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Review

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 cMET and HER3 [4,19–24]. 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

Overexpression of HER3 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

binding, (iv) preventing dimerization with other HER family members, (v) triggering internalization, and (vi) engaging the immune system for cancer cell killing. In addition to using mono-specific mAbs to target HER3 [61], other modalities are being developed such as bi-specific antibodies [62–64], mAbs with dual action Fab (DAF) [65–68], anti-HER3 vaccines [69], bi-specific ligand traps for EGFR and HER3 [70,71], HER3-locked nucleic acid-based RNA inhibitors [72], and small molecule inhibitors targeting the pseudokinase of HER3 [73]. Among all possible modalities, almost all of the HER3-targeting therapies in clinical development belong to the antibody class. A partial list of these therapies is described below and summarized in **Table 1** and **Fig. 1**.

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 HRG 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 HRG biomarker positive patients compared with control patients [78].

Patritumab 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 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 chemotherapies 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

Table 1. HER3-targeting antibodies in clinical development for cancer therapy

Compound (Company)	Description	Targeted indication				References
		NSCLC	BC	GC	Other cancers	
Patritumab (Daiichi Sankyo)	Human mAb from XenoMouse. HRG high only or not required	+erlotinib, P3	+trastuzumab, P1b/2, HER2+		+cetuximab, H&NC, P1	[74–80]
MM-121 (Merrimack)	Targeting high HRG patients only	+chemo, P2	Completed P2		Completed P2, OC	[61,81–87]
RG7116 (Roche)	High affinity to FcγRIIIa, resulting in ADCC. Ligand dependent	1st line, P1b/2	+pertuzumab, HER2 low, P1		+cetuximab or erlotinib, solid tumor, P1	[88–90]
LJM716 (Novartis)	Lock HER3 in an inactive conformation		HER2$^+$, P1: +tras only; or +BYL719 & tras	HER2$^+$, P1: +tras	P1/2: +cetuximab, H&NC; +BYL719, esophageal cancer	[91–96]
GSK2849330 (GlaxoSmithKline)	Immuno Positron Emission Tomography (PET) study				HER3$^+$ solid tumor, P1	[97,98]
KTN 3379 (Kolltan Pharmaceuticals)	Competes with HRG for the same binding site. Ligand dependent and independent	Multiple P1b studies in combination with cetuximab, erlotinib, vemurafenib, or trastuzumab in various solid tumors				[99]
AV-203 (Aveo Oncology)	Ligand dependent and independent. Neuregulin 1 as biomarker				Solid tumor, P1 completed	[100,101]
REGN1400 (Regeneron)	Ligand dependent				+cetuximab or erlotinib, P1 completed	[102,103]
MM-111 (Merrimack)	Bi-specific mAb with HER2 as docking arm and HER3 as a therapeutic. HRG$^+$ patients		Completed P1	+trastuzumab P2	Completed a P1 for solid tumors	[63,64]
MM-141 (Merrimack)	Bi-specific against both IGF-1R and HER3 inhibiting PI3K/AKT/mTOR				1st line pancreatic cancer, P2	[62,104]
RG7597 (Roche)	DAF inhibiting both EGFR and HER3; ADCC				+cobimetinib, KRAS-mutation + tumors, P1	[65–68,105,106]

BC, breast cancer; GC, gastric cancer; OC, ovarian cancer; H&NC, head and neck cancer; P, Phase; tras, trastuzumab.

