Review

HER3/ErbB3, an emerging cancer therapeutic target

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Abstract

HER3 is a member of the HER (EGFR/ErbB) receptor family consisting of four closely related type 1 transmembrane receptors (EGFR, HER2, HER3, and HER4). HER receptors are part of a complex signaling network intertwined with the Ras/Raf/MAPK, PI3K/AKT, JAK/STAT, and PKC signaling pathways. Aberrant activation of the HER receptors and downstream signaling molecules tips the balance on cellular events, leading to various types of cancers. Monoclonal antibodies (mAbs) and small molecule inhibitors targeting EGFR and HER2 tyrosine kinase activities exhibit clinical benefits in the treatment of several types of cancers, but their clinical efficacy is limited by the occurrence of drug resistance. HER3 is the preferred dimerization partner of HER2 and it is well established that HER3 plays an important role in drug resistance to EGFR- and HER2-targeting therapies. Since HER3 has limited kinase activity, mAbs are being explored to target HER3 for cancer therapy. Currently, approximately a dozen of anti-HER3 mAbs are at different stages of clinical development. However, the lack of established biomarkers has made it more challenging to stratify cancer patients to whom HER3-targeting therapies can be more effective. In this review, we focus on the validation of HER3 as a cancer drug target, the recent development in biomarker discovery for anti-HER3 therapies, and the progress made in the clinical development of HER3-targeting mAbs.

Key words: HER3/ErbB3, monoclonal antibody (mAb), biomarkers, cancer therapy

Introduction

The family of human epidermal growth factor receptors (EGFR/HER) is involved in multiple complex and tightly controlled signaling pathways for the regulation of various cellular functions including cell proliferation, organ development, and organ repair [1–8]. Aberrant HER signaling is associated with the development of various solid tumors [9,10]. Of the four members of the HER family, EGFR (HER1) and HER2 are well-documented proto-oncogenes. Their excessive signaling is known to contribute to the development of various types of cancers. At least nine cancer therapeutics [monoclonal antibodies (mAbs) and small molecule tyrosine kinase inhibitors (TKIs)] targeting EGFR and/or HER2 are currently in clinical use [5,9,11–14]. Although clinical benefits have been demonstrated, the patient responses to these drugs vary and drug resistance is often encountered. For example, HER2 overexpression (HER2+) typically accounts for approximately 25% of breast cancer patients in whom trastuzumab is indicated. However, about 70% of these patients may have primary resistance to trastuzumab [15]. In addition, the effectiveness of trastuzumab has been limited to breast or gastric cancer and not to other solid tumors [16]. Another example of this limitation is cetuximab, an anti-EGFR mAb indicated for the treatment of head and neck cancer (H&NC), and colorectal cancer (CRC) in patients with EGFR overexpression. While effective in patients with wild-type KRAS, cetuximab did not significantly affect the overall survival rates in CRC patients with KRAS mutated tumors [17,18]. The mechanisms resulting in refractory and acquired resistance to anti-HER agents are poorly understood. Different models have been proposed including genetic mutations of key genes in the HER pathway such as KRAS and...
PTEN as well as up-regulation of oncogenes such as c-MET and HER3 [4,19–34]. In contrast to EGFR, HER2, and HER4 that possess active tyrosine kinase domains, HER3 lacks intrinsic kinase activity [25] and a HER3 homodimer has not been reported [26]. HER3 activation relies on ligand binding and/or hetero-dimerization with other HER receptors. Among all the homodimer and heterodimer pairs necessary for EGFR/HER activity, the HER3/HER2 heterodimer is the most potent partner for activation of the PI3K/AKT signaling cascade through direct HER3 binding to the p85 subunit of PI3K [27,28] suggestive of the pivotal role HER3 plays in regulating the HER signaling cascade and in therapy resistance. HER3 activation has been recognized as one of the causes of gefitinib resistance in lung cancer cells [4]. In other instances, compensatory HER3 phosphorylation evades inhibition by TKIs targeting EGFR and/or HER2 [3]. Increased HER3 expression was associated with resistance to trastuzumab [21]. Many other cancer types such as melanoma, breast, pancreatic, prostate, ovarian, and gastric cancers are known to have HER3 activation [29–34]. The anti-HER2 mAb pertuzumab that blocks HER2 dimerization with HER3, induces HER3 dimerization with EGFR in both low and high HER2 expressing cancer cells [35]. Treatment of the low HER2 expressing MCF7 cancer cells with pertuzumab promoted cell proliferation and migration in the absence of HER3 ligand stimulation. This is the result of pertuzumab-induced HER3 signaling via EGFR/HER3 dimerization and activation of downstream AKT signaling pathways [35]. These results suggested that HER3 plays a key role in maintaining the equilibrium of the HER family member dimerization and signaling and in sensing its perturbations. Oncogenic HER3 gene gain of function mutations have been reported in colon and gastric cancers [36,37]. These studies have also provided evidence of oncogenic activity of Q809R HER3 mutation in gastric cancer [36]. Although HER3 mutation at V714M was identified in non-small cell lung cancer (NSCLC) patients and S846I mutation in patients with colon cancer, the oncogenic function of these mutants has not been tested [36]. Taken together, the emerging evidence from both laboratory and clinical observations strongly validates HER3 as a cancer drug target [4,19,21,24].

