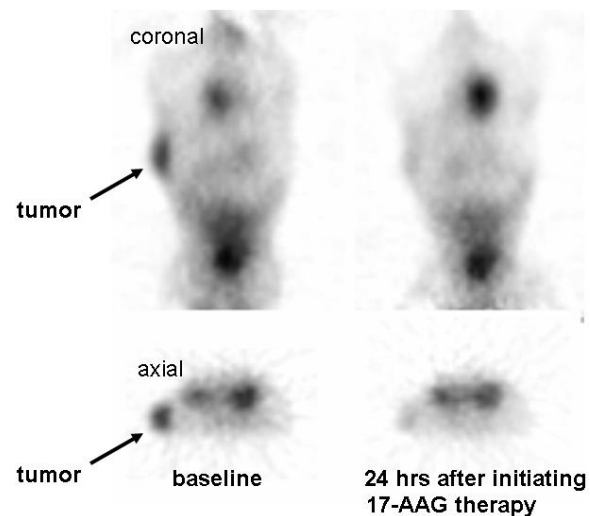
of pharmacokinetics with other enhancing drugs<sup>[62]</sup>. Perhaps one of the most important applications of this



**Figure 4** SPECT-CT imaging of [<sup>125</sup>I]DCIT in an LNCaP and PC-3 tumor-bearing SCID mouse. The PSMA-expressing LNCaP tumor displays high uptake while the PSMA non-expressing PC-3 tumor shows minimal uptake (adapted from reference [18]).

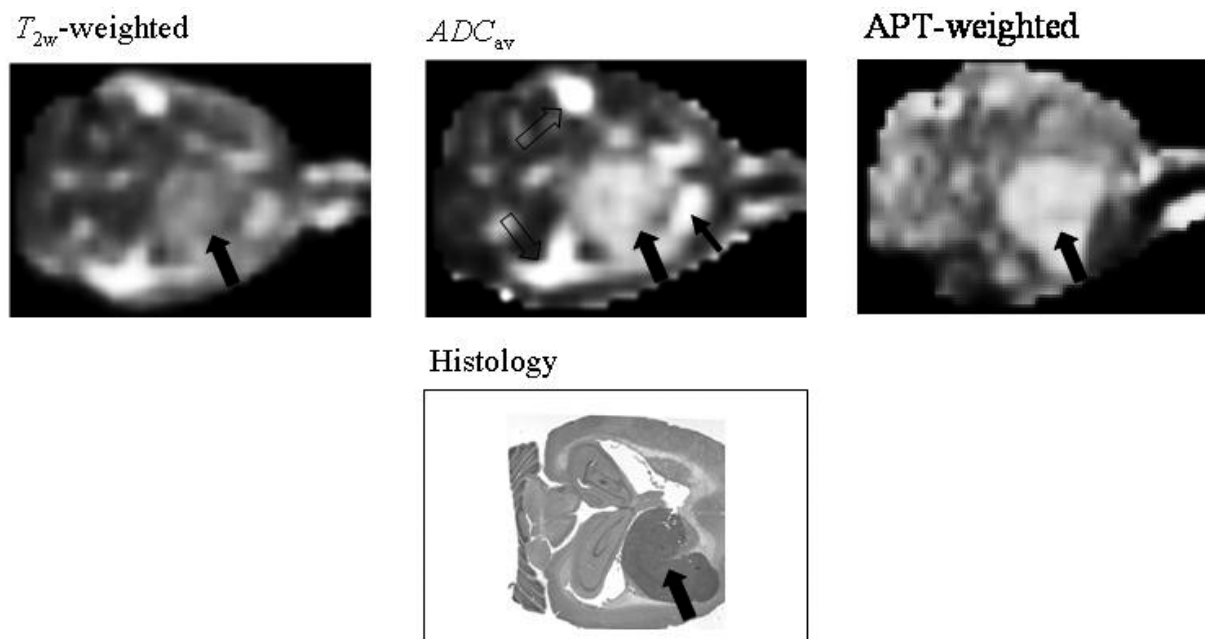
technology is to determine whether a patient will be a candidate for the corresponding chemotherapeutic agent based on the ability of the tumor to sequester the radiolabeled analog. Another key application will be to determine if the radiolabeled agent can be used for prediction of early cytostasis and cytotoxicity. A classic example of that application has been provided by Saleem *et al.* in which they showed how pharmacologic doses of eniluracil were able to improve the delivery of 5-[<sup>18</sup>F]fluorouracil to tumors quantitatively and noninvasively in human subjects<sup>[62]</sup>. Radiolabeled substrates for the multidrug resistance (MDR)-derived P-glycoprotein