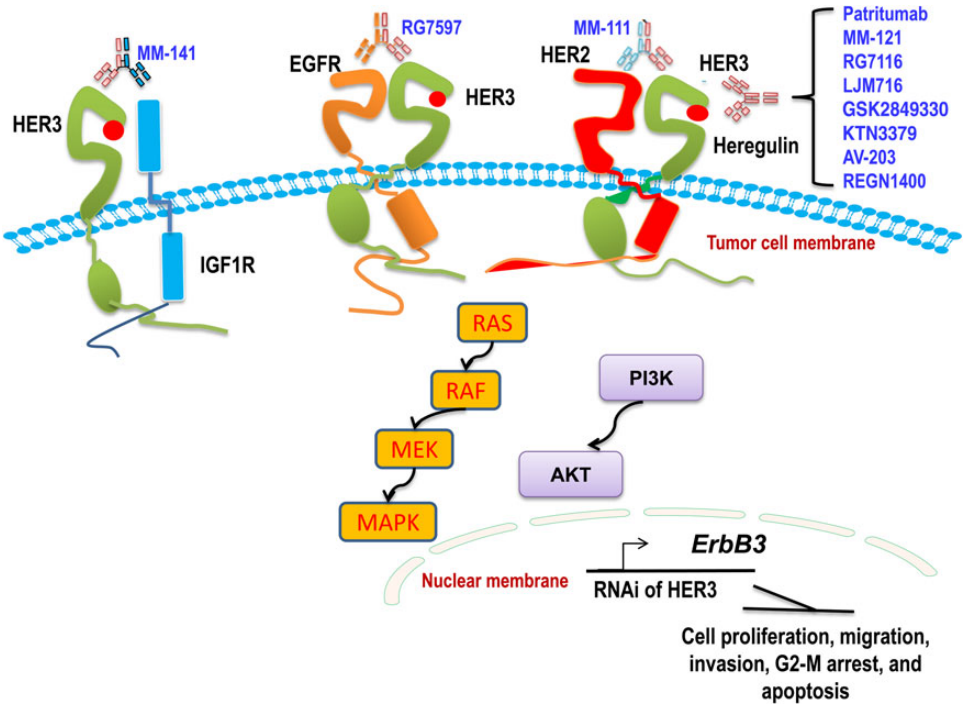

Figure 1. Key HER3 hetero-dimerization partners, signaling pathways, and anti-HER3 mAbs in clinical development for the treatment of various solid cancers
Eleven different mAbs that bind at various domains of HER3 to block HER3 signaling, cancer cell proliferation, migration, and invasion are currently in various stages of clinical trials. These anti-HER3 mAbs are being developed using one or the combination of the following mechanisms: blocking ligand HRG binding to HER3; locking HER3 in an inactive conformation; preventing dimerization with other HER or RTK family members; triggering HER3 internalization, and engaging the immune system for cancer cell killing. In addition to mono-specific mAbs to target HER3, other modalities are being developed such as bi-specific mAbs and mAbs with dual actions.

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 IC_{50} 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 I 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$^+$

carcinomas 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 potentiate 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 benefits 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 (duligotuzumab)

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-β3. 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 oncogenes 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 BT474M1 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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References