Due to the limited intrinsic kinase activity of HER3, mAbs have been the main drug modality to target HER3 through blocking HER3/ligand and/or other HER receptor interactions. Currently more than a dozen of anti-HER3 mAbs are in different stages of clinical development, and several more in preclinical development. Multiple comprehensive reviews on HER3 biology and regulation have been published in the recent years [38–52]. In this review, we focus on the recent progress in the discovery of clinical biomarkers for anti-HER3 antibody development and the status of HER3-targeting drugs in clinical development.

**Biomarkers for Anti-HER3 Antibody Development**

**Heregulin (HRG)**

Heregulins are the major class of soluble HER3 ligands playing an important role in HER3 activation and signaling. Meta-data analysis of three separate Phase 2 clinical studies in cancer patients with high HRG (HRG*), but low HER2 expression (HER2−) demonstrated a statistically significant 63%–74% reduction in the risk of disease progression in 38%–54% of the study population across three cancer types (breast, lung, and ovarian) treated with MM-121, an experimental HER3-targeting antibody being developed by Merrimack Pharmaceuticals. The hazard ratios (0.26–0.37) are better than most cancer therapies on the market today [53].

Cancer patients with high HRG represent about 30%–50% of solid tumor patients, depending on the cancer type [the Cancer Genome Atlas (TCGA), https://tcga-data.nci.nih.gov/tcga/]. For those cancers, the MM-121 antibody inhibition of HER3 has shown proof of concept of therapeutic activity in Phase 2 studies, and the potential target population is approximately 326,000 patients in the United States alone [53]. This finding is still preliminary and needs to be validated and proven in large clinical studies. More importantly, this selective targeting of HER3 among the HER2 low expressing patient segment may fill a major gap left by trastuzumab for which HER2 overexpression is one of the major selection criteria.

Currently there are no standard measurements to define a high or low HRG in tumor tissues. Merrimack defines high HRG as >5 by RT-qPCR (reverse transcription-quantitative polymerase chain reaction) or ≥1+ by RNA-ISH (RNA-based in situ hybridization) [53]. Daiichi Sankyo is conducting a Phase 3 study in the United States with two separate arms. In Arm A, HRG level is not required in selecting patients for the trial, while in Arm B, only patients with a high level of HRG are recruited.

**HER3 overexpression and HER3 mutations**

HER3 overexpression has been reported in both primary cancers and cultured cells of multiple cancer types including breast, ovarian, prostate, colon, pancreas, stomach, oral cavity, and lung cancers [48]. Fifty to seventy percent of breast cancers have detectable HER3 levels as evaluated by IHC. In breast cancers activated HER3 is usually co-overexpressed with HER2 [54–56]. It has been reported that overexpression of HER3 can transform a mammalian cell line such as CHO to possess cancer cell hallmarks including changes in proliferation and migration [57]. Even though it is logical to think that HER3 overexpression or amplification could be a biomarker for the clinical development of HER3-targeting therapies, currently there are no standard methods for measuring HER3 overexpression. Oncogenic mutations in HER3 gene were reported in human colon and gastric cancers and some of these mutations were shown to be gain of function mutations [36], but more studies are needed to validate if HER3 gain of function mutations can be developed as biomarkers for the clinical development of HER3-targeting therapies.

