

Aiming for a Direct Hit: Combining Molecular Imaging with Targeted Cancer Therapy

Oncogene addiction is an intriguing new term that has emerged in the lexicon of cancer biology (1). This term implies that tumor cells may have an extraordinary, almost complete dependence on the function of one or more genes (oncogenes) for their survival, whereas these same genes expressed in normal cells are not absolutely essential. The human epidermal growth factor receptor type 2 (*HER2*) is one such oncogene that encodes a transmembrane receptor tyrosine kinase overexpressed in about

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20% of breast cancers, primarily because of gene amplification (2). *HER2* overexpression confers an aggressive tumor phenotype that correlates with hormone insensitivity (3), chemotherapy resistance (4), and poor long-term survival (5). Trastuzumab (Herceptin; Roche Pharmaceuticals) is a humanized IgG₁ monoclonal antibody approved for the treatment of *HER2*-amplified metastatic breast cancer and as an adjuvant treatment for early-stage disease (6). The mechanisms through which trastuzumab exerts its antitumor effects are not completely understood, but an important predictor of response is tumor *HER2* overexpression (7). *HER2* positivity is assessed in a biopsy sample of primary breast cancer by immuno-

histochemical staining or probing for *HER2* gene amplification by fluorescence *in situ* hybridization. There is generally good concordance in *HER2* positivity between primary and metastatic lesions, but some discrepancies have been noted (8). Patients with *HER2*-amplified tumors (immunohistochemical staining 3+ or *HER2*-to-chromosome-17 control gene sequence ratios > 2.2) are expected to benefit most from trastuzumab (9).

A novel alternative strategy to interfere with the function of the *HER2* oncogene is the use of heat-shock protein (Hsp) inhibitors, in particular those aimed at Hsp90: 17(allylamino)-17-demethoxygeldanamycin (17-AAG; tanespimycin) and its orally bioavailable analog, 17(dimethylaminoethylamino)-17-demethoxygeldanamycin (17-DMAG). Hsp90 is a molecular chaperone that complexes with Hsp70 and Hsp40, as well as with cochaperone proteins (p23 and p50^{Cdc37}), to mediate the correct folding of numerous proteins in the cell, thereby protecting them from routing to the proteasome for degradation (10). 17-AAG and 17-DMAG block the function of Hsp90 by preventing its conversion to the active adenosine triphosphate-bound form (11). Hsp90 inhibitor treatment of breast cancer is based on the premise that the cell-surface expression of *HER2* can be diminished by rerouting newly synthesized receptors to the proteasome for proteolytic destruction. Reliance on Hsp90 may in itself represent a form of oncogene addiction (10). Moreover, there is evidence that Hsp90 permits recycling of internalized *HER2* back to the cell surface, thus preserving overexpression (12). Because trastuzumab has promoted *HER2* internalization in some studies (7), its combined use with Hsp90 inhibitors would be complemen-

tary and synergistic; in fact, one clinical trial has examined this combination for treatment of *HER2*-positive breast cancer, with promising results (13).

Fundamental to the success of any therapeutic strategy aimed at disrupting the influence of the *HER2* oncogene are an accurate assessment of the level of *HER2* density in tumors and the ability to sensitively measure a treatment effect on *HER2* expression. In this issue of *The Journal of Nuclear Medicine*, Kramer-Marek et al. report that small-animal PET with ¹⁸F-labeled Z_{HER2:342} Affibody (Affibody AB) specific for *HER2* can sensitively provide information on these 2 phenomena (14). Affibody is a new class of targeting vehicle generated from a phage display library of *Staphylococcus* protein A variants in which substitutions were made to a 13-amino-acid sequence within the Z-domain (15). Initial panning of the library for binding to the *HER2* extracellular domain yielded Z_{HER2:4} Affibody with good specificity but relatively low affinity (dissociation constant, 50 nM) (16). Subsequent modifications and rescreening (affinity maturation) identified the extremely high affinity Z_{HER2:324} Affibody (dissociation constant, 22 pM) used in this study (17). Successful imaging of *HER2*-overexpressing human tumor xenografts in mice has been achieved with Affibody labeled with ¹¹¹In, ^{99m}Tc, or ¹⁸F (18), and these novel radiopharmaceuticals were recently studied for the first time in humans (19). Kramer-Marek et al. showed that the exquisite affinity of the ¹⁸F-Z_{HER2:324} Affibody for *HER2* provided excellent tumor uptake ($\leq 21\%$ injected dose [%ID]/g) whereas the low molecular weight (6 kDa) caused rapid elimination from the blood. The resulting tumor-to-blood ratios exceeded 20:1 at only 1–2 h after injection, thus making it feasible

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to use the 110-min half-life positron-emitter, ¹⁸F, as a radiolabel.

