CLINICAL INVESTIGATION

HER2/neu: an increasingly important therapeutic target. Part 1: basic biology & therapeutic armamentarium

Edward L Nelson
University of California, Irvine, School of Medicine; Division of Hematology/Oncology; Department of Medicine; Department of Molecular Biology & Biochemistry; Sprague Hall, Irvine, CA 92697, USA
enelson@uci.edu

This is the first of a comprehensive three-part review of the foundation for and therapeutic targeting of HER2/neu. No biological molecule in oncology has been more extensively or more successfully targeted than HER2/neu. This review will summarize the pertinent biology of HER2/neu and the EGF receptor family to which it belongs, with attention to the biological foundation for the design and clinical development of the entire range of HER2/neu-targeted therapies, including efforts to mitigate resistance mechanisms. In conjunction with the subsequent two parts (HER2/neu tissue expression and current HER2/neu-targeted therapeutics), this comprehensive survey will identify opportunities and promising areas for future evaluation of HER2/neu-targeted therapies, highlighting the importance of HER2/neu as an increasingly important therapeutic target.

Keywords: c-erbB2 • EGF receptor • EGFR • EGFR ligand • expression modulation • HER2/neu • monoclonal antibody • signaling network • targeted therapeutic • tyrosine kinase inhibitor • vaccine

The history of the molecule known as HER2/neu dates back to the earliest studies of virus-associated oncogenes. In 1979, studies of avian erythroblastosis virus identified two putative viral oncogenes, v-erbA and v-erbB [1–3]. Subsequently, the Bishop group identified the normal avian (chicken, 'c') and mammalian homologs (c-erbA and c-erbB) [4,5], and that the encoded protein is a membrane glycoprotein [6]. This gene and its gene product were rapidly recognized to have a relationship to the recently identified, EGF receptor (EGFR) [7,8]. Simultaneous, but separate, studies in the Weinberg laboratory in chemical-induced rat tumors identified an oncogene – neu – from a rat neuroblastoma that could transform wild-type 3T3 cells. This oncogene was noted to have tyrosine kinase activity and sequence homology with c-erbB and the EGFR genes [9–12]. This relationship became clearer with the studies of Semba *et al.* in which the individual genes and the gene nomenclature for EGFR family members (c-erbB1 = EGFR, c-erbB2 = neu, c-erbB3 = EGFR-3, and eventually, c-erbB4 = EGFR-4) were established [13]. The fact that the neu molecule and coding sequence was originally identified in the rat species and only recently has a biological function been demonstrated for the murine gene locus [14–16] has complicated studies of the biology and fueled a separate nomenclature in humans; HER1, 2, 3 and 4 (Table 1). The high degree of homology between HER2 and rat neu (~85%) along with the recognition that the protein product of the *c-erbB2* gene was known in various circles as HER2 or as neu led to the commonly used terminology HER2/neu.

The structural elements (Figure 1 & Table 1) and the dimerization patterns (Figure 2) result in a complex biology for the HER family. These type 1 transmembrane proteins contain, in the extracellular portion of the molecule, three cysteine-rich Furin-like domains and two L domains. Each L domain consists of a right hand, single-stranded β-helix, which forms the ligand-binding site. It

FUTURE SCIENCE part of fsg

Table 1. Nomenclature of EGF receptor family members.

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

EGFR: EGF receptor.

remains unclear whether the variable arrangement of the three Furin-like domains has any biological significance. The intracellular domain contains a protein tyrosine kinase consensus domain and multiple phosphorylation sites that permit downstream signal transduction in all but HER3, which has an in active protein tyrosine kinase domain [17]. Receptor dimerization, as a homodimer or heterodimer, is essential for signal transduction and involves a portion of the transmembrane domain along with a portion of the membrane proximal, extracellular domain.

Although the ligands for each HER family member are relatively restricted, some ligands engage more than one HER family member (Figure 2 & Table 1) [18]. The ligands for HER1 (EGFR) include: EGF, amphiregulin, TGF-α and epigen [19,20]. Three ligands can bind to either HER1 or HER4 including: β-cellulin, epiregulin and HB-EGF. The remaining identified HER family ligands are members of the Neuregulin family. Neuregulin 1 and Neuregulin 2 both have α- and β-isoforms. Neuregulin 1 (aka Heregulin, NDF) and Neuregulin 2, both with α- and β-isoforms, can bind to HER3 or HER4, while Neuregulin 3 and Neuregulin 4 can only bind HER4. Importantly, HER2 has no established ligand.

Two facets of HER family biology limit the potential combinatorial diversity. HER3 is a dead kinase [17] and thus, any signaling that occurs is dependent upon the dimerizing partner for HER3. HER2 has no defined ligand and, thus, is dependent upon the dimerizing partner for ligand-dependent signaling. Recent structural studies suggest that ligand interaction with extracellular domains I and III result in alterations in domain II that permit heterodimerization [21,22], the relative activity of anti-HER2 antibodies (trastuzumab and pertuzumab) are related to binding to different domains. HER2 exists in a structurally receptive conformation that allows it to readily form dimers and heterodimers [23] and is the preferred dimerization partner for heterodimers involving HER1, HER3 and HER4. This conformation favors heterodimerization with HER2 in lieu of homodimerization where presumably two ligands would be required to convert two proteins into the permissive dimerization conformation [21,22]. Phosphorylation events occurring on the cytoplasmic tails of the HER proteins, either via auto- or trans-phosphorylation [24–26], are essential for signal transduction.

Signaling through the HER family members involves a limited number of major signal transduction pathways (Figure 2) [18,27]. The PI3K/AKT/mTOR pathway is the major pathway involved. Additional pathways include the ERK, the Ras/Raf, the Rho/Rac and the phospholipase C pathways [27–29]. An appreciation of the complexity of these signaling pathways is essential for understanding the resistance mechanisms that come into play to counteract therapies targeting the HER molecules, including HER2/neu [30–32]. These pathways impact a number of biological processes (Figure 2). Signaling through the HER family members has been demonstrated to influence the regulation of proliferation, transcription, autophagy, apoptosis and chemotaxis. Crosstalk between the various involved pathways results in more extensive propagation of the ligand-induced signal [18]. The overexpression of HER2/neu, with its intact kinase activity that is not dependent upon engagement of a distinct cognate ligand, is believed to perturb the balance of signaling within the HER family and contribute to dysregulated growth [33].

The HER2/neu molecule, which will be the focus of the remainder of this review, 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. The normal expression of HER2/neu appears to be primarily transcriptionally regulated [34–36]. A number of transcriptional elements and factors have been identified, which vary between normal and malignant transformed states and by tissue [35,36]. In the absence of gene amplification, transcription dysregulation is one mechanism for tumor overexpression of HER2/neu [37,38]. Post-transcriptional regulation of HER2/neu expression has also been described [39–41]. Recent work

Figure 1. Protein and coding sequences for HER1, HER2, HER3 and HER4 were retrieved from the US National Center for Biotechnology Information Entrez Gene database and submitted for evaluation in the context of the Conserved Domain Data Base at the NIH. (Summary graphic output for each HER molecule has been combined into this figure for comparative purposes).

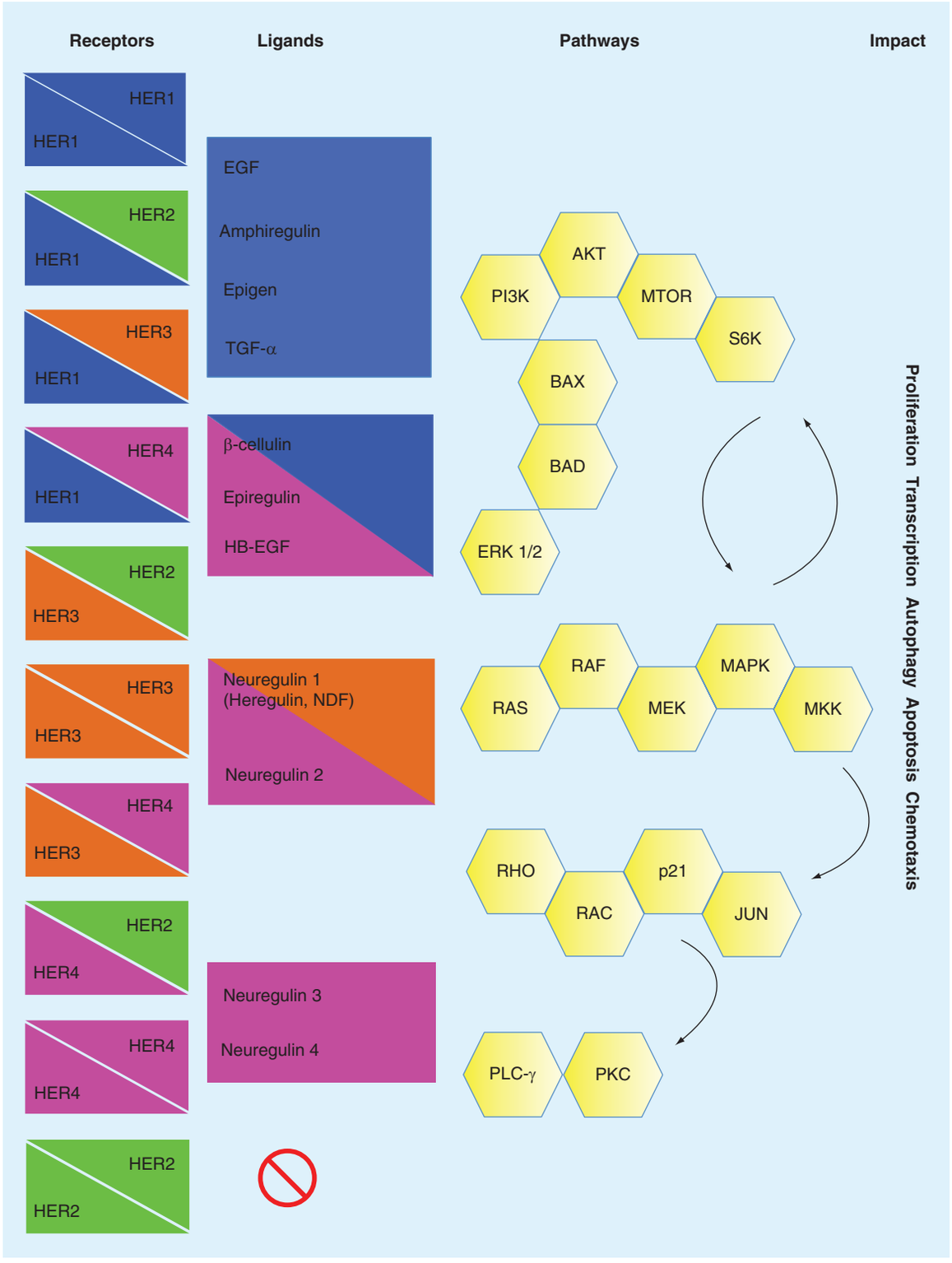

Figure 2. The individual HER heterodimers are depicted in the left hand column and their cognate ligands color coded for binding to their respective HER molecules. Blue: HER1 and its respective ligands; green: HER2; orange: HER3 and its respective ligands; fuschia: HER4 and its respective ligands. Ligands 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.

supports an important role for miRNAs in the biology and expression of HER2/neu [42–45]. Glucocorticoids have been reported to stabilize HER2/neu mRNA levels [39] and HSP90 stabilizes the expression levels of at least HER1 and HER2 by inhibiting their degradation via the ubiquitin proteasome pathway and by facilitating dimerization [46,47]. Thus, there are transcriptional and other nonclassical post-transcriptional mechanisms by which tumor overexpression of HER2/neu may occur in the absence of gene amplification. The exemplary work by Slamon *et al.* demonstrated that overexpression of HER2/neu by gene amplification in breast cancer imparted a poor prognosis [48] and was subsequently independently confirmed by Berger *et al.* [49]. HER2/neu overexpression without gene amplification has also been associated with prognosis in multiple tumor types, potentially related to the transcriptional dysregulation or other mechanisms.

Evidence for a critical biological function of HER2 has been derived from gene-targeting studies in mice, which occurred nearly 20 years after the original description of neu. Either constitutive kinase dead or *erbB2*-null mice demonstrate embryonic lethality secondary to gross cardiovascular abnormalities, most significantly abnormal development of the heart [14,16]. The *erbB2*-null mice or perinatal cre/lox deletion of erbB2 also revealed an important role for development and maintenance of the nervous system [14–16]. The fact that major defects in erbB2 compromised mice were somewhat limited, despite HER2 being the preferred dimerization partner for the HER family members, amplifies the complexity and redundancy present within the EGFR family, but also provides a foundation for understanding the unexpected cardiac toxicity experienced with initial therapeutics directed at HER2/neu.

