

Molecular Pathways: HER3 Targeted Therapy

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Abstract

The HER family of receptor tyrosine kinases, including EGF receptor (EGFR), HER2, HER3, and HER4, transduce growth-promoting signals in response to ligand binding to their extracellular domains (ECD). This family is deregulated in numerous cancers, with mutations in EGFR and HER2 often serving as "driver" events to activate key growth factor signaling pathways such as the RAS-ERK and PI3K-AKT pathways. Less attention has been paid to the oncogenic functions of HER3 due to its lack of intrinsic kinase activity. Recent work, however, has placed HER3 in the spotlight as a key signaling hub in several clinical contexts. First, HER3 has been shown to play a major role in mediating resistance to HER2 and phosphoinositide 3-kinase (PI3K) pathway-directed therapies due to its feedback regulation via AKT signaling. Second, activating mutations in HER3 have been identified in multiple cancer types, including gastric, colon, bladder, and non-small cell lung cancers. As a result, HER3 is now being examined as a direct therapeutic target. In the absence of a strong enzymatic activity to target, the focus has been on strategies to prevent HER3 activation including blocking its most relevant dimerization partner's kinase activity (erlotinib, gefitinib, and lapatinib), blocking its most relevant dimerization partner's ability to dimerize with HER3 (trastuzumab and pertuzumab), and directly targeting the HER3 ECD (MM-121, U3-1287, and LJM716). Although drugs targeting EGFR and HER2 have proven effective even as single agents, the preclinical and clinical data on the antibodies directly targeting HER3 suggest more limited potential for single-agent activity. Possible reasons for this include the lack of a suitable biomarker for activated HER3, the lack of potency of the antibodies, and the lack of relevance of HER3 for growth of some of the cancer types analyzed. Nevertheless, clear improvements in activity are being observed for many of these compounds when they are given in combination. In this snapshot, we will highlight the basis for HER3 activation in cancer, the different pharmacologic strategies being used, and opportunities for further development. *Clin Cancer Res*; 20(6); 1410–6. ©2014 AACR.

Background

The v-erb-b2 erythroblastic leukemia viral oncogene (ErbB)/HER family of receptor tyrosine kinases (RTK), consisting of HER1 [EGF receptor (EGFR)], HER2, HER3, and HER4, are key regulators of cell growth and differentiation. These receptors share a common domain structure consisting of an extracellular ErbB ligand-binding domain, an intracellular tyrosine kinase domain, and an intracellular C-terminal tail with multiple tyrosine residues which, when phosphorylated, activate downstream signaling cascades. Members of this family interact with a variety of ligands. As its name suggests, EGFR has been shown to interact with EGF as well as other ligands including betacellulin (BTC), epigen (EPG), epiregulin (EPR), amphiregulin (AR), hepa-

rin-binding EGF-like growth factor (HB-EGF), and TGF- α . HER3 has been shown to interact with neuregulin (NRG)-1 and -2. HER4 can interact with all four NRGs (NRG-1, -2, -3, and -4), EPR, HB-EGF, and BTC. HER2 is distinct in having no known ligand and is thought to not require ligand for its activation. Deregulation of ErbB kinase activity has been strongly implicated in tumorigenesis with mutational activation of EGFR and HER2 frequently observed in a variety of cancer histologies. Members of the family, particularly HER2 and EGFR, have made excellent therapeutic targets selectively in those tumors showing evidence of receptor activation (1). More recent attention has been placed on HER3 as a potential therapeutic target as there is mounting evidence for its frequent activation in RTK-driven tumors.

HER3 is distinguished from other ErbB family members by two attributes. First, HER3 lacks a functioning kinase domain (2). Although HER3 is able to bind ATP (3), multiple lines of evidence demonstrate that it is catalytically impaired for the phospho-transfer reaction (2). This likely contributed to HER3 being somewhat ignored as a therapeutic target while multiple drugs against EGFR and HER2 have moved forward. Second, HER3 is a potent inducer of the phosphoinositide 3-kinase (PI3K)-protein