There was a direct relationship between uptake of ¹⁸F-Z_{HER2:324} Affibody and tumor HER2 density in a panel of 5 subcutaneous human breast cancer xenografts in athymic mice, except for MCF-7/clone18 tumors. Uptake was lower for MCF-7/clone18 tumors than for BT-474 xenografts (12 %ID/g vs. 20 %ID/g, respectively), despite 1.5-fold higher HER2 expression measured ex vivo by enzyme-linked immunosorbent assay. MCF-7/clone18 is a HER2 gene-transfected variant of MCF-7 cells, which naturally have low HER2 density. This discrepancy was attributed to possible differences in the microenvironment of MCF-7/clone18 xenografts that may have affected the delivery of ¹⁸F-Z_{HER2:324} Affibody. Interestingly, we recently reported that strong associations between tumor HER2 density and uptake of ¹¹¹In-labeled trastuzumab in a panel of 5 HER2-positive breast cancer xenografts in athymic mice were obtained only if tumor uptake was corrected for nonspecific IgG localization or circulating blood-pool radioactivity, factors that presumably reflect differences in the tumor microenvironment (20). Reasonably good correlations between epidermal growth factor receptor density and the accumulation of ⁶⁴Cu-labeled anti-epidermal growth factor receptor cetuximab antibodies in epidermal growth factor receptor-positive xenografts in mice have similarly been described (21). However, Aerts et al. (22) have reported disparities in uptake of ⁸⁹Zr-labeled cetuximab by tumors and epidermal growth factor receptor density, again possibly due to differences in tumor physiology that control radiopharmaceutical delivery. Kramer-Marek et al. (14) visualized all breast cancer xenografts by small-animal PET using ¹⁸F-Z_{HER2:324} Affibody, but the tumor signal ranged from low for MCF-7 xenografts to intermediate for MCF-7/clone18 tumors and strong for BT-474 tumors.

The avid accumulation of ¹⁸F-Z_{HER2:324} Affibody in the MCF-7/

clone18 and BT-474 xenografts provided an opportunity to interrogate the effects of 17-DMAG Hsp90 inhibitor therapy on tumor HER2 expression. Tumor-bearing mice received a baseline PET scan and were then treated intravenously with 4 doses of 40 mg/kg of 17-DMAG over a 60-h period, followed by repeated imaging 12 h after the final dose. Region-of-interest analysis revealed a 71% and 33% significantly lower uptake of ¹⁸F-Z_{HER2:324} Affibody in the BT-474 and MCF-7/clone18 tumors, respectively, after 17-DMAG treatment. There was virtually complete agreement between decreased tumor uptake of ¹⁸F-Z_{HER2:324} Affibody and ex vivo analysis of tumor HER2 expression by enzyme-linked immunosorbent assay and Western blot. Unexpectedly, immunohistochemical staining for HER2 (HercepTest score; Dako) in the explanted tumors did not reveal decreases in HER2 positivity after 17-DMAG treatment, indicating that this technique does not appear to have the sensitivity required to detect these changes. We have also recently found that immunohistochemical staining does not have sufficient sensitivity to detect HER2 downregulation in athymic mice bearing subcutaneous human breast cancer xenografts treated with trastuzumab, whereas micro-SPECT with ¹¹¹In-labeled pertuzumab (a HER2 antibody that recognizes an epitope different from trastuzumab) was capable of monitoring such changes (23). Imaging of decreased tumor HER2 expression caused by Hsp90 inhibition was first reported by Smith-Jones et al. using ⁶⁸Ga-labeled trastuzumab F(ab')₂ fragments (24); this approach proved to be an earlier predictor of tumor response to 17-AAG therapy than was ¹⁸F-FDG PET (25).

It is important to appreciate that the effectiveness of 17-AAG in downregulating tumor HER2 expression in clinical trials is most commonly measured by Western blot analysis of readily accessible peripheral blood lymphocytes for Hsp90, Hsp70, or client proteins (e.g., RAF-1, cdk4, or LCK) rather than by sampling tumors

directly (26). These biomarkers, although convenient, have yielded inconsistent results that do not appear to correlate with tumor response (13,26,27). Molecular imaging using ¹⁸F-Z_{HER2:324} Affibody may provide a more sensitive, direct, and highly feasible means of evaluating tumor response *in situ* to these new therapies; it could also be invaluable in optimizing the dose. Phase I clinical trials of 17-AAG have used conventional dose-escalation protocols to reach the maximally tolerated dose for subsequent phase II studies, yet this may not be the lowest or optimal dose required to block Hsp90. These drugs are associated with significant toxicities, including hepatotoxicity (26,27), and thus, identifying the minimum dose that is effective in blocking Hsp90 would be desirable. PET with ¹⁸F-Z_{HER2:324} Affibody could probe the downstream effect of increasing doses of 17-AAG or 17-DMAG in patients enrolled in trials of Hsp90 inhibitors for the treatment of HER2-positive tumors. Evaluation of the kinetics of HER2 downregulation by Hsp90 inhibitors through repeated imaging either preclinically or clinically would be facilitated by the rapid biologic elimination of ¹⁸F-Z_{HER2:324} Affibody and the short physical half-life of the radionuclide. The results of such molecular imaging of HER2 expression may be particularly profound for combinations of trastuzumab and 17-AAG (13), in which there may be dramatic downregulation due to the potential synergy between these 2 drugs as discussed earlier. Molecular imaging is a sensitive tool that clearly has an important future role to play in predicting and monitoring response to new targeted cancer therapies that aim to exploit oncogene addiction (28). The study by Kramer-Marek et al. (14) stimulates our imagination about what could be achieved by combining these 2 exciting and continually evolving fields.

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