**E3 ubiquitin ligases NEDD4 and Nrdp1**

E3 ubiquitin ligases are known to regulate the HER family receptors. A RING finger E3 ubiquitin ligase Nrdp1 has been reported to interact with HER3 and promotes HER3 ubiquitination and degradation via proteasome in breast and prostate cancer cells [58,59]. Our recent study identified E3 ubiquitin ligase NEDD4 as a novel interaction partner of HER3 [60]. A negative correlation between NEDD4 and HER3 levels in prostate cancer cells and tissues suggests the importance of NEDD4 in HER3-driven cancers [60]. Further investigation of the role of NEDD4 and Nrdp1 in HER3 regulation and signaling should help determine whether levels of the E3 ubiquitin ligases can serve as a biomarker in the development of HER3-targeting cancer antibody therapies.

**Anti-HER3 Therapies in Clinical Development**

Based on our current understanding of the HER3 biology, an effective anti-HER3 therapy can be developed using any one or the combination of the following mechanisms: (i) locking HER3 in an inactive conformation, (ii) trapping the ligands, (iii) blocking ligand HRG...
Patritumab (AMG-888)
Patritumab is a human HER3-targeting mAb that is being developed by Daiichi Sankyo and has been investigated in various models of breast cancer and NSCLC [74–77]. Results from preclinical studies suggested that patritumab is more effective against HER2 or EGFR amplified cancers relative to the HERG driven cancers. A Phase 1 study of patritumab with erlotinib (a TKI against EGFR) in patients with advanced stage NSCLC showed that patritumab achieved a progression-free survival (PFS) hazard ratio (HR) of 0.32 with a P-value less than 0.003 in HERG biomarker positive patients compared with control patients [78].
Patriutumab is now in a 780-patient Phase 3 study for NSCLC in the United States. The study is divided into two groups: Group A’s primary endpoint is PFS, in which high HRG level is not required. Group B has a primary endpoint in overall survival; only patients with high HRG level are selected [78]. Patritumab is also being investigated in a Phase 1b/2 study in combination with trastuzumab plus paclitaxel in patients with newly diagnosed metastatic breast cancer [107]. Patritumab is also in a Phase 1 study for treating squamous cell carcinoma of the H&NC in combination with cetuximab, cisplatin, or carboplatin [79].

MM-121 (seribantumab)
MM-121 is a human mAb against HER3 designed to block heterodimerization of HER3 with the other HER receptors. Among all of the anti-HER3 programs in development, MM-121 is the most extensively studied anti-HER3 mAb by Merrimack [61,81–83]. MM-121 has been investigated in combination with different therapies for various types of cancers in multiple Phase 2 studies.
Phase 2 clinical trials of MM-121 in combination with TKIs or chemotherapy showed that low mRNA expression of HRG is associated with poor response in patients with platinum-resistant ovarian cancer (PROC), ER+/PR+/HER2+ breast cancer, or EGFR wild-type NSCLC. Subgroup analyses further demonstrated that targeting HRG-positive tumors with MM-121 sensitizes patients to exemestane, erlotinib and paclitaxel in metastatic breast, lung and ovarian cancers, respectively, and significantly lowers the risk of tumor progression. These clinical studies therefore identified HRG as a patient response biomarker for MM-121. Further, the clinical trials concluded that patients with low HER2 level expression exhibited the maximum benefit from MM-121 [53].
A meta-analysis of 128 HRG-positive patients had HRs for PFS between 0.26 and 0.37 [53]. This means that in 38%–54% of the study population, across three cancer types, there was a statistically significant 63%–74% reduction in risk of disease progression. These are considered remarkable results. Since more than 40% of prostate, liver, bladder, cervical, and H&NC patients have high HRG levels, MM-121 could have potential use in these cancers as well.

A global open-label, randomized Phase 2 clinical trial of MM-121 was recently initiated using HRG as the selection biomarker in patients with locally advanced or metastatic NSCLC [84]. This study will be the first clinical study to prospectively select patients based on HRG status. The trial will enroll 120 HRG-positive patients randomized (2:1) to receive either MM-121 plus the investigator’s choice of docetaxel or pemetrexed, or the investigator’s choice of docetaxel or pemetrexed alone. The primary endpoint of the trial is PFS.