pump (Pgp), such as [<sup>99m</sup>Tc]sestamibi, have been used to predict how patients may respond to therapy for lung cancer<sup>[63]</sup>. Radiolabeled taxanes have also been used to

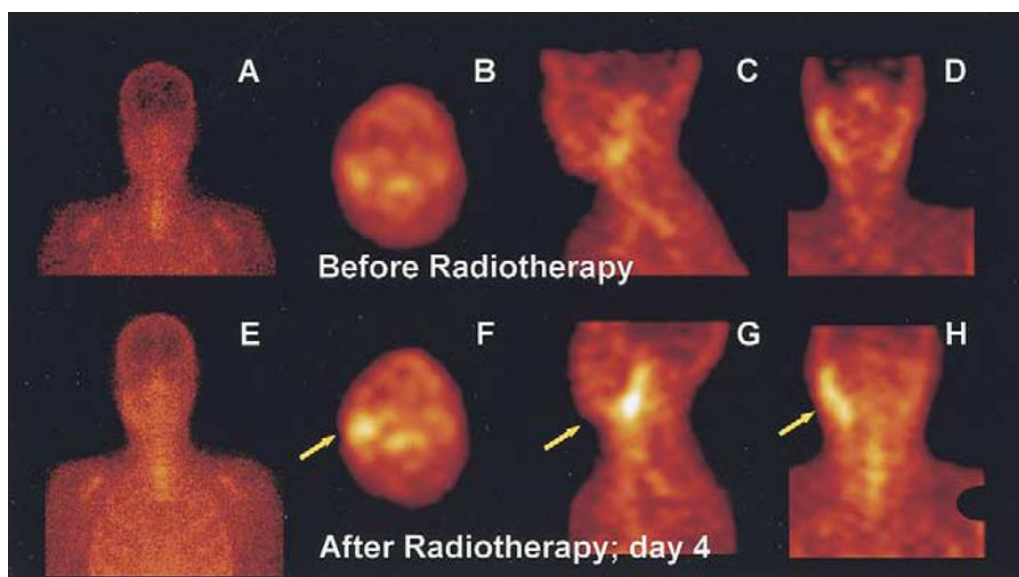


**Figure 5** MicroPET images obtained 3 h post injection with <sup>68</sup>Ga-F(ab)<sub>2</sub>-Herceptin in a mouse with a BT 474 breast tumor (images provided courtesy Steven Larson, Memorial Sloan Kettering Cancer Center). Note the early metabolic response to therapy with 17-allylamino-17-demethoxygeldanamycin (17-AAG).

quantify the modulation of Pgp, a new strategy in enhancing cancer chemotherapy, in nonhuman primates<sup>[60]</sup>. Small animal PET imaging has been used in this context, such as the study by Leyton *et al.* in which FLT was used to measure the effects of cisplatin treatment of fibrosarcoma tumor-bearing mice<sup>[64]</sup>. That study found FLT-PET to be superior to FDG-PET for predicting early



**Figure 6** Amide proton transfer imaging (APTI) in 9L tumors in rat. Both conventional (T2-weighted and apparent diffusion coefficient (ADC) maps) and APTI are shown. Note that the hyperintensity within the peritumoral tissue (small arrow) and cerebrospinal fluid (open arrows) in the ADC map become normal with APTI, adding to the clearer contour of the tumor (large arrow) on the latter images. APTI provides clearer tumor contour than the T2-weighted image as well (adapted from reference [92]).