HER2/neu-targeted therapeutics

Development of therapeutic strategies targeting HER2/neu have been driven by the underlying biology of the EGFR/HER family and HER2/neu, with a major focus on breast cancer [50]. Antibodies directed at the extracellular domain were generated [51], with both agonistic and antagonistic properties [33,52–54]. The absence of a cognate ligand for HER2/neu precluded development of either ligand-clearing antibodies or small-molecule decoy ligands or ligand-binding site inhibitors. However, with the launch of the small-molecule targeted therapy era, small-molecule tyrosine kinase inhibitors (TKIs) have been developed and approved for HER2/neu and other HER family members. Antitumor antigen-specific immunotherapeutic strategies (tumor vaccines) and modulators of HER2/neu expression, such as inhibitors of HSP90, are advancing through clinical studies and have shown promise. Future strategies targeting HER2/neu may well employ new small-molecule pathway inhibitors or combinations of HER2/neu therapeutic strategies or agents to minimize the development of resistance [55].

Antibody-based therapies

Trastuzumab (Herceptin®)

The clinical and commercial success of the anti-HER2/neu antibody trastuzumab (Herceptin®) is self-evident and establishes without question the proof-of-concept for therapeutic targeting of HER2/neu. The series of studies leading to the approval and broad application of this monoclonal antibody have been held up as a prototype for bench-to-bedside translation [56]. Initial studies characterizing the *erbB2* gene in human tumors demonstrated that it was amplified in breast [48] and subsequently in ovarian adenocarcinoma [57]. Because HER2/neu was a known cell-surface molecule and because antagonistic antibodies had been reported, the development of an antagonistic antibody for clinical application was logical, given the state of the art in the late 1980s [58,59]. The characterization of the antibody designated 4D5 demonstrated significant growth inhibition of SKBR3 breast carcinoma cells, in which the *erbB2* gene is amplified, and sensitized them to the cytotoxic effects of TNF-α [58] resulting in the initial patent (US Patent No 5,677,171). The epitope recognized by the 4D5 antibody resides within extracellular domain IV of HER2/neu [60], can elicit antibody-dependent cellular cytotoxicity, and importantly, disrupts HER2/neu-mediated signaling, with modest disruption of dimerization. Interestingly, this disruption is much more pronounced for the HER1:HER2 heterodimer than the HER2:HER3 heterodimer, even though HER3 is the preferred dimerization partner for HER2 [61]. This antibody would go on to be genetically engineered to create a chimeric, 'humanized' IgG1subclass monoclonal antibody [62], and take its place in the clinical armamentarium as trastuzumab (Herceptin) [63]. In the landmark clinical studies, associated cardiac toxicity was observed, particularly in patients previously treated with anthracyclines [64]. The aforementioned murine work identified the critical role for HER2/neu in cardiovascular development [14,16], wherein *erbB2*-null animals have an embryonic lethal phenotype secondary to abnormal heart development [14], and was reported only slightly before this unexpected toxicity was observed, in 27% of study participants.

The development of resistance and tumor progression on treatment was observed in the initial studies of trastuzumab in the advanced disease setting. Resistance can be generally classified as primary resistance

or as acquired/secondary resistance [65]. Given the complexity of the EGFR family and the extensive crosstalk between signaling pathways, it comes as no surprise that the mechanisms of trastuzumab resistance are diverse and likely to be multiple in any one patient [31,65,66]. Perhaps the easiest mechanism to understand arises from the recognition that the extracellular domain of HER2/neu can be proteolytically cleaved into a soluble form, can be detected in the circulation, and that levels of this soluble form have been variably associated with disease burden, prognosis and inversely with response to treatment [67–70]. Indirect studies suggest that the soluble ECD of HER2 could both act as a sink for trastuzumab [71,72] and may elicit or be associated with anti-HER immune responses, particularly antibody responses [73]. There are data supporting that the remaining portion of the cleaved HER2/neu molecule has increased endogenous constitutive signaling capacity [74], suggesting an additional mechanism of resistance. Additionally, an alternatively spliced form of HER2/neu has been described that is truncated, yielding a potentially secreted form of the molecule, but which appears to be sequestered in the perinuclear cytoplasm [75]; however, a role for this potential alternative fragment in normal or neoplastic tissues has not been documented. Interestingly, binding of trastuzumab to HER2/neu has been reported to inhibit the proteolytic cleavage of the extracellular domain [76,77]. Although there is no compelling evidence to suggest that HER2/neu undergoes mutation to abrogate the trustuzumab-recognized epitope, there is data that MUC-4 can mask the binding site and may be responsible for some primary resistance [78]. Perturbations of downstream signaling pathways can also yield resistance (Figure 2). Loss or low levels of expression of PTEN have been associated with a decreased response to trastuzumab, perhaps through release of the counter-regulatory input of PTEN on AKT [79,80]. Activating mutations of PI3K have also been associated with trastuzumab resistance [81,82], as has increased Rac activity [83,84]. There are also data to suggest that increased expression of other HER family members can overcome the trastuzumab-mediated inhibition of HER2/neu function, and thereby confer resistance [85]. Other receptor kinases can also function in a similar manner to HER2/neu and other HER family members, including IGF-1 receptor, Met and erythropoietin receptor [86–90].

Pertuzumab

In the initial screen that identified 4D5 as a potent therapeutic antibody for HER2/neu gene-amplified tumors, another antibody with a nonoverlapping epitope, 2C4, was identified [59]. In contrast to trastuzumab, this antibody binds to the extracellular domain II, resulting in enhanced steric blocking of dimerization via binding to the requisite domain for dimerization and signaling [91]. Thus, the humanized 2C4 antibody (pertuzumab) [92] is more broadly effective at inhibiting heterodimer formation with HER2/neu. Pertuzumab has activity with lower levels of expression of HER2/neu [93] and unlike trastuzumab, which has limited ability to inhibit heterodimerization between HER2/neu and HER3; pertuzumab inhibits signaling by HER3 ligands [94]. Pertuzumab may inhibit, but not abrogate HER2/neu HER3 heterodimerization and may have less activity inhibiting HER2/neu HER1 heterodimerization [95,96]. It is unclear if pertuzumab interferes with the heterodimerization of HER2/neu with alternate tyrosine kinase receptors such as IGF-1R, and Met.

Trastuzumab emtansine (T-DM1; Kadcyla®)

An alternate strategy for improving the efficacy of trastuzumab, in the setting of lower expression of HER2/neu or resistance to trastuzumab, is the conjugation of a cellular toxin or radioisotope in a manner analogous to that demonstrated for hematologic malignancies; Adcetris®, Zevalin®, Bexxar® and Mylotarg®. Trastuzumab emtansine (T-DM1) is a conjugate of trastuzumab and the cellular cytotoxic agent mertansine (DM1) that is a derivative of the microtubule disruptive macrolide maytansine, which, when conjugated to the trastuzumab antibody using the linking agent 4-(3-mercapto-2,5-dioxo-1-pyrrolidinylmethyl)-cylohexanecarboxylic acid, is known as emtansine [97]. The use of this linking agent substantially improved the release of mertansine by proteolytic cleavage once internalized, relative to more traditional reducible disulfide-linking chemistries that were used in the early studies of antibody conjugates of this cytotoxic agent [98,99]. Mertansine binds to tubulin at a site different from that of the vinca alkaloids and is substantially more potent at inhibiting microtubule formation [97]. 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 pharmacokinetic data suggested that this is not the case [100].

Other modified antibodies targeting HER2/neu have been designed for both therapeutics and imaging, although many suffer from the limitations of traditional disulfide-based conjugation chemistries [99]. These conjugates include affitoxin [101], pseudomonas exotoxin [102,103], ricin A chain [104], various probes (radioactive and nonradioactive) [104–112], photosensitizers [113–115], RNAse [116,117], cytokines [118] or chemotherapeutic agents [119–122]. A Phase II study of a novel

PET agent (zr89-labeled trastuzumab) as a predictor of response to trastuzumab emtansine (ZEPHIR) has recently been initiated in Europe (NCT01565200). Additionally, bispecific antibodies targeting HER2/neu have been developed [123–127] taking advantage of the intrinsic immunologic function of antibodies recognizing HER2/neu. Finally, aptamers selected for recognition and binding to the HER2/neu molecule can deliver cytotoxic agents to HER2/neu-positive cells as an alternative to antibody-based delivery [128].

Tyrosine kinase inhibitors

The groundbreaking work that identified Imatinib as an effective small-molecule inhibitor of the tyrosine kinase activity of the oncogene-derived fusion protein BCR/Abl launched the era of molecularly targeted therapeutics [129]. 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 and other targeted therapeutics [130–134]. An additional impetus for the development of this class of therapeutic agent was the observed increased incidence of CNS metastases in the setting of breast cancer patients treated with trastuzumab or other antibody-based HER2/neu-targeted therapies, with this often being the site of progression while other systemic disease remained under control. Well-designed small molecules might have better CNS penetration and potentially circumvent this problem. Generalizing from the Imatinib experience, it was anticipated that multiple TKIs would be needed as acquired mutations could lead to resistance. Although all TKIs are designed to inhibit receptor signaling, there are differences in spectrum, reversibility and potential secondary mechanisms [135]. Recent observations that many of these TKIs interact with various drug transporters including ABCB1, ABCG2 and the P-glycoprotein multidrug resistance transporter [136–138] in a semi-selective and dose-dependent manner, adds another complicating element to their development and clinical application.

The first TKI developed to target HER2/neu was lapatinib ditosylate (GW572016, Tykerb), which is a reversible inhibitor of both HER2/neu and HER1/EGFR [139]. In the initial Phase I studies of lapatinib ditosylate the toxicities matched those predicted from clinical experience with HER1/EGFR inhibitors erlotinib and gifitinib; rash, fatigue and gastrointestinal symptoms (diarrhea) [140–144]. Interestingly, no additional or unexpected cardiotoxicities were observed when lapatinib ditosylate was combined with trastuzumab in early-phase trials [141–144]. The clinical efficacy observed in several Phase II studies and a landmark Phase III trial led to US FDA approval of lapatinib ditosylate [145]. Whether this represents additional or more effective targeting of HER2/neu versus the addition of HER1/EGFR inhibition remains to be determined. Either would be expected to have clinical impact given the mechanisms of resistance to trastuzumab and the crosstalk between HER family member signaling pathways described above. Recently, acquired mutations in HER2/neu have been identified in association with resistance to lapatinib ditosylate [146] suggesting that, as in the case of imatinib, other inhibitors targeting the same molecule will be necessary to treat those tumors with acquired resistance. An additional concern arises from the observation that lapatinib ditosylate increases the shedding of HER2/neu [147], a potential mechanism for resistance particularly to HER2/neu-directed antibody-based therapies.

Other TKIs targeting HER2/neu have been generated and are at various stages of development. In contrast to lapatinib ditosylate, which is a reversible inhibitor of HER1 and HER2, the pipeline of TKIs targeting HER2/neu contains second-generation irreversible inhibitors [148], which can be categorized as either pan-HER or dual HER1/HER2 inhibitors. These TKIs, like essentially all drugs in this class, are oral, multikinase inhibitors inhibiting HER2/neu, other members of the HER family, and other receptor tyrosine kinases (Table 2).

Afatinib (Gilotrif, BIBW 2992, Tomtovok) is in the class of irreversible TKIs that target HER2/neu along with other HER family members. This 'second generation' of TKIs with irreversible inhibitory activity are felt to not only have increased potency, but also the capacity to inhibit tumors that have progressed on the first-generation reversible inhibitors, typically by acquiring mutations that confer resistance to first generation TKIs [132,148,149]. The majority of the effort in development of afatinib has been directed toward its activity inhibiting EGFR (HER1), but it was rapidly recognized to also inhibit HER2/neu [150,151]. Preclinical work also suggested that afatinib would be synergistic with radiation therapy [151,152]. Pharmacokinetic [150,153] and Phase I studies have led to a number of administration schedules and doses with acceptable toxicities including: 2 weeks with daily administration of 70 mg with 2 weeks off [154]; continuous 50 mg daily dosage [155,156]; continuous 40 mg daily dosage [157]; 3 weeks of daily 40 mg and 1 week off [158]; afatinib 90 mg days 2–4 in combination with paclitaxel 75 mg/m^2 on day 1 every 3 weeks [159]; and, more multidrug regimens containing cisplatin and either paclitaxel or 5-fluorouracil at 20 or 39 mg daily, respectively [160]. The dose-limiting toxicities are, as expected from the first-generation HER family-targeting TKIs,

Table 2. Tyrosine kinase inhibitors with HER2/neu inhibitory activity.

