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UNDERSTANDING THE PATHWAY

Two Dimensions in Targeting HER2

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The endeavor to develop human epidermal growth factor receptor 2 (HER2) –targeting agents for cancer therapy now spans more than two decades, with four drugs already on the market and numerous others in the pharmaceutical pipelines. The interest in this mode of cancer therapy continues to intensify, particularly in the era of personalized medicine, because *HER2* amplification underlies the biology of subsets of a large variety of cancers, including breast, gastric, esophageal, endometrial, ovarian, colorectal, bladder, head and neck, and others.

HER2 is a receptor tyrosine kinase located at the cell membrane with a large extracellular domain (ECD) and an intracellular catalytic kinase domain (KD) and signaling tail. Signal generation by HER2 occurs through heterodimerization with its HER family siblings (epidermal growth factor receptor, HER3, HER4), particularly HER3 (Fig 1). This process is prompted by ligand binding to HER3, which reconfigures its ECD exposing the interface that mediates dimerization with HER2. The proximation leads to the allosteric activation of the HER2 KD by the HER3 KD. The activated HER2 KD then phosphorylates the c-tail of HER3, leading to recruitment of several proteins and initiating a series of parallel signaling cascades that ultimately execute the phenotypic changes in cell behavior.

Numerous cell cultured and mouse transgenic models have confirmed that the overexpression of HER2 is tumorigenic and continues to be a driver of the tumors that it generates.[1,2] It is now also apparent from several cell-based, xenograft, and transgenic mouse models that HER3 is an essential partner and codriver for HER2 in tumorigenesis.[3-5] HER3 functions both upstream and downstream of HER2. It functions upstream because its own KD, although catalytically inactive, is a highly competent allosteric activator of the HER2 KD.[6] It functions downstream because it is a key substrate of HER2, particularly competent at recruiting and activating PI3K, and HER2 activates this pathway through the phosphorylation of the HER3 c-tail.[7,8]

The 25-year endeavor to develop targeted therapies for this type of cancer has had an evolutionary course closely following the trail of scientific developments. The monoclonal antibody trastuzumab was developed in the early days following the discovery of HER2 and is now known to bind the juxta-membrane region of the HER2 ECD.[9,10] Pertuzumab was designed much later to interfere with HER2 signaling and binds the dimerization interface of the HER2 ECD (Fig 1).[11,12] These agents exhibit only limited activity in the monotherapy of advanced-stage *HER2*-amplified breast or gastric cancers.[13-17] But they do enhance the efficacies of active chemotherapy regimens and have become staples of combination regimens for the management of advanced breast and gastric cancers.[18-20] The efficacy enhancement afforded by trastuzumab is even more pronounced in early-stage breast cancer, with significant survival benefits,[21,22] and the neoadjuvant data available thus far suggest further enhancement by the addition of pertuzumab.[23]

The antibody trastuzumab was developed on the basis of 1980s understanding of HER2, and it is now clear that it does not actually inhibit HER2 signaling functions very well. A mixed literature has precluded finality in this debate, because some investigators find profound trastuzumab effects on HER2 expression or signaling.[24-26] But the majority of investigators, including our own group, see only partial, minimal, or no effects on HER2 expression or signaling, even at high concentrations of trastuzumab.[27-41] The antibody pertuzumab, which was specifically designed to interfere with the ECD-mediated dimerization of HER2, does in fact inhibit this dimerization function in its physiologic setting of ligand-induced HER2 signaling when HER2 levels are normal.[12] But it shows no such effects in the pathologic scenario of constitutive HER2 signaling seen in cancer cells with massive HER2 overexpression.[27,35,36,42] The failure of these antibodies to inactivate HER2 signaling in *HER2*-amplified cancers reflects our naive understanding of how constitutive signaling is generated in these cancers. It is plausible that massive overexpression of HER2 leads to KD interactions and constitutive signaling without the requirement for ligand-driven ECD dimerization, and the conformation and interactions of the ECD may be irrelevant in this disease state of overexpression. If true, this would suggest that targeting the KDs directly would be a much more effective therapeutic strategy.

Advances in small-molecule discovery platforms and sophisticated structure-guided chemistries have enabled the development of potent and selective kinase inhibitors, and lapatinib is at the pinnacle of these accomplishments. Lapatinib inhibits the HER2 kinase with low nanomolar potency,[43] in part because of a slow off-rate,[44] near singular selectivity for the HER family,[45] and excellent pharmacokinetic properties,[46] making it one of the most potent and selective clinical tyrosine kinase inhibitors (TKIs) yet developed. Despite its truly remarkable chemical and pharmacologic attributes, lapatinib has only limited single-agent activity in patients with *HER2*-amplified breast, gastric, or gastroesophageal cancers.[47-51] The irreversible HER2 TKI neratinib shows only slightly higher activity at significant cost in toxicity profile,[52,53] and there is little evidence that any of the plethora of other TKIs in the pharmaceutical pipelines are able to substantially improve on these TKIs. In patients with *HER2*-amplified breast cancer, the incremental activity of lapatinib is more clinically

