Title
HER2/neu: An increasingly important therapeutic target: Part 3 Clinical Applications & Investigations

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Author
Nelson, EL

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HER2/neu is the molecule that has been most successfully targeted for therapeutic intent in the field of oncology. This extraordinary clinical impact has been driven by basic and translational investigations along with astute development of a range of therapeutic agents. This is the third part of a comprehensive three-part review. The clinical applications of HER2/neu-targeted therapies will be reviewed for individual tumor types, including early and late phase clinical studies, building on the prior reviews of the biology of the EGF receptor family and the biological distribution of HER2/neu overexpression. This comprehensive survey will identify opportunities for therapeutic development and promising areas for future clinical investigations of HER2/neu-targeted therapies, highlighting HER2/neu as an increasingly important therapeutic target.

Keywords: c-erbB2 • clinical studies • EGF receptor (EGFR) family • expression modulation • HER2/neu • monoclonal antibodies • resistance • targeted therapeutics • tyrosine kinase inhibitors • vaccine

The HER2/neu molecule is expressed in a wide range of normal tissues, overexpressed in a variety of tumor types, with or without gene amplification and is an established target for antitumor therapeutics. No molecule in the field of oncology has been more extensively or more successfully targeted for therapeutic intent than this product of the c-erbB2 gene. The studies characterizing the biology of this molecule and related family members, along with the therapies developed to target HER2/neu have been reviewed in Part 1 [1]. The tissue and tumor distribution of HER2/neu overexpression and gene amplification is reviewed in Part 2 [2]. Part 3 will review clinical investigations and applications of HER2/neu-targeted therapeutic strategies, but will only selectively address preclinical work.

The protein product of the c-erb B2 gene is a member of the EGF receptor (EGFR) family, which comprises four distinct, type I transmembrane proteins known as; human EGFR (HER) 1, 2, 3 and 4 (Table 1). This receptor family has a complex biology, which is influenced in part by the presence or absence of identified ligands, presence or absence of kinase activity and the combinatorial diversity imparted by multiple dimerization partners (Figure 1). There are multiple phosphorylation sites that permit downstream signal transduction in all but HER3, where the protein tyrosine kinase (PTK) domain is inactive [1]. Receptor dimerization is essential for signal transduction, either as a homodimer or heterodimer, and generally involves ligand interactions that result in permissive structural alterations [1]. Signaling through phosphorylation events involves a number of major signal transduction pathways influencing the regulation of proliferation, transcription, autophagy, apoptosis and chemotaxis (Figure 1 & Table 1). The overexpression of HER2/neu is believed to perturb the balance of signaling within the HER family and contribute to dysregulated growth [1]. Evidence for a critical biological function of HER2 has been derived from gene-targeting studies...
in mice. Either constitutive kinase dead or erbB2 null mice demonstrate embryonic lethality secondary to gross cardiovascular abnormalities and a critical role in neurologic development [1]. Appreciation of the tissue distribution, degree of gene amplification (Table 2), the complexity of involved signaling pathways and the role of erbB2 in development [1,2], is essential for understanding the full extent of resistance mechanisms and toxicities that come into play along with rationale for investigation of the HER2/neu-targeted therapies in specific tumor types that will be the focus of the remainder of this review.

**Breast adenocarcinoma**

The vast majority of therapies targeting HER2/neu have been developed and evaluated in breast adenocarcinoma. The recognition that c-erbB2 gene amplification and associated overexpression of HER2/neu was a critical prognostic factor [3] led to the initial therapy targeting HER2/neu, trastuzumab (Herceptin®), and provided the proof of concept for the therapeutic targeting of HER2/neu. The development and clinical studies leading to the approval and broad application of this monoclonal antibody have been held up as a prototype for bench-to-bedside translation [4]. Trastuzumab elicits antibody-dependent cellular cytotoxicity and importantly disrupts HER2/neu-mediated signaling through disruption of heterodimer formation. Interestingly, this disruption is much more pronounced for the HER1:HER2 heterodimer than the HER2:HER3 heterodimer, even though HER3 is the preferred partner [5], and would foreshadow the development of another anti-HER2 antibody, pertuzumab [6].

Early phase clinical studies of trastuzumab in metastatic breast cancer [7] were initiated in the mid-1990s, including Phase II studies conducted in patients with heavily pretreated metastatic breast adenocarcinoma overexpressing HER2/neu, first as a single agent [8] and subsequently in combination with chemotherapy [9], resulting in objective response rates of 11.6 and 24.3%, respectively. It is worthwhile to note that immunohistochemical (IHC) demonstration of HER2/neu expression was adequate for enrollment of subjects in many of these early phase trials and was not restricted to 2+ or 3+ staining. Thus, the response rates are believed to underestimate the clinical activity in patients with erbB2 gene amplification. A subsequent multinational, multi-institutional Phase II study of over 200 women with progressive disease after one or two chemotherapy regimens whose tumors had HER2/neu expression at the 2+ and 3+ level demonstrated a response rate of 15% including a small proportion of complete responses (CRs) [10], although investigators’ reported a preliminary 23% response rate. It was in this trial that the associated cardiac toxicity was first observed, particularly in patients previously treated with anthracyclines. Concomitant animal work identified the critical role for HER2/neu in cardiovascular development providing a biological basis for the unanticipated risk of cardiotoxicity observed with trastuzumab [2].

A definitive Phase III randomized clinical study for patients with metastatic breast cancer with 2+ or 3+ HER2/neu staining demonstrated benefit for the combination of chemotherapy plus trastuzumab over chemotherapy alone, in terms of response rate (50% vs 32%; \( p < 0.001 \)), median freedom from progression (7.4 vs 4.6 months; \( p < 0.001 \)) and median survival (25.1 vs 20.3 months; \( p = 0.046 \)) [11]. In this study, the risk of cardiotoxicity (with anthracycline, cyclophosphamide and trastuzumab exposure) was significant, occurring in 27% of participants randomized to this combination. Nevertheless, this Phase III study demonstrated significant improvement in overall survival in patients with metastatic breast cancer and provided critical data for US FDA approval of trastuzumab. The observation that overexpression of HER2/neu is associated with resistance to hormonal therapy in hormone receptor-positive breast tumors provided a rationale for the study of trastuzumab in so-called dual-positive breast tumors, the TAnDEM trial [12], where it was found to have clinical benefit when added

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**Table 1. Nomenclature of EGF receptor family members.**

<table>
<thead>
<tr>
<th>Gene nomenclature</th>
<th>EGFR nomenclature</th>
<th>HER nomenclature</th>
<th>Common protein nomenclature</th>
<th>Kinase signaling capacity</th>
<th>Ligand(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>erb-B1</td>
<td>EGFR</td>
<td>HER1</td>
<td>EGFR</td>
<td>Active</td>
<td>EGFR, amphiregulin, TGF-α, epigen, β-cellulin, HB-EGF</td>
</tr>
<tr>
<td>erb-B2</td>
<td>EGFR-2</td>
<td>HER2/neu</td>
<td>HER2/neu</td>
<td>Active</td>
<td>–</td>
</tr>
<tr>
<td>erb-B3</td>
<td>EGFR-3</td>
<td>HER3</td>
<td>EGFR-3</td>
<td>Inactive</td>
<td>Neuregulin 1, Neuregulin 2</td>
</tr>
<tr>
<td>erb-B4</td>
<td>EGFR-4</td>
<td>HER4</td>
<td>EGFR-4</td>
<td>Active</td>
<td>Neuregulin 1, Neuregulin 2, Neuregulin 3, Neuregulin 4, β-cellulin, HB-EGF</td>
</tr>
</tbody>
</table>

EGFR: EGF receptor.
to anastrozole for metastatic hormone and HER2/neu-positive breast cancer. Thus, trastuzumab provides clinical benefit for the treatment of all metastatic HER2/neu-overexpressing breast tumors.

Given the efficacy of trastuzumab in the metastatic setting and the poor prognosis for HER2/neu-positive breast cancer patients, investigation of primary, neoadjuvant, treatment of locally advanced breast cancer and in the adjuvant setting was indicated, but the increased cardiotoxicity was of concern. The National Surgical Adjuvant Breast and Bowel Project (NSABP) initiated an adjuvant clinical study in women with HER2/neu-overexpressing breast tumors as defined by 3+ IHC or FISH positive for erbB2 gene amplification, in 2000 comparing two arms doxorubicin, cyclophosphamide followed by paclitaxel ± trastuzumab, NSABP-B31. A similar study was initiated later in 2000 by the North Central Cancer Treatment Group (NCCTG) with identical HER2/neu inclusion criteria comparing three arms doxorubicin, cyclophosphamide followed by paclitaxel ± trastuzumab initiated either after completion of the 12 weeks of weekly paclitaxel or concurrent with paclitaxel. The 3351 participants in the concordant arms of these two studies have been reported together [14,15]. Kaplan–Meier estimates revealed a significant difference in disease-free survival and separation of the overall survival curves with only 154 deaths when first reported [16]. Which chemotherapy is better, AC-T versus the AC-T standard therapy arm. Not unexpectedly, greater cardiac toxicity was encountered in the AC-T arm (2%) relative to the AC-T arm (0.7%) and the TCH arm (0.4%). Which chemotherapy is the best partner for trastuzumab remains to be seen as evidenced by the BCIRG 007 study examining the role of docetaxel ± carboplatin, which did not demonstrate any benefit to the addition of carboplatin [12]. More recently, the HERA and PHARE trials have been reported, along with an ongoing Italian study [18], have defined the optimal duration of trastuzumab treatment to 1 year in the adjuvant setting given the potential inferiority of a shorter (6 months) treatment interval [14,19–22]. Several small clinical studies reported increased pathological CR rates with the inclusion of trastuzumab in neoadjuvant treatment for HER2/neu 2+ or 3+ IHC or FISH gene-amplified breast cancer [19,23,24]. Subsequently, larger Phase III clinical trials have been reported [25,26], which together with the accumulated early phase data [27,28], firmly support the inclusion of trastuzumab in neoadjuvant treatment regimens for patients with HER2/neu-positive breast cancer. Trastuzumab is now an integral part of the therapeutic armamentarium for all stages of breast cancer with overexpression of HER2/neu.