Tyrosine kinase inhibitor	Reversibility	Documented receptor targets†	Clinical trial status‡	Tumor types§
Gifitinib	Reversible	**EGFR** (HER1), HER2/neu	Approved (1)	Multiple
Erlotinib	Reversible	**EGFR** (HER1), HER2/neu	US FDA approved (2)	Mulitiple
Lapatinib	Reversible	**HER2/neu**, EGFR (HER1)	FDA approved (3)	Breast
TAK-285	Reversible	**HER2/neu**, EGFR (HER1)	Phase I	Multiple
Afatinib	Irreversible	**EGFR** (HER1), HER2/neu, HER4	FDA approved (4)	Lung (NSCLC), breast, colorectal
Neratinib	Irreversible	**HER2/neu**, EGFR (HER1)	Phase III	Breast, lung (NSCLC), colon
Pelitinib	Irreversible	**EGFR** (HER1), HER2/neu	Phase II (no Phase III registered)	Lung (NSCLC), colorectal
AST1306	Irreversible	**HER2/neu**, EGFR (HER1)	Preclinical only	
Canertinib	Irreversible	**EGFR** (HER1), HER2, HER4	Phase II (no Phase III registered)	Breast, lung (NSCLC), ovarian
Dacomitinib	Irreversible	**EGFR** (HER1), HER2, HER4	Phase II (no Phase III registered)	Lung (NSCLC), head and neck squamous cell and glioblastoma
BMS-599626	Irreversible	**EGFR** (HER1), HER2, HER4	Phase I (no Phase II or III registered)	HER2-positive tumors
BMS-690514	Reversible	**EGFR** (HER1), HER2, VEGFR-1, 2 and 3	Phase II (no Phase III registered)	Breast, lung (NSCLC)
AEE788	Reversible	**EGFR** (HER1), **HER2**, VEGFR-1 and 2	Phase I (no Phase II or III registered)	Multiple, brain (GBM)

†Target molecules in bold represent the primary molecular target for the respective tyrosine kinase inhibitor.
‡(1): approved outside of the USA, for first-line lung NSCLC, with mutated EGFR; (2): first-, second- and third-line lung NSCLC, with mutated EGFR; first line for advanced pancreatic adenocarcinoma; (3): second-line advanced or metasatic HER2/neu+ breast adenocarcinoma; (4) first-, second- and third-line lung NSCLC, with mutated EGFR.
§Tumor types that have been evaluated in either Phase II or III clinical studies with the exception of TAK-285, BMS599626 and AEE788.
EGFR: EGF receptor; GBM: Glioblastoma multiforme; NSLC: Non-small-cell lung cancer.

diarrhea and rash. Afatinib was given fast track status for NSCLC by the FDA [161] leading to recent FDA approval. Afatinib has been reported to inhibit HER4 [162], which would indicate that it resides in the class of irreversible pan-HER inhibitors. Other members of this class of pan-HER irreversible TKIs include canertinib (CI-1033), which has TKI activity across the entire HER family, HER1, HER2 and HER4 and was noted to have radiosensitizing activity [163,164]. Phase I studies conducted by different groups using different schedules including oral continuous, oral for 14 days with 7 off, oral for 7 days with 14 off, and intravenous, came to entirely different maximum-tolerated doses [165–170] with dose-limiting toxicities being rash, nausea, diarrhea or fatigue depending, in part, on schedule and route. Dacomitinib (PF-00299804) is a second-generation irreversible pan-HER TKI related to canertinib, but with better pharmacokinetic and bioavailability properties [171,172]. Although HER2/neu is subject to inhibition by this agent, the overwhelming bulk of its development has focused on its inhibition of EGFR (HER1) [171–173]. In Phase I studies, dacomitinib did not appear to have the same variability in maximum-tolerated dose, established at 60 mg daily, by schedule and route as in the first-generation pan-HER inhibitor canertinib [174–176]. Dacomitinib, at 45 mg daily the dose carried forward to Phase II studies, is able to inhibit HER family members with acquired mutations that confer resistance, particularly to erlotinib and gefitinib, but amplification of the mutated HER sequence

can lead to dacomitinib resistance [177]. BMS599626 (AC480) is also a second-generation pan-HER inhibitor [178,179] that appears to be a radiosensitizing agent [179]. Somewhat surprisingly, the dose-limiting toxicities were not the usual mucocutaneous toxicities, which did occur, but rather QT prolongation and elevation of liver transaminases with a maximum-tolerated dose of 600 mg daily [180].

A subset of pan-HER inhibitors were designed to include significant inhibitory activity of one or more of the VEGFRs. AEE788 is a pan-HER inhibitor with capacity to inhibit the three VEGF receptors, which has roughly equivalent activity inhibiting HER1 and HER2 [181]. Preclinical work with AEE788 suggests antiangiogenesis activity, radiosensitizing capacity, and potential synergy with aromatase inhibition in breast cancer models [182,183]. The initial Phase I study demonstrated the expected dose-limiting toxicities rash and diarrhea with the maximum-tolerated dose of 450 mg/day, but based on biomarker data suggested that in humans there was limited antiangiogenic activity [184]. BMS-690514, is another pan-HER inhibitor that includes inhibitory activity for all three VEGF receptors [185]. Pharmacokinetic and Phase I studies have been reported identifying the maximum-tolerated dose of 200 mg daily [186–190]. In contrast to AEE788, BMS-690514 appears to have significant antiangiogenic activity by toxicity profile and biomarker analyses [186,191]. There is also evidence for synergism with radiation when BMS-690514 is administered in sequence with radiation [192].

Neratitib (HKI-272, WAY-179272) is an irreversible, dual EGFR and HER2 TKI moving through the development pipeline [193]. The initial Phase I study identified a maximum-tolerated dose of 320 mg daily, with the expected dose-limiting toxicity being gastrointestinal (diarrhea). In subsequent Phase I and II studies, conducted in breast and lung adenocarcinoma, this toxicity proved to be excessive resulting in a dose of 240 mg daily being carried forward into more advanced clinical studies [194–197]. Pelitinib (EKB-569) is another analog of established dual HER1 and HER2 inhibitors [198,199]. Preclinical and Phase I studies suggest that pelitinib has greater activity in inhibiting EGFR/HER1, is capable of overcoming resistance to other EGFR-targeted TKIs due to acquired mutations, and also inhibits signaling through HER2/neu [198–200]. Phase I studies have been reported establishing a maximum-tolerated dose of 75 mg/day with gastrointestinal toxicity (diarrhea) being limiting [200–204]. AST1306 is another recently developed dual HER1 and HER2 TKI, which has yet to progress to early-phase clinical studies [205]. Several other dual targeting TKIs have been developed and entered into clinical studies but abandoned, including: MP-412 (AV-412), XL647, CP-724,714 and PPI-166 [206].

HER2/neu antigen-specific immunotherapy

The work of Slamon *et al.* in defining the critical role for HER2/neu gene amplification in identifying a group of breast cancer patients with particularly poor prognosis [48], the awareness of the role of HER2 in receptor signaling for all HER molecules [18], and the clinical success of an exogenous antibody recognizing HER2/neu (trastuzumab) [63] made HER2/neu an appealing target for development of antigen-specific, antitumor vaccines [207,208]. The high degree of homology with other receptor tyrosine kinases, including other members of the HER family, and the expression of HER2/neu in normal tissues, pose significant challenges that need to be addressed, including overcoming the anticipated immunological tolerance while maintaining specificity of the elicited immune response. Much of the preclinical work was performed in HER2/neu transgenic mouse models due, in part, to the fact that a murine homolog was not confirmed until the early 2000s [14–16], although other models were also employed (dog, guinea pig, rat, primate) [209–214]. The majority of these have focused on the extracellular domain of HER2/neu due, in large part to the high degree of homology of the intracellular kinase domain with other receptor tyrosine kinases both in and outside of the HER family [209,215–219], although some strategies have included elements from the intracellular domain [213–215]. Indeed, cytotoxic T-lymphocytes directed against HER2/neu have been documented to react with HER3 and HER4 [220].

In the mid-1990s, preclinical work demonstrated a number of potential peptides derived from HER2/neu that could be viable target antigens giving rise to peptide-based immunotherapy strategies [221–223]. These moved into early-phase clinical studies in the first decade of the 2000s including: several early-phase clinical studies of the immunodominant E75 peptide with granulocyte macrophage colony-stimulating factor as a biological adjuvant (now referred to as NeuVax™) [221–223]; of an improved E75 vaccine, AE37 (NCT00524277) [224–227]; and of the subdominant G2 peptide (NCT00524277) [228–230]. Other groups have ongoing Phase I and II clinical studies examining other peptides, other adjuvants and/or combinations of immunomodulatory agents (NCT00058526, NCT00194714, NCT00791037, NCT00952692, NCT01355393, NCT01376505 and NCT01632332). Early data suggest synergy between peptide-based anti-HER2/neu vaccine and other HER2/neu-targeted therapies, for example trastuzumab [231,232] and lapatinib ditosylate [233]. However, in some cases,

there is substantial toxicity arising from combining or incorporated adjunctive elements [221].

A multitude of other vaccination strategies and immunotherapeutic methodologies have been evaluated in both preclinical and early clinical studies [207,208]. Dendritic cells (DCs) are the most potent antigen presenting and immune-stimulating cells within the immune system, thus, DC-based immunotherapeutic strategies directed against HER2/neu have been investigated, including DCs loaded with various fragments or peptides from the HER2/neu sequence [234–237] (NCT00266110 and NCT00923143) and transduced DCs [213,237–243] including adenoviral transduced autologous DCs expressing the extracellular and transmembrane domains of HER2/neu (NCT01730118). A preparation analogous to Sipuleucel-T (the first FDA-approved cellular based-immunotherapy) [244] is being developed, Lapuleucel-T, consisting of a HER2/neu truncated fusion with granulocyte macrophage colony-stimulating factor that is used to generate an autologous mixture of antigen-presenting cells targeting the HER2/neu component [245].

HER2/neu has been targeted using polynucleotide or DNA vaccine strategies involving both syngeneic and xenogeneic sequences, the latter to improve the magnitude of elicited immune responses [246–254], in prime boost strategies combining DNA vaccines with viral vector-based vaccines [255] and with gene-modified allogeneic cellular vaccines (NCT00095862) [256]. Viral vector vaccine strategies using adenovirus vectors [257–260], alphavirus vectors [212,213,261], vaccinia virus vectors (NCT00485277 and NCT01152398), vesicular stomatitis virus [262] and polyoma virus systems [263,264] have all been used to elicit anti-HER2/neu immune responses. The intracellular bacteria *Listeria monocytogenes* has also been adapted as an immunotherapeutic vector system and studied in strategies to elicit anti-HER2/neu immune responses [215,265].

The broad strategy of adoptive cellular therapy has also been investigated. Typically, this has involved clones of autologous T-lymphocytes derived from tumor-infiltrating lymphocytes or from peripheral circulation [266]. Some groups have combined peptide vaccination, in an effort to increase the number of circulating HER2/neu reactive T-lymphocytes, to provide an alternative source of T-lymphocytes for expansion and adoptive transfer back to the patient (NCT00791037). An alternative approach to generate HER2/neu-reactive T-lymphocytes is to genetically modify autologous lymphocytes with chimeric antigen receptors (CARs) [267,268]. The history of CAR strategies for targeting HER2/neu is quite substantial. Soon after the identification of antibodies that recognized HER2/neu, they were leveraged to try to target T-lymphocytes to HER2/neu-expressing tumors [269–271]. Initial efforts involved electroporation of a murine T-cell hybridoma with cDNA-encoding antigen-binding domain from a HER2/neu-recognizing antibody and the CD3 ζ-chain transmembrane and intracellular signaling domain [269]. Although this provided proof-of-principle for imparting functional capacity to T-lymphocytes enabling them to recognize and respond to the HER2/neu antigen, electroporation and the use of T-cell hybridomas was not readily translatable to the clinical arena. The development of retrovirus/lentivirus vectors provided for more efficient transduction of T-lymphocytes and NK cells [272–280]. Various groups have sought to refine the CAR constructs, including tuning affinity of the HER2/neu antigen-recognition domain [275], utilizing the T-cell receptor peptide recognition domain instead of an antibody-based recognition domain [281] and incorporation of costimulatory elements [282] within the construct. With the identification of Heregulin as a ligand for heterodimers involving HER3 or HER4, including those with HER2/neu, a chimeric ligand molecule (heregulin and intracellular signaling component from the CD3 ζ-chain) was developed [283]. Novel transduction methods [284] and the application of allogeneic CAR-modified cells [285], in a bone marrow transplant-like setting, have been developed. Within the antitumor immunotherapy community there is enthusiasm for the potential of CARs to overcome some of the major hurdles encountered in the clinical application of adoptive cellular therapy [286–290], due in large part to the work of Carl June with CAR-modified T-lymphocytes in the setting of chronic lymphocytic leukemia [291]. However, recent data on the adoptive transfer of CAR engineered T-lymphocytes has raised some concerns as to toxicity and potential differences between targeting cell surface molecules on hematopoietic versus solid tumor cells [286,292].