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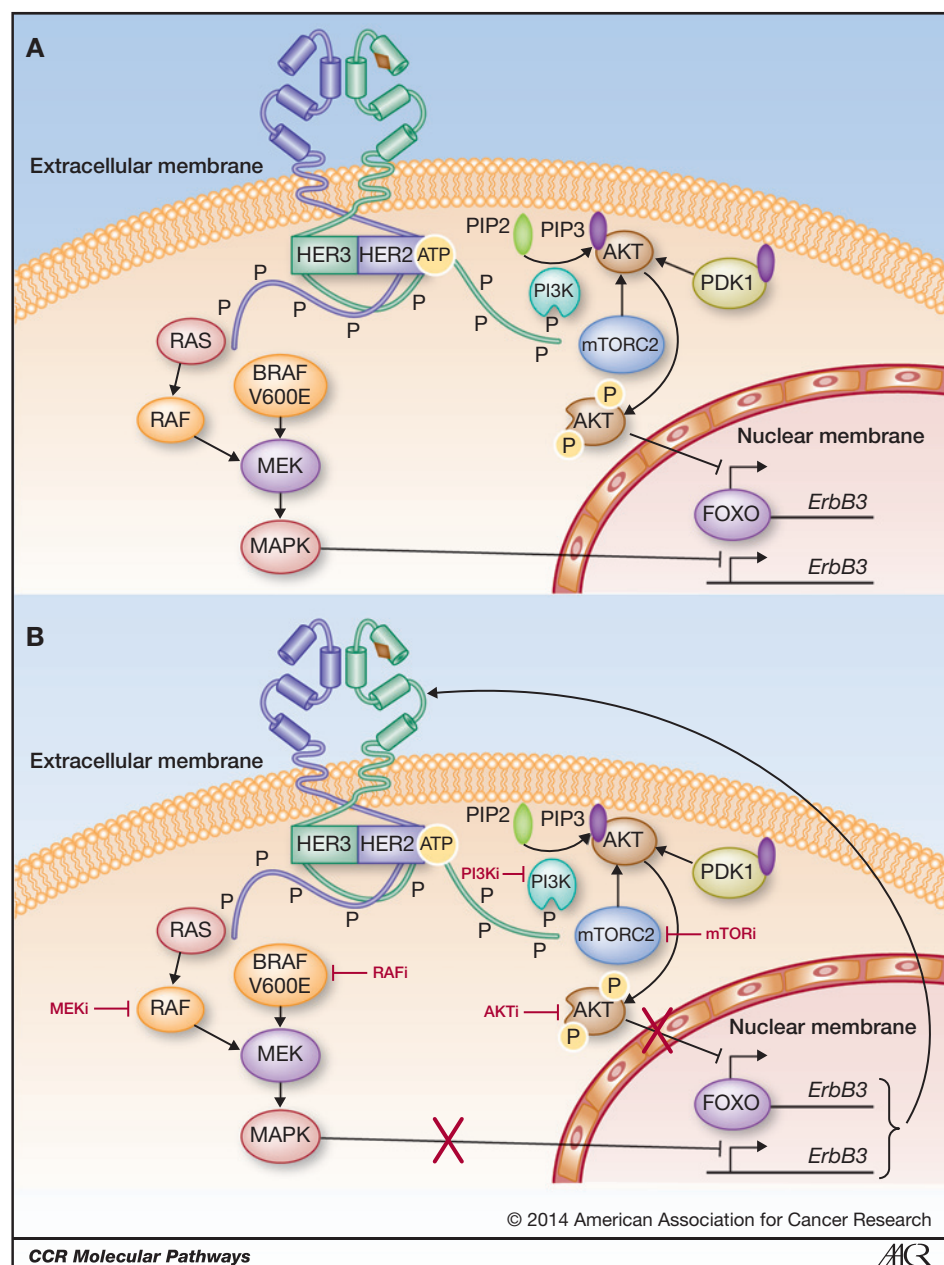
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kinase B (AKT) pathway through six consensus phosphotyrosine sites on its C-terminal tail, which bind the PI3K p85 subunit (Fig. 1; refs. 4–6). Binding of p85 to tyrosine phosphorylated HER3 induces PI3K activity, which then potentiates multiple signals essential for the transformed phenotype including activation of AKT (7). In many tumors it seems that HER3 functions as the major link between RTK and PI3K activation and thus, it has more recently gained attention as a selective means of inhibiting PI3K signaling in RTK-driven tumors.