Pertuzumab (2C4) provides enhanced steric hindrance impeding heterodimerization of HER2/neu [29], has activity with lower levels of expression of HER2/neu [30] and, unlike trastuzumab that has limited ability to inhibit heterodimerization between HER2/neu and HER3, significantly inhibits signaling by HER3 ligands [31]. But, pertuzumab may have less activity inhibiting HER2 HER1 heterodimerization [32,33]. It is unclear if pertuzumab interferes with the heterodimerization of HER2/neu with alternate tyrosine kinase receptors such as IGF-1R, and Met. Based on preclinical evidence that the combination of trastuzumab and pertuzumab had increased activity over each agent individually [34] and disappointing results in an early phase single agent studies of pertuzumab [35], the combination of trastuzumab and pertuzumab was evaluated in early phase clinical studies. These studies demonstrated no increase in cardiotoxicity and clinical efficacy in both the advanced and neoadjuvant settings [36–39]. The multi-institutional Phase III CLEOPATRA study [40] yielded a statistically significant improvement in progression-free survival from 12.4 to 18.5 months with the addition of pertuzumab [41], which resulted in the FDA approval, in June 2012, for the use of pertuzumab in combination with trastuzumab and docetaxel. Subsequently, an improvement in overall survival in addition to improved progression-free survival, was also reported [42]. NEOSPHERE, a four-arm study, demonstrated an increase in pathologic CR, defined as absence of viable tumor in the breast, rate from 21.5 to 39.3% with the addition of pertuzumab [43], which resulted in the FDA ODAC briefing document [44]. It is worthwhile to note that these response rates are lower than those reported in the peer-reviewed manuscript of 29.0 and 45.8%, respectively [44]. A small three-arm Phase II study of combined neoadjuvant and adjuvant therapy, with a primary end point of cardiac safety and a secondary end point of pathologic CR rate, TRYPHAENA, yielded pathological CR rates between 57 and 66% although this study was not powered to
Clinical Trial Outcomes

Neuregulin 1 (Heregulin, NDF)
Neuregulin 2

Neuregulin 3
Neuregulin 4

β-cellulin
Epiregulin
HB-EGF

EGF
Amphiregulin
Epigen
TGF-α

HER1
HER1
HER1
HER2
HER1
HER3
HER1
HER4
HER3
HER2
HER3
HER4
HER4
HER4
HER2
HER2

Impact

Proliferation
Transcription
Autophagy
Apoptosis
Chemotaxis

Pathways

PI3K
AKT
MTOR
S6K

BAX
BAD

ERK 1/2

RAS
MEK
MAPK
MKK

RHO
RAC
p21
JUN

PLC-γ
PKC

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Clinical Trial Outcomes

Neuregulin 1 (Heregulin, NDF)
Neuregulin 2

Neuregulin 3
Neuregulin 4

β-cellulin
Epiregulin
HB-EGF

EGF
Amphiregulin
Epigen
TGF-α

HER1
HER1
HER1
HER2
HER1
HER3
HER1
HER4
HER3
HER2
HER3
HER4
HER4
HER4
HER2
HER2

Impact

Proliferation
Transcription
Autophagy
Apoptosis
Chemotaxis

Pathways

PI3K
AKT
MTOR
S6K

BAX
BAD

ERK 1/2

RAS
MEK
MAPK
MKK

RHO
RAC
p21
JUN

PLC-γ
PKC

CLIN. INVEST. (LOND.) (2014) 4(9)

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**Figure 1.** HER family dimerization patterns, ligands, engaged pathways and biological effects (see facing page). The individual HER heterodimers are depicted in the left hand column and their cognate ligands color coded for binding to their respective HER molecules: HER1 and its respective ligands, blue background; HER2, green background; HER3 and its respective ligands, orange background; HER4 and its respective ligands, fuscia background. Ligands with two colors bind to both HER molecules. The major engaged signaling pathways are depicted with arrows indicating well-established nodes for ‘cross-talk.’ The major biological impacts of HER family mediated signaling are depicted on the right.

demonstrate superiority of any one arm with respect to pathologic CR and there were significant imbalances between arms with respect to hormonal receptor status and HER2 IHC 2+ tumors [43,45]. Although there was an increased incidence of left ventricular dysfunction in arms that included anthracycline containing combination chemotherapy, this study provided additional support for the dual antibody approach. Together, these data led to FDA approval for use of this dual antibody therapy in the neoadjuvant setting, in September 2013. Additional Phase III clinical studies are underway in the adjuvant (ClinicalTrials.gov identifier [ID] #NCT01358877) and neoadjuvant setting (ClinicalTrials.gov ID #NCT01583426).

<table>
<thead>
<tr>
<th>Tumors with HER2/neu overexpression</th>
<th>Approximate frequency of overexpression (%)†</th>
<th>Approximate frequency of gene amplification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast adenocarcinoma</td>
<td>17–35</td>
<td>15–25</td>
</tr>
<tr>
<td>Esophageal</td>
<td>≥30</td>
<td>15 to ≥30</td>
</tr>
<tr>
<td>Gastric</td>
<td>30–40</td>
<td>10–20</td>
</tr>
<tr>
<td>Colon</td>
<td>10–20</td>
<td>≤10–30†</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>20–30</td>
<td>2–16</td>
</tr>
<tr>
<td>Carcinoid, bowel (not gastric)</td>
<td>90</td>
<td>40</td>
</tr>
<tr>
<td>Lung, non-small-cell carcinoma</td>
<td>20–30</td>
<td>2–3</td>
</tr>
<tr>
<td>Head and neck</td>
<td>15–40</td>
<td>10–20</td>
</tr>
<tr>
<td>Salivary, mucoepidermoid adenocarcinoma</td>
<td>&gt;75</td>
<td>50–80</td>
</tr>
<tr>
<td>Ovarian, epithelial</td>
<td>10–20</td>
<td>4–10</td>
</tr>
<tr>
<td>Ovarian, mucinous epithelial</td>
<td>18–35</td>
<td>16–18</td>
</tr>
<tr>
<td>Ovarian, Müllerian</td>
<td>40–52</td>
<td>68†</td>
</tr>
<tr>
<td>Endometrial adenocarcinoma</td>
<td>16–52</td>
<td>3–63†</td>
</tr>
<tr>
<td>Endometrial carcinosarcoma</td>
<td>20–30</td>
<td>14–43†</td>
</tr>
<tr>
<td>Cervical squamous cell</td>
<td>10–30</td>
<td>14–17</td>
</tr>
<tr>
<td>Osteosarcoma</td>
<td>&gt;40‡</td>
<td>14–26</td>
</tr>
<tr>
<td>Synovial sarcoma</td>
<td>10–42</td>
<td>31‡</td>
</tr>
<tr>
<td>Transitional cell carcinoma</td>
<td>35</td>
<td>0–32</td>
</tr>
<tr>
<td>Prostate</td>
<td>55</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Thyroid, papillary</td>
<td>34–70</td>
<td>3–14</td>
</tr>
<tr>
<td>Meningioma</td>
<td>30 to &gt;50</td>
<td>13</td>
</tr>
<tr>
<td>Gliomas</td>
<td>0–50</td>
<td>0†</td>
</tr>
<tr>
<td>Childhood medulloblastosomas</td>
<td>40–85</td>
<td>0†</td>
</tr>
<tr>
<td>Wilms – epithelial differentiation</td>
<td>&gt;40‡</td>
<td>0†</td>
</tr>
</tbody>
</table>

†Immunohistochemical (IHC) scoring and primary antibodies vary, generally considered 3+ on a scale of 0–3. A small proportion of IHC 2+ will also have gene amplification. Inclusion of IHC 2+ as overexpression contributes to the high end of the ranges.

‡Gene amplification is reported to be more frequent than protein overexpression in some reports.

§A large proportion of tumors overexpress cytoplasmic HER2/neu with a smaller proportion <10% with membrane overexpression.

¶Only a single report describes evaluation of gene amplification and this report did not detect overexpressed protein.

NR: Not reported.
A recent addition to the antibody therapeutic armamentarium targeting HER2/neu is trastuzumab emtansine (T-DM1; Kadcyla®). Early phase studies evaluating T-DM1 in the setting of tumor progression while on trastuzumab treatment, demonstrated promising pharmacokinetics (PKs), significant antitumor activity and restricted toxicities that were readily manageable [46–49]. These promising randomized Phase II data, were insufficient for FDA approval in the initial 2010 application. However, the recent report from the EMILIA trial [50], having reached its predetermined early stop parameters, showed an increase in overall survival from 25.1 to 30.9 months, improved response rates and a lower incidence of grade III or IV toxicity, over that observed in the capecitabine and lapatinib ditosylate comparison arm. These data led to FDA approval of T-DM1 for recurrent or metastatic HER2+ breast adenocarcinoma, in February 2013. It is worthwhile noting that the comparator arm of the EMILIA trial does not represent current best practice, there are data supporting clinical efficacy for the extended use of trastuzumab while changing chemotherapeutic agents in the setting of progressive advanced HER2+ breast cancer [51], data from the CLEOPATRA study [40] and FDA approval for combined pertuzumab, trastuzumab, docetaxel in the metastatic and neoadjuvant settings; all suggest that future studies should include direct comparison with trastuzumab, regardless of progression status on prior trastuzumab containing therapy. Interestingly, the West German Study Group recently launched a randomized multicenter, open-label Phase II study comparing preoperative T-DM1 with or without standard endocrine therapy versus trastuzumab with standard endocrine therapy in the small population of breast cancer patients whose tumors are both hormone receptor and HER2/neu positive and also eligible for neoadjuvant therapy. This study, with a target cohort of 380 patients, is being conducted within the ADAPT umbrella trial and is the first to directly compare T-DM1 to trastuzumab in any setting (ClinicalTrials.gov ID #NCT01745965). There is a recent report comparing T-DM1 to trastuzumab plus docetaxel in patients with metastatic HER2/neu + breast cancer that demonstrated an improved progression-free survival, 14.2 months for T-DM1 versus 9.2 months for trastuzumab/docetaxel [52]. Thus, it is likely that the direct comparison in the ADAPT trial will show benefit for T-DM1 over trastuzumab. Given the observed cardiac toxicity with trastuzumab, a more potent agent potentially targeting lower level HER2/neu expression might be expected to have greater cardiotoxicity, but early PK data suggested that this might not be the case [53]. Nevertheless, several Phase II studies are underway with various agents including anthracyclines specifically designed to address this issue (ClinicalTrials.gov IDs #NCT00679341, NCT00875979, NCT01120184, NCT01120561 and NCT01196052). Additionally, the Phase III MARIANNE clinical study is currently examining T-DM1 in combination with pertuzumab as a frontline treatment for patients with metastatic breast cancer. Thus, the ultimate place of T-DM1 in the sequence of therapeutic agents targeting HER2/neu remains to be firmly established, despite the FDA approvals noted above. Other modified antibodies targeting HER2/neu have been designed for HER2/neu-positive breast cancer therapies. An antibody conjugated to ricin A chain [54], has been developed and tested in breast and ovarian systems. Similarly, bispecific antibodies targeting HER2/neu have been developed and tested in breast and ovarian cancer [55,56].