Other modulators of HER2 expression or activity

Less direct targeting of HER2 has been explored. Since the early 2000s, inhibition of HSP90 has been documented to inhibit HER2 and HER1-mediated cell signaling, along with increasing ubiquitin-mediated proteosomal degradation of HER2/neu [46,47]. The diverse activities and clients of HSP90 result in a number of biological pathways being impacted by HSP90 inhibition [47], several of which involve, directly or indirectly, the HER2/neu signaling pathways. Thus, it is not surprising that a substantial effort is being put forth by numerous groups and companies to develop HSP90 inhibitors with attractive therapeutic to toxicity ratios and pharmacokinetics [293–304]. One of the more impor-

tant activities associated with HSP90 inhibition is the reversal of resistance to both trastuzumab and hormonal agents [305,306]. Importantly, there is preclinical data indicating that HSP90 inhibitors are synergistic with both trastuzumab [307–309] and lapatinib ditosylate [309,310]. Phase I and II clinical studies of first-generation HSP90 inhibitors have been published [311–313]. As with most biologic or targeted therapies, the effectiveness is quite schedule-dependent. Another compound, tephrosin, can downregulate both HER1 and HER2 expression [314], providing an alternative pathway to HSP90 for the downregulation of HER2/neu expression. There are scattered studies of 'natural products' and pharmacologic agents that modulate signaling or expression of HER2 [314–317]. Preclinical studies in a murine breast cancer model of an adenovirus encoding kinase dead HER2/neu have been conducted as a potential mechanism to downregulate HER family signaling and take advantage of the promiscuity of heterodimerization with HER2/neu [318]; based on the biology of the HER family it is predicted that this strategy would have its greatest effect in tumors with high HER3 expression. The role of these pathway modulators remains to be determined, but the HSP90 inhibitors in particular are showing substantial promise and have the potential to circumvent resistance to other therapeutic agents.

With the clinical success of immune checkpoint inhibitors targeting CTLA-4 and the PD1 pathway [319] in melanoma, the incorporation of other adjunctive maneuvers to enhance tumor immunogenicity and immunotherapeutic efficacy [320] are indicated for HER2/neu-directed immunotherapeutic strategies. The co-targeting of other biological processes, such as the autophagy pathway, or other signaling pathways, such as the HedgeHog pathway, may be beneficial and could be simultaneously impacted by agents such as the HSP90 inhibitors [321–323]. Similarly targeting the microenvironment in which tumors survive may be a complimentary approach [324,325]. There are recent data that support a role for HER2/neu in the biology of cancer stem cells, which opens the potential for application of HER2/neu-targeted therapy in combination with therapeutic strategies targeting the cancer stem cell population [326].

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. 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. 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. 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. 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 making HER2/neu an increasingly important therapeutic target.

Future perspective

Our increasing appreciation of the complexity of intracellular signaling pathways, particularly with respect to crosstalk between pathways and the impact of other nonsignaling biological pathways, is dramatically driving the field towards systems biology and network analyses. The basic laboratory work elucidating these biological interactions that began well over a decade ago, including those involving the HER signaling pathways, is now poised to make an impact in the clinical arena in the next decade. Although there will still be a role for determining HER2/neu overexpression, at the protein and genetic level, network analyses from expression profiling (proteomic or genomic) of individual tumors will reveal both resistance mechanisms and identify potential nodes for combining other agents to improve the efficacy of targeting HER2/neu. It is quite possible, given recent and ongoing technical advances, that tissue obtained from small needle biopsies will be sufficient to provide expression profiling of primary and metastatic tumors enabling network analyses to make way into routine clinical use to direct the design and adaptation of individualized treatment plans for patients over the course of their disease. In this future setting, synergistic modulation of multiple biological pathways, not necessarily limited to the primary target signaling pathway, will become the norm. Given the success of immune checkpoint inhibitors in melanoma and lung cancer, the combination of molecularly targeted therapies and immunotherapy/immunomodulatory therapies will be evaluated in the setting of HER2/neu overexpression. In the short term, the next 5 years, the robust pipeline of small-molecule

inhibitors will make its way forward; defining the clinical utility of individual agents and providing a larger armamentarium for the practicing clinician.

Financial & competing interests disclosure

The author has no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Executive summary

History & biology of HER2/neu

- Member of EGF receptor (EGFR) family.
- Complex heterodimer and ligand signaling network.
- Involved in normal development of cardiovascular and neurologic systems.

HER2/neu-targeted therapeutics

- Antibody-based therapies are the most advanced and broadly employed:
  - Trastuzumab
  - Pertuzumab
  - Trastuzumab emtansine (T-DM1)
- Small-molecule tyrosine kinase inhibitors have been approved and have a role in the treatment armamentarium:
  - Lapatinib
  - Afatinib
- Immunotherapeutic strategies
  - Tumor vaccines have advanced into clinical trials with suggestion of clinical benefit in some settings.
  - Adoptive cellular therapy and chimeric antigen receptor engineered T cells are in the pipeline.
- Modulators of HER2/neu expression and downstream signaling:
  - HSP90 inhibitors

References

1 Bister K, Duesberg PH. Structure and specific sequences of avian erythroblastosis virus RNA: evidence for multiple classes of transforming genes among avian tumor viruses. *Proc. Natl Acad. Sci. USA* 76(10), 5023–5027 (1979).

2 Lai MM, Hu SS, Vogt PK. Avian erythroblastosis virus: transformation-specific sequences form a contiguous segment of 3.25 kb located in the middle of the 6 kb genome. *Virology* 97(2), 366–377 (1979).

3 Roussel M, Saule S, Lagrou C *et al.* Three new types of viral oncogene of cellular origin specific for haematopoietic cell transformation. *Nature* 281(5731), 452–455 (1979).

4 Vennstrom B, Bishop JM. Isolation and characterization of chicken DNA homologous to the two putative oncogenes of avian erythroblastosis virus. *Cell* 28(1), 135–143 (1982).

5 Sergeant A, Saule S, Leprince D, Begue A, Rommens C, Stehelin D. Molecular cloning and characterization of the chicken DNA locus related to the oncogene erbB of avian erythroblastosis virus. *EMBO J.* 1(2), 237–242 (1982).

6 Privalsky ML, Sealy L, Bishop JM, Mcgrath JP, Levinson AD. The product of the avian erythroblastosis virus erbB locus is a glycoprotein. *Cell* 32(4), 1257–1267 (1983).

7 Downward J, Yarden Y, Mayes E *et al.* Close similarity of epidermal growth factor receptor and v-erb-B oncogene protein sequences. *Nature* 307(5951), 521–527 (1984).

8 Lin CR, Chen WS, Kruiger W *et al.* Expression cloning of human EGF receptor complementary DNA: gene amplification and three related messenger RNA products in A431 cells. *Science* 224(4651), 843–848 (1984).

9 Schechter AL, Stern DF, Vaidyanathan L *et al.* The neu oncogene: an erb-B-related gene encoding a 185,000-Mr tumour antigen. *Nature* 312(5994), 513–516 (1984).

10 Schechter AL, Hung MC, Vaidyanathan L *et al.* The neu gene: an erbB-homologous gene distinct from and unlinked to the gene encoding the EGF receptor. *Science* 229(4717), 976–978 (1985).

11 Bargmann CI, Hung MC, Weinberg RA. The neu oncogene encodes an epidermal growth factor receptor-related protein. *Nature* 319(6050), 226–230 (1986).

12 Akiyama T, Sudo C, Ogawara H, Toyoshima K, Yamamoto T. The product of the human c-erbB-2 gene: a 185-kilodalton glycoprotein with tyrosine kinase activity. *Science* 232(4758), 1644–1646 (1986).

13 Semba K, Kamata N, Toyoshima K, Yamamoto T. A v-erbB-related protooncogene, c-erbB-2, is distinct from the c-erbB-1/epidermal growth factor-receptor gene and is amplified in a human salivary gland adenocarcinoma. *Proc. Natl Acad. Sci. USA* 82(19), 6497–6501 (1985).

14 Lee KF, Simon H, Chen H, Bates B, Hung MC, Hauser C. Requirement for neuregulin receptor erbB2 in neural and cardiac development. *Nature* 378(6555), 394–398 (1995).

15 Garratt AN, Voiculescu O, Topilko P, Charnay P, Birchmeier C. A dual role of erbB2 in myelination and in expansion of the Schwann cell precursor pool. *J. Cell Biol.* 148(5), 1035–1046 (2000).

16 Chan R, Hardy WR, Laing MA, Hardy SE, Muller WJ. The catalytic activity of the ErbB-2 receptor tyrosine kinase is essential for embryonic development. *Mol. Cell. Biol.* 22(4), 1073–1078 (2002).

17 Guy PM, Platko JV, Cantley LC, Cerione RA, Carraway KL 3rd. Insect cell-expressed p180erbB3 possesses an impaired tyrosine kinase activity. *Proc. Natl Acad. Sci. USA* 91(17), 8132–8136 (1994).

18 Zaczek A, Brandt B, Bielawski KP. The diverse signaling network of EGFR, HER2, HER3 and HER4 tyrosine kinase receptors and the consequences for therapeutic approaches. *Histol. Histopathol.* 20(3), 1005–1015 (2005).

19 Strachan L, Murison JG, Prestidge RL, Sleeman MA, Watson JD, Kumble KD. Cloning and biological activity of epigen, a novel member of the epidermal growth factor superfamily. *J. Biol. Chem.* 276(21), 18265–18271 (2001).

20 Kochupurakkal BS, Harari D, Di-Segni A *et al.* Epigen, the last ligand of ErbB receptors, reveals intricate relationships between affinity and mitogenicity. *J. Biol. Chem.* 280(9), 8503–8512 (2005).

21 Landgraf R. HER2 therapy. HER2 (ERBB2): functional diversity from structurally conserved building blocks. *Breast Cancer Res.* 9(1), 202 (2007).

22 Ferguson KM. Structure-based view of epidermal growth factor receptor regulation. *Annu. Rev. Biophys.* 37, 353–373 (2008).

23 Garrett TP, McKern NM, Lou M *et al.* The crystal structure of a truncated ErbB2 ectodomain reveals an active conformation, poised to interact with other ErbB receptors. *Mol. Cell* 11(2), 495–505 (2003).

24 Margolis BL, Lax I, Kris R *et al.* All autophosphorylation sites of epidermal growth factor (EGF) receptor and HER2/neu are located in their carboxyl-terminal tails. Identification of a novel site in EGF receptor. *J. Biol. Chem.* 264(18), 10667–10671 (1989).

25 Walton GM, Chen WS, Rosenfeld MG, Gill GN. Analysis of deletions of the carboxyl terminus of the epidermal growth factor receptor reveals self-phosphorylation at tyrosine 992 and enhanced *in vivo* tyrosine phosphorylation of cell substrates. *J. Biol. Chem.* 265(3), 1750–1754 (1990).

26 Wallasch C, Weiss FU, Niederfellner G, Jallal B, Issing W, Ullrich A. Heregulin-dependent regulation of HER2/neu oncogenic signaling by heterodimerization with HER3. *EMBO J.* 14(17), 4267–4275 (1995).

27 Hynes NE, Macdonald G. ErbB receptors and signaling pathways in cancer. *Curr. Opin. Cell. Biol.* 21(2), 177–184 (2009).

28 Reese DM, Slamon DJ. HER-2/neu signal transduction in human breast and ovarian cancer. *Stem Cells* 15(1), 1–8 (1997).

29 Balz LM, Bartkowiak K, Andreas A *et al.* The interplay of HER2/HER3/PI3K and EGFR/HER2/PLC-gamma1 signalling in breast cancer cell migration and dissemination. *J. Pathol.* 227(2), 234–244 (2012).

30 Jain A, Penuel E, Mink S *et al.* HER kinase axis receptor dimer partner switching occurs in response to EGFR tyrosine kinase inhibition despite failure to block cellular proliferation. *Cancer Res.* 70(5), 1989–1999 (2010).

31 Kruser TJ, Wheeler DL. Mechanisms of resistance to HER family targeting antibodies. *Exp. Cell. Res.* 316(7), 1083–1100 (2010).

32 Yamaguchi H, Chang SS, Hsu JL, Hung MC. Signaling cross-talk in the resistance to HER family receptor targeted therapy. *Oncogene* 33(9), 1073–1081(2013).

33 Hudziak RM, Schlessinger J, Ullrich A. Increased expression of the putative growth factor receptor p185HER2 causes transformation and tumorigenesis of NIH 3T3 cells. *Proc. Natl Acad. Sci. USA* 84(20), 7159–7163 (1987).

34 Suen TC, Hung MC. Multiple cis- and trans-acting elements involved in regulation of the neu gene. *Mol. Cell. Biol.* 10(12), 6306–6315 (1990).

35 Bates NP, Hurst HC. Transcriptional regulation of type I receptor tyrosine kinases in the mammary gland. *J. Mammary Gland Biol. Neoplasia* 2(2), 153–163 (1997).

36 Vernimmen D, Gueders M, Pisvin S, Delvenne P, Winkler R. Different mechanisms are implicated in ERBB2 gene overexpression in breast and in other cancers. *Br. J. Cancer* 89(5), 899–906 (2003).

37 Bosher JM, Williams T, Hurst HC. The developmentally regulated transcription factor AP-2 is involved in c-erbB-2 overexpression in human mammary carcinoma. *Proc. Natl Acad. Sci. USA* 92(3), 744–747 (1995).

38 Vernimmen D, Begon D, Salvador C, Gofflot S, Grooteclaes M, Winkler R. Identification of HTF (HER2 transcription factor) as an AP-2 (activator protein-2) transcription factor and contribution of the HTF binding site to ERBB2 gene overexpression. *Biochem. J.* 370(Pt 1), 323–329 (2003).