1. Tebbutt N, Pedersen MW, Johns TG. Targeting the ERBB family in cancer: couples therapy. *Nat Rev Cancer* 2013, 13: 663–673.
2. Lee-Hoeflich ST, Crocker L, Yao E, Pham T, Munroe X, Hoeflich KP, Sliwkowski MX, *et al*. A central role for HER3 in HER2-amplified breast cancer: implications for targeted therapy. *Cancer Res* 2008, 68: 5878–5887.
3. Sergina NV, Rausch M, Wang D, Blair J, Hann B, Shokat KM, Moasser MM. Escape from HER-family tyrosine kinase inhibitor therapy by the kinase-inactive HER3. *Nature* 2007, 445: 437–441.
4. Engelman JA, Zejnullahu K, Mitsudomi T, Song Y, Hyland C, Park JO, Lindeman N, *et al*. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. *Science* 2007, 316: 1039–1043.
5. Sordella R, Bell DW, Haber DA, Settleman J. Gefitinib-sensitizing EGFR mutations in lung cancer activate anti-apoptotic pathways. *Science* 2004, 305: 1163–1167.
6. Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S, Herman P, *et al*. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* 2004, 304: 1497–1500.
7. Casalini P, Iorio MV, Galmozzi E, Menard S. Role of HER receptors family in development and differentiation. *J Cell Physiol* 2004, 200: 343–350.
8. Lee KF, Simon H, Chen H, Bates B, Hung MC, Hauser C. Requirement for neuregulin receptor erbB2 in neural and cardiac development. *Nature* 1995, 378: 394–398.
9. Albanell J, Baselga J. Trastuzumab, a humanized anti-HER2 monoclonal antibody, for the treatment of breast cancer. *Drugs Today (Barc)* 1999, 35: 931–946.
10. Amann J, Kalyankrishna S, Massion PP, Ohm JE, Girard L, Shigematsu H, Peyton M, *et al*. Aberrant epidermal growth factor receptor signaling and enhanced sensitivity to EGFR inhibitors in lung cancer. *Cancer Res* 2005, 65: 226–235.
11. Rusnak DW, Alligood KJ, Mullin RJ, Spehar GM, Arenas-Elliott C, Martin AM, Degenhardt Y, *et al*. Assessment of epidermal growth factor receptor (EGFR, ErbB1) and HER2 (ErbB2) protein expression levels and response to lapatinib (Tykerb, GW572016) in an expanded panel of human normal and tumour cell lines. *Cell Prolif* 2007, 40: 580–594.
12. Valentini AM, Pirrelli M, Caruso ML. EGFR-targeted therapy in colorectal cancer: does immunohistochemistry deserve a role in predicting the response to cetuximab? *Curr Opin Mol Ther* 2008, 10: 124–131.
13. Friess T, Scheuer W, Hasmann M. Combination treatment with erlotinib and pertuzumab against human tumor xenografts is superior to monotherapy. *Clin Cancer Res* 2005, 11: 5300–5309.
14. Easley C, Kirkpatrick P. Panitumumab. *Nat Rev Drug Discov* 2006, 5: 987–988.
15. Chung A, Cui X, Audeh W, Giuliano A. Current status of anti-human epidermal growth factor receptor 2 therapies: predicting and overcoming herceptin resistance. *Clin Breast Cancer* 2013, 13: 223–232.
16. Hudis CA. Trastuzumab-mechanism of action and use in clinical practice. *N Engl J Med* 2007, 357: 39–51.
17. Petrelli F, Borgonovo K, Cabiddu M, Ghilardi M, Barni S. Cetuximab and panitumumab in KRAS wild-type colorectal cancer: a meta-analysis. *Int J Colorectal Dis* 2011, 26: 823–833.
18. Paliga A, Onerheim R, Gologan A, Chong G, Spatz A, Niazi T, Garant A, *et al*. EGFR and K-ras gene mutation status in squamous cell anal carcinoma: a role for concurrent radiation and EGFR inhibitors? *Br J Cancer* 2012, 107: 1864–1868.
19. Wheeler DL, Huang S, Kruser TJ, Nechrebecki MM, Armstrong EA, Benavente S, Gondi V, *et al*. Mechanisms of acquired resistance to cetuximab: role of HER (ErbB) family members. *Oncogene* 2008, 27: 3944–3956.
20. Frattini M, Saletti P, Romagnani E, Martin V, Molinari F, Ghisletta M, Camponovo A, *et al*. PTEN loss of expression predicts cetuximab efficacy in metastatic colorectal cancer patients. *Br J Cancer* 2007, 97: 1139–1145.
21. Narayan M, Wilken JA, Harris LN, Baron AT, Kimbler KD, Maihle NJ. Trastuzumab-induced HER reprogramming in "resistant" breast carcinoma cells. *Cancer Res* 2009, 69: 2191–2194.
22. Shattuck DL, Miller JK, Carraway KL III, Sweeney C. Met receptor contributes to trastuzumab resistance of Her2-overexpressing breast cancer cells. *Cancer Res* 2008, 68: 1471–1477.
23. Nagata Y, Lan KH, Zhou X, Tan M, Esteva FJ, Sahin AA, Klos KS, *et al*. PTEN activation contributes to tumor inhibition by trastuzumab, and loss of PTEN predicts trastuzumab resistance in patients. *Cancer Cell* 2004, 6: 117–127.
24. Kong A, Calleja V, Leboucher P, Harris A, Parker PJ, Larijani B. HER2 oncogenic function escapes EGFR tyrosine kinase inhibitors via activation of alternative HER receptors in breast cancer cells. *PLoS One* 2008, 3: e2881.
25. Sierke SL, Cheng K, Kim HH, Koland JG. Biochemical characterization of the protein tyrosine kinase homology domain of the ErbB3 (HER3) receptor protein. *Biochem J* 1997, 322 (Pt 3): 757–763.
26. Berger MB, Mendrola JM, Lemmon MA. ErbB3/HER3 does not homodimerize upon neuregulin binding at the cell surface. *FEBS Lett* 2004, 569: 332–336.
27. Suenaga A, Takada N, Hatakeyama M, Ichikawa M, Yu X, Tomii K, Okimoto N, *et al*. Novel mechanism of interaction of p85 subunit of phosphatidylinositol 3-kinase and ErbB3 receptor-derived phosphotyrosyl peptides. *J Biol Chem* 2005, 280: 1321–1326.
28. Mattoon DR, Lamothe B, Lax I, Schlessinger J. The docking protein Gab1 is the primary mediator of EGF-stimulated activation of the PI-3K/Akt cell survival pathway. *BMC Biol* 2004, 2: 24.
29. Reschke M, Mihic-Probst D, van der Horst EH, Knyazev P, Wild PJ, Hutterer M, Meyer S, *et al*. HER3 is a determinant for poor prognosis in melanoma. *Clin Cancer Res* 2008, 14: 5188–5197.

