

Chaperoning Drug Development with PET

In recent years, numerous molecular abnormalities have been identified in cancer cells that may be used as targets for therapeutic interventions (1). In parallel, the introduction of combinatorial chemistry and high-throughput technology (2) has led to the development of a large number of lead compounds that inhibit these target molecules (3). The ultimate goal is personalized therapeutics that inhibit the specific molecular alterations driving tumor growth in an individual patient. However, the annual number of new drug approvals has not changed significantly over the last 30 y. During

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this time, the cost of drug development has increased by a factor of 8 (4), primarily because of a 4-fold increase in the preclinical evaluation (to more than \$330 million for a single drug) and an 8-fold increase for clinical trial expenditures (to more than \$450 million) (4). Only about 20% of drugs tested in phase I trials eventually reach the market (4). Improving the “hit rate” from 20% to 30% could reduce development costs per drug by almost 50% (5). Considering the huge costs of drug development and the multitude of new drug candidates, there is an enormous need for new techniques that increase the efficiency of the drug development process.

In the early clinical development of a new drug, several key issues that have been called the “pharmacologi-

cal audit trail” need to be addressed (6). First, it is important to establish that intratumoral drug concentrations are sufficient to potentially achieve biologic activity. Even then, the drug may not hit the target in vivo for various reasons. For example, the affinity of the drug to the target protein may be different from the model systems studied preclinically. Therefore, it is necessary to determine whether the drug is actually inhibiting the targeted biochemical pathway. However, successful inhibition of the pathway does not necessarily mean that the desired biologic effect will be achieved, as independent pathways may drive tumor growth. For example, in breast cancer, gefitinib, an inhibitor of the epidermal growth factor receptor (EGFR) kinase, has been shown to completely suppress EGFR phosphorylation at a dose of 500 mg/d. Nevertheless, tumor proliferation was not inhibited and none of the patients demonstrated a clinical response (7). Similar observations have been made for EGFR antibodies, where at a dose of 1,200 mg EGFR kinase activity was completely inhibited in 92% of the patients, whereas a tumor response was achieved in only 23% of the patients (8).

Similar questions are also relevant for the clinical use of targeted drugs. Because common solid tumors are genetically highly heterogeneous, drugs targeting a specific molecular alteration are likely to be efficient in only a subgroup of the treated patients. For example, small-molecule inhibitors of the EGFR kinase have recently been approved for treatment of advanced non-small cell lung cancer. However, only 10%–20% of the treated patients appear to benefit from these new drugs. Therefore, it will become more important to select patients for specific treatments or to identify nonresponding patients after a brief course of therapy.

PET has enormous potential for improving the efficiency of the drug development process and the clinical use of targeted drugs, by demonstrating noninvasively their pharmacokinetic or pharmacodynamic properties. One excellent example is the clinical evaluation of drugs targeting heat shock protein 90 (Hsp90). The heat shock response was discovered serendipitously in 1962 by Ritossa (9), who observed changes in *Drosophila* salivary gland chromosome “puffs” (regions of gene transcription) after the temperature of the incubator had been increased accidentally. Extensive subsequent studies have shown that the heat shock response is highly conserved from bacteria to humans and represents an essential defense mechanism from a wide range of harmful conditions, including heat shock, inhibition of energy metabolism, and oxidative stress (10). Under these conditions, the redox state and hydration of the cells are frequently changed, which causes increased levels of misfolded proteins with altered and potentially harmful biologic activities (10). Cells respond to this stress by induced synthesis of a variety of so-called Hsps. Proteases are one class of Hsps, which degrade damaged proteins (10). “Molecular chaperons” are another class of Hsps, which refold the altered proteins. Chaperon proteins not only are induced by cellular stress but also are expressed constitutively and function in the correct folding and translocation of newly synthesized proteins (11). Without chaperons, native proteins tend to interact with other proteins in the cytosol, which prevents them from folding into their correct 3-dimensional structure. Chaperon proteins bind to the newly synthesized proteins as soon as they emerge from the ribosomes and prevent their aggregation with other proteins. Furthermore,

Received Dec. 16, 2005; revision accepted Dec. 28, 2005.

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they can actively help the proteins to fold correctly through several cycles of binding and release (11).

A variety of human tumors have been shown to express high levels of Hsps and this likely represents a mechanism to maintain homeostasis under the stress of hypoxia and acidosis. Because cancer cells may be more sensitive than normal tissues to inhibition of Hsp function, the targeting of Hsps has been extensively studied as a new approach for cancer therapy. One particularly attractive target is Hsp90, a Hsp with a molecular weight of 90 kDa. Hsp90 is a molecular chaperon that is involved in the folding of many proteins important for cellular signaling, proliferation, invasion, and angiogenesis. These include various receptor tyrosine kinases, steroid receptor hormones, telomerase, hypoxia-inducible factor- α , AKT, and matrix metalloproteinase 2 (12). Thus, inhibition of Hsp90 may affect multiple cellular processes considered as the "hallmarks of cancer" (13). Hsp90 function is inhibited by the natural antibiotics radicicol and geldanamycin, which bind to the adenosine triphosphate-binding pocket of the molecule. Several Hsp90 inhibitors have been developed on the basis of these molecules (14). Clinical testing is most advanced for 17-allylamino-geldanamycin (17-AAG) (15). Hsp90 derived from cancer cells has a 100-fold higher binding affinity to 17-AAG than does Hsp90 from normal cells (16). This high affinity is explained by the presence of activated Hsp90 complexes and makes 17-AAG selectively toxic for cancer cells (16). Furthermore, it has been shown that wild-type Hsp90 client proteins are less sensitive to 17-AAG than their mutated counterparts expressed by cancer cells (17).

