Toward Molecular Imaging–Driven Drug Development in Oncology

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Summary: With current testing strategies, the number of novel targeted anticancer agents will exceed our drug selection capacity. Molecular imaging is a powerful additional tool that can assist us in selecting effective drugs and help patients benefit from targeted agents. Moreover, measurement of the functional effects of such targeted agents could permit dynamic tuning of treatment selection at the earliest time point at which loss of functional effects is observed. Cancer Discovery. 1(1); 25–8. ©2011 AACR.

Cancer remains a leading cause of death in developed countries; because cancer mortality is expected to continue rising worldwide, new drugs are urgently required. An unprecedented number of cancer drugs, often molecularly targeted and rationally designed, are currently becoming available for clinical use. This abundance of agents has resulted in a major challenge for those involved in selecting compounds during the drug development process. If current drug development practices continue, numerous clinical trials involving large numbers of patients will be required. These studies will be labor intensive and expensive; the research and development costs for each newly approved drug are currently estimated at $1 billion (1). In addition, many of the newly developed targeted agents will be more effective when combined with other drugs. This possibility makes optimal research on the best indications for a drug or the most rational drug combinations even more relevant.

Another challenge in current drug development practice is the high level of heterogeneity that has been discovered within specific tumor types. Heterogeneity calls for personalized treatment based on careful characterization of the individual patient’s tumor; this practice will make treatment with costly targeted agents more effective and economically sustainable. Moreover, de Bono and Ashworth (2) concluded recently that drug development remains painfully slow, despite some recent successes. They and others envision an increasing shift to integrated cancer research and biomarker-driven adaptive and hypothesis-testing clinical trials. It is therefore essential for early-phase trials to incorporate novel strategies for selection of suitable patients and to show that anticancer activity of the agent is based on the targeted mechanism. If the functional effects of such targeted agents could be measured, it would permit dynamic tuning of treatment selection at the earliest time point at which loss of these effects becomes apparent. In such events—for example, those resulting from the activation of compensatory pathways—other rationally chosen targeted drugs can be used.

It is a major challenge to implement this frequently heard call for better-designed clinical trials incorporating preclinical know-how and novel approaches. For example, blood samples or tumor tissues are usually collected for biomarker development. However, blood samples do not necessarily reflect what is happening at the level of the tumor, and tumor biopsies provide only static information on the status of a marker in a small part of the tumor and disregard the remaining tumor tissue and possible metastases. Therefore, it would be a major advantage if the drug behavior, drug target, and functional effects of novel targeted agents could be evaluated in the entire tumor and all metastases over time. Molecular imaging is a promising method for accomplishing this objective.

Molecular imaging is the in vivo characterization and measurement of biological processes at the cellular and molecular levels. It can be performed with several techniques, such as radionuclide, optical, and magnetic resonance imaging. Molecular imaging will likely have the capability to assist in novel drug development strategies and has indeed been widely acknowledged as an approach with great potential. In the Critical Path Initiative, launched by the FDA in 2004 to optimize development, evaluation, and manufacturing of FDA-regulated products, molecular imaging was considered as a technology to support this process. Unfortunately, this and other initiatives have not yet had a major impact on this field, which can be attributed to several causes (3). First, molecular imaging requires investment to overcome such technical hurdles as tracer production, image quantification, clinical validation, and standardization. Second, clinical translation and implementation of this process require a strong cross-disciplinary team of physicians, chemists, pharmacists, biologists, physicists, and engineers. Third, generation of a highly innovative drug development process requires strong links with industrial partners.

Most experience in the clinical translation of molecular imaging in oncology has been acquired with positron emission tomography (PET) and single-photon emission computed tomography (SPECT). Initially, the focus in oncology was on the visualization of general tumor processes such as glucose consumption with $^{18}$F-fluorodeoxyglucose PET and DNA proliferation with $^{18}$F-fluorothymidine PET. However, in recent years, molecular imaging has matured significantly, and more specific tracers have been developed. For example, the estrogen receptor can be visualized with $^{18}$F-fluoroestradiol PET (FES-PET) and the androgen receptor with $^{18}$F-dihydrotestosterone PET (FDHT-PET). These hormone receptors are relevant drug targets in breast and prostate cancer, respectively, and FES-PET...
and FDHT-PET imaging can detect their presence in tumors. Clinical trials have shown striking heterogeneity in tumor tracer uptake between patients and within patients. In addition, partial blockade of the estrogen receptor by the antiestrogen agent tamoxifen was visualized by FES-PET (4). Similar results were found with FDHT-PET, which visualized partial blockade of the androgen receptor by the nonsteroidal androgen drugs flutamide and MDV3100 (5, 6).