30. Frolov A, Schuller K, Tzeng CW, Cannon EE, Ku BC, Howard JH, Vickers SM, *et al*. ErbB3 expression and dimerization with EGFR influence pancreatic cancer cell sensitivity to erlotinib. *Cancer Biol Ther* 2007, 6: 548–554.
31. van der Horst EH, Murgia M, Treder M, Ullrich A. Anti-HER-3 MAbs inhibit HER-3-mediated signaling in breast cancer cell lines resistant to anti-HER-2 antibodies. *Int J Cancer* 2005, 115: 519–527.
32. Jiang X, Borgesi RA, McKnight NC, Kaur R, Carpenter CL, Balk SP. Activation of nonreceptor tyrosine kinase Bmx/Etk mediated by phosphoinositide 3-kinase, epidermal growth factor receptor, and ErbB3 in prostate cancer cells. *J Biol Chem* 2007, 282: 32689–32698.
33. Nagumo Y, Faratian D, Mullen P, Harrison DJ, Hasmann M, Langdon SP. Modulation of HER3 is a marker of dynamic cell signaling in ovarian cancer: implications for pertuzumab sensitivity. *Mol Cancer Res* 2009, 7: 1563–1571.
34. Yoon YK, Kim HP, Han SW, Hur HS, Oh do Y, Im SA, Bang YJ, *et al*. Combination of EGFR and MEK1/2 inhibitor shows synergistic effects by suppressing EGFR/HER3-dependent AKT activation in human gastric cancer cells. *Mol Cancer Ther* 2009, 8: 2526–2536.
35. Choi B-K, Fan X, Deng H, Zhang N, An Z. HER3 (ERBB3) is a key sensor in the regulation of ERBB-mediated signaling in both low and high HER2 (ERBB2) expressing cancer cells. *Cancer Med* 2012, 1: 28–38.
36. Jaiswal BS, Kljavin NM, Stawiski EW, Chan E, Parikh C, Durinck S, Chaudhuri S, *et al*. Oncogenic ERBB3 mutations in human cancers. *Cancer Cell* 2013, 23: 603–617.
37. Jeong EG, Soung YH, Lee JW, Lee SH, Nam SW, Lee JY, Yoo NJ, *et al*. ERBB3 kinase domain mutations are rare in lung, breast and colon carcinomas. *Int J Cancer* 2006, 119: 2986–2987.
38. Baselga J, Swain SM. Novel anticancer targets: revisiting ERBB2 and discovering ERBB3. *Nat Rev Cancer* 2009, 9: 463–475.
39. Aurisicchio L, Marra E, Roscilli G, Mancini R, Ciliberto G. The promise of anti-ErbB3 monoclonals as new cancer therapeutics. *Oncotarget* 2012, 3: 744–758.
40. Campbell MR, Amin D, Moasser MM. HER3 comes of age: new insights into its functions and role in signaling, tumor biology, and cancer therapy. *Clin Cancer Res* 2010, 16: 1373–1383.
41. Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. *Nat Rev Mol Cell Biol* 2001, 2: 127–137.
42. Yarden Y, Pines G. The ERBB network: at last, cancer therapy meets systems biology. *Nat Rev Cancer* 2012, 12: 553–563.
43. Mendelsohn J, Baselga J. Status of epidermal growth factor receptor antagonists in the biology and treatment of cancer. *J Clin Oncol* 2003, 21: 2787–2799.
44. Holbro T, Civenni G, Hynes NE. The ErbB receptors and their role in cancer progression. *Exp Cell Res* 2003, 284: 99–110.
45. Olayioye MA, Neve RM, Lane HA, Hynes NE. The ErbB signaling network: receptor heterodimerization in development and cancer. *EMBO J* 2000, 19: 3159–3167.
46. Burgess AW, Cho HS, Eigenbrot C, Ferguson KM, Garrett TP, Leahy DJ, Lemmon MA, *et al*. An open-and-shut case? Recent insights into the activation of EGF/ErbB receptors. *Mol Cell* 2003, 12: 541–552.
47. Carraway KL III. E3 ubiquitin ligases in ErbB receptor quantity control. *Semin Cell Dev Biol* 2010, 21: 936–943.
48. Sithanandam G, Anderson LM. The ERBB3 receptor in cancer and cancer gene therapy. *Cancer Gene Ther* 2008, 15: 413–448.
49. Amin DN, Sergina N, Ahuja D, McMahon M, Blair JA, Wang D, Hann B, *et al*. Resiliency and vulnerability in the HER2-HER3 tumorigenic driver. *Sci Transl Med* 2010, 2: 16ra17.
50. Gala K, Chandarlapaty S. Molecular pathways: HER3 targeted therapy. *Clin Cancer Res* 2014, 20: 1410–1416.
51. Kol A, Terwisscha van Scheltinga AG, Timmer-Bosscha H, Lamberts LE, Bensch F, de Vries EG, Schroder CP. HER3, serious partner in crime: therapeutic approaches and potential biomarkers for effect of HER3-targeting. *Pharmacol Therapeut* 2014, 143: 1–11.
52. Mujoo K, Choi BK, Huang Z, Zhang N, An Z. Regulation of ERBB3/HER3 signaling in cancer. *Oncotarget* 2014, 5: 10222–10236.