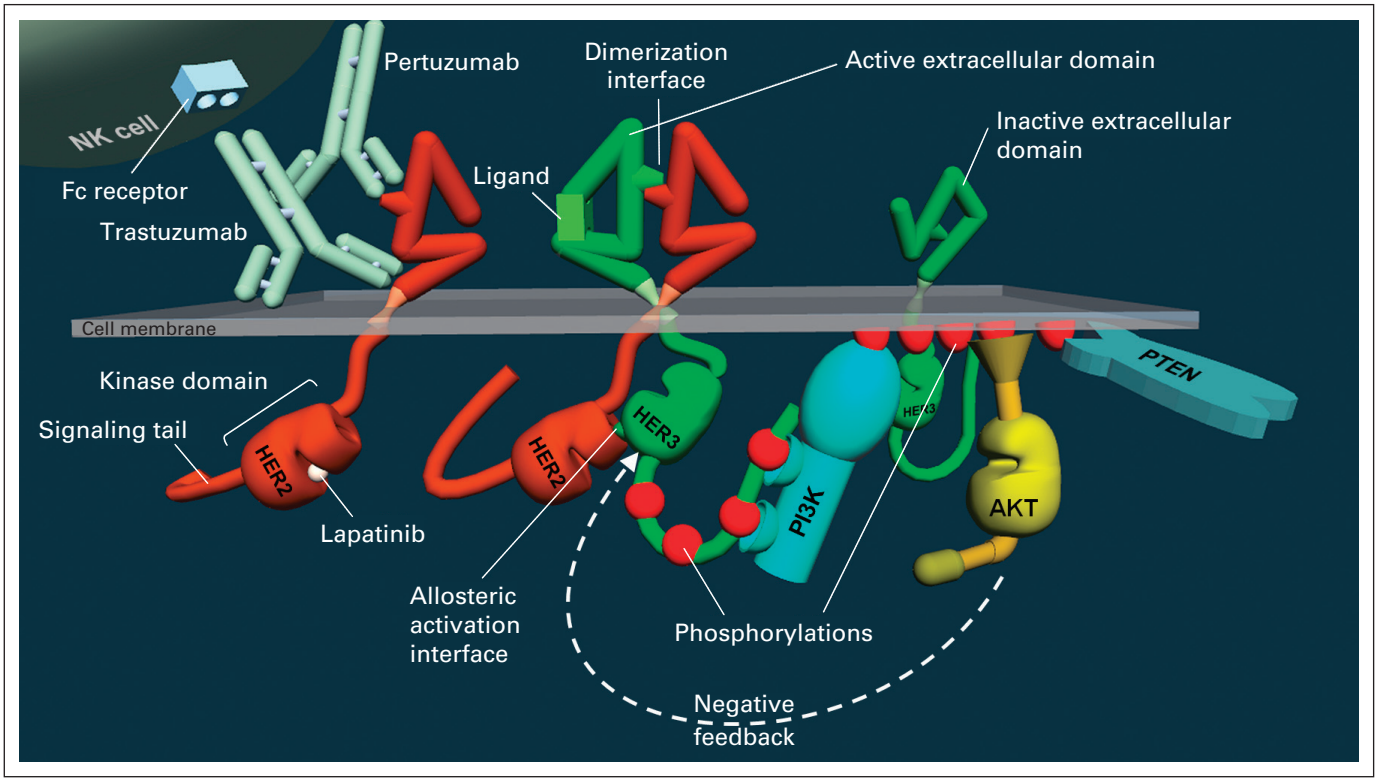

Fig 1. Structure of the human epidermal growth factor receptor 2 (HER2) and HER3 receptors and their mode of activation through dimerization and activation of PI3K/Akt signaling and binding sites of trastuzumab, pertuzumab, and lapatinib, showing both an inactive and ligand-activated HER3. Binding of ligand reconfigures the extracellular domain of HER3, exposing the dimerization interface. The extracellular domain of HER2 is always in the active configuration and does not require ligand. The phosphorylated signaling tail of HER3 binds and activates PI3K, leading to phosphorylation of membrane lipids, which is reversed by the phosphatase PTEN. These membrane phospholipids recruit and activate Akt, which regulates many downstream events. In HER2-driven cancer cells, it also regulates HER3 in a feedback loop shown by the arrow.