The appreciation of the signaling capacity and tyrosine kinase activity of HER2/neu made identification of small molecule inhibitors an obvious priority, ultimately resulting in the development of lapatinib ditosylate, along with other small molecule kinase inhibitors that are at various points in the pipeline (Table 3) [57–62]. The first use of tyrosine kinase inhibitor (TKIs) in therapeutic strategies targeting HER2/neu involved combinations designed to inhibit EGFR (HER1) with trastuzumab. The clinical development of erlotinib and gefitinib has been well documented and even though directed at inhibition of EGFR (HER1) these agents inhibit, albeit to a lesser extent, HER2/neu [63,64]. The addition of erlotinib to trastuzumab, in Phase I studies, resulted in some responses in patients who had progressed on trastuzumab containing regimens, suggesting the capacity to overcome resistance to trastuzumab [65].

The first TKI developed to target predominantly HER2/neu was lapatinib ditosylate (GW572016, Tykerb), which is a reversible inhibitor of both HER2/neu and HER1/EGFR [66]. The initial Phase I studies of lapatinib ditosylate reported toxicities from clinical experience with HER1/EGFR inhibitors [67–71], without additional or unexpected cardiotoxicities when lapatinib ditosylate was combined with trastuzumab in early phase trials [68–71]. Several Phase II studies were rapidly initiated in multiple settings, which ultimately demonstrated efficacy in treating tumors that progressed on trastuzumab, but were quickly supplanted by the interim analysis of a landmark Phase III trial [72,73]. The interim analysis of the seminal Phase III study [72,73], reported in 2006, showed an increased median time to progression of 8.4 months with the combination of lapatinib ditosylate and capecitabine versus 4.4 months with monotherapy. This along with...
acquired Phase II data led to the FDA approval of lapatinib ditosylate in combination with capecitabine for the treatment of HER2/neu-overexpressing breast cancer, which had progressed on trastuzumab containing therapy [74]. A subsequent Phase III study (EGFI0907-LAP107692/LETLOB) [75,76], demonstrated an improvement in median progression-free survival 8.2 versus 3.0 months with the addition of lapatinib ditosylate to letrozole, and led to approval for treatment in combination with letrozole for metastatic hormone receptor-positive, HER2/neu-positive breast cancer patients in 2010. Recent data in the neoadjuvant setting suggest that trastuzumab and lapatinib ditosylate are not entirely interchangeable, as a clinical study reported significantly inferior pathologic CR rate in the lapatinib ditosylate arm, 22.7 versus 30.3% in the trastuzumab arm [77], although the NSABP B-41, NeoALLTO and CHER-LOB clinical studies support equivalence [78–80]. More recently an interim analysis of the National Cancer Institute of Canada trial, MA.31, was reported at the 2012 Annual ASCO meeting and demonstrated quite pronounced inferiority of lapatinib ditosylate versus trastuzumab when either was combined with a taxane in the first-line setting [81]. The NeoAllto and CHER-LOB reports suggest that the combination of trastuzumab and lapatinib ditosylate with nondose dense neoadjuvant chemotherapy increases the pathologic CR rates [78,79], but relapse and overall survival data remain to be established. However, when both trastuzumab and lapatinib ditosylate

<p>| Table 3. Tyrosine kinase inhibitors with HER2/neu inhibitory activity. |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|</p>
<table>
<thead>
<tr>
<th>Tyrosine kinase inhibitor</th>
<th>Reversibility</th>
<th>Documented receptor targets</th>
<th>Clinical trial status</th>
<th>Tumor types</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gifitinib</td>
<td>Reversible</td>
<td><strong>EGFR</strong> (HER1), <strong>HER2/neu</strong></td>
<td>Approved§</td>
<td>Multiple</td>
</tr>
<tr>
<td>Erlotinib</td>
<td>Reversible</td>
<td><strong>EGFR</strong> (HER1), <strong>HER2/neu</strong></td>
<td>US FDA approved¶</td>
<td>Multiple</td>
</tr>
<tr>
<td>Lapatinib</td>
<td>Reversible</td>
<td><strong>HER2/neu, EGFR (HER1)</strong></td>
<td>FDA approvedµ</td>
<td>Breast</td>
</tr>
<tr>
<td>TAK-285</td>
<td>Reversible</td>
<td><strong>HER2/neu, EGFR (HER1)</strong></td>
<td>Phase I</td>
<td>Multiple</td>
</tr>
<tr>
<td>Afatinib</td>
<td>Irreversible</td>
<td><strong>EGFR</strong> (HER1), <strong>HER2/neu, HER4</strong></td>
<td>FDA approved††</td>
<td>Lung (NSCLC), breast, colorectal</td>
</tr>
<tr>
<td>Neratinib</td>
<td>Irreversible</td>
<td><strong>HER2/neu, EGFR (HER1)</strong></td>
<td>Phase III</td>
<td>Breast, lung (NSCLC), colon</td>
</tr>
<tr>
<td>Pelitinib</td>
<td>Irreversible</td>
<td><strong>EGFR</strong> (HER1), <strong>HER2/neu</strong></td>
<td>Phase II (no Phase III registered)</td>
<td>Lung (NSCLC), colorectal</td>
</tr>
<tr>
<td>AST1306</td>
<td>Irreversible</td>
<td><strong>HER2/neu, EGFR (HER1)</strong></td>
<td>preclinical only</td>
<td></td>
</tr>
<tr>
<td>Canertinib</td>
<td>Irreversible</td>
<td><strong>EGFR</strong> (HER1), <strong>HER2, HER4</strong></td>
<td>Phase II (no Phase III registered)</td>
<td>Breast, lung (NSCLC), ovarian</td>
</tr>
<tr>
<td>Dacomitinib</td>
<td>Irreversible</td>
<td><strong>EGFR</strong> (HER1), <strong>HER2, HER4</strong></td>
<td>Phase II (no Phase III registered)</td>
<td>Lung (NSCLC), head and neck squamous cell, and glioblastoma</td>
</tr>
<tr>
<td>BMS-599626</td>
<td>Irreversible</td>
<td><strong>EGFR</strong> (HER1), <strong>HER2, HER4</strong></td>
<td>Phase I (no Phase II or III registered)</td>
<td>HER2-positive tumors</td>
</tr>
<tr>
<td>BMS-690514</td>
<td>Reversible</td>
<td><strong>EGFR</strong> (HER1), <strong>HER2, VEGFR-1, 2 and 3</strong></td>
<td>Phase II (no Phase III registered)</td>
<td>Breast, lung (NSCLC)</td>
</tr>
<tr>
<td>AEE788</td>
<td>Reversible</td>
<td><strong>EGFR</strong> (HER1), <strong>HER2, VEGFR-1 and 2</strong></td>
<td>Phase I (no Phase II or III registered)</td>
<td>Multiple, brain (GBM)</td>
</tr>
</tbody>
</table>

§Target molecules in bold represent the primary molecular target for the respective tyrosine-kinase inhibitor.
††Tumor types that have been evaluated in either Phase II or Phase III clinical studies with the exception of TAK-285, BMS599626 and AEE788.
¶First-, second- and third-line lung NSCLC, with mutated EGFR.
§Approved outside of the USA, for first-line lung NSCLC with mutated EGFR.
¶First-, second- and third-line lung NSCLC, with mutated EGFR; first line for advanced pancreatic adenocarcinoma.
†Approved outside of the USA, for first-line NSCLC with mutated EGFR.
#Second-line advanced or metastatic HER2/neu breast adenocarcinoma.
††First-, second- and third-line lung NSCLC, with mutated EGFR.
EGFR: EGF receptor; GBM: Glioblastoma multiforme; NSCLC: Non-small-cell lung carcinoma.
were used in combination with dose-dense neoadjuvant chemotherapy; unacceptable gastrointestinal toxicity was observed [82], even though the nondose dense combination did not yield excessive toxicity in Phase I studies and had benefit in heavily pretreated metastatic HER2/neu-positive tumors [83]. This is important given the accumulating evidence for the benefit of dose-dense neoadjuvant chemotherapy in breast cancer. Just recently, the initial results from the ALTTO trial were presented; comparing trastuzumab to the combinations with sequential or concurrent lapatinib ditosylate, in a Phase III randomized study in the adjuvant setting, with the primary end point of disease-free interval [84]. The entire study cohort had fewer events than predicted, at the planned primary analysis at 4.5-year median follow-up. The study arms containing lapatinib ditosylate failed to cross the threshold for a statistically significant benefit. With further follow-up, it is possible that there will be a significant benefit, but as in other studies the combination was significantly more toxic. Another concern regarding the combination of lapatinib ditosylate and trastuzumab arises from the observation that lapatinib ditosylate increases the shedding of HER2/neu [85], a potential mechanism for resistance to trastuzumab. Thus, at this point in time, lapatinib ditosylate is primarily employed in second-line therapy of HER2/neu-overexpressing breast tumors after progression on trastuzumab. Recently, acquired mutations in HER2/neu have been identified in association with resistance to lapatinib ditosylate [86] suggesting that other TKIs targeting the same molecule will be necessary to treat tumors with acquired resistance.