FDHT was used during development of 2 drugs, including MDV3100, in a competition assay to measure relative androgen receptor–binding affinity (7). This elegant study showed that both drugs bound to the androgen receptor in human prostate cancer cells with 5 to 8 times greater affinity than the nonsteroidal androgen bicalutamide and had only 2 to 3 times reduced affinity relative to the derivative of the native ligand FDHT.

For molecular imaging to fulfill its role in drug selection and in tumor characterization in individual patients, many novel tracers and imaging techniques are required. Indeed, in recent years, new detection systems have come into use, and many more novel targeted tracers have become available for preclinical and clinical use. With these tools, a wide variety of specific tumor characteristics can be visualized. The data on tracers and techniques used for preclinical molecular imaging are enormously rich. However, before patients can benefit, it will be crucial to translate these tracers to use in the clinic. Preclinical models often fail in predicting tumor responses in patients. In addition, preclinical studies can, at best, address the behavior of a human tumor in an animal. With increasing importance placed on the role of tumor–host microenvironment interaction in tumor behavior, the need to study this role clinically is even more relevant.

New tracers are slowly entering the clinic. Many use clinically relevant targeted antibodies that have recently become available. For example, our group developed the SPECT and PET radiopharmaceuticals indium-111- and zirconium-89–labeled bevacizumab and ranibizumab to image VEGF and trastuzumab to image HER2 in tumors (8, 9). The 89Zr-based antibody PET tracers are especially promising because they result in highly informative images with excellent quantitative properties. Moreover, because 89Zr residualizes in cells upon tracer internalization and endosomal degradation, the resulting signal indicates both tumor target expression and target dynamics (for the proposed mechanism behind visualization with 89Zr-trastuzumab, see Supplementary Movie S1). These new 89Zr-labeled tracers have been successfully tested preclinically and clinically, and they clearly illustrate the potential of molecular imaging. For example, 89Zr-bevacizumab and 89Zr-trastuzumab whole-body PET provided a quantitative indication of the distribution of these antibodies in the human body and showed specific accumulation in tumors. In our research, we found 89Zr-trastuzumab uptake in brain metastases to an extent comparable to that in bone metastases. This was new information; the degree to which antibodies enter brain metastases was previously unknown. Moreover, 89Zr-trastuzumab imaging showed the highly dose-dependent and tumor load–dependent pharmacokinetics of trastuzumab (10). Future early-phase studies with similar antibodies should consider a more patient-tailored antibody dosing schedule. In such studies, PET imaging with an 89Zr antibody can be used to assess whether dosing based on tumor burden results in more effective antibody levels in all lesions than does dosing based on a patient’s body weight.

Detecting the drug target and visualizing drug biodistribution are already valuable techniques, but drug development and personalized treatment would be aided substantially if molecular imaging could also be used to measure the functional effects of targeted agents (Fig. 1).
measure functional targeting with molecular imaging, efforts should be directed toward identification of what we refer to as “effect sensors.” These are proteins whose expression is modulated in response to the functional inhibition of a drug target. For molecular imaging purposes, these effect sensors should be preferably localized to the extracellular surface of plasma membranes or excreted into the extracellular matrix. This strategy would allow detection with antibodies, Fab fragments, or other high-molecular-weight imaging ligands. Preclinical studies with HSP90 inhibitors and the tyrosine kinase inhibitor sunitinib have shown that effect sensors are powerful tools to measure functional drug effects. For example, HER2 and VEGF are known to be downregulated after HSP90 inhibition, and their downregulation could indeed be visualized in vivo after treatment with an HSP90 inhibitor in human tumor-bearing mice (11, 12). This approach is currently being explored in the clinic using 89Zr-trastuzumab and 89Zr-bevacizumab (http://www.clinicaltrials.gov NCT01081600 & NCT01081613). In addition, the effect of sunitinib treatment and withdrawal on the tumor was investigated using 89Zr-ranibizumab, an engineered Fab fragment of bevacizumab. 89Zr-ranibizumab PET showed dynamic changes within the tumor during sunitinib treatment, with a strong decline in signal in the tumor center but only minimal reduction in the tumor rim, and with a pronounced rebound after sunitinib discontinuation (13). These results corresponded to tumor growth and immunohistochemical vascular and tumor markers. This finding highlights the potential for using molecular imaging to conduct serial analysis in different areas within a tumor after relevant effect sensors are identified. The results of these studies also mandate identification of optimal effect sensors for other targeted agents and underscore the need for imaging techniques that allow simultaneous visualization of drug behavior and functional effects.