useful in combination regimens with capecitabine, paclitaxel, or trastuzumab.[49,54,55] In the accompanying article, Satoh et al report that lapatinib only modestly enhances the efficacy of paclitaxel in patients with *HER2*-amplified gastric cancer. It remains to be determined whether lapatinib can perform better when combined with more active gastric cancer chemotherapy regimens. The clinical activity of lapatinib does not compare favorably with HER2-targeting antibodies in randomized studies.[56,57] However, unlike HER2-targeting antibodies, lapatinib potently inactivates constitutive HER2 signaling in *HER2*-amplified cancer cells.[34,58,59]

These developments in HER2-targeting have brought about two key conundrums: First, considering the overwhelming evidence that HER2 is a disease-driving oncogene, why do HER2-targeting agents not have much higher clinical activity in monotherapy? Second, why are the HER2-targeting antibody therapies more active than the TKIs clinically, if they are much poorer inhibitors of HER2 signaling? Hypothesis-driven experimental science has provided resolutions to these dilemmas and identified new directions for pursuit and renewed hope in the development of far more effective HER2-directed therapies.

It is now recognized that the HER2-HER3 complex, which is the functionally relevant tumor driver in *HER2*-amplified cancers, is much more resilient to inhibition than had been anticipated. This is because tumor cells will not tolerate the loss of Akt activity, and negative feedback signaling loops induced by the loss of Akt activity can execute a marked increase in HER3 signaling output to preserve this critical signaling throughput[60,61] (Fig 1). The highly dynamic nature of HER3 signaling endows the HER2-HER3 complex with the ability to increase its signaling output approximately 100-fold in response to pharmaceutical inhibitors, overpowering and undermining the activity of all such HER2 or HER3 inhibitors.[62] Although inhibitors such as lapatinib can inactivate HER2-HER3 signaling at clinically relevant concentrations, the inhibition lasts less than 24 hours and is ultimately overpowered by the compensatory mechanisms. These findings have redefined the HER2-HER3 complex as the true driver of *HER2*-amplified cancer and the effective inactivation of this complex is the new bar for pharmaceutical drug development. None of the current agents rise to this bar, entirely consistent with their limited activities as monotherapy.

The fact that HER2-targeting antibodies are far weaker inhibitors of oncogenic HER2 signaling than TKIs, even though they exhibit greater clinical efficacy than TKIs in *HER2*-amplified cancers, seems paradoxical at first glance. But the key to the paradox lies in the immunologic dimension encompassed by antibody therapies that are completely lacking in TKIs. The massive cell-surface expression of HER2 in *HER2*-amplified cancer cells enables abundant binding of engineered HER2-targeting antibodies, thus providing a therapeutic index for endogenous immunologic responses that would ordinarily be lacking against self-antigens. Trastuzumab and pertuzumab are

both fully competent human antibodies capable of mediating immune functions such as antibody-dependent cell-mediated cytotoxicity as well as potentially other immune effector functions, and there is now considerable evidence in support of this mode of action. The in vivo antitumor efficacy of trastuzumab or its murine predecessor in a xenograft model is almost entirely lost by a single mutation or a proteolytic disruption in the Fc region of trastuzumab or elimination of the mouse Fc receptor gamma, all of which impair the ability of the mouse to mount an immunologic response to the trastuzumab-coated xenograft tumor.[63,64] Immunocompetent models of *neu*-driven mammary tumorigenesis further elucidate the roles of both innate and adaptive immunities underlying the antitumor activities of HER2/*neu*-targeting antibodies.[65] There is an abundance of clinical evidence in patients treated with trastuzumab that further supports an immunologic mode of action, including the induction of antibody-dependent cellular cytotoxicity,[66] endogenous humoral and enhanced T-cell–mediated immune responses,[67] suppression of regulatory T cells and induction of Th17 cells,[68,69] and increased tumor infiltration with immune effectors including natural killer cells.[70-72] The endogenous immunity induced by trastuzumab therapy appears to last beyond the cessation of therapy and has been detected in patients enrolling onto subsequent vaccine studies.[73] It is widely believed that the greatest potential in the immunologic approach to cancer therapy lies in the treatment of microscopic residual disease. Consistent with this, the greatest impact of trastuzumab therapy has been seen in the adjuvant setting with improvements in outcomes surpassing other systemic modalities.[21,22]

The hypothesis that *HER2*-amplified cancers can be effectively treated through the inactivation of their HER2-HER3 drivers remains a solid treatment hypothesis vigorously being pursued through numerous innovative pharmaceutical approaches. The fact that massive HER2 expression adorns the outside surface of *HER2*-amplified cancer cells, forsaking their ability to camouflage themselves, creates the opportunity for an entirely separate dimension of pharmaceutical immunotherapy and immunodelivery approaches. Although much of the promise of the HER2-inhibiting dimension remains in its future, HER2 immunotherapy approaches have made a seismic impact already, even without their appropriate label and accolades as the true pioneers of the cancer immunotherapy era.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author indicated no potential conflicts of interest.

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