Second-generation, irreversible TKIs have been investigated for the treatment of HER2/neu-positive breast cancer. Afatinib (BIBW 2992, Tomtovik™) targets HER2/neu along with other HER family members. Early phase clinical trials involving multiple solid tumor types, including breast adenocarcinoma, have been reported [59,87–91] with some activity in breast adenocarcinoma [59,88,90]. A Phase II study [92] and a Phase III study in breast cancer have been reported, the latter in abstract form [93]. However the majority of the effort for this TKI has been directed toward other solid tumor types. Canertinib (CI-1033) is another irreversible, pan-HER, inhibitor whose toxicities are exquisitely schedule dependent, as noted in Part 1 [1]. Only one of the five Phase I studies that included breast cancer patients [94–98] provided evidence of activity [97]. A Phase II clinical study of canertinib in metastatic breast cancer [99] has been conducted. Dacomitinib (PF-00299804) a second-generation irreversible pan-HER TKI with preclinical activity in breast cancer and showed some activity in a Phase I study that included breast cancer patients [100], but no more advanced studies are registered that include breast cancer. The pan-HER inhibitor, BMS 599626 (AC480), has been evaluated in early phase trials that include breast cancer patients (ClinicalTrials.gov IDs #NCT002071012, NCT00095537 and NCT00093730). BMS-690514 is a TKI that includes pan-HER and VEGF receptor activity, which is under development. Pharmacokinetic and Phase I studies have been reported, with a maximum tolerated dose of 200 mg daily [101–105] with others yet to be reported; as a single agent (ClinicalTrials.gov IDs #NCT00420186 and NCT00329004) or in combination with established combinations of cytotoxic chemotherapeutic agents (ClinicalTrials.gov ID #NCT00479583) and a Phase II clinical study evaluating BMS-690514 with letrozole in breast cancer (ClinicalTrials.gov ID #NCT01068704) is registered. Another irreversible TKI, neratinib (HKI-272, WAY-179272), which is a dual HER1 HER2 inhibitor, has been evaluated at a dose of 240 mg daily in early phase trials [106,107] (ClinicalTrials.gov IDs #NCT00397046, NCT00768469 and NCT00266877), in the setting of CNS metastases (ClinicalTrials.gov ID #NCT01494662); in combination with trastuzumab (ClinicalTrials.gov ID #NCT00398567); in combination with trastuzumab and paclitaxel [108]; in combination with paclitaxel (ClinicalTrials.gov ID #NCT00445458); in comparison to trastuzumab. The NEFERTT trial (ClinicalTrials.gov ID #NCT00915018) is comparing neratinib to trastuzumab and the NSABP FB-7 trial (ClinicalTrials.gov ID #NCT01008150); in comparison to the FDA approved combination of lapatinib ditosylate and Capcitabine (ClinicalTrials.gov ID #NCT00777101). Neratinib is also being evaluated as an element of the I-SPY 2 multi-investigational agent breast cancer trial (ClinicalTrials.gov ID #NCT01042379). An international, multi-institutional, Phase III study evaluating neratinib after adjuvant trastuzumab has completed enrollment (ClinicalTrials.gov ID #NCT00878709) and was reported in abstract form to have a positive Internal Data Safety Monitoring Committee interim analysis in favor of continued enrollment [109], but no results have been released.

Immunotherapeutic strategies have progressed to clinical investigation for the treatment of breast cancer. HER2/neu peptide-based strategies are the most developed [110] with conduct of several Phase I studies [111–113] and two concurrent Phase II studies [114,115] of the immunodominant E75 peptide with granulocyte macrophage colony-stimulating factor (GM-CSF) as a biological adjuvant (now referred to as NeuVax). The Phase II study directed by George Peoples demonstrated an improved recurrence rate,
have entered into early phase clinical studies including cancer patients who had progressed on trastuzumab. Phase I and Phase II of heat shock protein (HSP)90 is the most advanced another strategy that is being investigated. Inhibition dependent. Administration of 17-AAG at 450 mg/m² targeted therapies, the effectiveness is quite schedule irrespective, and has led to the current ongoing Phase III trial (ClinicalTrials.gov ID #NCT01479244). There are also completed Phase I and ongoing Phase II studies of the subdominant G2 peptide (ClinicalTrials.gov ID #NCT00524277) [116]; an improved E75 vaccine, AE37 (ClinicalTrials.gov ID #NCT00524277) [117,118]; other peptides, other adjuvants and/or combinations of immunomodulatory agents (ClinicalTrials.gov IDs #NCT00058526, NCT00194714, NCT00791037, NCT00952692, NCT01355393, NCT01376505 and NCT01632332), including combining protein vaccination with trastuzumab [119] or lapatinib ditosylate [120]. A number of other immunotherapeutic strategies have entered into early phase clinical studies including: HER2/neu peptide-loaded dendritic cell (DC) preparations [121,122]; adenoviral transduced autologous DCs expressing the extracellular and transmembrane HER2/neu domains (ClinicalTrials.gov ID #NCT01730118); lapuleucel-T [123]; various genetic vaccines (ClinicalTrials.gov IDs #NCT00485277 and NCT01152398) [124]; a gene modified allogeneic cellu

Gastric & gastroesophageal junction adenocarcinoma

The recognition that gastric adenocarcinomas and adenocarcinomas arising at the gastroesophageal junction overexpress HER2/neu (Part 2) [2], led to early phase (predominantly Phase II) studies of trastuzumab that have been reported in abstract form [132–134] and two published studies [135,136]. These early phase studies provided the foundation for the multinational ToGA trial [137] reported in 2010, that demonstrated a 2.7-month improvement in overall survival with the addition of trastuzumab to the combination of cisplatin plus either fluorouracil or capcitabine, compared with chemotherapy alone. In this study, initiated in 2005, of 594 patients with locally advanced or metastatic HER2/neu-overexpressing (IHC 3+ or gene amplification by FISH) adenocarcinoma of the stomach or GE junction, the median survival was 13.8 months for patients treated with trastuzumab and chemotherapy and 11.1 months for patients treated with chemotherapy alone (p = 0.0046). An updated survival analysis after greater than 200 events in each arm of the ToGA trial, demonstrated a median survival of 13.1 months for patients receiving trastuzumab and chemotherapy and 11.7 months for patients receiving chemotherapy alone (HR: 0.8; 95% CI: 0.67–0.97). Exploratory overall survival analyses in subgroups defined by protein expression (IHC testing) suggest that trastuzumab was most effective in prolonging survival in the 294-patient subgroup with HER2/neu IHC 3+ tumors (HR: 0.66; 95% CI: 0.50–0.87) and less effective in the 160-patient subgroup with IHC 2 + tumors (HR: 0.78; 95% CI: 0.55–1.10). No trastuzumab treatment effect was apparent in the 133-patient subgroup with ICH 0 or 1+ tumors (HR: 1.33; 95% CI: 0.92–1.92) regardless of gene amplification. Interestingly, cardiac toxicity did not differ between groups and may be related to the different trastuzumab dosing schedules. In 2012, trastuzumab received FDA approval for the treatment of HER2/neu-overexpressing, metastatic gastric or gastroesophageal adenocarcinoma. More recent reports support combining trastuzumab with the current state-of-the-art combination chemotherapy (FOLFIRI or FLOT) [138–141] and a study incorporating trastuzumab in the second line setting was recently reported [142]. There is a single case report of the successful use of trastuzumab in combination with capecitabine and cisplatin in the neoadjuvant setting with para-aortic lymph node involvement with gastric adenocarcinoma [143]. Anti-HER2/neu antibodies
conjugated in some manner to toxins or other binding entities, have been explored in early phase studies for gastric adenocarcinoma [144,145]. The logical extension of the work on conjugated antibodies is the testing of T-DM1 [146]. Additionally, a Phase II clinical study for patients with HER2/neu-positive advanced gastric adenocarcinoma testing the combination of pertuzumab, trastuzumab, cisplatin and capcitabine, is currently underway (ClinicalTrials.gov ID #NCT01461057). A Phase III randomized trial for metastatic gastric or gastroesophageal cancer testing is expected to begin accrual in April 2013 (ClinicalTrials.gov ID #NCT01774786) and will test the addition of pertuzumab to FDA approved trastuzumab containing chemotherapy regimens for HER2/neu-positive gastric adenocarcinoma.

In addition to evaluation of anti-HER2/neu antibody-based strategies, the TKIs have also been evaluated in gastric cancer. Including Phase I [105] and Phase II trials of; lapatinib ditosylate as first-line therapy in advanced or metastatic HER2/neu-overexpressing gastric carcinoma [147] and dacomitinib in HER2/neu-overexpressing gastric adenocarcinoma progressing on first-line chemotherapy [148]. Results of two other clinical studies were presented at the 2013 ASCO GI conference, a Phase II study (HER-BIS-1) of lapatinib ditosylate in combination with the fluoropyrimidine S-1 and cisplatin that demonstrated clinical efficacy with this combined therapy [149] and a Phase III study (TyTAN) evaluating the addition of lapatinib ditosylate to paclitaxel in the setting of second-line treatment for advanced gastric cancer, which did not show statistically significant benefit over paclitaxel alone [150], although overall survival was extended by 2 months in the combination arm. Although preclinical work supports combining a TKI and trastuzumab for greater efficacy than either alone [151,152], this strategy has not advanced to clinical studies. Afatinib has been evaluated in a Phase I study treating gastric adenocarcinoma (ClinicalTrials.gov ID #NCT01649271) but not reported, and in a Phase II study [87]. Similarly, Dacomitinib (PF-00299804) another second-generation irreversible pan-HER TKI has been evaluated in an unscheduled Phase I clinical studies (ClinicalTrials.gov IDs #NCT00207012, NCT00955537 and NCT0093730). Interestingly, Canertinib (CI-1033) a related pan-HER inhibitor, did not have any suggestion of activity against gastric or esophageal adenocarcinoma in its Phase I studies [95,96]. BMS599626 (AC480) a dual HER1 HER2 inhibitor has been evaluated in Phase I clinical studies that include gastric adenocarcinoma (ClinicalTrials.gov IDs #NCT00207012, NCT00955537 and NCT0093730).

Other treatment strategies targeting HER2/neu, which are less developed, have also been evaluated in gastric adenocarcinoma. In the early 1990s, it was recognized that T lymphocytes from patients with gastric cancer recognized antigenic peptides from HER2/neu [153] leading to reported [154] and registered early phase clinical trials of anti-HER2/neu immunotherapeutic strategies (ClinicalTrials.gov IDs #NCT01123473, NCT01649271 and NCT01705340). HER2/neu peptide-loaded DC preparations also entered clinical trials [154]. HSP90 inhibitors have been investigated in a gastric adenocarcinoma (ClinicalTrials.gov IDs #NCT01402401 and NCT01084330) including an ongoing study combining HSP90 inhibitor with a PI3K inhibitor, both of which would impact HER2/neu signaling (ClinicalTrials.gov ID #NCT01613950).