Despite these very encouraging pre-clinical data, the clinical development of 17-AAG has been challenging. 17-AAG demonstrates limited stability and tends to form complexes. It needs to be activated in vivo by a polymorphic enzyme (NQO1/DT-diaphorase), is a substrate of P-glycoproteins, and is metabolized by polymorphic P450 enzymes (6). All these factors influ-

ence the plasma and intratumoral drug concentrations in an individual patient. This is particularly relevant for 17-AAG therapy, as 17-AAG has only a modest potency for Hsp90 inhibition and a limited therapeutic index. In summary, numerous factors make it difficult to predict how much 17-AAG gets into the tumor and what 17-AAG does to the tumor cells (18).

Smith-Jones et al. have recently reported an innovative technique to study noninvasively the pharmacodynamics of 17-AAG by PET (19). This approach uses ^{68}Ga -labeled F(ab')₂ fragments of the antibody herceptin to image HER2 expression of tumors (19). HER2 expression is highly dependent on Hsp90 function, and treatment with 17-AAG causes rapid degradation of HER2 (20). In a tumor model with high HER2 expression, Smith-Jones et al. have shown that the loss of HER2, induced by 17-AAG, can be quantified noninvasively by microPET with ^{68}Ga -DOTA-F(ab')₂-herceptin (DOTA is 1,4,7,10-tetraazacyclododecane-*N,N',N'',N'''*-tetraacetic acid) and thus allows noninvasive assessment of the pharmacodynamics of 17-AAG (19).

In an article in this issue of *The Journal of Nuclear Medicine* (21), the same group has further evaluated monitoring 17-AAG treatment with ^{68}Ga -DOTA-F(ab')₂-herceptin. Mice bearing xenografts of the HER2-expressing breast cancer cell line BT474 were imaged by microPET with ^{68}Ga -DOTA-F(ab')₂-herceptin and ^{18}F -FDG before and after treatment with 17-AAG. Within 24 h, treatment with 150 mg/kg 17-AAG caused a dramatic decrease in tumor uptake of ^{68}Ga -DOTA-F(ab')₂-herceptin, which remained below baseline levels for up to 10 d. This was paralleled by a significant inhibition of tumor growth lasting for up to 3 wk. In contrast, 17-AAG did not cause a measurable reduction of tumor ^{18}F -FDG uptake at any of the time points studied.

These findings further support the concept that downregulation of ^{68}Ga -DOTA-F(ab')₂-herceptin uptake by 17-AAG is a specific readout of Hsp90 inhibition, and not simply due

to treatment-induced cell death, as cell death would lead to a decrease of ^{18}F -FDG uptake as well. It is also quite remarkable that 17-AAG did not decrease tumor ^{18}F -FDG uptake despite the fact that it inhibited tumor growth for up to 3 wk. This is a surprising finding, as it is frequently assumed that cell growth is one of the major factors responsible for the increased glucose metabolic activity of cancer cells. Although clinical studies have frequently shown only a weak correlation between tumor cell proliferation and ^{18}F -FDG uptake (22), one would still have expected to see some decrease in ^{18}F -FDG uptake if tumor growth is almost completely inhibited as in the study by Smith-Jones et al. (21). Therefore, it will be interesting to further study the mechanisms of growth inhibition by 17-AAG in BT474 tumors and their relationship to glucose metabolism. For example, it is known that 17-AAG itself causes a heat shock response, and one may speculate that energy is needed for this repair mechanism (12,15). In addition, a technical factor should be considered: because of the high metabolic activity of normal murine tissues, contrast between tumor xenografts and normal tissues is frequently low in ^{18}F -FDG PET studies of mice. This can make quantitative assessment of treatment-induced changes in ^{18}F -FDG uptake challenging (23). Therefore, it will be important to confirm, by ex vivo tissue sampling and cell culture studies, that treatment with 17-AAG inhibits tumor growth without affecting glucose metabolism.

It will also be interesting to compare the reduction of ^{68}Ga -DOTA-F(ab')₂-herceptin uptake during treatment with 17-AAG with changes in choline metabolism. Cell culture studies have indicated that reduction of choline uptake may also be used as a readout for Hsp90 inhibition (24). Furthermore, alterations in phosphocholine levels during treatment with 17-AAG have been observed by magnetic resonance spectroscopy and may also represent a marker for the pharmacodynamic effects of 17-AAG (25).

Currently, the link between choline metabolism and Hsp90 function is not as well established as the link between Hsp90 and HER2 expression. Therefore, choline metabolism may be a less specific marker for Hsp90 inhibition. On the other hand, it has been well established in patients that many malignant tumors demonstrate markedly increased choline uptake (26). Thus, one can expect a robust baseline signal before 17-AAG treatment and it should be relatively straightforward to measure changes in choline uptake during therapy. In contrast, it still needs to be established whether HER2 expression can be imaged by PET with ^{68}Ga -DOTA-F(ab')₂-herceptin in patients.

In conclusion, the study of Smith-Jones et al. (21) provides very encouraging data for the clinical testing of PET with ^{68}Ga -DOTA-F(ab')₂-herceptin in patients undergoing treatment with Hsp90 inhibitors. However, it also suggests that ^{18}F -FDG PET may be limited in detecting a cytostatic response to targeted therapies. This underlines the fact that we should not assume glucose metabolism to represent the optimal readout for all targeted therapies, despite the excitement in using ^{18}F -FDG PET for monitoring imatinib treatment of gastrointestinal stromal tumors (27). Instead, preclinical studies should test whether cellular processes that can be imaged by PET are significantly modulated by a new class of drugs. On the basis of these data, the optimal imaging probe should be selected for monitoring treatment effects in the clinic. Using such an approach, PET is likely to become a powerful chap-

eron of the drug development process in the future.

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