39 Karlan BY, Jones J, Slamon DJ, Lagasse LD. Glucocorticoids stabilize HER-2/neu messenger RNA in human epithelial ovarian carcinoma cells. *Gynecol. Oncol.* 53(1), 70–77 (1994).

40 Bae CD, Juhnn YS, Park JB. Post-transcriptional control of c-erb B-2 overexpression in stomach cancer cells. *Exp. Mol. Med.* 33(1), 15–19 (2001).

41 Issaenko OA, Bitterman PB, Polunovsky VA, Dahlberg PS. Cap-dependent mRNA translation and the ubiquitin-proteasome system cooperate to promote ERBB2-dependent esophageal cancer phenotype. *Cancer Gene Ther.* 19(9), 609–618 (2012).

42 Giles KM, Barker A, Zhang PM, Epis MR, Leedman PJ. MicroRNA regulation of growth factor receptor signaling in human cancer cells. *Methods Mol. Biol.* 676, 147–163 (2011).

43 Gong C, Yao Y, Wang Y *et al.* Up-regulation of miR-21 mediates resistance to trastuzumab therapy for breast cancer. *J. Biol. Chem.* 286(21), 19127–19137 (2011).

44 Chen F, Yu Z, Baoyu G. MiR-199b-5p targets HER2 in breast cancer cells. *J. Cell. Biochem.* 114(7), 1457–1463 (2013).

45 Lehmann TP, Korski K, Ibbs M, Zawierucha P, Grodecka-Gazdecka S, Jagodzinski PP. rs12976445 variant in the pri-miR-125a correlates with a lower level of hsa-miR-125a and ERBB2 overexpression in breast cancer patients. *Oncol. Lett.* 5(2), 569–573 (2013).

46 Citri A, Kochupurakkal BS, Yarden Y. The Achilles heel of ErbB-2/HER2: regulation by the Hsp90 chaperone machine and potential for pharmacological intervention. *Cell Cycle* 3(1), 51–60 (2004).

47 Zhang H, Burrows F. Targeting multiple signal transduction pathways through inhibition of Hsp90. *J. Mol. Med. (Berl.)* 82(8), 488–499 (2004).

48 Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, Mcguire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science* 235(4785), 177–182 (1987).

49 Berger MS, Locher GW, Saurer S *et al.* Correlation of c-erbB-2 gene amplification and protein expression in human breast carcinoma with nodal status and nuclear grading. *Cancer Res.* 48(5), 1238–1243 (1988).

50 Murphy CG, Modi S. HER2 breast cancer therapies: a review. *Biologics* 3, 289–301 (2009).

51 Mckenzie SJ, Marks PJ, Lam T *et al.* Generation and characterization of monoclonal antibodies specific for the human neu oncogene product, p185. *Oncogene* 4(5), 543–548 (1989).

52 Drebin JA, Link VC, Weinberg RA, Greene MI. Inhibition of tumor growth by a monoclonal antibody reactive with an oncogene-encoded tumor antigen. *Proc. Natl Acad. Sci. USA* 83(23), 9129–9133 (1986).

53 Yarden Y. Agonistic antibodies stimulate the kinase encoded by the neu protooncogene in living cells but the oncogenic mutant is constitutively active. *Proc. Natl Acad. Sci. USA* 87(7), 2569–2573 (1990).

54 Sarup JC, Johnson RM, King KL *et al.* Characterization of an anti-p185HER2 monoclonal antibody that stimulates receptor function and inhibits tumor cell growth. *Growth Regul.* 1(2), 72–82 (1991).

55 Awada A, Bozovic-Spasojevic I, Chow L. New therapies in HER2-positive breast cancer: a major step towards a cure of the disease? *Cancer Treat. Rev.* 38(5), 494–504 (2012).

56 Pegram MD, Konecny G, Slamon DJ. The molecular and cellular biology of HER2/neu gene amplification/overexpression and the clinical development of herceptin (trastuzumab) therapy for breast cancer. *Cancer Treat. Res.* 103, 57–75 (2000).

57 Slamon DJ, Godolphin W, Jones LA *et al.* Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. *Science* 244(4905), 707–712 (1989).

58 Hudziak RM, Lewis GD, Winget M, Fendly BM, Shepard HM, Ullrich A. p185HER2 monoclonal antibody has antiproliferative effects *in vitro* and sensitizes human breast tumor cells to tumor necrosis factor. *Mol. Cell. Biol.* 9(3), 1165–1172 (1989).

59 Fendly BM, Winget M, Hudziak RM, Lipari MT, Napier MA, Ullrich A. Characterization of murine monoclonal antibodies reactive to either the human epidermal growth factor receptor or HER2/neu gene product. *Cancer Res.* 50(5), 1550–1558 (1990).

60 Schmitz KR, Ferguson KM. Interaction of antibodies with ErbB receptor extracellular regions. *Exp. Cell. Res.* 315(4), 659–670 (2009).

61 Wehrman TS, Raab WJ, Casipit CL, Doyonnas R, Pomerantz JH, Blau HM. A system for quantifying dynamic protein interactions defines a role for Herceptin in modulating ErbB2 interactions. *Proc. Natl Acad. Sci. USA* 103(50), 19063–19068 (2006).

62 Carter P, Presta L, Gorman CM *et al.* Humanization of an anti-p185HER2 antibody for human cancer therapy. *Proc. Natl Acad. Sci. USA* 89(10), 4285–4289 (1992).

63 Slamon DJ, Leyland-Jones B, Shak S *et al.* Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N. Engl. J. Med.* 344(11), 783–792 (2001).

64 Baselga J. Clinical trials of Herceptin(R) (trastuzumab). *Eur. J. Cancer* 37(Suppl. 1), 18–24 (2001).

65 Vu T, Claret FX. Trastuzumab: updated mechanisms of action and resistance in breast cancer. *Front. Oncol.* 2, 62 (2012).

66 Pohlmann PR, Mayer IA, Mernaugh R. Resistance to trastuzumab in breast cancer. *Clin. Cancer Res.* 15(24), 7479–7491 (2009).

67 Kandl H, Seymour L, Bezwoda WR. Soluble c-erbB-2 fragment in serum correlates with disease stage and predicts for shortened survival in patients with early-stage and advanced breast cancer. *Br. J. Cancer* 70(4), 739–742 (1994).

68 Tsigris C, Karayiannakis AJ, Syrigos KN *et al.* Clinical significance of soluble c-erbB-2 levels in the serum and urine of patients with gastric cancer. *Anticancer Res.* 22(5), 3061–3065 (2002).

69 Chen CH, Lin YS, Lin CC, Yang YH, Ho YP, Tsai CC. Elevated serum levels of a c-erbB-2 oncogene product in oral squamous cell carcinoma patients. *J. Oral Pathol. Med.* 33(10), 589–594 (2004).

70 Leyland-Jones B, Smith BR. Serum HER2 testing in patients with HER2-positive breast cancer: the death knell tolls. *Lancet Oncol.* 12(3), 286–295 (2011).

71 Brodowicz T, Wiltschke C, Budinsky AC, Krainer M, Steger GG, Zielinski CC. Soluble HER-2/neu neutralizes biologic effects of anti-HER-2/neu antibody on breast cancer cells *in vitro*. *Int. J. Cancer* 73(6), 875–879 (1997).

72 Troise F, Cafaro V, Giancola C, D'alessio G, De Lorenzo C. Differential binding of human immunoagents and Herceptin to the ErbB2 receptor. *FEBS J.* 275(20), 4967–4979 (2008).

73 Visco V, Bei R, Moriconi E, Gianni W, Kraus MH, Muraro R. ErbB2 immune response in breast cancer patients with soluble receptor ectodomain. *Am. J. Pathol.* 156(4), 1417–1424 (2000).

74 Esparis-Ogando A, Diaz-Rodriguez E, Pandiella A. Signalling-competent truncated forms of ErbB2 in breast cancer cells: differential regulation by protein kinase C and phosphatidylinositol 3-kinase. *Biochem. J.* 344(Pt 2), 339–348 (1999).

75 Scott GK, Robles R, Park JW *et al.* A truncated intracellular HER2/neu receptor produced by alternative RNA processing affects growth of human carcinoma cells. *Mol. Cell. Biol.* 13(4), 2247–2257 (1993).

76 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.* 61(12), 4744–4749 (2001).

77 Cho HS, Mason K, Ramyar KX *et al.* Structure of the extracellular region of HER2 alone and in complex with the Herceptin Fab. *Nature* 421(6924), 756–760 (2003).

78 Nagy P, Friedlander E, Tanner M *et al.* Decreased accessibility and lack of activation of ErbB2 in JIMT-1, a herceptin-resistant, MUC4-expressing breast cancer cell line. *Cancer Res.* 65(2), 473–482 (2005).

79 Nagata Y, Lan KH, Zhou X *et al.* PTEN activation contributes to tumor inhibition by trastuzumab, and loss of PTEN predicts trastuzumab resistance in patients. *Cancer Cell* 6(2), 117–127 (2004).

80 Junttila TT, Akita RW, Parsons K *et al.* Ligand-independent HER2/HER3/PI3K complex is disrupted by trastuzumab and is effectively inhibited by the PI3K inhibitor GDC-0941. *Cancer Cell* 15(5), 429–440 (2009).

81 Berns K, Horlings HM, Hennessy BT *et al.* A functional genetic approach identifies the PI3K pathway as a major determinant of trastuzumab resistance in breast cancer. *Cancer Cell* 12(4), 395–402 (2007).

82 Kataoka Y, Mukohara T, Shimada H, Saijo N, Hirai M, Minami H. Association between gain-of-function mutations in PIK3CA and resistance to HER2-targeted agents in HER2-amplified breast cancer cell lines. *Ann. Oncol.* 21(2), 255–262 (2010).

83 Dokmanovic M, Hirsch DS, Shen Y, Wu WJ. Rac1 contributes to trastuzumab resistance of breast cancer cells: Rac1 as a potential therapeutic target for the treatment of trastuzumab-resistant breast cancer. *Mol. Cancer Ther.* 8(6), 1557–1569 (2009).

84 Zhao Y, Wang Z, Jiang Y, Yang C. Inactivation of Rac1 reduces Trastuzumab resistance in PTEN deficient and insulin-like growth factor I receptor overexpressing human breast cancer SKBR3 cells. *Cancer Lett.* 313(1), 54–63 (2011).

85 Ritter CA, Perez-Torres M, Rinehart C *et al.* Human breast cancer cells selected for resistance to trastuzumab *in vivo* overexpress epidermal growth factor receptor and ErbB ligands and remain dependent on the ErbB receptor network. *Clin. Cancer Res.* 13(16), 4909–4919 (2007).

86 Lu Y, Zi X, Pollak M. Molecular mechanisms underlying IGF-I-induced attenuation of the growth-inhibitory activity of trastuzumab (Herceptin) on SKBR3 breast cancer cells. *Int. J. Cancer* 108(3), 334–341 (2004).

87 Nahta R, Yuan LX, Zhang B, Kobayashi R, Esteva FJ. Insulin-like growth factor-I receptor/human epidermal growth factor receptor 2 heterodimerization contributes to trastuzumab resistance of breast cancer cells. *Cancer Res.* 65(23), 11118–11128 (2005).

88 Shattuck DL, Miller JK, Carraway KL 3rd, Sweeney C. Met receptor contributes to trastuzumab resistance of Her2-overexpressing breast cancer cells. *Cancer Res.* 68(5), 1471–1477 (2008).

89 Liang K, Esteva FJ, Albarracin C *et al.* Recombinant human erythropoietin antagonizes trastuzumab treatment of breast cancer cells via Jak2-mediated Src activation and PTEN inactivation. *Cancer Cell* 18(5), 423–435 (2010).

90 Zhang C, Duan X, Xu L, Ye J, Zhao J, Liu Y. Erythropoietin receptor expression and its relationship with trastuzumab response and resistance in HER2-positive breast cancer cells. *Breast Cancer Res. Treat.* 136(3), 739–748 (2012).

91 Franklin MC, Carey KD, Vajdos FF, Leahy DJ, De Vos AM, Sliwkowski MX. Insights into ErbB signaling from the structure of the ErbB2-pertuzumab complex. *Cancer Cell* 5(4), 317–328 (2004).

92 Adams CW, Allison DE, Flagella K *et al.* Humanization of a recombinant monoclonal antibody to produce a therapeutic HER dimerization inhibitor, pertuzumab. *Cancer Immunol. Immunother.* 55(6), 717–727 (2006).

93 Agus DB, Akita RW, Fox WD *et al.* Targeting ligand-activated ErbB2 signaling inhibits breast and prostate tumor growth. *Cancer Cell* 2(2), 127–137 (2002).

94 Liu J, Kern JA. Neuregulin-1 activates the JAK–STAT pathway and regulates lung epithelial cell proliferation. *Am. J. Respir. Cell Mol. Biol.* 27(3), 306–313 (2002).