Despite the controversies surrounding the incidence and prognostic significance of HER2/neu overexpression in gastric cancer, the FDA approval of trastuzumab for incorporation into the treatment of HER2/neu-positive gastric cancer, the demonstration that this therapeutic strategy is cost effective [155], and the adoption into national treatment guidelines [156,157], all but assures continued development of the entire range of anti-HER2/neu therapeutic strategies for the treatment of gastric and gastroesophageal cancer [158–160]. However, the role of agents targeting HER2/neu other than trastuzumab, remains to be defined.

**Esophagus**

A smaller but yet significant percentage of esophageal cancers with squamous cell histology, located proximal to the gastroesophageal junction, also overexpress HER2/neu (Part 2) [2]. Preclinical studies and the success of the ToGA study have resulted in more activity in the evaluation of small molecule TKIs in esophageal cancer [88,101,162]. Other advanced phase clinical studies are underway evaluating combinations of anti-HER1 and anti-HER2/neu antibodies and/or dual HER1 HER2 TKIs [87,163]. Immunotherapeutic strategies for targeting HER2/neu have not been extensively explored in esophageal cancer, although there has been a Phase I study of an anti-HER2/neu antibody/IL-12 fusion protein [149].

**Pancreatic**

Pancreatic adenocarcinoma is a particularly challenging neoplasm with a dismal overall survival rate beyond 2 years, regardless of treatment. Early phase clinical studies of trastuzumab with various chemotherapeutic agents in patients with metastatic HER2/neu positive (IHC 2+ or 3+) pancreatic adenocarcinoma failed to demonstrate a significant improvement in overall survival or progression-free...
survival over that expected for chemotherapy alone [145,164,165]. However, two Phase II studies have yet to be reported; the first examining direct therapeutic benefit (ClinicalTrials.gov ID #NCT00003797) [166] and the second examining the potential synergy with radiation therapy (ClinicalTrials.gov ID #NCT00005926). Antibody-targeted delivery of radionuclide or other cytotoxic agents has also been investigated, but in completed studies has not demonstrated significant benefit [167–169]. A Phase I study of a novel trastuzumab-based radioimmunotherapeutic is ongoing (ClinicalTrials.gov ID #NCT01384253). Early phase clinical studies support the activity of TKIs [170,171]; a yet to be reported (ClinicalTrials.gov ID #NCT000034218) and an ongoing Phase II trial (ClinicalTrials.gov ID #NCT01204372) will provide additional data. The capacity of HER2/neu to heterodimerize with HER1/EGFR and its proposed role in resistance to antibodies directed against HER1/EGFR [172–175], led to the evaluation of pertuzumab [38] and dual HER1 HER2 blockade either by antibody treatment (cetuximab and trastuzumab, respectively) [176,177] or trastuzumab in combination with erlotinib or lapatinib [180]. A group recently reported preclinical studies of a ‘triple’ therapy, incorporating trastuzumab, panitumumab and trametinib (an MEK1/2 inhibitor), with antitumor efficacy in human pancreatic adenocarcinoma xenograft models and a high degree of in vitro Ras signaling blockade [179].

Other therapeutic strategies targeting HER2/neu in pancreatic adenocarcinoma have been examined in early phase trials. A Phase II investigation of HSP90 inhibition in combination with gemcitabine has recently been reported, but failed to yield any tumor responses [188] and there is an ongoing Phase II study (ClinicalTrials.gov ID #NCT01484860). As noted above, one of the Phase I studies focusing on the immune system uses a strategy incorporating a trastuzumab conjugate with the cytokine IL-12 [145]. A Phase I study employing the α-virus replicon vector targeting HER2/neu in pancreatic cancer patients is currently enrolling patients (ClinicalTrials.gov ID #NCT01526473).

As with the majority of therapeutic strategies for pancreatic adenocarcinoma, the results of HER2/neu-targeted therapy have been underwhelming. Although there is controversy as to the degree of overexpression of HER2/neu in pancreatic adenocarcinoma, or lack thereof, the preponderance of evidence supports a subset of tumors with at least low-level expression that would be expected to be amenable to targeted therapeutics. However, the enthusiasm for anti-HER2/neu therapeutics for pancreatic carcinoma is not nearly at the level seen in the gastric and esophageal carcinomas.

Hepatocellular & cholangiocarcinoma
A small percentage of extrahepatic cholangiocarcinomas overexpresses HER2/neu. Preclinical data for antibodies directed at HER2/neu have been obtained for cholangiocarcinoma [181–183] and there is a case report of response to trastuzumab in cholangiocarcinoma [184]. Afatinib (BIBW 2992, Tomtovik) has been evaluated in a Phase I study for cholecystic adenocarcinoma (ClinicalTrials.gov ID #NCT01679405). Perhaps because of the rarity of this tumor there has been a paucity of clinical investigations of therapy targeting HER2/neu in cholangiocarcinoma. Interestingly, even though hepatocellular carcinoma is not considered to have a significant subset of HER2/neu-expressing tumors (Box 1), a multi-institutional Phase II study of lapatinib ditosylate in advanced hepatocellular carcinoma was conducted, not surprisingly it reported little clinical activity [185,186].

Colorectal adenocarcinoma
Colorectal adenocarcinoma has a small subset, between 10 and 20%, that overexpress HER2/neu (Part 2) [2]. Most studies have not discriminated between colon and rectal adenocarcinoma. Given the relative low-level expression, the relative lack of gene amplification of HER2/neu, and the mixed preclinical data in colorectal cancer, it is not surprising that the one reported early phase study of trastuzumab had unimpressive results [187]. There is a case report of response to trastuzumab in rectal cancer [188].

Box 1. Tumors without HER2/neu overexpression.

- Larynx
- Salivary (adenoidcystic)
- Lung, small cell
- Thymoma
- Peripheral neuroendocrine tumors, excluding small bowel carcinoid
- Ewings sarcoma
- Small bowel adenocarcinoma
- Anal carcinoma
- Hepatocellular
- Cholecysitic
- Renal cell carcinoma
- Germ cell neoplasms
- Common soft tissue sarcomas (rhabdomyo-, leiomyo-, fibro-, angio-, lipo-, chondro- sarcomas and malignant fibrous histiocytoma, among others)
- Desmoid
- Melanoma
- Basal cell carcinoma
- Lymphoma, non-Hodgkins and Hodgkin’s
- Acute and chronic leukemias (myeloid and lymphoid)
The lack of activity of antibodies directed at HER2/neu and the recognition that HER1 and HER2 expression patterns are associated with resistance to therapies targeting either molecule alone, has provided a rationale for exploring dual blockade [173–175], but primarily through the use of TKIs. Preclinical data support dual HER1 HER2 inhibition in colon cancer cell lines [189–191], even though a compensatory increase in HER2 activation has been observed with HER1 inhibition [192]. An early phase clinical study of lapatinib has been reported [193]. Afatinib (BIBW 2992, Tomtovok) has been evaluated in a Phase II study in colorectal cancer [187,194]. Neratinib has been evaluated in a Phase I study [62]. Peltinib (EBK-569), another dual HER1 and HER2 inhibitor has been tested in a Phase II study in advanced colorectal cancer (ClinicalTrials.gov ID #NCT00072748). Canertinib (CI-1033) an irreversible pan-HER inhibitor has demonstrated activity in three of the five Phase I clinical studies that included colorectal adenocarcinoma [94,95,97]. BMS599626 (AC480) another dual HER1 HER2 inhibitor has been evaluated in early phase trials that included colon adenocarcinoma (ClinicalTrials.gov IDs #NCT00207012, NCT00095537 and NCT00093730) [171].

Immunotherapeutic strategies to elicit endogenous anti-HER2/neu immune responses have been explored in colorectal cancer. HER2/neu peptide-loaded DC preparations are also in clinical trials [195] and lapuleucel-T has advanced to a Phase I study [123,195], as has a polypeptide vaccine containing B lymphocyte/antibody recognized epitopes from HER2/neu (ClinicalTrials.gov ID #NCT01376505) [196], along with other peptide-based vaccine strategies (ClinicalTrials.gov ID #NCT00091286). An early phase clinical study of bispecific antibody has been reported [197] without progression to more advanced studies. Gene-modified or CAR-modified T cells have been used in colon cancer patients in early phase trials. It is noteworthy that the severe toxicity observed in CAR-modified T cells occurred in a patient with colon cancer [198].

Indirect modulation of the activity or expression of HER2/neu in colorectal cancer has been investigated. Preclinical studies of HSP90 inhibitors have been reported [199,200] and a Phase I study, in various solid tumors including colorectal carcinoma, of the HSP90 inhibitor AUY922 with capecitabine has been reported in abstract form [201], with four of 14 subjects having partial responses two of which had colorectal cancer. Colon cancer cell lines were used in preclinical studies for the demonstration that the novel retinoid, Tephrosin, that can downregulate both HER1 and HER2 expression [202], providing an alternative pathway to HSP90 for the modulation of HER2/neu signaling.

### Lung

Approximately 30% of non-small-cell lung carcinomas (NSCLC) overexpress HER2/neu, in contrast to small cell carcinoma that does not overexpress HER2/neu to any meaningful degree (Box 1) [2]. Initial work examined antibody-based inhibition with both pertuzumab [203] and trastuzumab including; one case report [204], an early phase trial of antibody monotherapy [205], combined antibody and cytotoxic therapy [206–211], was conducted, but did not yield significant efficacy [212]. The co-involvement of HER1/EGFR and HER2/neu in NSCLC biology suggested that dual HER1 HER2 inhibition might have greater clinical benefit [213,214] and drove the conduct of early phase studies evaluating combinations of agents targeting HER1/EGFR and HER2/neu [215] along with studies of dual (HER1, HER2) TKIs [88,216–219] and pan-HER inhibitors [220–228].

Aftinib (BIBW 2992, Tomtovok) an irreversible pan-HER TKI has been developed primarily in the setting of NSCLC. Early phase studies with activity for patients with NSCLC have been reported [87,88,90,91,229–231]. Based upon early demonstration of clinical efficacy in Phase II clinical studies and the capacity to overcome resistance to the FDA-approved reversible TKIs targeting the HER family [232], Aftainib was given ‘fast track’ status for NSCLC by the FDA [87]. The Boehringer Ingelheim corporation organized LUX clinical trials network [233] has accelerated the clinical development of afatinib through multiple Phase IIb and III clinical studies primarily in NSCLC [234,235]. The data from the LUX-Lung 3 clinical study demonstrated a significant improvement in median progression-free survival (13.6 vs 6.9 months) [236]. Additional Phase III data have been reported in abstract form [93,237–240]. Data from the LUX-3 and LUX 4 studies led to FDA approval for first-line treatment of patients with metastatic NSCLC whose tumors have EGFR exon 19 deletions or exon 21 (L858R) substitution mutations based on improved progression-free survival in July 2013. Currently, 40 Phase III clinical trials involving afatinib are registered with ClinicalTrials.gov.