53. Macbeath G, Adiwijaya B, Liu J, Sequist LV, Pujade-Lauraine E, Higgins M, Tabah-Fisch I, *et al*. A meta-analysis of biomarkers in three randomized, Phase 2 studies of MM-121, a ligand-blocking anti-erbB3 antibody, in patients with ovarian, lung, and breast cancers. *Annal Oncol* 2014, 25 (suppl_4): iv58–iv84.
54. Naidu R, Yadav M, Nair S, Kutty MK. Expression of c-erbB3 protein in primary breast carcinomas. *Br J Cancer* 1998, 78: 1385–1390.
55. Lemoine NR, Barnes DM, Hollywood DP, Hughes CM, Smith P, Dublin E, Prigent SA, *et al*. Expression of the ERBB3 gene product in breast cancer. *Br J Cancer* 1992, 66: 1116–1121.
56. Witton CJ, Reeves JR, Going JJ, Cooke TG, Bartlett JM. Expression of the HER1-4 family of receptor tyrosine kinases in breast cancer. *J Pathol* 2003, 200: 290–297.
57. Choi BK, Cai X, Yuan B, Huang Z, Fan X, Deng H, Zhang N, *et al*. HER3 intracellular domains play a crucial role in HER3/HER2 dimerization and activation of downstream signaling pathways. *Protein Cell* 2012, 3: 781–789.
58. Chen L, Siddiqui S, Bose S, Mooso B, Asuncion A, Bedolla RG, Vinall R, *et al*. Nrdp1-mediated regulation of ErbB3 expression by the androgen receptor in androgen-dependent but not castrate-resistant prostate cancer cells. *Cancer Res* 2010, 70: 5994–6003.
59. Qiu XB, Goldberg AL. Nrdp1/FLRF is a ubiquitin ligase promoting ubiquitination and degradation of the epidermal growth factor receptor family member, ErbB3. *Proc Natl Acad Sci USA* 2002, 99: 14843–14848.
60. Huang Z, Choi BK, Mujoo K, Fan X, Fa M, Mukherjee S, Owiti N, *et al*. The E3 ubiquitin ligase NEDD4 negatively regulates HER3/ErbB3 level and signaling. *Oncogene* 2014, 56: 1–11.
61. Schoeberl B, Faber AC, Li D, Liang MC, Crosby K, Onsum M, Burenkova O, *et al*. An ErbB3 antibody, MM-121, is active in cancers with ligand-dependent activation. *Cancer Res* 2010, 70: 2485–2494.
62. Fitzgerald JB, Johnson BW, Baum J, Adams S, Iadevaia S, Tang J, Rimkunas V, *et al*. MM-141, an IGF-IR- and ErbB3-directed bispecific antibody, overcomes network adaptations that limit activity of IGF-IR inhibitors. *Mol Cancer Ther* 2014, 13: 410–425.
63. McDonagh CF, Huhalov A, Harms BD, Adams S, Paragas V, Oyama S, Zhang B, *et al*. Antitumor activity of a novel bispecific antibody that targets the ErbB2/ErbB3 oncogenic unit and inhibits heregulin-induced activation of ErbB3. *Mol Cancer Ther* 2012, 11: 582–593.
64. Kirouac DC, Du JY, Lahdenranta J, Overland R, Yarar D, Paragas V, Pace E, *et al*. Computational modeling of ERBB2-amplified breast cancer identifies combined ErbB2/3 blockade as superior to the combination of MEK and AKT inhibitors. *Sci Signal* 2013, 6: ra68.
65. Schaefer G, Haber L, Crocker LM, Shia S, Shao L, Dowbenko D, Totpal K, *et al*. A two-in-one antibody against HER3 and EGFR has superior inhibitory activity compared with monospecific antibodies. *Cancer Cell* 2011, 20: 472–486.
66. Kamath AV, Lu D, Gupta P, Jin D, Xiang H, Wong A, Leddy C, *et al*. Preclinical pharmacokinetics of MEHD7945A, a novel EGFR/HER3 dual-action antibody, and prediction of its human pharmacokinetics and efficacious clinical dose. *Cancer Chemother Pharmacol* 2012, 69: 1063–1069.
67. Huang S, Li C, Armstrong EA, Peet CR, Saker J, Amler LC, Sliwkowski MX, *et al*. Dual targeting of EGFR and HER3 with MEHD7945A overcomes acquired resistance to EGFR inhibitors and radiation. *Cancer Res* 2013, 73: 824–833.
68. Tao JJ, Castel P, Radosevic-Robin N, Elkabets M, Auricchio N, Aceto N, Weitsman G, *et al*. Antagonism of EGFR and HER3 enhances the response to inhibitors of the PI3K-Akt pathway in triple-negative breast cancer. *Sci Signal* 2014, 7: ra29.
69. Miller MJ, Foy KC, Overholser JP, Nahta R, Kaumaya PT. HER-3 peptide vaccines/mimics: combined therapy with IGF-1R, HER-2, and HER-1 peptides induces synergistic antitumor effects against breast and pancreatic cancer cells. *Oncoimmunol* 2014, 3: e956012.
70. Huang Z, Brdlik C, Jin P, Shepard HM. A pan-HER approach for cancer therapy: background, current status and future development. *Expert Opin Biol Ther* 2009, 9: 97–110.