With the exception of HER2, ErbB family members are activated by ligand binding to the extracellular domain (ECD), which promotes conformational changes that

enable the receptors to homo- or heterodimerize. Dimerization is followed by allosteric activation of one dimer partner by the other, after which the activated receptor can phosphorylate the C-terminal tyrosine residues of its binding partner (8). The phosphotyrosine residues then bind and recruit proteins with SH2 domains as well as PTB-binding proteins, eventually resulting in activation of downstream pathways. Because of its lack of kinase activity, HER3 cannot activate signaling within homodimers; however, in the presence of HER3 ligands, HER3 may promote the kinase activity of EGFR or HER2 and thereby induce phosphorylation of the HER3 C-terminal tail. In the absence of ligand(s), it is thought that the HER3 C-terminal

Figure 1. Negative feedback inhibition of HER3. **A**, the HER2–HER3 dimer potentially activates both the PI3K–AKT and MAPK pathways, both of which result in inhibition of HER3 transcription. Activation of PI3K by phosphorylated (P) HER3 results in the PI3K-mediated conversion of PIP2 to PIP3. The production of PIP3 recruits AKT to the extracellular membrane where it can become phosphorylated by mTORC2 as well as PDK1. Phosphorylated AKT can then inhibit the FOXO family of transcription factors that activate RTK gene expression. Signaling through the MAPK pathway begins with RTK-mediated activation of RAS which signals downstream to RAF, MEK, and eventually, MAPK. Specifically in the context of mutant V600E BRAF expression in thyroid cancer, it has been shown that MAPK signaling can negatively regulate transcription of HER3. **B**, inhibition of the PI3K–AKT pathways, with either PI3K, AKT, or mTOR inhibitors, as well as inhibition of the MAPK pathway with vemurafinib (BRAF inhibitor) has been shown to result in transcriptional upregulation of HER3, eventually leading to reactivation of its downstream targets.



tail acts *in trans* to block its activation domain, preventing HER3 from inappropriate activation by partner kinases (2). It should be noted that studies by Junttila and colleagues suggest a mechanism of ligand-independent HER2–HER3 dimerization in HER2-amplified cells. In this setting, the abundance of HER2 forces a HER2–HER3 dimer, without the need for ligand, in a conformation distinct from that which is taken during ligand-dependent dimerization (9). Although the HER2–HER3 dimer is the most potent HER family dimer, HER3 has been shown to dimerize with EGFR, as well as non-HER family members such as c-MET (10, 11). This complex mechanism of activation of ErbB family members presents multiple aspects that can be pharmacologically targeted including inhibition of ligand binding to the ECD, inhibition of receptor dimerization, and inhibition of the partner tyrosine kinase activity, all of which have been exploited and proven to be successful in a variety of oncologic contexts.

The essential function of HER3 in linking RTK and PI3K activation has been most readily demonstrated in the context of HER2-amplified breast cancer. Within such tumors, the HER2–HER3 dimer has been shown to be essential for tumor formation and tumor maintenance (12–15). The significance of this finding has been most powerfully illustrated by the benefit of targeting the HER2–HER3 dimer

using the HER2-targeting antibodies, trastuzumab and pertuzumab. Although trastuzumab promotes several antitumor actions, including antibody-dependent cell-mediated cytotoxicity (ADCC), a major portion of its action is to block dimers between HER2 and HER3 that occur in the absence of ligand by binding to domain IV of the HER2 ECD (Fig. 2; refs. 9, 16). Meanwhile, pertuzumab acts almost exclusively through its blockade of ligand-dependent HER2–HER3 dimers by binding to domain II of the HER2 ECD (Fig. 2; refs. 9, 17). Preclinically, the combination of these two antibodies has been shown to be more potent at down-regulating HER3–PI3K signaling than either alone (18), which has translated to an increased response rate and overall survival for administration of the combination (19). The studies on this combination have largely focused on HER2⁺ breast cancers; however, the potential exists for effective blockade of HER3 activity using this doublet in other tumor types driven by HER2 (20).