Canertinib (CI-1033), an irreversible pan-HER TKI, had antitumor activity observed in five of six Phase I studies, which included NSCLC patients [94–98,241] and led to a Phase II study in NSCLC [242]. Dacomitinib (PF-00299804), also an irreversible pan-HER TKI, has also been studied in lung cancer including Phase I and II studies [237,238,239,240], the ARCHER 1042 study (ClinicalTrials.gov ID #NCT01465802), and the ARCHER-1009 study (ClinicalTrials.gov ID #NCT01360554). Finally, BMS-690514, also a pan-HER inhibitor that includes anti-VEGFR activity has
been studied in early phase trials involving NSCLC patients [101,103,105,248], including a Phase II clinical study (ClinicalTrials.gov ID #NCT01167244) reported in abstract form [248] and a Phase I/II study (ClinicalTrials.gov ID #NCT00329004). There is also evidence for synergism with radiation when BMS-690514 is administered in sequence with radiation [246].

Dual HER1 HER2 inhibitors have also been evaluated in NSCLC. Neratinib (HKI-272, WAY-179272) has undergone early phase testing in lung cancer patients [226,247]. A Phase I study of pelitinib (EKB-569), a newer dual HER1/HER2 inhibitor, with temsirolimus has been completed [248], which demonstrated some activity in NSCLC. A Phase II study of pelitinib has been completed in non-small-cell carcinoma of the lung (ClinicalTrials.gov ID #NCT00067548), but not yet reported. BMS 599626 (AC480) has been evaluated in Phase I studies (ClinicalTrials.gov IDs #NCT00207012, NCT00095537 and NCT00093730) [171]. Dual TKI inhibition can have activity in the setting of HER2/neu expression with activating mutations [249]. Mechanisms of resistance to dual HER1 HER2 inhibition in NSCLC have been characterized and are being recognized [250]. The data above and the recognition of evolving resistance mechanisms have driven the development of a robust pipeline (>500 compounds) of potential agents for NSCLC including those targeting HER2/neu [251,252].

Data demonstrating recognition of shared antigens between NSCLC and other tumors, specifically those derived from HER2/neu, provide the rationale for the evaluation of immunotherapeutic strategies [253–256]. A Phase I clinical study of peptide vaccination has been reported that included a few NSCLC patients [257,258]. A 16-patient early phase clinical study of autologous DCs loaded with allogeneic NSCLC cell line lysates has also been reported to have elicited antigen-specific responses in six NSCLC patients, which did not segregate with clinical outcome [259], and two additional clinical studies of antitumor vaccines composed of HER2/neu-derived peptides and the biological adjuvant GM-CSF (ClinicalTrials.gov IDs #NCT00003002 and NCT00005023) have been completed. An approach with lymphodepletion and adoptive transfer of autologous HER2/neu-reactive T cells is about to be initiated (ClinicalTrials.gov ID #NCT00228358).

Despite substantial preclinical data for HSP90 inhibitors, the clinical studies in lung cancer have not specifically targeted HER2/neu. Development of this alternative strategy for targeting HER2/neu would have to be conducted in the face of the successful adoption of afatinib, EGFR inhibitors, other molecularly targeted therapies and immune checkpoint blockade strategies.

### Head & neck

The story of HER2/neu-targeted therapy in head and neck cancers parallels that of NSCLC in many ways. Head and neck cancers including some salivary tumors (excluding adenoid cystic histology; Box 1) have been variably reported to have HER2/neu expression across multiple subtypes (anatomic locations and histology; Part 2) [2]. Phase II clinical studies of trastuzumab have been completed in head and neck squamous cell carcinoma, in conjunction with cisplatin and paclitaxel, but no benefit was observed for the addition of trastuzumab, perhaps because less than 7% of patients had membrane staining of HER2/neu [260]. A Phase II clinical study in salivary gland cancer is registered (ClinicalTrials.gov ID #NCT00004163). HER2/neu expression has been associated with cisplatin and fluorouracil resistance in primary head and neck tumors [261] and activation of the HER2/neu pathway has been associated with cetuximab resistance [175]. A completed Phase I clinical study of the combination of trastuzumab and recombinant IL-12 in patients with HER2/neu-overexpressing tumors, in which head and neck cancers were eligible (ClinicalTrials.gov ID #NCT00004074), has yet to be reported. LJM716, a HER3 directed antibody that inhibits both HER2/neu and HER3, is being evaluated in an ongoing Phase I study (ClinicalTrials.gov ID #NCT01598077).

Preclinical work with TKIs has suggested that targeting HER2/neu may improve the clinical activity of cisplatin-based chemotherapy [262,263]. This preclinical work has led to a number of early phase clinical studies, only a few of which have been reported. Phase I clinical studies of lapatinib ditosylate suggest some activity, particularly in salivary gland tumors [264–266] and a Phase II study [267] reported modest activity. However, another Phase II study reported a negative result [268] and a third has been completed but remains to be reported (ClinicalTrials.gov ID #NCT0095563). A Phase II clinical study of the oral regimen of capcitabine and lapatinib ditosylate is ongoing (ClinicalTrials.gov ID #NCT01044433). A Phase III clinical study of lapatinib ditosylate in the postsurgical adjuvant setting has completed enrollment, passed its predicted primary end point completion date and should be reported soon (ClinicalTrials.gov ID #NCT00424255). A recent study has implicated HER3 as an important element for any potential for clinical benefit with targeting HER2/neu [269], as its expression and one of its cognate ligands, neuregulin 1, has been associated with responsiveness to the HER2/neu TKI lapatinib ditosylate. A
natural product has been described with the capacity to inhibit both HER1/EGFR and HER2/neu signaling and induce cellular apoptosis in head and neck squamous cell carcinoma [270]. Additional preclinical studies support synergy of TKIs [271] with radiation therapy, although this has not been tested clinically against chemoradiation. Lapatinib ditosylate in combination with chemoradiation has been tested in a Phase I [272] and in Phase II studies [273] (ClinicalTrials.gov IDs #NCT00490061 and NCT00387127), one of which has reported no additional clinical benefit to the addition of lapatinib ditosylate [273].

Studies of irreversible and pan-HER TKIs have been initiated [274] and are progressing through Phase III clinical studies. Afatinib has been evaluated in Phase I clinical studies (ClinicalTrials.gov IDs #NCT01721525, NCT01732640 and NCT01783587). Afatinib is being evaluated as a single agent in a completed but not yet reported study (ClinicalTrials.gov ID #NCT00514943), an ongoing study as a single agent for treatment of unresectable, recurrent or metastatic head and neck carcinoma (ClinicalTrials.gov ID #NCT01469546), an ongoing study in patients with advanced head and neck cancer (ClinicalTrials.gov ID #NCT014695466), and in an ongoing study in the neoadjuvant setting (ClinicalTrials.gov ID #NCT01538381). Another randomized Phase II study evaluating afatinib in comparison to cetuximab, in the metastatic or recurrent head and neck squamous cell carcinoma, has been presented in abstract form reporting improved response rates and progression-free survival, 16 versus 10 weeks, for afatinib over cetuximab [275,276]. Afatinib has been incorporated into several Phase III clinical studies including the LUX series which includes: LUX-Head & Neck-1, a trial comparing afatinib to methotrexate in recurrent or metastatic head and neck squamous cell carcinoma after platinum-based chemotherapy (ClinicalTrials.gov ID #NCT01345682) [239]; LUX-Head & Neck-2, a Phase III study comparing afatinib with placebo after treatment with chemoradiotherapy (ClinicalTrials.gov ID #NCT01345669); and a double-blind placebo-controlled trial evaluating afatinib as maintenance therapy after postoperative chemoradiation (ClinicalTrials.gov ID #NCT01427478). Afatinib is also being evaluated in a Phase I study combining it with postoperative radiation (ClinicalTrials.gov ID #NCT01783587). A Phase I study of another pan-HER inhibitor dacomitinib has revealed clinical activity as a single agent [277] and Phase II studies are ongoing (ClinicalTrials.gov IDs #NCT01449201 and NCT00768664). Dacomitinib is being characterized for its PK parameters when administered through a feeding tube as is commonly employed in the management of these patients (ClinicalTrials.gov ID #NCT01484847). A future clinical study of dacomitinib with and without cisplatin is registered but not yet initiated (ClinicalTrials.gov ID #NCT01737008). BMS999626 (AC480) a second-generation pan-HER inhibitor has activity as a radiosensitizing agent [271] similar to another pan-HER inhibitor canertinib (CI-1033) [278], which suggested activity in a Phase I study [99].

Other avenues of targeting HER2/neu in head and neck cancers have not been evaluated to any significant degree. Antibody-directed targeting of toxins has progressed to a Phase I study [279,280]. As with pancreatic adenocarcinoma, defective antigen processing with decreased cytotoxic T lymphocyte (CTL) recognition has been described for head and neck cancer [281], perhaps explaining the lack of work in and success of immunotherapeutic strategies. Use of HSP90 inhibitors has not been reported. However, preclinical work with a histone deacetylase inhibitor, vorinostat, has demonstrated decreased expression of both HER1/EGFR and HER2/neu, and enhancement of gefitinib activity [282].

**Nervous system**

A small percentage of gliomas of varying grades, up to and including glioblastoma multiforme (GBM), express HER/neu, despite the fact that developing neurologic tissue expresses high levels of HER2/neu [2]. Despite this, there have been efforts to target HER2/neu including; preclinical work with the pan-HER inhibitor CI-1033 [283], preclinical work with the irreversible dual inhibitor Bay846 [284], and a reported Phase I/II study of lapatinib ditosylate, which demonstrated no clinical efficacy [285]. Afatinib has been evaluated in a Phase I clinical study (ClinicalTrials.gov ID #NCT00977431) and a Phase II clinical study [87] for GBM. Dacomitinib (PF-00299804) has similarly been evaluated in a Phase II study (ClinicalTrials.gov ID #NCT01520870) also for GBM. Additionally, there is a completed study of the dual HER1, HER2 and VEGFR inhibitor AEE788, in recurrent GBM (ClinicalTrials.gov ID #NCT0016376) [286] although unacceptable toxicity and minimal activity was observed. There is also an ongoing clinical study of HER2/neu-specific CAR-modified CTls (ClinicalTrials.gov ID #NCT01109095) and an ongoing study of trastuzumab in combination with pegylated doxorubicin (ClinicalTrials.gov ID #NCT0136580). HER2/neu peptide-loaded DC preparations are also in clinical trials [287].