In breast cancer, MM-121 has demonstrated that it is more effective in the cancer patients with high HRG but low level of HER2 expression. As more than 70% of the breast cancer patients are HER2+ [108], MM-121 has the potential to target a large population of breast cancer patients as well as other cancer types if the clinical trial validates the use of HRG/HER2+ as a biomarker.

RG7116 (lumretuzumab, RO-5479599)
RG7116 is a glyco-engineered humanized mAb by Roche and is designed to inhibit the activation and signaling of HER3 [109]. RG7116 engages the immune system when bound to tumor cells and elicits enhanced antibody-dependent cellular cytotoxicity (ADCC) [109]. There are three ongoing clinical trials of RG7116 targeting NSCLC, breast cancer, and other solid tumors. In a Phase 1b/2 trial targeting first line metastatic NSCLC of squamous histology, RG7116 is investigated in combination with carboplatin and paclitaxel with overall response rate (ORR) as the primary endpoint [88]. RG7116 is being investigated in combination with pertuzumab and paclitaxel in a Phase 1 trial targeting HER2+/HER3+ metastatic breast cancer [89]. RG7116 is also being investigated in combination with cetuximab or erlotinib in another Phase 1 trial targeting HER3+ solid tumors.

LJM716
LJM716 is a human anti-HER3 IgG1 antibody that is being developed by Novartis. LJM716 is selective for an epitope on domains II and IV of the HER3 extracellular domain (ECD) [91,92] and locks HER3 in an inactive conformation, preventing both ligand-dependent and ligand-independent activation of HER3 [91]. LJM716 treatment resulted in significant growth inhibition in various xenograft models. Ligand-driven models such as the FaDu xenograft model also showed significant in vivo growth inhibition with LJM716 [91,92]. There are currently four reported clinical trials of LJM716 targeting various cancer types: (i) a Phase 1b/2 study of LJM716 in combination with cetuximab in patients with platinum-pretreated recurrent/metastatic SCCHN [93]; (ii) a Phase 1 study evaluating the safety and tolerability of LJM716 in combination with BYL719 (alpelisib, a PI3Ka inhibitor) and trastuzumab in patients with metastatic HER2+ breast cancer [94]; (iii) a Phase 1 trial in combination with trastuzumab for the treatment of metastatic HER2+ breast cancer or gastric cancer [95]; and (iv) a Phase 1b/2 study of LJM716 in combination with BYL719, compared with taxane or irinotecan in patients with previously treated esophageal squamous cell carcinoma (ESCC) [96].

GSK2849330
GlaxoSmithKline is conducting a 155-patient Phase 1 study of a HER3-targeting mAb (GSK2849330) in HER3+ cancer patients. The dosing is 100 mg/ml administered over a 1-h infusion [97]. In addition, they are...
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<tr>
<th>Compound (Company)</th>
<th>Description</th>
<th>Targeted indication</th>
<th>References</th>
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<tbody>
<tr>
<td>Patritumab (Daichi Sankyo)</td>
<td>Human mAb from XenoMouse. HRG high only or not required</td>
<td>+erlotinib, P3 +trastuzumab, P1b/2, HER2+</td>
<td>[74–80]</td>
</tr>
<tr>
<td>MM-121 (Merrimack)</td>
<td>Targeting high HRG patients only</td>
<td>+chemo, P2 Completed P2</td>
<td>Completed P2, OC +cetuximab or erlotinib, solid tumor, P1</td>
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<tr>
<td>RG7116 (Roche)</td>
<td>High affinity to FcyRIIIa, resulting in ADCC. Ligand dependent</td>
<td>1st line, P1b/2 +pertuzumab, HER2 low, P1</td>
<td>[88–90]</td>
</tr>
<tr>
<td>LJM716 (Novartis)</td>
<td>Lock HER3 in an inactive conformation</td>
<td>HER2*, P1: +tras only; or +BYL719 &amp; tras</td>
<td>[91–96]</td>
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<tr>
<td>GSK2849330 (GlaxoSmithKline)</td>
<td>Immuno Positron Emission Tomography (PET) study</td>
<td>Multiple P1b studies in combination with cetuximab, erlotinib, vemurafenib, or trastuzumab in various solid tumors</td>
<td>[97,98]</td>
</tr>
<tr>
<td>KTN 3379 (Kolltan Pharmaceuticals)</td>
<td>Competes with HRG for the same binding site. Ligand dependent and independent</td>
<td>Solid tumor, P1 completed</td>
<td>[99]</td>
</tr>
<tr>
<td>AV-203 (Aveo Oncology)</td>
<td>Ligand dependent and independent. Neuregulin 1 as biomarker</td>
<td>+cetuximab or erlotinib, P1 completed</td>
<td>[100,101]</td>
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<tr>
<td>REGN1400 (Regeneron)</td>
<td>Ligand dependent</td>
<td></td>
<td>[102,103]</td>
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<tr>
<td>MM-111 (Merrimack)</td>
<td>Bi-specific mAb with HER2 as docking arm and HER3 as a therapeutic. HRG+ patients</td>
<td>Completed P1</td>
<td>Completed a P1 for solid tumors</td>
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<tr>
<td>MM-141 (Merrimack)</td>
<td>Bi-specific against both IGF-1R and HER3 inhibiting PI3K/AKT/mTOR</td>
<td>1st line pancreatic cancer, P2</td>
<td>[62,104]</td>
</tr>
<tr>
<td>RG7597 (Roche)</td>
<td>DAF inhibiting both EGF and HER3; ADCC</td>
<td>+cobimetinib, KRAS-mutation + tumors, P1</td>
<td>[65–68,105,106]</td>
</tr>
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BC, breast cancer; GC, gastric cancer; OC, ovarian cancer; H&NC, head and neck cancer; P, Phase; tras, trastuzumab.
also conducting an imaging study in 15–20 patients with HER3+ solid tumors to characterize the distribution of GSK2849330 [98].