The significance of HER3 in HER2-driven tumors has been further underscored by multiple studies implicating upregulation of HER3 in resistance to HER2-targeted therapy. Moreover, these studies have shown that upregulation of HER3 may promote resistance to a number of signaling inhibitors that are designed to directly or indirectly antagonize activated PI3K signaling (21–25). As growth factor

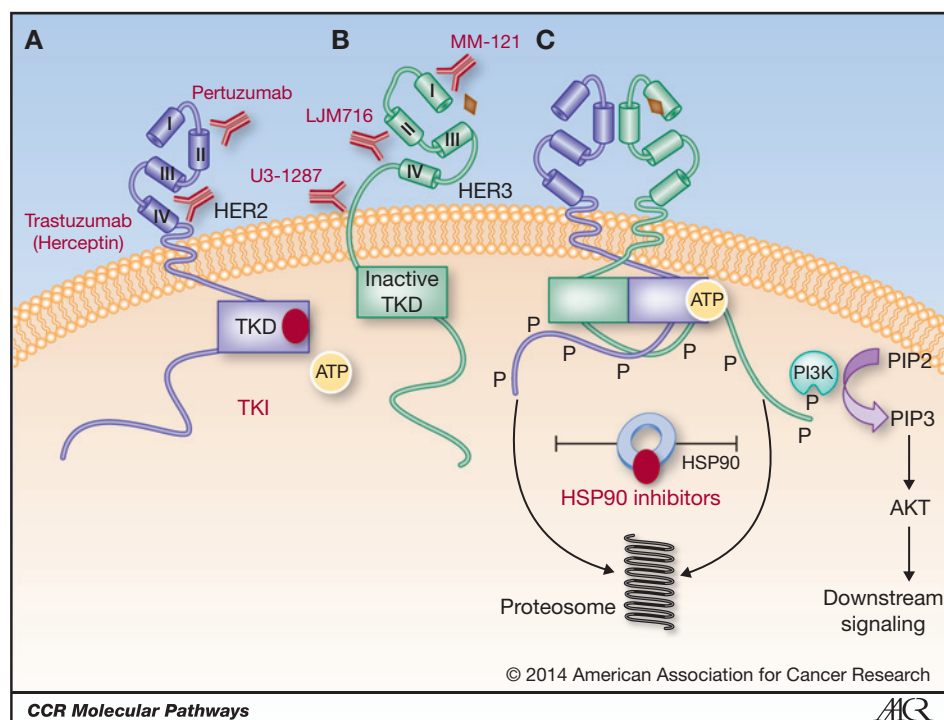


Figure 2. Multiple modes of inhibiting HER3 pathway activation. Inhibitors are depicted in red. A, targeted therapy against HER2 prevents HER2-dependent activation of HER3. Pertuzumab, a HER2 monoclonal antibody, binds domain II of the HER2 ECD and prevents ligand-dependent dimerization between HER2 and HER3. Trastuzumab binds domain IV of the HER2 ECD and prevents ligand-independent dimerization between HER2 and HER3 in the context of HER2-amplified breast cancer. HER2 TKIs, including lapatinib, afatinib, and neratinib, bind the tyrosine kinase domain (TKD), compete with ATP-binding and prevent phosphorylation (P) of the HER3 C-terminal tail. B, the monoclonal HER3 antibody, MM-121 prevents ligand binding while LJM716 specifically binds an epitope created by ECD domains II and IV in the HER3 closed conformation. U3-1287 is another HER3 monoclonal antibody that binds the ECD. C, HSP90, a chaperone protein, inhibits the proteasome-mediated degradation of phosphorylated HER2 and HER3. Inhibitors of HSP90, therefore, promote RTK degradation.

signaling is physiologically regulated by negative feedback, mutationally activated growth factor signaling seen in tumors is associated with elevated levels of negative feedback. The consequence is that, in the steady state, the tumors feature suppression of upstream signals such as RTKs. We and others have observed that inhibitors of oncoprotein-driven signaling pathways cause "relief" of this elevated feedback. This "relief of feedback" is manifest as increased expression and activity of RTKs that themselves have oncogenic functions. The induced RTK activity can function as a mechanism of resistance to the drug. Among RTKs, HER3 is very frequently observed as an RTK induced in this setting (25–27), as it seems to be a key node for feedback regulation of PI3K/AKT signaling in most cells as well as MEK/ERK signaling in more select cellular contexts.