**Figure 7** SPECT imaging of  $^{99m}\text{Tc}$ -annexin V in a patient with follicular lymphoma. Note high uptake in tumor-bearing lymph nodes after radiation therapy (from reference [98]).

changes due to chemotherapy, lending support to the use of FLT-PET for early detection of chemotherapy-induced tumor metabolic changes. In this sense, small animal PET imaging can be used to aid in the translation not only of diagnostic but also new therapeutic agents or drugs, which are increasingly of the cytostatic variety. A cytostatic drug may not produce a decrease in tumor size readily detectable by anatomic measures,

such as by CT diameter, the current clinical standard. A metabolic technique should be tailored to a new metabolic therapy, as shown for a recent study performed in an effort to provide information on 3-bromopyruvic acid as a putative treatment for sarcoma<sup>[48]</sup> (Fig. 3). Badly needed in conjunction with all of these animal PET studies is concurrent validation *in vitro* using histologic techniques that reflect the underlying biological changes

that are expected with therapy, i.e. validation of mechanism.

### *Recent and near-term translation of receptor-based imaging agents*

The prostate specific membrane antigen (PSMA) has been a target for molecular imaging for the last ten years using the monoclonal antibody ProstaScint®<sup>[65]</sup>. Because of the inherent difficulty of antibody-based imaging, small molecule ligands for PSMA are being pursued actively<sup>[18,66,67]</sup>. Animal models reveal high tumor target selectivity, providing the impetus for translation of agents of this class to the clinic<sup>[18]</sup> (Fig. 4). Although the ultimate clinical success of those agents for prostate cancer remain to be seen, steroid receptor-based imaging has proved clinically useful in studies dating back nearly 20 years<sup>[38]</sup>. First, estrogen<sup>[38,68]</sup> and more recently androgen receptor<sup>[44]</sup> imaging have been shown to provide biologically meaningful images in patients with receptor-positive breast and prostate cancer, respectively. HER-2/neu, a receptor up-regulated in certain forms of breast cancer, primarily due to amplification, has been a target for multimodality molecular imaging<sup>[69–71]</sup>. Artemov *et al.* have pursued an MR-based approach using avidin-biotin technology to image successfully HER-2/neu overexpressing tumors in an experimental model<sup>[69]</sup>. Although not yet ready for clinical translation, that study provided the proof-of-principle that receptor-based MR imaging *in vivo* was possible provided that appropriate signal amplification techniques are employed. On the same theme, Larson *et al.* have developed a positron-emitting anti-HER-2/neu antibody labeled with gallium-68. That construct has been used preclinically and is currently under assessment for clinical translation<sup>[72]</sup> (Fig. 5). The use of a positron-emitting analog has certain advantages over an MR-based agent in that it will be administered in subpharmacologic, i.e. ‘tracer’, doses, enabling a smoother path to clinical translation. In addition to early diagnosis or detection of metastatic disease, receptor-based imaging techniques can also be used to check the efficacy of receptor-based therapies. Although not performed to date, receptor occupancy studies, in analogy with those that are performed for neuropsychiatric drugs<sup>[17,73]</sup>, could be performed in oncology research as well. Notably both PSMA and HER-2/neu have been targets for the development of nanodiagnosics, illustrating the wide variety of potential new agents that can be developed once a suitable target is chosen<sup>[74,75]</sup>.

Radiolabeled RGD peptides, directed toward the angiogenic marker,  $\alpha_v\beta_3$  integrin receptor, have shown great utility in imaging experimental tumor models and are beginning to be used in the clinic<sup>[8,9,36,37]</sup>. Much effort has been expended in pharmacokinetic optimization of these agents, including incorporation of

a polyethylene glycol moiety and coupling with a long-lived positron emitter such as copper-64<sup>[76]</sup>. The RGD peptides represent an excellent example of a class of compounds that are likely to find widespread clinical use in the near future for imaging a variety of cancers.