Additionally, medulloblastoma is a subset of nervous system tumors that has been documented to have a high proportion of overexpression of HER2/neu [288,289]. A preclinical study of adoptive transfer of HER2/neu reactive CAR-modified T lymphocytes induced regression.
of established medulloblastomas in an orthotopic xenograft model [290]. Medulloblastomas were included in a completed Phase II clinical study of lapatinib ditosylate in pediatric patients with recurrent or refractory medulloblastoma, malignant glioma or ependymoma (ClinicalTrials.gov ID #NCT00095940), in which there were no clinical responses in any of the three tumor types. Studies of second-generation agents have not been registered or initiated to date.

Neuroendocrine tumors
Although a small subset of carcinoid tumors arising outside of the colon have been variably described as overexpressing HER2/neu (Part 2) [2]. However, given the rarity of these tumors and the small subset that overexpresses HER2/neu (Box 1), it is unlikely that a clinical study of antibody-based HER2/neu-targeted therapy will be undertaken in this setting. Whether TKIs will show activity in these tumors is yet to be determined.

Sarcomas
The common soft-tissue sarcomas do not overexpress HER2/neu (Box 1), with the exception of osteosarcoma, synovial sarcoma, rhabdomyosarcoma, carcinosarcoma and mixed Müllerian tumors, the latter two originating in the gynecologic tract (Part 2) [2]. A Phase II study of trastuzumab in combination with chemotherapy in metastatic pediatric osteosarcoma has recently been published, which demonstrated no difference in outcome with the addition of trastuzumab [291], but must be interpreted with caution given the observation of loss of HER2/neu expression in metastatic lesions [292]. Preclinical work with osteosarcoma cell lines revealed that the HER2/neu-specific inhibitor AG825 did not alter motility, invasiveness or colony formation [293]; however, the demonstration that early passage osteosarcoma cells have constitutive phosphorylation of HER2/neu that could be inhibited by the pan-HER inhibitor canertinib (CI-1033), supports the biological activity of cytoplastic HER2/neu [294] and further testing of TKIs in this setting. A Phase I clinical study of the broad-spectrum pan-HER inhibitor that also targets the VEGF receptors, BMS-690514, has shown activity in cases of leiomyosarcoma, but it is unclear if this is HER2 related given the expression profile of HER2/neu (Box 1) [295]. Preclinical work has been reported for both immunotoxin conjugates [295,296] and CAR-modified T cells [297,298] targeting HER2/neu in osteosarcoma xenograft models with activity reported for both strategies.

Urinary tract
Normal kidney tissues, particularly the terminal collecting duct epithelium, express HER2/neu along with transitional cell carcinomas (TCC) [2]. For TCC, use of single agent trastuzumab has been reported, with varying degrees of efficacy, in a small series of less than ten patients [299,300]. There is also a completed, yet to be reported, Phase II study (ClinicalTrials.gov ID #NCT00004856). Interestingly, one group from Germany recently reported a higher incidence of bladder dysfunction associated with trastuzumab administration in breast cancer patients [301], suggesting HER2/neu has important biological functions in the bladder and makes it more likely that HER2/neu is a viable target in bladder tumors. A completed Phase II clinical study [302], has been reported with a reasonable response rate (57% confirmed). There is an ongoing RTOG Phase II clinical trial (ClinicalTrials.gov ID #NCT00238420) examining trastuzumab in the setting of chemoradiation (paclitaxel). TKI targeting in TCC has also been investigated. A Phase I study of lapatinib ditosylate has been reported [303] along with a single-arm Phase II study in TCC that had progressed on platinum-based therapy that showed promising clinical activity, with progression-free and overall survival being 8.6 and 17.9 weeks, respectively, and increased activity in patients with tumors overexpressing both HER1/EGFR and HER2/neu [304]. There is an ongoing Phase II/III clinical trial (ClinicalTrials.gov ID #NCT00949455) being conducted out of Queen Mary University of London evaluating lapatinib ditosylate versus placebo in metastatic TCC progressing after first-line treatment. BMS599626 (AC480) has been evaluated in Phase I clinical studies that include transitional cell cancer of the bladder (ClinicalTrials.gov IDs #NCT00207012, NCT00095537 and NCT00093730) [171], but activity has not been reported. Preclinical studies have been reported for second-generation HER2/neu targeting TKIs in which the ability to overcome resistance to cetuximab [305] and TKI radiosensitization have been demonstrated [306].

Investigations of HSP90 inhibitors have been described as sensitizing to chemoradiation [307], enhancing HER2/neu DNA vaccines [308], and have been evaluated as single agents in the preclinical setting [309,310]. However, in these studies there is no description of modulation in HER2/neu expression. The HER2/neu molecule has been used for targeted delivery of a γ-retrovirus encoding IL-12 [311] and the HER2/neu promoter for driving expression of an oncolytic virus [312], investigated in part because of the accessibility of bladder TCC. There is an ongoing Phase II clinical study of lapuvelucel-T (Neuveng™), (ClinicalTrials.gov ID #NCT01353222), in the adjuvant setting of high-risk HER2-positive TCC.

The experience with renal cell carcinoma is less promising, due in part to the absence of convincing
evidence of HER2/neu overexpression in renal adenocarcinoma (Box 1). There has been a Phase III randomized clinical study comparing lapatinib ditosylate to megestrol acetate in progressive renal cell carcinoma that failed to demonstrate superiority of lapatinib ditosylate [313]. Efficacious immunological targeting of HER2/neu for the treatment of renal cell carcinoma remains elusive despite substantial preclinical evidence for the capacity to elicit anti-HER2/neu immune responses. Other, nonadenocarcinoma renal cell tumors have also been investigated. In preclinical work in Wilms tumors, trastuzumab demonstrated some activity in a xenograft animal model [314]. This work has not advanced into early phase clinical studies. Additionally, there is one case report of a significant response of a refractory collecting duct carcinoma to treatment with the combination of lapatinib ditosylate, trastuzumab and capcitabine [315].

**Prostate**

The study of HER2/neu-targeted therapies for prostate adenocarcinomas is complicated by the expression pattern of HER2/neu that includes normal prostatic epithelium (Part 2) [2]. Early phase clinical studies include Phase II studies of trastuzumab as a single agent in hormone refractory prostate cancer (ClinicalTrials.gov ID #NCT00003740) [316], and a Phase II study of trastuzumab in combination with docetaxel [317], the former demonstrating little clinical benefit (10%) and the latter reported too low of accrual for meaningful data to be obtained. A Phase I clinical study of pertuzumab as a single agent in advanced cancers, including HER2/neu-positive prostate adenocarcinoma, has been reported [38]. Three Phase II clinical studies of pertuzumab were conducted for patients with hormone-refractory HER2/neu-positive prostate cancer. In two of these, disease stabilization was observed, but no objective responses were reported [318,319], while in the third, a Phase IIa study of pertuzumab and docetaxel, more than half of the 18 subjects experienced stable disease with one subject experiencing a radiological partial response and a greater than 50% decline in PSA [320]. The completed Genentech-sponsored Phase II clinical study of pertuzumab (ClinicalTrials.gov ID #NCT00058539) is yet to be reported. There is also a Phase I clinical study underway for the ‘Fc-optimized’ anti-HER2/neu antibody MGAH22, (ClinicalTrials.gov ID #NCT01195935). Various anti-HER2/neu antibody conjugates, including a Phase I study of single chain immunotoxin, anti-HER2/neu pseudomonas exotoxin A conjugate [280], have been tested. Bispecific antibodies have been evaluated including a Phase I [321], PK evaluation [322] and a Phase II study of MDXH210 (an anti-HER2/neu CD64 fusion) [323] that did not use RECIST response criteria, but did note improved pain control, decreased PSA velocity in 83% of subjects and a decrease in PSA greater than 50% from enrollment levels, in 35% of subjects.

TKIs that target HER2/neu have been explored for the treatment of prostate adenocarcinoma [324,325] driven in part by the recognition of the cross talk between the HER2/neu and androgen receptor pathways (Part 1) [1]. Phase II studies of lapatinib ditosylate in hormone naïve or sensitive prostate adenocarcinoma [326,327] have demonstrated no significant activity, but a separate Phase II study in hormone-resistant prostate adenocarcinoma as a single agent demonstrated limited (~10%) activity for lapatinib ditosylate [328]. There is an ongoing Phase I/II clinical study of lapatinib ditosylate in the setting of multifaceted hormonal therapy (ketoconazole, hydrocortisone and dutasteride; ClinicalTrials.gov ID #NCT00953576). There is an ongoing Phase II study being conducted in Germany of afatinib in hormone-resistant prostate adenocarcinoma (ClinicalTrials.gov ID #NCT01320280) [87].

T lymphocytes from prostate cancer patients can recognize HER2/neu peptides [256,329–331] suggesting the capacity to elicit anti-HER2/neu immune responses. A Phase I clinical study of the E75 HER2/neu peptide vaccine has been reported [332] and with continued follow-up, the data support a delay in prostate cancer progression/recurrence [333]. A separate Phase I study of a vaccine strategy using HER2/neu-derived peptides and GM-CSF has been shown to be safe and elicit immune responses [334]. DC-based vaccine strategies have, by and large, been supplanted by sipuleucel-T and have not been pursued.