The role of HER3 in feedback regulation of PI3K signaling emerged from work done by the Moasser and colleagues showing that inhibition of HER2–HER3 signaling by EGFR/HER2 tyrosine kinase inhibitors (TKI) caused only transient downregulation of HER3 phosphorylation. A rebound in Her3 activity was demonstrated to occur and mediate resistance to EGFR/HER2 TKIs (26). We examined feedback regulation of PI3K/AKT signaling by studying the effects of AKT inhibition upon RTK activation and found multiple RTKs [HER3, insulin-like growth factor-I receptor (IGF-IR), and insulin receptor] to be transcriptionally regulated by AKT signaling (21). Notably, these RTKs are known to be powerful activators of PI3K/AKT signaling, pointing to a feedback response. These effects on HER3 were also predictably noted in examining the effects of an mTOR kinase inhibitor (22) and PI3K inhibitor (23) both of which result in AKT inhibition. Thus, various mechanisms that result in AKT inhibition subsequently result in a FOXO-dependent induction of transcription of HER3 (Fig. 1; refs. 21–23, 25–27). Beyond the induction of HER3 expression, it has become clear that other mechanisms contribute to HER3 activation in response to PI3K/AKT inhibition including effects on HER3 localization (26) and on the expression of HER3 ligands. Thus, inhibition of PI3K/AKT signaling serves as a powerful stimulus to drive HER3 activation and this may subsequently promote reactivation of PI3K/AKT signaling or other growth factor signaling cascades, such as the Ras pathway. In several of these studies, it was shown that blocking the induced HER3 via RNA interference (RNAi), antibodies, EGFR/HER2 kinase inhibitors, or even HSP90 inhibitors could significantly improve the antitumor effects (21–24, 26, 27).

Although the aforementioned studies focused on feedback regulation of PI3K/AKT signaling in tumors featuring PI3K pathway activation, HER3 was also implicated in feedback regulation of MEK/ERK signaling in the specific context of BRAF V600E–driven thyroid cancer. Montero-Conde and colleagues noted that treatment of such tumors with the RAF inhibitor vemurafinib only led to very transient inhibition of downstream targets such as phosphorylated mitogen-activated protein/extracellular signal-regulated kinase kinase (pMEK) and phosphory-

lated extracellular signal-regulated kinase (pERK) with concomitant induction of several RTKs including HER2 and HER3 (Fig. 1; ref. 28). This study along with others in BRAF-driven tumors suggested that combined inhibition of RAF/MEK along with ErbB kinases (that can activate the feedback induced HER3) led to superior antitumor effects (29, 30). What is less clear from these studies is the specific role of HER3 activation in mediating resistance, as blockade of EGFR or HER2 may reasonably be enough to augment inhibition of RAS/RAF/MEK signaling irrespective of blocking HER3 activation. Further studies on selective HER3 ablation in this context will be needed to tease out the function of HER3 in these tumors.

A function for HER3 in tumor initiation and maintenance has been somewhat elusive until recently. Several studies examined steady state tumoral expression of HER3 (reviewed in ref. 31) and found little meaningful correlation with prognosis. Indeed, it does not seem that there is a clear correlation between levels of HER3 expression in tumor cell lines and its function in proliferation. Sheng and colleagues analyzed the effects of RNAi against HER3 in multiple ovarian cancer cell lines and found that HER3 knockdown only curtailed the proliferation of cell lines in which HER3 was activated despite relatively equal levels of HER3 protein expression between cell lines (32). More recently, evidence has begun to emerge on possible activation of HER3 through mutations mainly in the ECD (33–39). Jaiswal and colleagues surveyed multiple cancers and found recurrent mutations in the ERBB3 coding region that had transforming capabilities *in vivo* and *in vitro* (40). The authors found the ERBB3 mutations in 12% and 11% of gastric and colon cancers, respectively, and also found the mutations in 1% non-small cell lung cancers (NSCLC). Most of the recurrent mutations are within in the ECD (with a few located in the kinase domain); however, the mechanism(s) by which the ECD mutations activate HER3 is unknown. The authors speculate that the ECD mutations may promote an untethered, open conformation for HER3 that is primed for dimerization and activation, but further structural studies are needed to support such a hypothesis. It is important to note that none of the HER3 mutations remove the need for an active kinase to phosphorylate HER3, and therefore, it remains relevant to consider drugs that inhibit dimerization or drugs that inhibit the partner kinase as means to therapeutically target the HER3 mutants. In fact, the authors show that cells expressing both the HER3 mutants and HER2 can be effectively arrested by antibodies targeting the ECD of HER2 or HER3, as well as with an EGFR/HER2 TKI or a PI3K inhibitor. The functional significance of the kinase domain mutants is less clear. The authors speculate that these mutations may enhance phosphotransferase activity to the typically inactive HER3 kinase domain or may promote a new conformation that promotes HER3 dimerization with partner kinases; however, there has yet to be data confirming either of these hypotheses. In fact, under the assays conducted by the authors, they failed to see an increase in kinase activity by the kinase domain mutants.