### *Metabolic imaging agents*

FDG-PET imaging for cancer has been reviewed extensively elsewhere<sup>[19,47,77]</sup>. FDG is the only FDA-approved positron-emitting radiopharmaceutical in widespread clinical use and only for cancer and Alzheimer disease. As suggested above in the discussion of radiolabeled chemotherapeutic agents, perhaps the most important role for imaging in therapeutic monitoring is in the early prediction of patient outcome with a particular therapeutic agent. For example, FDG-PET was able to predict the outcome of patients with gastrointestinal stromal tumors treated with imatinib within several days of initiating treatment<sup>[78]</sup>. A similar result, indicating early prediction of tumor response to chemotherapy, has recently been shown in breast cancer using MR spectroscopy<sup>[79]</sup>. The article cited above by Leyton *et al.* suggests the improved utility of FLT over FDG for predicting tumor response<sup>[64]</sup>. FLT is the result of many years of development of other thymidine analogs for cancer imaging and is based on the fact that thymidine kinase, the enzyme of which FLT is a substrate, is regulated by the cell cycle, which often goes awry in cancer<sup>[80]</sup>. Because of the salutary metabolic characteristics and direct link of the mechanism of action to FLT uptake to cancer, FLT will soon gain widespread use and quite possibly displace FDG as the primary metabolic tumor imaging agent. Other promising metabolic cancer molecular imaging agents include the family of radiolabeled choline analogs for prostate cancer<sup>[49,81]</sup> as well as radiolabeled analogs of ATSM for imaging tumor hypoxia<sup>[30,82]</sup>. Agents of both of these classes recently entered the clinic and are beginning to demonstrate utility in cancer detection and monitoring. The metabolic imaging technique of magnetic resonance spectroscopy, which has been in clinical use for about 15 years, has been applied both to central nervous system malignancies as well as to prostate cancer to good advantage<sup>[22,83–85]</sup>. In the former, it can be used to distinguish radiation necrosis versus recurrent neoplasm, an important problem in brain tumor imaging<sup>[86]</sup>, and in the latter it can be used to direct biopsy toward the most malignant elements of a prostate tumor<sup>[87]</sup>. That indication is important because current prostate cancer biopsies are random and highly subject to sampling error.

### *Recent and near-term translation of novel molecular imaging agents and methods*

The abovementioned examples tend to focus on radionuclide-based techniques, however MR-based meth-

ods are also proving useful. After more than ten years of meticulous preclinical optimization, Weissleder *et al.* have shown the ability for iron oxide nanoparticles to differentiate benign from malignant lymph nodes with prostate cancer<sup>[25]</sup>. One significant facet of that study was that lesions smaller than what could be detected by PET, touted to be the much more sensitive technique (1 million-fold), were detectable. Also, significant about that work is that it represents the first practical clinical application of nanotechnology to imaging in that the particles used to generate contrast were engineered, primarily inorganic, substances. Similar technology has also been used recently to delineate the margins of gliomas, using a multifunctional reporter for intraoperative management<sup>[88]</sup>. Other multifunctional nanoparticles have also been developed using antibody-based approaches as well as for the visualization of angiogenesis with  $\alpha_v\beta_3$  as the target, in analogy to the RGD peptides<sup>[89]</sup>. Although nanoparticle technology suffers from the relatively large size of the resultant imaging agents, suggesting that they may be limited to intravascular applications, that is not necessarily the case as they may be linked to various peptides that promote internalization<sup>[90]</sup> or may be introduced to the cells of interest, such as in tracking studies using MR, through *ex vivo* techniques such as microelectroporation<sup>[91]</sup>. Another, new MR-based molecular imaging technique that will find clinical use shortly is amide proton transfer imaging (APTI). APTI obviates the use of exogenously administered contrast media and is performed in analogy to magnetization transfer imaging for visualization of proteins within malignant tissue<sup>[92]</sup> (Fig. 6)<sup>[93]</sup>. While the MR-based techniques may suffer, in many cases, from less sensitivity than the radionuclide-based methods, they do have the advantage of ready clinical translation, if no exogenous contrast is administered, as in the case of APTI. So far the only clinical example of molecular-genetic imaging of cancer is the study performed by Jacobs *et al.*, now nearly 4 years old, in which patients undergoing ganciclovir therapy were imaged with a radiolabeled nucleoside analog<sup>[94]</sup>.