71. Sarup J, Jin P, Turin L, Bai X, Beryt M, Brdlik C, Higaki JN, *et al*. Human epidermal growth factor receptor (HER-1:HER-3) Fc-mediated heterodimer has broad antiproliferative activity *in vitro* and in human tumor xenografts. *Mol Cancer Ther* 2008, 7: 3223–3236.
72. Wu Y, Zhang Y, Wang M, Li Q, Qu Z, Shi V, Kraft P, *et al*. Downregulation of HER3 by a novel antisense oligonucleotide, EZN-3920, improves the antitumor activity of EGFR and HER2 tyrosine kinase inhibitors in animal models. *Mol Cancer Ther* 2013, 12: 427–437.
73. Xie T, Lim SM, Westover KD, Dodge ME, Ercan D, Ficarro SB, Udayakumar D, *et al*. Pharmacological targeting of the pseudokinase Her3. *Nat Chem Biol* 2014, 10: 1006–1012.
74. Wakui H, Yamamoto N, Nakamichi S, Tamura Y, Nokihara H, Yamada Y, Tamura T. Phase 1 and dose-finding study of patritumab (U3-1287), a human monoclonal antibody targeting HER3, in Japanese patients with advanced solid tumors. *Cancer Chemother Pharmacol* 2014, 73: 511–516.
75. Nishio M, Horiike A, Murakami H, Yamamoto N, Kaneda H, Nakagawa K, Horinouchi H, *et al*. Phase I study of the HER3-targeted antibody patritumab (U3-1287) combined with erlotinib in Japanese patients with non-small cell lung cancer. *Lung cancer* 2015, 88: 275–281.
76. Yonesaka K, Hirotani K, Kawakami H, Takeda M, Kaneda H, Sakai K, Okamoto I, *et al*. Anti-HER3 monoclonal antibody patritumab sensitizes refractory non-small cell lung cancer to the epidermal growth factor receptor inhibitor erlotinib. *Oncogene* 2015, doi: 10.1038/onc.2015.142.
77. Kawakami H, Okamoto I, Yonesaka K, Okamoto K, Shibata K, Shinkai Y, Sakamoto H, *et al*. The anti-HER3 antibody patritumab abrogates cetuximab resistance mediated by heregulin in colorectal cancer cells. *Oncotarget* 2014, 5: 11847–11856.
78. Sankyo D. Study of patritumab in combination with erlotinib in subjects with locally advanced or metastatic non-small cell lung cancer (NSCLC). ClinicalTrialsgov 2015: NCT02134015.
79. Sankyo D. A study using patritumab in combination with cetuximab and a platinum containing therapy for patients with head and neck cancer. ClinicalTrialsgov 2015: NCT02350712.
80. LoRusso P, Janne PA, Oliveira M, Rizvi N, Malburg L, Keedy V, Yee L, *et al*. Phase I study of U3-1287, a fully human anti-HER3 monoclonal antibody, in patients with advanced solid tumors. *Clin Cancer Res* 2013, 19: 3078–3087.
81. Wang S, Huang J, Lyu H, Cai B, Yang X, Li F, Tan J, *et al*. Therapeutic targeting of erbB3 with MM-121/SAR256212 enhances antitumor activity of paclitaxel against erbB2-overexpressing breast cancer. *Breast Cancer Res* 2013, 15: R101.
82. Huang J, Wang S, Lyu H, Cai B, Yang X, Wang J, Liu B. The anti-erbB3 antibody MM-121/SAR256212 in combination with trastuzumab exerts potent antitumor activity against trastuzumab-resistant breast cancer cells. *Mol Cancer* 2013, 12: 134.
83. Jiang N, Wang D, Hu Z, Shin HJ, Qian G, Rahman MA, Zhang H, *et al*. Combination of anti-HER3 antibody MM-121/SAR256212 and cetuximab inhibits tumor growth in preclinical models of head and neck squamous cell carcinoma. *Mol Cancer Ther* 2014, 13: 1826–1836.
84. Merrimack. A study of MM-121 in combination with chemotherapy versus chemotherapy alone in heregulin positive NSCLC. ClinicalTrialsgov 2015: NCT02387216.
85. Schoeberl B, Pace EA, Fitzgerald JB, Harms BD, Xu L, Nie L, Linggi B, *et al*. Therapeutically targeting ErbB3: a key node in ligand-induced activation of the ErbB receptor-PI3K axis. *Sci Signal* 2009, 2: ra31.
86. Merrimack. A study of investigational SAR256212 in combination with SAR245408 in patients with solid tumor cancers. ClinicalTrialsgov 2014: NCT01436565.
87. Merrimack. Phase I safety study of the drug MM-121 in patients with advanced solid tumors resisting ordinary Treatment. ClinicalTrialsgov 2014: NCT00734305.
88. Roche. A study evaluating RO5479599 in combination with carboplatin and paclitaxel in patients with advanced or metastatic non-small cell lung cancer (NSCLC) of squamous histology. ClinicalTrialsgov 2015: NCT02204345.