Clinical-Translational Advances

Given the key roles for HER3 in both serving as a conduit between RTK activation and the PI3K pathway and in functioning as a feedback regulator that can contribute to resistance to PI3K/AKT-directed therapy across a wide variety of tumors, interest in drugging this receptor has been very high. However, targeting this protein has proven to be a challenge as HER3 lacks enzymatic activity. Therefore, attention has been placed on targeting the HER3 ECD through antibodies. The various antibodies being developed have unique ways of inhibiting HER3 pathway activation reflecting the various mechanisms by which HER3 can become activated. For example, MM-121, a human anti-HER3 monoclonal antibody, is thought to compete with ligand binding and specifically prevent ligand-dependent HER3 heterodimerization (41). LJM716, another monoclonal antibody, is selective for an epitope created by domains II and IV of the HER3 ECD (Fig. 2; ref. 42). These two domains are specifically responsible for mediating the closed and inactive conformation of HER3 (3). In theory, LJM716 can lock HER3 in a closed conformation and thereby prevent both ligand-dependent and -independent activation (42). The mechanism of action of U3-1287, another monoclonal antibody targeting the HER3 ECD, is less clear; however, it does seem to downregulate HER3 expression, possibly through increased endocytosis of the antibody-bound HER3 protein (43).

Another challenge in the attempt to therapeutically target HER3 is that there is no effective biomarker to define HER3 activation in patients. This issue has proven to be a difficult hurdle for effective patient selection. Consideration has been given to markers for activation/overexpression of partner kinases, such as HER2, as assayed by immunohistochemistry of tumor biopsies, markers for the expression levels of HER3, and even markers for the expression levels of ligands that activate HER3. For example, the researchers who developed MM-121 quantified expression levels of NRG-1 and BTC to distinguish cell lines that would respond to MM-121 between those that would not use computational modeling. Their data suggest that the use of ligand expression as a biomarker has the potential to identify a select group of patients in which MM-121 might prove to be effective over the use of receptor expression as biomarkers (44). However, while their computational modeling data implicate BTC and NRG-1 as the most potent inducers of phosphorylated HER3, it does not exclude the potential for other HER family ligands to continue to be relevant in the clinical setting. In addition, ligand expression is likely quite limited as an effective biomarker as multiple other routes to HER3 activation exist, such as mutation and ligand-independent dimers. Therefore, the presence of activated HER3 may be an even more powerful biomarker for efficacy than the presence of ligand alone. As such, a caveat for all of the compounds targeting HER3 in the clinic is that it is unknown how well patients with *bona fide* HER3-activated/driven disease may fare on a HER3 selective therapy.

Although multiple phase I and II trials have been opened for various HER3-targeted antibodies, clinical benefits of the antibodies as single agents have not been reported. The lack of early signals of single-agent activity suggests a revisiting of the preclinical data to ascertain whether this is a case of a limited target, limited set of drugs, or limited biomarkers to match the right patients and drugs. Models of HER2⁺ breast cancer are an obvious genotype to assess the efficacy of HER3-targeted therapy as the importance of HER3 has been very well defined in this context. Activated HER2 can drive HER3 signaling in both ligand-dependent and -independent manners, and thus anti-HER3 therapy may need to inhibit both of these dimer types. In this regard, LJM716 stands out as it was designed to inhibit both ligand-dependent and -independent activation. In support of this type of activity, LJM716 has shown growth inhibitory effects in HER2⁺- and NRG-1-expressing models as measured by cell proliferation assays as well as xenograft models (42, 45) with more than 80% growth inhibition in HER2⁺ BT474 xenografts. However, thus far, LJM716 has mainly been demonstrated to have tumor regression efficacy *in vivo* against ligand-driven models like FaDu (42).