95 Cai Z, Zhang G, Zhou Z *et al.* Differential binding patterns of monoclonal antibody 2C4 to the ErbB3-p185her2/neu and the EGFR-p185her2/neu complexes. *Oncogene* 27(27), 3870–3874 (2008).

96 Garrett J, Sutton CR, Kuba MG, Cook RS, Arteaga CL. Dual blockade of HER2 in HER2-overexpressing tumor cells does not completely eliminate HER3 function. *Clin. Cancer Res.* 19(3), 610–619 (2012).

97 Junttila TT, Li G, Parsons K, Phillips GL, Sliwkowski MX. Trastuzumab-DM1 (T-DM1) retains all the mechanisms of action of trastuzumab and efficiently inhibits growth of lapatinib insensitive breast cancer. *Breast Cancer Res. Treat.* 128(2), 347–356 (2011).

98 Kovtun YV, Audette CA, Ye Y *et al.* Antibody–drug conjugates designed to eradicate tumors with homogeneous and heterogeneous expression of the target antigen. *Cancer Res.* 66(6), 3214–3221 (2006).

99 Erickson HK, Lewis Phillips GD, Leipold DD *et al.* The effect of different linkers on target cell catabolism and pharmacokinetics/pharmacodynamics of trastuzumab maytansinoid conjugates. *Mol. Cancer Ther.* 11(5), 1133–1142 (2012).

100 Lu D, Burris HA 3rd, Wang B *et al.* Drug interaction potential of trastuzumab emtansine (T-DM1) combined with pertuzumab in patients with HER2-positive metastatic breast cancer. *Curr. Drug Metabol.* 13(7), 911–922 (2012).

101 Zielinski R, Lyakhov I, Jacobs A *et al.* Affitoxin – a novel recombinant, HER2-specific, anticancer agent for targeted therapy of HER2-positive tumors. *J. Immunother.* 32(8), 817–825 (2009).

102 Batra JK, Kasprzyk PG, Bird RE, Pastan I, King CR. Recombinant anti-erbB2 immunotoxins containing Pseudomonas exotoxin. *Proc. Natl Acad. Sci. USA* 89(13), 5867–5871 (1992).

103 Kasprzyk PG, Sullivan TL, Hunt JD *et al.* Activity of anti-erbB-2 recombinant toxin OLX-209 on lung cancer cell lines in the absence of erbB-2 gene amplification. *Clin. Cancer Res.* 2(1), 75–80 (1996).

104 Xu F, Leadon SA, Yu Y *et al.* Synergistic interaction between anti-p185HER-2 ricin A chain immunotoxins and radionuclide conjugates for inhibiting growth of ovarian and breast cancer cells that overexpress HER-2. *Clin. Cancer Res.* 6(8), 3334–3341 (2000).

105 Chopra A. ^{64}Cu-labeled oxo-DO3A-conjugated trastuzumab and PCTA-conjugated trastuzumab. In: *Molecular Imaging and Contrast Agent Database (MICAD)*, Bethesda, MD, USA (2004).

106 Orlova A, Rosik D, Sandstrom M, Lundqvist H, Einarsson L, Tolmachev V. Evaluation of [(111/114m)In]CHX-A"-DTPA-ZHER2:342, an affibody ligand coniugate for targeting of HER2-expressing malignant tumors. *Q. J. Nucl. Med. Mol. Imaging* 51(4), 314–323 (2007).

107 Tran T, Engfeldt T, Orlova A *et al.* (99m)Tc-maEEE-Z(HER2:342), an Affibody molecule-based tracer for the detection of HER2 expression in malignant tumors. *Bioconjug. Chem.* 18(6), 1956–1964 (2007).

108 Ogawa M, Regino CA, Seidel J *et al.* Dual-modality molecular imaging using antibodies labeled with activatable fluorescence and a radionuclide for specific and quantitative targeted cancer detection. *Bioconjug. Chem.* 20(11), 2177–2184 (2009).

109 Orlova A, Tran TA, Ekblad T, Karlstrom AE, Tolmachev V. (186)Re-maSGS-Z (HER2:342), a potential Affibody conjugate for systemic therapy of HER2-expressing tumours. *Eur. J. Nucl. Med. Mol. Imaging* 37(2), 260–269 (2010).

110 Reddy S, Shaller CC, Doss M *et al.* Evaluation of the anti-HER2 C6.5 diabody as a PET radiotracer to monitor HER2 status and predict response to trastuzumab treatment. *Clin. Cancer Res.* 17(6), 1509–1520 (2011).

111 Lindberg H, Hofstrom C, Altai M *et al.* Evaluation of a HER2-targeting affibody molecule combining an N-terminal HEHEHE-tag with a GGGC chelator for 99mTc-labelling at the C terminus. *Tumour Biol.* 33(3), 641–651 (2012).

112 Malmberg J, Perols A, Varasteh Z *et al.* Comparative evaluation of synthetic anti-HER2 Affibody molecules site-specifically labelled with ^{111}In using N-terminal DOTA, NOTA and NODAGA chelators in mice bearing prostate cancer xenografts. *Eur. J. Nucl. Med. Mol. Imaging* 39(3), 481–492 (2012).

113 Carcenac M, Dorvillius M, Garambois V *et al.* Internalisation enhances photo-induced cytotoxicity of monoclonal antibody–phthalocyanine conjugates. *Br. J. Cancer* 85(11), 1787–1793 (2001).

114 Kuimova MK, Bhatti M, Deonarain M *et al.* Fluorescence characterisation of multiply-loaded anti-HER2 single chain Fv-photosensitizer conjugates suitable for photodynamic therapy. *Photochem. Photobiol. Sci.* 6(9), 933–939 (2007).

115 Stuchinskaya T, Moreno M, Cook MJ, Edwards DR, Russell DA. Targeted photodynamic therapy of breast cancer cells using antibody-phthalocyanine-gold nanoparticle conjugates. *Photochem. Photobiol. Sci.* 10(5), 822–831 (2011).

116 De Lorenzo C, Arciello A, Cozzolino R *et al.* A fully human antitumor immunoRNase selective for ErbB-2-positive carcinomas. *Cancer Res.* 64(14), 4870–4874 (2004).

117 Borriello M, Laccetti P, Terrazzano G, D'alessio G, De Lorenzo C. A novel fully human antitumour immunoRNase targeting ErbB2-positive tumours. *Br. J. Cancer* 104(11), 1716–1723 (2011).

118 Lyu MA, Kurzrock R, Rosenblum MG. The immunocytokine scFv23/TNF targeting HER-2/neu induces synergistic cytotoxic effects with 5-fluorouracil in TNF-resistant pancreatic cancer cell lines. *Biochem. Pharmacol.* 75(4), 836–846 (2008).

119 Suzuki S, Tanaka M, Masuko T, Hashimoto Y. Immunoselective cell growth inhibition by antibody-adriamycin conjugates targeting c-erbB-2 product on human cancer cells. *Biol. Pharm. Bull.* 18(9), 1279–1282 (1995).

120 Goren D, Horowitz AT, Zalipsky S, Woodle MC, Yarden Y, Gabizon A. Targeting of stealth liposomes to erbB-2 (Her/2) receptor: *in vitro* and *in vivo* studies. *Br. J. Cancer* 74(11), 1749–1756 (1996).

121 Coyne CP, Jones T, Pharr T. Synthesis of a covalent gemcitabine-(carbamate)-[anti-HER2/neu] immunochemotherapeutic and its cytotoxic anti-neoplastic activity against chemotherapeutic-resistant SKBr-3 mammary carcinoma. *Bioorg. Med. Chem.* 19(1), 67–76 (2011).

122 Gianolio DA, Rouleau C, Bauta WE *et al.* Targeting HER2-positive cancer with dolastatin 15 derivatives conjugated to trastuzumab, novel antibody–drug conjugates. *Cancer Chemoth. Pharmacol.* 70(3), 439–449 (2012).

123 Valone FH, Kaufman PA, Guyre PM *et al.* Phase Ia/Ib trial of bispecific antibody MDX-210 in patients with advanced breast or ovarian cancer that overexpresses the proto-oncogene HER-2/neu. *J. Clin. Oncol.* 13(9), 2281–2292 (1995).

124 Kaufman PA, Wallace PK, Valone FH, Wells WA, Memoli VA, Ernstoff MS. Bispecific antibody MDX-210 for treatment of advanced ovarian and breast cancer. *Methods Mol. Med.* 39, 793–806 (2001).

125 Schwaab T, Lewis LD, Cole BF *et al.* Phase I pilot trial of the bispecific antibody MDXH210 (anti-Fc gamma RI X anti-HER-2/neu) in patients whose prostate cancer overexpresses HER-2/neu. *J. Immunother.* 24(1), 79–87 (2001).

126 Repp R, Van Ojik HH, Valerius T *et al.* Phase I clinical trial of the bispecific antibody MDX-H210 (anti-Fc gamma RI x anti-HER-2/neu) in combination with Filgrastim (G-CSF) for treatment of advanced breast cancer. *Br. J. Cancer* 89(12), 2234–2243 (2003).

127 Steffen AC, Wikman M, Tolmachev V *et al. In vitro* characterization of a bivalent anti-HER-2 affibody with potential for radionuclide-based diagnostics. *Cancer Biother. Radiopharm.* 20(3), 239–248 (2005).

128 Liu Z, Duan JH, Song YM *et al.* Novel HER2 aptamer selectively delivers cytotoxic drug to HER2-positive breast cancer cells *in vitro*. *J. Transl. Med.* 10, 148 (2012).

129 Mauro MJ, Druker BJ. STI571: a gene product-targeted therapy for leukemia. *Curr. Oncol. Rep.* 3(3), 223–227 (2001).

130 Xia W, Mullin RJ, Keith BR *et al.* Anti-tumor activity of GW572016: a dual tyrosine kinase inhibitor blocks EGF activation of EGFR/erbB2 and downstream Erk1/2 and AKT pathways. *Oncogene* 21(41), 6255–6263 (2002).

131 Zhou H, Kim YS, Peletier A, Mccall W, Earp HS, Sartor CI. Effects of the EGFR/HER2 kinase inhibitor GW572016 on EGFR- and HER2-overexpressing breast cancer cell line proliferation, radiosensitization, and resistance. *Int. J. Radiat. Oncol. Biol. Phys.* 58(2), 344–352 (2004).

132 Ocana A, Amir E. Irreversible pan-ErbB tyrosine kinase inhibitors and breast cancer: current status and future directions. *Cancer Treat. Rev.* 35(8), 685–691 (2009).

133 Saini KS, Azim HA, Jr., Metzger-Filho O *et al.* Beyond trastuzumab: new treatment options for HER2-positive breast cancer. *Breast* 20(Suppl. 3), S20–S27 (2011).

134 Majem M, Pallares C. An update on molecularly targeted therapies in second- and third-line treatment in non-small-cell lung cancer: focus on EGFR inhibitors and anti-angiogenic agents. *Clin. Transl. Oncol.* 15(5), 343–357 (2013).

135 Sanchez-Martin M, Pandiella A. Differential action of small molecule HER kinase inhibitors on receptor heterodimerization: therapeutic implications. *Int. J. Cancer* 131(1), 244–252 (2012).

136 Abbas R, Hug BA, Leister C, Sonnichsen D. A double-blind, randomized, multiple-dose, parallel-group study to characterize the occurrence of diarrhea following two different dosing regimens of neratinib, an irreversible pan-ErbB receptor tyrosine kinase inhibitor. *Cancer Chemother. Pharmacol.* 70(1), 191–199 (2012).

137 Hegedus C, Truta-Feles K, Antalffy G *et al.* Interaction of the EGFR inhibitors gefitinib, vandetanib, pelitinib and neratinib with the ABCG2 multidrug transporter: implications for the emergence and reversal of cancer drug resistance. *Biochem. Pharmacol.* 84(3), 260–267 (2012).

138 Zhao XQ, Xie JD, Chen XG *et al.* Neratinib reverses ATP-binding cassette B1-mediated chemotherapeutic drug resistance *in vitro*, *in vivo*, and *ex vivo*. *Mol. Pharmacol.* 82(1), 47–58 (2012).

139 Wood ER, Truesdale AT, McDonald OB *et al.* A unique structure for epidermal growth factor receptor bound to GW572016 (Lapatinib): relationships among protein conformation, inhibitor off-rate, and receptor activity in tumor cells. *Cancer Res.* 64(18), 6652–6659 (2004).

140 Burris HA 3rd, Storniolo AM, Overmoyer EA *et al.* A Phase I, open-label study of the safety, tolerability and pharmacokinetics of lapatinib (GW572016) in combination with trastuzumab (Abstract 3043). Presented at: *San Antonio Breast Cancer Symposium*, San Antonio, TX, USA, 8–11 December 2004.

141 Burris HA 3rd. Dual kinase inhibition in the treatment of breast cancer: initial experience with the EGFR/ErbB-2 inhibitor lapatinib. *Oncologist* 9(Suppl. 3), 10–15 (2004).