**KTN3379**

Kolltan’s KTN3379 is an anti-HER3 mAb with a dual mechanism of action that blocks activity of HER3 when activated by HRG or by other receptor tyrosine kinase such as HER2 in the absence of HRG. The mechanism of KTN3379 is distinct from other antibodies in that it blocks HER3 activation by HRG by directly competing for the same binding site [110]. Kolltan has an ongoing Phase 1b study evaluating KTN3379 in combination with each of four targeted therapies approved for advanced lung, colorectal, breast, melanoma, and H&NC [99]. In May 2015, Kolltan reported the interim results that demonstrated good tolerability and showed early signs of activity (stable disease) in combination with other targeted agents. In addition, according to Kolltan, Pharmacodynamic biomarker analyses showed that soluble circulating HER3 levels were increased in all patients at all doses, indicating that KTN3379 binds HER3, and is not influenced by combination treatment [110].

**AV-203**

AV-203 is a HER3-targeting IgG1 mAb designed to inhibit both ligand-dependent and ligand-independent HER3 signaling [100]. AV-203 showed preclinical activity in a number of different tumor models including breast, head and neck, lung, ovarian, and pancreatic cancers [100]. AVEO has completed a Phase 1 safety study showing no dose-limiting toxicities at maximum dose of 20 mg/kg and CLIA (Clinical Laboratory Improvements Amendment) validation has been completed for a biomarker for potential patient selection [101].

**REGN1400**

The HER3-targeting antibody REGN1400 was generated by immunizing the VelocImmune mice with the HER3 ECD protein. The antibody potently blocked the binding of HRG1 to HER3 with an IC₅₀ of 0.14 nm [102]. A Phase 1 study sponsored by Regeneron was completed in January 2015. It was an open-label, multicenter, ascending multiple dose study of REGN1400 alone and in combination with erlotinib or cetuximab administered to patients with certain unresectable or metastatic types of cancer [103].

**MM-111**

Merrimack’s MM-111 is a bi-specific mAb that forms trimeric complex with HER2 and HER3. The HER2 arm is responsible for initial tumor cell targeting and docking, while the HER3 arm is designed to block HRG-induced cell signaling [63,64]. MM-111 is designed to allow the specific inhibition of HER3 signaling in cancer cells that have elevated HER2 expression, a subtype representing a large population of gastric and breast cancers. Additionally, opportunities exist to combine MM-111 with other HER2-targeted therapies, such as trastuzumab and lapatinib, where MM-111 could function to prevent the development of HER3-mediated resistance to these therapies. There is a strong rationale for this mechanism, given the success of pertuzumab, which prevents HER2 and HER3 dimerization [111]. Two Phase 1 clinical trials have been conducted for MM-111. One was in combination with trastuzumab in patients with advanced HER2 amplified, HRG-positive breast cancer [112]. The other Phase 1 clinical trial was in patients with advanced, refractory HER2 amplified, HRG-positive solid tumors [113]. MM-111 was also in a Phase 2 clinical trial in combination with paclitaxel and trastuzumab for patients with HER2+...
cancers of the distal esophagus, gastroesophageal junction, and stomach [114]. However, this Phase 2 study is currently on hold.