Preclinical data on U3-1287 show more limited ability to induce tumor regressions as a single agent in xenograft studies featuring both HER2⁺- and NRG-1-driven models, including A549 and FaDu cells (46, 47). With the A549 model, U3-1287 was able to achieve tumor stasis; however, with the FaDu model, the antibody only partially inhibited growth of the tumor cells potentially indicating increased effectiveness in HER2⁺/EGFR⁺-amplified models over NRG-1-driven models. Interestingly, MM-121 showed little benefit in HER2⁺ models, suggesting HER2 amplification as a mechanism of resistance to antibodies, such as MM-121, which are specifically relevant in competing away ligand binding (44). MM-121 did significantly reduce tumor growth in multiple xenograft models including ovarian (OVCAR8), prostate (DU145), and kidney (ACHN) cancer but did not lead to tumor regressions (32, 41, 44). In these contexts, the lack of tumor regression observed may have been attributable to a lack of dependence of the model on HER3 or a lack of potency of the compound against activated HER3. Sheng and colleagues attempted to address this key issue by characterizing the importance of a NRG/HER3 autocrine loop in certain models of ovarian cancer (32). They showed that both MM-121 as well as HER3-targeted short hairpin RNA (shRNA) slowed growth of OVCAR8 cells *in vitro* and *in vivo*. One of the shRNAs could indeed induce regression while MM-121 treatment did not seem to do so, suggesting that single-agent treatment with MM-121 may be insufficient in the clinical setting despite the importance of the HER3/NRG loop in select tumors types.

Given the limited single-agent activity of the HER3-targeting antibodies and the key role of HER3 as a mechanism of feedback regulation and resistance, therapeutic targeting of HER3 in combination with primary drivers of the tumor has been evaluated in multiple contexts. For instance, we showed that combining an AKT inhibitor with either lapatinib or an inhibitor against the chaperone protein HSP90

prevented the feedback-mediated induction of phosphorylated HER3 and led to significantly greater antitumor effects than did either drug alone in BT474 xenografts (21). Even though the use of lapatinib or the HSP90 inhibitor provide indirect inhibition of HER3 (Fig. 2), their effectiveness in combination with the AKT inhibitor validates the idea that targeted therapy against HER3 will prove to be synergistic with multiple PI3K and possibly even mitogen-activated protein kinase (MAPK) pathway inhibitors. Recently, more direct evidence has come in the form of a study examining the benefit of LJM716 in combination with trastuzumab and lapatinib. In this setting, feedback induction of HER3 would be expected to limit the efficacy of the anti-HER2 antibody doublet. Addition of LJM716 was shown to significantly improve survival of mice when added to the doublet and synergistically induce cell death when given in combination with the PI3K inhibitor, BYL719 (45). These data support the addition of a pure HER3 antagonist to a disease in which HER3 is well established to mediate the driver oncoprotein signal and also be the key node of feedback regulation.

Overall, the data on the activity of HER3 selective drugs may be viewed as modest. Despite several reagents developed to target the protein, tumor regressions in mice and single-agent activity in humans has not been robustly demonstrated. There seems to be more than one reason for this. First, unlike other RTKs that have been successfully targeted in the clinic, HER3 is predominantly serving as a scaffold with little enzymatic activity. Antibody targeting has the potential to lower surface expression and block selected sets of dimers, but is less likely to achieve rapid and potent downregulation. Second and perhaps more significantly, the lack of a suitable biomarker for activated HER3 remains a major hurdle in moving forward with HER3-

targeted therapy. Most of the studies on the HER3 antibodies thus far have focused on tumors with either HER2 amplification or NRG expression, both of which are only surrogates for actual HER3 activity. Fortunately, there exist modalities that may enable us to better gauge where HER3 is activated. For example, using either the collaborative enzyme enhanced reactive (CEER) immunoassay (Prometheus) or reverse-phase protein microarrays (RPPA) with antibodies against phosphorylated HER3, we may be able to quantitate levels of active HER3 in patient samples. In addition, the development of radioactively or fluorescently labeled probes against activated HER3 may facilitate a noninvasive method for ascertaining where the receptor is important. Beyond these methods, a more rigorous assessment of genetic contexts where we anticipate HER3 to be essential (HER3 ECD mutants, HER2 amplification, in combination with PI3K inhibitors in PI3K-driven tumors) is likely to prove fruitful.

Disclosure of Potential Conflicts of Interest

S. Chandralapaty is a consultant/advisory board member for Glaxo-SmithKline. No potential conflicts of interest were disclosed by the other author.

Authors' Contributions

Conception and design: S. Chandralapaty

Writing, review, and/or revision of the manuscript: K. Gala, S. Chandralapaty

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): S. Chandralapaty

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