142 Storniolo AM, Burris Iii HA, Overmoyer B, Al E. A Phase I, open-label study of the safety, tolerability and pharmacokinetics of lapatinib (GW572016) in combination with trastuzumab. *Breast Cancer Res. Treat.* 94(Suppl. 1), A-1040 (2005).

143 Burris HA 3rd, Hurwitz HI, Dees EC *et al.* Phase I safety, pharmacokinetics, and clinical activity study of lapatinib (GW572016), a reversible dual inhibitor of epidermal growth factor receptor tyrosine kinases, in heavily pretreated patients with metastatic carcinomas. *J. Clin. Oncol.* 23(23), 5305–5313 (2005).

144 Storniolo AM, Pegram MD, Overmoyer B *et al.* Phase I dose escalation and pharmacokinetic study of lapatinib in combination with trastuzumab in patients with advanced ErbB2-positive breast cancer. *J. Clin. Oncol.* 26(20), 3317–3323 (2008).

145 Geyer CE, Forster J, Lindquist D *et al.* Lapatinib plus capecitabine for HER2-positive advanced breast cancer. *N. Engl. J. Med.* 355(26), 2733–2743 (2006).

146 Trowe T, Boukouvala S, Calkins K *et al.* EXEL-7647 inhibits mutant forms of ErbB2 associated with lapatinib resistance and neoplastic transformation. *Clin. Cancer Res.* 14(8), 2465–2475 (2008).

147 Vazquez-Martin A, Oliveras-Ferraros C, Cufi S, Del Barco S, Martin-Castillo B, Menendez JA. Lapatinib, a dual HER1/HER2 tyrosine kinase inhibitor, augments basal cleavage of HER2 extracellular domain (ECD) to inhibit HER2-driven cancer cell growth. *J. Cell. Physiol.* 226(1), 52–57 (2011).

148 Ou SH. Second-generation irreversible epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs): a better mousetrap? A review of the clinical evidence. *Crit. Rev. Oncol. Hematol.* 83(3), 407–421 (2012).

149 Bridges AJ. The rationale and strategy used to develop a series of highly potent, irreversible, inhibitors of the epidermal growth factor receptor family of tyrosine kinases. *Curr. Med. Chem.* 6(9), 825–843 (1999).

150 Spicer JF, Rudman SM. EGFR inhibitors in non-small-cell lung cancer (NSCLC): the emerging role of the dual irreversible EGFR/HER2 inhibitor BIBW 2992. *Target. Oncol.* 5(4), 245–255 (2010).

151 Schutze C, Dorfler A, Eicheler W *et al.* Combination of EGFR/HER2 tyrosine kinase inhibition by BIBW 2992 and BIBW 2669 with irradiation in FaDu human squamous cell carcinoma. *Strahlenther. Onkol.* 183(5), 256–264 (2007).

152 Tsai YC, Yeh CH, Tzen KY *et al.* Targeting epidermal growth factor receptor/human epidermal growth factor receptor 2 signalling pathway by a dual receptor tyrosine kinase inhibitor afatinib for radiosensitisation in murine bladder carcinoma. *Eur. J. Cancer* 49(6), 1458–1466 (2012).

153 Stopfer P, Marzin K, Narjes H *et al.* Afatinib pharmacokinetics and metabolism after oral administration to healthy male volunteers. *Cancer Chemother. Pharmacol.* 69(4), 1051–1061 (2012).

154 Eskens FA, Mom CH, Planting AS *et al.* A Phase I dose escalation study of BIBW 2992, an irreversible dual inhibitor of epidermal growth factor receptor 1 (EGFR) and 2 (HER2) tyrosine kinase in a 2-week on, 2-week off schedule in patients with advanced solid tumours. *Br. J. Cancer* 98(1), 80–85 (2008).

155 Yap TA, Vidal L, Adam J *et al.* Phase I trial of the irreversible EGFR and HER2 kinase inhibitor BIBW 2992 in patients with advanced solid tumors. *J. Clin. Oncol.* 28(25), 3965–3972 (2010).

156 Murakami H, Tamura T, Takahashi T *et al.* Phase I study of continuous afatinib (BIBW 2992) in patients with advanced non-small-cell lung cancer after prior chemotherapy/erlotinib/gefitinib (LUX-Lung 4). *Cancer Chemother. Pharmacol.* 69(4), 891–899 (2012).

157 Gordon MS, Mendelson DS, Gross M *et al.* A Phase I, open-label, dose-escalation study of continuous once-daily oral treatment with afatinib in patients with advanced solid tumors. *Invest. New Drugs* 31(2), 409–416 (2012).

158 Marshall J, Hwang J, Eskens FA *et al.* A Phase I, open-label, dose escalation study of afatinib, in a 3-week-on/1-week-off schedule in patients with advanced solid tumors. *Invest. New Drugs* 31(2), 399–408 (2012).

159 Awada AH, Dumez H, Hendlisz A *et al.* Phase I study of pulsatile 3-day administration of afatinib (BIBW 2992) in combination with docetaxel in advanced solid tumors. *Invest. New Drugs* 31(3), 734–741 (2012).

160 Vermorken JB, Rottey S, Ehrnrooth E *et al.* A Phase Ib, open-label study to assess the safety of continuous oral treatment with afatinib in combination with two chemotherapy regimens: cisplatin plus paclitaxel and cisplatin plus 5-fluorouracil, in patients with advanced solid tumors. *Ann. Oncol.* 24(5), 1392–1400 (2013).

161 Minkovsky N, Berezov A. BIBW-2992, a dual receptor tyrosine kinase inhibitor for the treatment of solid tumors. *Curr. Opin. Investig. Drugs* 9(12), 1336–1346 (2008).

162 Solca F, Dahl G, Zoephel A *et al.* Target binding properties and cellular activity of afatinib (BIBW 2992), an irreversible ErbB family blocker. *J. Pharmacol. Exper. Ther.* 343(2), 342–350 (2012).

163 Smaill JB, Rewcastle GW, Loo JA *et al.* Tyrosine kinase inhibitors. 17. Irreversible inhibitors of the epidermal growth factor receptor: 4-(phenylamino)quinazoline- and 4-(phenylamino)pyrido[3,2-d]pyrimidine-6-acrylamides bearing additional solubilizing functions. *J. Med. Chem.* 43(7), 1380–1397 (2000).

164 Slichenmyer WJ, Elliott WL, Fry DW. CI-1033, a pan-erbB tyrosine kinase inhibitor. *Semin. Oncol.* 28(5 Suppl. 16), 80–85 (2001).

165 Calvo E, Tolcher AW, Hammond LA *et al.* Administration of CI-1033, an irreversible pan-erbB tyrosine kinase inhibitor, is feasible on a 7-day on, 7-day off schedule: a Phase I pharmacokinetic and food effect study. *Clin. Cancer Res.* 10(21), 7112–7120 (2004).

166 Nemunaitis J, Eiseman I, Cunningham C *et al.* Phase 1 clinical and pharmacokinetics evaluation of oral CI-1033 in patients with refractory cancer. *Clin. Cancer Res.* 11(10), 3846–3853 (2005).

167 Chiappori AA, Ellis PM, Hamm JT *et al.* A Phase I evaluation of oral CI-1033 in combination with paclitaxel and carboplatin as first-line chemotherapy in patients with advanced non-small-cell lung cancer. *J. Thorac. Oncol.* 1(9), 1010–1019 (2006).

168 Garland LL, Hidalgo M, Mendelson DS *et al.* A Phase I clinical and pharmacokinetic study of oral CI-1033 in combination with docetaxel in patients with advanced solid tumors. *Clin. Cancer Res.* 12(14 Pt 1), 4274–4282 (2006).

169 Simon GR, Garrett CR, Olson SC *et al.* Increased bioavailability of intravenous versus oral CI-1033, a pan erbB tyrosine kinase inhibitor: results of a Phase I pharmacokinetic study. *Clin. Cancer Res.* 12(15), 4645–4651 (2006).

170 Zinner RG, Nemunaitis J, Eiseman I *et al.* Phase I clinical and pharmacodynamic evaluation of oral CI-1033 in patients with refractory cancer. *Clin. Cancer Res.* 13(10), 3006–3014 (2007).

171 Engelman JA, Zejnullahu K, Gale CM *et al.* PF00299804, an irreversible pan-ERBB inhibitor, is effective in lung cancer models with EGFR and ERBB2 mutations that are resistant to gefitinib. *Cancer Res.* 67(24), 11924–11932 (2007).

172 Gonzales AJ, Hook KE, Althaus IW *et al.* Antitumor activity and pharmacokinetic properties of PF-00299804, a second-generation irreversible pan-erbB receptor tyrosine kinase inhibitor. *Mol. Cancer Ther.* 7(7), 1880–1889 (2008).

173 Kalous O, Conklin D, Desai AJ *et al.* Dacomitinib (PF-00299804), an irreversible Pan-HER inhibitor, inhibits proliferation of HER2-amplified breast cancer cell lines resistant to trastuzumab and lapatinib. *Mol. Cancer Ther.* 11(9), 1978–1987 (2012).

174 Kelly RJ, Carter C, Giaccone G. Personalizing therapy in an epidermal growth factor receptor-tyrosine kinase inhibitor-resistant non-small-cell lung cancer using PF-00299804 and trastuzumab. *J. Clin. Oncol.* 28(28), e507–e510 (2010).

175 Takahashi T, Boku N, Murakami H *et al.* Phase I and pharmacokinetic study of dacomitinib (PF-00299804), an oral irreversible, small molecule inhibitor of human epidermal growth factor receptor-1, -2, and -4 tyrosine kinases, in Japanese patients with advanced solid tumors. *Invest. New Drugs* 30(6), 2352–2363 (2012).

176 Ruiz-Garcia A, Janne PA, Park K *et al.* EGFR status and daily dose: effect on tumor growth inhibition in cancer patients treated with dacomitinib (PF-00299804). *J. Clin. Oncol.* 30(Suppl.) Abstract e18093 (2012).

177 Ercan D, Zejnullahu K, Yonesaka K *et al.* Amplification of EGFR T790M causes resistance to an irreversible EGFR inhibitor. *Oncogene* 29(16), 2346–2356 (2010).

178 Gavai AV, Fink BE, Fairfax DJ *et al.* Discovery and preclinical evaluation of [4-[[1-(3-fluorophenyl)methyl]-1H-indazol-5-ylamino]-5-methylpyrrolo[2,1-f][1,2,4]triazin-6-yl]carbamic acid, (3S)-3-morpholinylmethyl ester (BMS-599626), a selective and orally efficacious inhibitor of human epidermal growth factor receptor 1 and 2 kinases. *J. Med. Chem.* 52(21), 6527–6530 (2009).

179 Torres MA, Raju U, Molkentine D, Riesterer O, Milas L, Ang KK. AC480, formerly BMS-599626, a pan Her inhibitor, enhances radiosensitivity and radioresponse of head and neck squamous cell carcinoma cells *in vitro* and *in vivo*. *Invest. New Drugs* 29(4), 554–561 (2011).

180 Soria JC, Cortes J, Massard C *et al.* Phase I safety, pharmacokinetic and pharmacodynamic trial of BMS-599626 (AC480), an oral pan-HER receptor tyrosine kinase inhibitor, in patients with advanced solid tumors. *Ann. Oncol.* 23(2), 463–471 (2012).

181 Traxler P, Allegrini PR, Brandt R *et al.* AEE788: a dual family epidermal growth factor receptor/ErbB2 and vascular endothelial growth factor receptor tyrosine kinase inhibitor with antitumor and antiangiogenic activity. *Cancer Res.* 64(14), 4931–4941 (2004).

182 Oehler-Janne C, Jochum W, Riesterer O *et al.* Hypoxia modulation and radiosensitization by the novel dual EGFR and VEGFR inhibitor AEE788 in spontaneous and related allograft tumor models. *Mol. Cancer Ther.* 6(9), 2496–2504 (2007).

183 Evans AH, Pancholi S, Farmer I *et al.* EGFR/HER2 inhibitor AEE788 increases ER-mediated transcription in HER2/ER-positive breast cancer cells but functions synergistically with endocrine therapy. *Br. J. Cancer* 102(8), 1235–1243 (2010).

184 Baselga J, Mita AC, Schoffski P *et al.* Using pharmacokinetic and pharmacodynamic data in early decision making regarding drug development: a Phase I clinical trial evaluating tyrosine kinase inhibitor, AEE788. *Clin. Cancer Res.* 18(22), 6364–6372 (2012).

185 Pennell NA, Lynch TJ Jr. Combined inhibition of the VEGFR and EGFR signaling pathways in the treatment of NSCLC. *Oncologist* 14(4), 399–411 (2009).

186 Bahleda R, Felip E, Herbst RS *et al.* Phase I multicenter trial of BMS-690514: Safety, pharmacokinetic profile, biological effects, and early clinical evaluation in patients with advanced solid tumors and non-small-cell lung cancer. *J. Clin. Oncol.* 26(Suppl.) Abstract 2564 (2008).