**Gynecologic tumors**

Uterine carcinomas, specifically adenocarcinomas, are relatively uncommon tumors that overexpress HER2/neu, as do squamous cell carcinoma of the uterine cervix with approximately half of these demonstrating gene amplification (Part 2) [2]. Three independent case reports of use of trastuzumab further support the possible efficacy of trastuzumab in endometrial adenocarcinoma [335–337]. However, a Phase II study of trastuzumab in cases of advanced or recurrent, gene-amplified endometrial adenocarcinoma was terminated with insufficient accrual (226 screened, 34 enrolled), although 12 of the 34 had stabilization of their disease [338]. There is an ongoing Phase II clinical study evaluating the addition of trastuzumab to the combination of carboplatin and paclitaxel in patients with advanced or recurrent endometrial adenocarcinoma (ClinicalTrials.gov ID #NCT01367002). There are no reports of the clinical evaluation of pertuzumab
in endometrial tumors. The evaluation of TKIs in has included, a Phase I study of the pan-HER inhibitor CI-1033 in which a CR was noted in a cervical cancer patient [96], a Phase I study that is ongoing examining the combination of lapatinib ditosylate and ixabepilone in patients with progressive endometrial carcinoma and carcinosarcoma (ClinicalTrials.gov ID NCT01454479), a Phase II study of lapatinib ditosylate in recurrent endometrial adenocarcinoma in which a limited response rate was observed [339] and a Phase II study of pazopanib and lapatinib ditosylate as single agents or in combination for patients with advanced and recurrent cervical cancer [340] that revealed increased toxicity of the combination and statistically significant improved progression-free survival (18.1 vs 17.1 weeks) pazopanib over lapatinib ditosylate or the combination, thus not supporting the combination. Methods for HER2/neu-targeted immunotherapy have not been explored in this setting nor have strategies for modification of HER2/neu expression or activity. The incidence and age distributions of endometrial adenocarcinoma, carcinosarcoma and cervical carcinoma along with the existing very limited activity in early phase clinical studies, pose significant challenges to future studies of HER2/neu-targeted therapy.

Ovarian

Starting with the initial studies of Slamon et al. [341], the overexpression of HER2/neu has been described and extensively studied in ovarian cancer in parallel with breast adenocarcinoma (Part 2), with the consensus supporting HER2/neu overexpression in 10–20% of ovarian epithelial neoplasms with a higher frequency of both gene amplification and HER2/neu overexpression in the mucinous subset [2]. The disparity in clinical success in targeting HER2/neu in ovarian epithelial tumors versus breast adenocarcinomas may be due to the fact that gene amplification drives a lower percentage of HER2/neu overexpression in ovarian epithelial neoplasms, occurring in less than half of the cases overexpressing HER2/neu, along with the presence of cytoplasmic HER2/neu, which confounds the evaluation of overexpression.

A number of antibody-based therapeutic strategies have been explored for HER2/neu-overexpressing ovarian epithelial cancers. A Phase II clinical study of trastuzumab in pretreated patients with progressive ovarian epithelial neoplasms was limited by both a lower frequency of HER2/neu overexpression (11.4%) and lower than expected response rate (7.3%) [342]. Interestingly, in a study conducted in mucinous ovarian carcinoma in which there is an increased frequency of HER2/neu overexpression and gene amplification (nearly 20%; Part 2), the use of trastuzumab alone or with standard chemotherapy yielded an objective response in one of six patients with HER2/neu-overexpressing and gene-amplified recurrent mucinous ovarian carcinoma, while another had an isolated CNS metastatic progression [343]. These data suggest that mucinous ovarian carcinoma maybe a better study population for clinical evaluation of anti-HER2/neu therapies in ovarian epithelial neoplasms.

Pertuzumab has been evaluated for treatment of HER2/neu-expressing ovarian epithelial carcinomas. Reported Phase I studies included three subjects with ovarian epithelial tumors, one of which experienced a response to pertuzumab as a single agent [38] and one of two subjects experienced a response in another [344]. Phase II studies of pertuzumab have been conducted as a single agent, with stable disease or a partial response in 11% of 117 subjects [345], and in combination with second-line chemotherapy (gemcitabine) in platinum-resistant tumors, with an objective response rate of 13.8% for the combination compared with 4.6% for gemcitabine alone [346]. It is possible that the benefit observed with pertuzumab may be maximal in the HER2/neu high expressing and HER3 low level expressing tumors [347]. The rationale for and preclinical benefit of combining trastuzumab and pertuzumab for the treatment of HER2/neu-expressing ovarian epithelial carcinomas has been described [348]. There is support for biological synergy between HER2/neu and HER1/EGFR, with preclinical data supporting synergy between pertuzumab and cetuximab for sensitizing ovarian cancer cell lines to the proapoptotic effect of docetaxel [349].

The published work examining the use of HER family TKIs in ovarian epithelial carcinomas is extremely limited. Three Phase II studies have been reported examining lapatinib ditosylate, none of which documented sufficient activity to proceed forward in recurrent or platinum-resistant carcinomas [350–352]. Canertinib (CI-1033) has had modest activity against ovarian adenocarcinoma in Phase I studies and has also been evaluated in a Phase II study with similar disappointing results [353]. However, there was a paucity of mucinous or clear cell histology in these studies. BMS559626 (AC480), a dual HER1 HER2 inhibitor, has also been evaluated in Phase I clinical studies that include ovarian adenocarcinoma (ClinicalTrials.gov IDs #NCT00207012, NCT00095537 and NCT00093730) [171].

There is a long history of targeting HER2/neu with an antigen-specific immune response in ovarian epithelial carcinoma. HER2/neu peptides recognized by CTLs from patients with ovarian epithelial carcinoma were described [1]. Early Phase I studies of HER2/neu peptide vaccination demonstrated peptide-specific
immune responses [354–356], but in some cases this immune response did not contain a detectable cytotoxic component for HER2/neu-overexpressing tumor [358]. There is an ongoing Phase I peptide vaccine trial (ClinicalTrials.gov ID #NCT01376505). A Phase I study investigating a B lymphocyte epitope from HER2/neu with a promiscuous T lymphocyte helper epitope in six subjects with gynecologic tumors, yielded stable disease and partial response in each of two subjects with ovarian epithelial tumors and a partial response in one subject with endometrial adenocarcinoma [196]. A Phase I clinical study of peptide-loaded DCs demonstrated robust antigen-specific immune responses [357] and a Phase I/II study of DCs loaded with multiple peptides and treated with low-dose cyclophosphamide to ameliorate tumor-associated immune suppression, demonstrated improved survival despite the absence of a robust immune response [358]. A Phase I study of lapuleucel-T contained four subjects with recurrent, refractory ovarian epithelial carcinoma, who were in the two lower dose cohorts, had two of the four experiencing 15–18 weeks of stable disease [123].

In ovarian epithelial carcinoma, a wider range of strategies for downregulating HER2/neu expression have been evaluated than in other tumor types. Preclinical studies of antisense oligonucleotides either alone [359] or in liposomes with anti-HER2/neu antibody incorporation for targeting have been reported [360]. Hammerhead RNA [361] and ribozyme [361,362] constructs have been used to downregulate the expression of HER2/neu, the latter of which demonstrated the unexpected finding that resistance to paclitaxel was associated with decreased HER2/neu expression, which was also seen when the ribozyme was combined with trastuzumab and a HER2/neu-specific TKI [363]. Adenoviral encoded single chain anti-HER2/neu antibody has demonstrated good preclinical activity, downregulating HER2/neu expression [364–367] and increasing radiosensitivity [368]. This approach has progressed to early clinical studies [369,370] where it has been shown to be safe [371,372]. Three Phase I clinical studies of incorporation of the adenoviral E1A coding sequence into cationic liposomes, two single institution and one multicenter, yielded decreased HER2/neu expression, increased apoptosis and tumor regression in the preclinical setting and has been reported to be safe [371,373,374]. A dramatic response, resolution of ascites and pleural effusion for 2 years, was recently reported for one of the three subjects with ovarian epithelial tumors enrolled in a Phase I study of the HSP90 inhibitor, alvespimycin, combined with trastuzumab [127]. The unique biology and anatomic distribution of ovarian epithelial carcinoma in conjunction with the reported studies above, suggest that downregulation of HER2/neu expression may be more productive that either antibody- or TKI-based inhibition of HER2/neu signaling.

Conclusion
The breadth of potential therapeutic agents targeting HER2/neu far exceeds that directed at any other tumor-associated, biological molecule. The development of this repertoire is driven in large part by the fact that HER2/neu is overexpressed in a broad range of tumor types and the biological role HER2/neu plays in the broader HER family signaling network. However, the proportion of tumors with concordant overexpression and gene amplification varies greatly by tumor type. The ongoing characterization of the biology of HER2/neu and other HER family members continues to provide critical insight into mechanisms of resistance to individual therapeutic agents or strategies and also illuminates novel targetable nodes within the HER2/neu signaling network. Lessons learned from the work reviewed above reiterate a common theme in clinical research; the strategic selection of study populations, study end points and careful matching of agent or strategy with the underlying biology within a given tumor type is critical for success. Although FDA approval of antibodies, antibody conjugates and TKIs targeting HER2/neu has been obtained for breast, gastric and esophageal adenocarcinomas, there are other clear opportunities in other tumor types that have been identified. There is reason to be encouraged that other therapeutic approaches to targeting HER2/neu will find a place in the therapeutic armamentarium, including immunotherapeutic strategies and modulators of HER2/neu expression such as the HSP90 inhibitors. These alternate therapeutic approaches will likely broaden the tumor types in which targeting HER2/neu results in clinical benefit. The basic science, preclinical and clinical work reviewed above substantiates the proposition that targeting HER2/neu has and continues to be the consummate translational research success story and results in HER2/neu being an increasingly important therapeutic target.

Future perspective
The immediate and longer-term future for HER2/neu-targeted therapies is exceptionally bright. There is a robust pipeline, in active clinical development, of small molecule inhibitors of HER signaling. Additionally, the place in the therapeutic armamentarium for antibody conjugates, resistance-modulating agents and immunotherapeutic/immunomodulatory strategies will be revealed over the next decade. The field
will experience a dramatic shift in treatment planning paradigms as the impact of systems biology, expression profiling and network analyses are felt across the entire range of tumor types. With these advances in molecular profiling, additional tumor types and tumor subsets that are viable targets for therapeutic targeting of HER2/neu will be identified. The challenge for clinicians will be to determine which therapeutic strategies yield benefit in which tumor type and subset. It is likely that the field will move from histology-based to expression profile-based therapeutic planning. In the short term, 5 years, the evaluation of combinations of targeted therapies and the integration of resistance modulating agents will be a major thrust in the field. The challenge for widespread clinical impact of new combinatorial strategies targeting HER2/neu and directed by tumor expression profiles, is how to move from early phase limited cohort size studies to definitive, registration studies, which have traditionally been large Phase III studies. The field is fortunate to have a multitude of therapeutic options and diverse tumor types in which to evaluate these HER2/neu-targeted therapies, the trick will be to find the gem(s) in this very rich ore.

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