Finally, molecular imaging often involves radioactivity, which places limits on repeated imaging because of the radiation burden to the patient. However, improved technology is bringing nonradioactive imaging for drug development within reach. We now have available a battery of novel detection systems that use nonionizing electromagnetic radiation, such as visible light and radio frequencies, which pose no health hazard. These systems are suitable for imaging small animals and for imaging in clinical settings. Examples of such systems are an intraoperative multispectral system; diffuse optical tomography; a custom-made fluorescence endoscope, confocal laser endomicroscopy (CLE), which provides insight into in vivo histology; and a hand-held photo-acoustic–based imaging system with quantification and 3-dimensional properties. In contrast to PET and SPECT scanning, these systems will not be able to visualize the body as a whole. However, they could find great application in determining tumor characteristics. This optical imaging strategy has not yet been combined extensively with tumor-specific fluorescent tracers. Proof-of-principle of this concept is sought in a clinical study using an intraoperative tumor-targeted imaging approach. In this study, the small ligand folate is linked to a fluorophore [i.e., fluorescein isothiocyanate (FITC)], which specifically targets the folate-α receptor in ovarian cancer (Netherlands Trial Register #1980; http://www.trialregister.nl). This receptor is often overexpressed in ovarian cancers and currently serves as a drug target. Another example is a specific FITC-labeled heptapeptide sequence, VRPMLQ, developed with phage display peptide libraries for screening against fresh human colon adenomas. With CLE, this tracer showed specific binding to colonic dysplasia during endoscopy, after topical administration (14). Because of tissue-scattering properties, autofluorescence, and absorption of light in the 400- to 650-nm range, which is the range of FITC, a tracer will perform even better in the near-infrared (NIR) fluorescence spectrum, which uses light in the 650- to 900-nm range. In this range, autofluorescence signals are minimal and tissue absorption of light is lowest, resulting in optimal tissue penetration. The few NIR fluorescence probes currently available for clinical use are not nontargeted, but targeted NIR tracers are now within reach. The NIR fluorophore IRDye 800CW has good characteristics for clinical use and allows binding to antibodies, Fab fragments, and small proteins. A preclinical toxicity study with IRDye 800CW carboxylate showed no toxicity with 920 mg/kg administered i.v. or intradermally, and interesting preclinical results with IRDye 800CW labeled to trastuzumab have recently been reported (15, 16).

In addition to the clinical potential of these optical techniques, their use for drug development may be attractive, as these nonionizing imaging modalities allow more frequent follow-up. In addition, with multispectral optical imaging, several tracers could potentially be followed up at the same time.

In conclusion, molecular imaging can be a powerful additional strategy to bridge the gap in current drug development. Direct molecular imaging, with the labeled drug itself as an imaging tracer, provides insight into drug behavior, including elementary pharmacokinetic information like biodistribution and tumor accumulation. In addition, this approach would be useful for target visualization, and indirect molecular imaging has great potential as an effect sensor during early clinical drug trials. Finally, measuring the functional effects of targeted agents could permit dynamic tuning of treatment selection when loss of these effects is first observed.

Disclosure of Potential Conflicts of Interest

The authors received research grants from Roche, Novartis, and Human Genome Sciences. In addition, E.G.E. de Vries was on an advisory board for Roche.

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