89. Roche. A study to evaluate RO5479599 in combination with perjeta (Pertuzumab) and paclitaxel in patients with metastatic breast cancer expressing HER3 & HER2 protein. ClinicalTrialsgov 2015: NCT01918254.
90. Roche. A study of RO5479599 alone or in combination with cetuximab or erlotinib in patients with metastatic and/or locally advanced malignant HER3-positive solid tumors. ClinicalTrialsgov 2015: NCT01482377.
91. Garner AP, Bialucha CU, Sprague ER, Garrett JT, Sheng Q, Li S, Sineshchekova O, *et al*. An antibody that locks HER3 in the inactive conformation inhibits tumor growth driven by HER2 or neuregulin. *Cancer Res* 2013, 73: 6024–6035.
92. Garrett JT, Sutton CR, Kurupi R, Bialucha CU, Ettenberg SA, Collins SD, Sheng Q, *et al*. Combination of antibody that inhibits ligand-independent HER3 dimerization and a p110alpha inhibitor potently blocks PI3K signaling and growth of HER2$^+$ breast cancers. *Cancer Res* 2013, 73: 6013–6023.
93. Novartis. Study of efficacy and safety of LJM716 and cetuximab in head and neck squamous cell carcinoma patients. ClinicalTrialsgov 2014: NCT02143622.
94. Novartis. Open-label study evaluating the safety and tolerability of LJM716, BYL719 and trastuzumab in patients with metastatic HER2+ breast cancer. ClinicalTrialsgov 2015: NCT02167854.
95. Novartis. Phase I study LJM716 combined with trastuzumab in patients with HER2 overexpressing metastatic breast or gastric cancer. ClinicalTrialsgov 2015: NCT01602406.
96. Novartis. Study of safety & efficacy of the combination of LJM716 & BYL719 in patients with previously treated esophageal squamous cell carcinoma (ESCC). ClinicalTrialsgov 2015: NCT01822613.
97. GlaxoSmithKline. Dose escalation study to investigate the safety, pharmacokinetics, and pharmacodynamics of GSK2849330 in subjects with advanced Her3-positive solid tumors. ClinicalTrialsgov 2015: NCT01966445.
98. GlaxoSmithKline. Immuno positron emission tomography study of GSK2849330 in subjects with human epidermal growth factor receptor 3-positive solid tumors. ClinicalTrialsgov 2015: NCT02345174.
99. Kolltan. A phase 1 study to evaluate the safety and pharmacokinetics of KTN3379 in adult subjects with advanced tumors. ClinicalTrialsgov 2015: NCT02014909.
100. Meetze K, Vincent S, Tyler S, Mazsa EK, Delpero AR, Bottega S, McIntosh D, *et al*. Neuregulin 1 expression is a predictive biomarker for response to AV-203, an ERBB3 inhibitory antibody, in human tumor models. *Clin Cancer Res* 2015, 21: 1106–1114.
101. AVEO. A phase 1 dose escalation study of AV-203, an ERBB3 inhibitory antibody, in subjects with advanced solid tumors. ClinicalTrialsgov 2015: NCT01603979.
102. Zhang L, Castanaro C, Luan B, Yang K, Fan L, Fairhurst JL, Rafique A, *et al*. ERBB3/HER2 signaling promotes resistance to EGFR blockade in head and neck and colorectal cancer models. *Mol Cancer Ther* 2014, 13: 1345–1355.
103. Regeneron. Study of REGN1400 alone and in combination with erlotinib or cetuximab in patients with certain types of cancer. ClinicalTrialsgov 2015: NCT01727869.
104. Xu L, Kohli N, Rennard R, Jiao Y, Razlog M, Zhang K, Baum J, *et al*. Rapid optimization and prototyping for therapeutic antibody-like molecules. *MAbs* 2013, 5: 237–254.
105. Genentech. A study of MEHD7945A and cobimetinib (GDC-0973) in patients with locally advanced or metastatic cancers with mutant KRAS. ClinicalTrialsgov 2015: NCT01986166.
106. Genentech. A study of MEHD7945A + FOLFIRI versus cetuximab + FOLFIRI in second line in patients with KRAS wild-type metastatic colorectal cancer. ClinicalTrialsgov 2015: NCT01652482.
107. Sankyo D. Phase 1b/2 study of U3-1287 in combination with trastuzumab plus paclitaxel in newly diagnosed metastatic breast cancer (MBC). ClinicalTrialsgov 2015: NCT01512199.
108. Ross JS, Slodkowska EA, Symmans WF, Pusztai L, Ravdin PM, Hortobagyi GN. The HER-2 receptor and breast cancer: ten years of targeted anti-HER-2 therapy and personalized medicine. *Oncologist* 2009, 14: 320–368.