### Hurdles to overcome for translational molecular imaging

Although molecular imaging is a relatively new field, there is a sense that clinical translation of molecular imaging agents is not happening sufficiently rapidly. Although the scientific hurdles, such as cell penetration of imaging agents, appropriate pharmacokinetic profiles, etc. are being steadily overcome, regulatory hurdles persist. The US Food and Drug Administration (FDA) has recently recommended a relaxation in what were relatively stringent requirements for toxicity testing of new imaging agents, many of which can now be assessed using a microdosing protocol. However, increased burden

has been placed on individual laboratories for assuring good manufacturing practice (GMP) through heavy documentation of each step en route to the clinical examination. As discussed above, the DCIDE program at the NCI provides funds for toxicology and a supportive staff for the dissemination of promising new molecular imaging agents, however, despite these measures, few new molecular imaging agents have gained widespread clinical use over the last several years.

### Clinical translation: the cautionary tail of Apomate™

As stated at the outset, one of the most promising targets for molecular imaging in cancer is the process of apoptosis, which is the mechanism by which many chemotherapeutic agents produce their tumoricidal effect. Accordingly, Theseus Imaging Corporation (a wholly owned subsidiary of North American Scientific, Inc., Chatsworth, CA) invested significant manpower and funds into the clinical development of an early biomarker for cancer therapy based on apoptosis imaging technology using single photon emission computed tomography (SPECT) of annexin V<sup>[95,96]</sup>. Because the imaging agent was a recombinant DNA-derived protein, the US FDA required extensive preclinical testing to assure the lack of immunogenicity of the protein and required an assay to be in place during the clinical trial for further assurance that there would be no deleterious immune-mediated effects<sup>[97]</sup>. The development of those assays is challenging, time-consuming and caused a delay in obtaining approval for clinical apoptosis imaging in the US. Furthermore, the results of the first phase II trial were difficult to interpret, largely based on the design of the trial that combined broad based cytotoxic agents such as Taxol and Cisplatin, as well as steroids to counterbalance the side-effects of the chemotherapy. The results proved of great scientific interest, particularly with baseline imaging, and in some cases produced the counterintuitive result that patients who responded to conventional chemotherapy tended to demonstrate *lower* uptake of the radiolabeled annexin relative to the baseline scan. Those results suggest that the tumor environment studied by this imaging agent was much more complex than expected and included possibly lymphocytes and phagocytic cells in addition to tumor cells. Taken together, these findings indicate that a straightforward clinical protocol, in which patients were chosen at the outset based on a more targeted pro-apoptotic therapy, such as radiation therapy, would have possibly provided more readily interpretable data<sup>[98]</sup> (Fig. 7). Therefore, regulatory hurdles surrounding a challenging initial product such as those that are protein-based, and, in retrospect, a suboptimal phase II imaging trial design, may have significantly limited the dissemination of clinical apoptosis imaging.

## Perspective

Conventional clinical molecular imaging, i.e. with radionuclide-based probes, has been practiced for many years and is a growing field with the development of ever more selective receptor-, enzyme- and transporter-based imaging agents. We are beginning to see the first applications of MR- and optically-based clinical molecular imaging, particularly for cell trafficking studies and intra-operative guidance. One of the great promises of molecular imaging research, i.e. molecular-genetic imaging, will gain clinical use in parallel with the acceptance of gene therapy, which has proved challenging, and as more sensitive, biocompatible reporter-probe combinations are discovered. Regulatory hurdles remain prominent for translation of the most novel agents, but microdosing protocols are being adopted for some and programs such as DCIDE will continue to shepherd the most promising new contrast agents and probes to the clinic.

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