MM-141

MM-141 is a tetravalent bi-specific antibody developed by Merrimack targeting HER3 and IGF-1R [62]. Both IGF-1R and HER3 are receptors on the surface of tumor cells that can drive tumor growth and are commonly co-expressed in solid tumors. Previous studies indicated an aberrant activation of IGF-1R in many cancers associated with HER-targeted therapies [115]. Since the HER3 pathway can compensate for IGF-1R inhibition, it is believed that a combined inhibition of both IGF-1R and HER3 is required to inhibit tumor growth [116]. MM-141 has been shown to block the binding of HRG to HER3 and IGF-1/2 binding to IGF-1R, causing inhibition of PI3K/AKT/mTOR pro-survival signaling in preclinical cancer models [62]. MM-141 has also been proven to inhibit pancreatic tumor cell growth and potentiates the effect of gemcitabine in various preclinical models [62]. A multi-arm Phase 1 study showed an acceptable safety profile for MM-141 as both a monotherapy and in combination with everolimus or with nab-paclitaxel and gemcitabine in patients with advanced solid tumors [117]. The patients who had elevated pre-treatment IGF levels seemed to have more potential benefit with the treatment of MM-141 [116].

Merrimack has just initiated a Phase 2 front line study examining MM-141 in combination with nab-paclitaxel and gemcitabine in patients with metastatic pancreatic cancer who have high serum levels of free IGF-1 [118]. Eligible patients for the trial must have received no other IGF-1 [118]. The patients who had elevated pre-treatment IGF levels seemed to have more potential benefit with the treatment of MM-141 [116].

Merrimack has just initiated a Phase 2 front line study examining MM-141 in combination with nab-paclitaxel and gemcitabine in patients with metastatic pancreatic cancer who have high serum levels of free IGF-1 [118]. Eligible patients for the trial must have received no prior radiotherapy, surgery, chemotherapy, or investigational therapy for the treatment of metastatic disease. The primary endpoint of the trial is PFS. Merrimack plans to conduct the trial at multiple sites in the United States, Canada, and Europe.

RG7597 (dulogotuzumab)

Roche’s RG7597 is a phage-derived human IgG1 mAb with a DAF targeting EGFR and HER3. RG7597 was examined in a number of xenograft models with significant efficacy [65–67]. Furthermore, a recent study in triple-negative breast cancer (TNBC) showed that by antagonizing EGFR and HER3 using RG7597, an enhanced response to PI3K inhibitor (GDC-0941) and AKT inhibitor (GDC-0068) was observed [68]. These observations emphasized that the concomitant blockade of EGFR, PI3K, and AKT pathway should be investigated in the clinic. This antibody has also entered Phase 1 and 2 trials for H&NC and metastatic CRC [105,106].

Other Anti-HER3 Approaches for Cancer Therapy

Sym013 (Pan-HER)

Sym013 is a preclinical candidate in Symphogen’s oncology pipeline. Sym013 is a mixture of six humanized mAbs targeting EGFR, HER2, and HER3, which is designed to induce simultaneous down-modulation of all three targets and prevents compensatory receptor up-regulation [119]. This simultaneous targeting of all three receptors has been demonstrated in animal models to have better efficacy than targeting of a single receptor or any combination of two receptors in the HER family. Symphogen has recently stated that they plan to initiate a Phase 1 study in 2015.

Ligand traps

It is well established that targeting one receptor such as EGFR develops resistance to the single therapies by activation of compensatory receptors such as HER3 [3,4]. A HER ligand-binding molecule that sequesters multiple ligands for multiple receptors may circumvent these limitations. RB200 is a bi-specific ligand trap that is composed of full-length ECDs of EGFR and HER3, and Fc-mediated heterodimer of native EGFR and HER3 ligand-binding domains. RB200 can bind to EGFR ligands, including transforming growth factor-α and heparin-binding EGF, and HER3 ligands HRG1-α and HRG1-β. It inhibits cancer cell proliferation in vitro, and suppresses tumor growth and metastases in mouse xenograft models [70,71]. Despite a promising approach, its use as a therapeutic has not been validated in the clinic.