109. Meneses-Lorente G, Friess T, Kolm I, Holzlwimmer G, Bader S, Meille C, Thomas M, *et al*. Preclinical pharmacokinetics, pharmacodynamics, and efficacy of RG7116: a novel humanized, glycoengineered anti-HER3 antibody. *Cancer Chemother Pharmacol* 2015, 75: 837–850.
110. Bauer TM, Infante JR, Eder JP, LoRusso P, LaVallee T, Gedrich R, Sidor C, *et al*. A phase 1, open-label study to evaluate the safety and pharmacokinetics of the anti ErbB3 antibody, KTN3379, alone or in combination with targeted therapies in patients with advanced tumors. *J Clin Oncol* 2015, 33: (suppl: abstr 2598).
111. Lynce F, Swain SM. Pertuzumab for the treatment of breast cancer. *Cancer Invest* 2014, 32: 430–438.
112. Merrimack. MM-111 in combination with herceptin inpatients with advanced Her2 amplified, heregulin positive breast cancer. ClinicalTrialsgov 2014: NCT01097460.
113. Merrimack. A study of MM-111 in patients with advanced, refractory Her2 amplified, heregulin positive cancers (monotherapy). ClinicalTrialsgov 2014: NCT00911898.
114. Merrimack. A study of MM-111 and paclitaxel with trastuzumab in patients HER2 positive carcinomas of the distal esophagus, gastroesophageal (GE) junction and stomach. ClinicalTrialsgov 2015: NCT01774851.
115. Desbois-Mouthon C, Baron A, Blivet-Van Eggelpoel MJ, Fartoux L, Venot C, Bladt F, Housset C, *et al*. Insulin-like growth factor-1 receptor inhibition induces a resistance mechanism via the epidermal growth factor receptor/HER3/AKT signaling pathway: rational basis for cotargeting insulin-like growth factor-1 receptor and epidermal growth factor receptor in hepatocellular carcinoma. *Clin Cancer Res* 2009, 15: 5445–5456.
116. Isakoff SJ, Arnedos M, Soria J-C, Bahleda R, Shields A, LoRusso PM, Saleh M. Pre-clinical characterization and first in human study of MM-141, an antibody inhibitor of IGF-1R and ErbB3. AACR 2015: Abstract #CT237.
117. Merrimack. A phase 1 study of MM-141 in patients with advanced solid tumors. ClinicalTrialsgov 2014: NCT01733004.
118. Merrimack. A phase 2 study of MM-141 plus Nab-paclitaxel and gemcitabine in front-line metastatic pancreatic cancer (CARRIE). ClinicalTrialsgov 2015: NCT02399137.
119. Francis D, Huang S, Werner L, Lantto J, Horak ID, Kragh M, Harari PM. Sym013, novel pan-HER monoclonal antibody mixture, augments radiation response in human lung and head and neck tumors. *Cancer Res* 2014, 74: 4495.
120. Kaumaya PT. Bridging oncology and immunology: expanding horizons with innovative peptide vaccines and peptidomimetics. *Immunotherapy* 2013, 5: 1159–1163.
121. Molina MA, Codony-Servat J, Albanell J, Rojo F, Arribas J, Baselga J. Trastuzumab (herceptin), a humanized anti-Her2 receptor monoclonal antibody, inhibits basal and activated Her2 ectodomain cleavage in breast cancer cells. *Cancer Res* 2001, 61: 4744–4749.
122. Farooqi AA, Rehman ZU, Muntane J. Antisense therapeutics in oncology: current status. *OncoTargets Ther* 2014, 7: 2035–2042.