Peptide vaccines

In an attempt to develop a peptide vaccine that can induce the HER3 neutralizing antibodies, Miller et al. [69] evaluated HER3 peptide epitopes encompassing residues 99–122, 140–162, 237–269, and 461–479 of the HER3 ECD as putative B-cell epitopes for active immunotherapy against HER3-positive cancers. The results showed that the HER3 vaccine antibodies and HER3 peptide mimics induced antitumor responses: inhibition of cancer cell proliferation, inhibition of receptor phosphorylation, and induction of apoptosis and antibody-dependent cell-mediated cytotoxicity (ADCC) [69]. Two of the HER3 epitopes 237–269 (domain II) and 461–479 (domain III) significantly inhibited growth of xenografts originating from both pancreatic (BxPC3) and breast (JIMT-1) cancers [69]. These studies are still in early preclinical stages, but a peptide vaccine can stimulate the patients’ own immune system to develop high affinity antibodies targeting oncoproteins such as HER3, which represents a promising approach for cancer therapy [120].

Antisense oligonucleotides

Since the cytoplasmic tail of HER3 can be phosphorylated and thereby hyper-activated by other growth factors [4], HER3 antibodies may not be effective in all patients. Resistance to these mAb agents is also highly likely. In addition, isoforms of HER2 that are devoid of the ECD and expressed in many breast cancers have been documented [121], and thus may prohibit the use of antibodies that target HER2 or HER2/3 heterodimers. Therefore, an RNA antagonist to HER3 offers a unique solution to control HER3-mediated tumor growth. EZN-3920, a locked nucleic acid (LNA)-based HER3 antisense oligonucleotide, specifically down-modulated the expression of HER3, HER3-driven PI3K/AKT signaling pathway, and growth in tumors derived from BT474/M1 breast and HCC827 lung carcinoma cell lines [72]. Furthermore, co-administration of EZN-3920 with gefitinib or lapatinib enhanced antitumor activity compared with the effect of the monotherapy [72]. More importantly, EZN-3920 sustained its anti-proliferative effect in trastuzumab-resistant cells and three independently derived gefitinib-resistant cells [72]. Although RNAi therapeutics offers a promising drug modality for cancer and other diseases, the lack of efficient delivery systems hampers its applications in the clinic [122].

Small molecules targeting the pseudokinase HER3

A recent study showed that a selective small molecule HER3 ligand, TX1-85-1, forms a covalent bond with Cys721 located in the ATP-binding site of HER3 [73]. Subsequent derivatization with a hydrophobic adamantane moiety demonstrates that the resultant bivalent ligand (TX2-121-1) enhances the inhibition of HER3-dependent signaling. Treatment of cells with TX2-121-1 results in partial degradation of HER3 and serendipitously interferes with productive heterodimerization of HER3 with either HER2 or c-Met. These results
suggested that small molecules may also be capable of perturbing the biological function of HER3. Further studies are needed to validate this strategy for the development of HER3-targeting therapeutics [73].

**Perspective**

Drug resistance is a major challenge in cancer treatment, which limits clinical efficacy of many molecular targeted therapies including mAbs targeting HER family receptors such as EGFR and HER2. Both basic and clinical evidence indicate that HER3 plays an important role in the overall HER signaling pathway and in drug resistance. Currently, there are no marketed HER3-targeting therapies and clinical development of HER3 therapeutics is progressing slowly due to the lack of biomarkers. Recent clinical studies from experimental HER3-targeting antibodies such as MM-121 revealed that targeting HRG-positive tumors sensitized patients to TKI and chemotherapeutic drugs, and significantly lowered the risk of tumor progression. These encouraging clinical studies indicate that HRG is a promising candidate to serve as a responding biomarker for anti-HER3 mAbs such as MM-121. There are several promising HER3 antibodies in the clinical trials, and greater clinical benefits may be attained by combining the HER3 antibodies with other cancer-targeting antibody and/or small molecule TKIs. More studies are needed to investigate other potential biomarkers and to optimize combination strategies. Finally, ongoing clinical studies of bi-specific antibodies such as MM-111, RG7597, and MM-141 are promising and should demonstrate the capability to simultaneously target HER3 and other TKR partners such as HER2, EGFR, and IGF-1R.

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