187 Christopher LJ, Hong H, Vakkalagadda BJ *et al.* Metabolism and disposition of [14C]BMS-690514, an ErbB/vascular endothelial growth factor receptor inhibitor, after oral administration to humans. *Drug Metab. Dispos.* 38(11), 2049–2059 (2010).

188 Nokihara H, Yamamoto N, Yamada Y *et al.* A Phase I study of BMS-690514 in Japanese patients with advanced or metastatic solid tumors. *Cancer Chemother. Pharmacol.* 70(4), 559–565 (2012).

189 Vakkalagadda B, Park JS, Ahlers CM *et al.* Food increased the bioavailability of BMS-690514, an orally active EGFR/HER2/VEGF receptor kinase inhibitor, in healthy subjects. *J. Clin. Pharmacol.* 52(9), 1350–1356 (2012).

190 Chow LQ, Laurie SA, Belani CP *et al.* Phase I trial of BMS-690514 in combination with paclitaxel/carboplatin (PC) in patients with advanced or metastatic solid tumors. *J. Clin. Oncol.* 28(Suppl. 15), Abstract 2547 (2010).

191 Bahleda R, Soria J, Harbison C *et al.* Tumor regression and pharmacodynamic (PD) biomarker validation in non-small-cell lung cancer (NSCLC) patients treated with the ErbB/VEGFR inhibitor BMS-690514. *J. Clin. Oncol.* 27(15), Abstract 8098 (2009).

192 Loriot Y, Mordant P, Dorvault N *et al.* BMS-690514, a VEGFR and EGFR tyrosine kinase inhibitor, shows anti-tumoural activity on non-small-cell lung cancer xenografts and induces sequence-dependent synergistic effect with radiation. *Br. J. Cancer* 103(3), 347–353 (2010).

193 Rabindran SK, Discafani CM, Rosfjord EC *et al.* Antitumor activity of HKI-272, an orally active, irreversible inhibitor of the HER-2 tyrosine kinase. *Cancer Res.* 64(11), 3958–3965 (2004).

194 Sequist LV, Besse B, Lynch TJ *et al.* Neratinib, an irreversible pan-ErbB receptor tyrosine kinase inhibitor: results of a Phase II trial in patients with advanced non-small-cell lung cancer. *J. Clin. Oncol.* 28(18), 3076–3083 (2010).

195 Burstein HJ, Sun Y, Dirix LY *et al.* Neratinib, an irreversible ErbB receptor tyrosine kinase inhibitor, in patients with advanced ErbB2-positive breast cancer. *J. Clin. Oncol.* 28(8), 1301–1307 (2010).

196 Ito Y, Suenaga M, Hatake K *et al.* Safety, efficacy and pharmacokinetics of neratinib (HKI-272) in Japanese patients with advanced solid tumors: a Phase 1 dose-escalation study. *Jpn. J. Clin. Oncol.* 42(4), 278–286 (2012).

197 Awada A, Dirix L, Manso Sanchez L *et al.* Safety and efficacy of neratinib (HKI-272) plus vinorelbine in the treatment of patients with ErbB2-positive metastatic breast cancer pretreated with anti-HER2 therapy. *Ann. Oncol.* 24(1), 109–116 (2013).

198 Wissner A, Brawner Floyd MB, Rabindran SK *et al.* Syntheses and EGFR and HER-2 kinase inhibitory activities of 4-anilinoquinoline-3-carbonitriles: analogues of three important 4-anilinoquinazolines currently undergoing clinical evaluation as therapeutic antitumor agents. *Bioorg. Med. Chem. Lett.* 12(20), 2893–2897 (2002).

199 Wissner A, Mansour TS. The development of HKI-272 and related compounds for the treatment of cancer. *Arch. Pharm. (Weinheim)* 341(8), 465–477 (2008).

200 Yoshimura N, Kudoh S, Kimura T *et al.* EKB-569, a new irreversible epidermal growth factor receptor tyrosine kinase inhibitor, with clinical activity in patients with non-small-cell lung cancer with acquired resistance to gefitinib. *Lung Cancer* 51(3), 363–368 (2006).

201 Erlichman C, Hidalgo M, Boni JP *et al.* Phase I study of EKB-569, an irreversible inhibitor of the epidermal growth factor receptor, in patients with advanced solid tumors. *J. Clin. Oncol.* 24(15), 2252–2260 (2006).

202 Folprecht G, Tabernero J, Kohne CH *et al.* Phase I pharmacokinetic/pharmacodynamic study of EKB-569, an irreversible inhibitor of the epidermal growth factor receptor tyrosine kinase, in combination with irinotecan, 5-fluorouracil, and leucovorin (FOLFIRI) in first-line treatment of patients with metastatic colorectal cancer. *Clin. Cancer Res.* 14(1), 215–223 (2008).

203 Laheru D, Croghan G, Bukowski R *et al.* A Phase I study of EKB-569 in combination with capecitabine in patients with advanced colorectal cancer. *Clin. Cancer Res.* 14(17), 5602–5609 (2008).

204 Bryce AH, Rao R, Sarkaria J *et al.* Phase I study of temsirolimus in combination with EKB-569 in patients with advanced solid tumors. *Invest. New Drugs* 30(5), 1934–1941 (2012).

205 Xie H, Lin L, Tong L *et al.* AST1306, a novel irreversible inhibitor of the epidermal growth factor receptor 1 and 2, exhibits antitumor activity both *in vitro* and *in vivo*. *PLoS ONE* 6(7), e21487 (2011).

206 Rabindran SK. Antitumor activity of HER-2 inhibitors. *Cancer Lett.* 227(1), 9–23 (2005).

207 Disis ML, Schiffman K. Cancer vaccines targeting the HER2/neu oncogenic protein. *Semin. Oncol.* 28(6 Suppl. 18), 12–20 (2001).

208 Curigliano G, Spitaleri G, Dettori M, Locatelli M, Scarano E, Goldhirsch A. Vaccine immunotherapy in breast cancer treatment: promising, but still early. *Expert Rev. Anticancer Ther.* 7(9), 1225–1241 (2007).

209 Fendly BM, Kotts C, Vetterlein D *et al.* The extracellular domain of HER2/neu is a potential immunogen for active specific immunotherapy of breast cancer. *J. Biol. Response Mod.* 9(5), 449–455 (1990).

210 Peruzzi D, Mesiti G, Ciliberto G, La Monica N, Aurisicchio L. Telomerase and HER-2/neu as targets of genetic cancer vaccines in dogs. *Vaccine* 28(5), 1201–1208 (2010).

211 Fattori E, Aurisicchio L, Zampaglione I *et al.* ErbB2 genetic cancer vaccine in nonhuman primates: relevance of single nucleotide polymorphisms. *Hum. Gene Ther.* 20(3), 253–265 (2009).

212 Laust AK, Sur BW, Wang K, Hubby B, Smith JF, Nelson EL. VRP immunotherapy targeting neu: treatment efficacy and evidence for immunoediting in a stringent rat mammary tumor model. *Breast Cancer Res. Treat.* 106(3), 371–382 (2007).

213 Nelson EL, Prieto D, Alexander TG *et al.* Venezuelan equine encephalitis replicon immunization overcomes intrinsic tolerance and elicits effective anti-tumor immunity to the 'self' tumor-associated antigen, neu in a rat mammary tumor model. *Breast Cancer Res. Treat.* 82(3), 169–183 (2003).

214 Manjili MH, Wang XY, Chen X *et al.* HSP110-HER2/neu chaperone complex vaccine induces protective immunity against spontaneous mammary tumors in HER-2/neu transgenic mice. *J. Immunol.* 171(8), 4054–4061 (2003).

215 Singh R, Dominiecki ME, Jaffee EM, Paterson Y. Fusion to Listeriolysin O and delivery by Listeria monocytogenes enhances the immunogenicity of HER-2/neu and reveals subdominant epitopes in the FVB/N mouse. *J. Immunol.* 175(6), 3663–3673 (2005).

216 Esserman LJ, Lopez T, Montes R, Bald LN, Fendly BM, Campbell MJ. Vaccination with the extracellular domain of p185neu prevents mammary tumor development in neu transgenic mice. *Cancer Immunol. Immunother.* 47(6), 337–342 (1999).

217 Peoples GE, Goedegebuure PS, Smith R, Linehan DC, Yoshino I, Eberlein TJ. Breast and ovarian cancer-specific cytotoxic T-lymphocytes recognize the same HER2/neu-derived peptide. *Proc. Natl Acad. Sci. USA* 92(2), 432–436 (1995).

218 Kaumaya PT, Foy KC, Garrett J *et al.* Phase I active immunotherapy with combination of two chimeric, human epidermal growth factor receptor 2, B-cell epitopes fused to a promiscuous T-cell epitope in patients with metastatic and/or recurrent solid tumors. *J. Clin. Oncol.* 27(31), 5270–5277 (2009).

219 Dela Cruz JS, Lau SY, Ramirez EM *et al.* Protein vaccination with the HER2/neu extracellular domain plus anti-HER2/neu antibody-cytokine fusion proteins induces a protective anti-HER2/neu immune response in mice. *Vaccine* 21(13–14), 1317–1326 (2003).

220 Conrad H, Gebhard K, Kronig H *et al.* CTLs directed against HER2 specifically cross-react with HER3 and HER4. *J. Immunol.* 180(12), 8135–8145 (2008).

221 Baxevanis CN, Papamichail M, Perez SA. Toxicity profiles of HER2/neu peptide anticancer vaccines: the picture from Phase/I and II clinical trials. *Expert Rev. Vaccines* 11(6), 637–640 (2012).

222 Baxevanis CN, Voutsas IF, Gritzapis AD, Perez SA, Papamichail M. HER-2/neu as a target for cancer vaccines. *Immunotherapy* 2(2), 213–226 (2010).

223 Wiedermann U, Davis AB, Zielinski CC. Vaccination for the prevention and treatment of breast cancer with special focus on Her-2/neu peptide vaccines. *Breast Cancer Res. Treat.* 138(1), 1–12 (2013).

224 Mittendorf EA, Holmes JP, Murray JL, Von Hofe E, Peoples GE. $CD4^+$ T cells in antitumor immunity: utility of an li-key HER2/neu hybrid peptide vaccine (AE37). *Expert Opin. Biol. Ther.* 9(1), 71–78 (2009).

225 Holmes JP, Benavides LC, Gates JD *et al.* Results of the first Phase I clinical trial of the novel II-key hybrid preventive HER-2/neu peptide (AE37) vaccine. *J. Clin. Oncol.* 26(20), 3426–3433 (2008).

226 Sears AK, Perez SA, Clifton GT *et al.* AE37: a novel T-cell-eliciting vaccine for breast cancer. *Expert Opin. Biol. Ther.* 11(11), 1543–1550 (2011).

227 Perez SA, Kallinteris NL, Bisias S *et al.* Results from a Phase I clinical study of the novel Ii-Key/HER-2/neu(776–790) hybrid peptide vaccine in patients with prostate cancer. *Clin. Cancer Res.* 16(13), 3495–3506 (2010).

228 Carmichael MG, Benavides LC, Holmes JP *et al.* Results of the first Phase 1 clinical trial of the HER-2/neu peptide (GP2) vaccine in disease-free breast cancer patients: United States Military Cancer Institute Clinical Trials Group Study I-04. *Cancer* 116(2), 292–301 (2010).

229 Clive KS, Tyler JA, Clifton GT *et al.* The GP2 peptide: a HER2/neu-based breast cancer vaccine. *J. Surg. Oncol.* 105(5), 452–458 (2012).

230 Mittendorf EA, Storrer CE, Foley RJ *et al.* Evaluation of the HER2/neu-derived peptide GP2 for use in a peptide-based breast cancer vaccine trial. *Cancer* 106(11), 2309–2317 (2006).

231 Disis ML, Wallace DR, Gooley TA *et al.* Concurrent trastuzumab and HER2/neu-specific vaccination in patients with metastatic breast cancer. *J. Clin. Oncol.* 27(28), 4685–4692 (2009).

232 Mittendorf EA, Storrer CE, Shriver CD, Ponniah S, Peoples GE. Investigating the combination of trastuzumab and HER2/neu peptide vaccines for the treatment of breast cancer. *Ann. Surg. Oncol.* 13(8), 1085–1098 (2006).

233 Hamilton E, Blackwell K, Hobeika AC *et al.* Phase I clinical trial of HER2-specific immunotherapy with concomitant HER2 kinase inhibtion. *J. Transl. Med.* 10, 28 (2012).

234 Kavanagh B, Ko A, Venook A *et al.* Vaccination of metastatic colorectal cancer patients with matured dendritic cells loaded with multiple major histocompatibility complex class I peptides. *J. Immunother.* 30(7), 762–772 (2007).

235 Phuphanich S, Wheeler CJ, Rudnick JD *et al.* Phase I trial of a multi-epitope-pulsed dendritic cell vaccine for patients with newly diagnosed glioblastoma. *Cancer Immunol. Immunother.* 62(1), 125–135 (2013).

236 Sharma A, Koldovsky U, Xu S *et al.* HER-2 pulsed dendritic cell vaccine can eliminate HER-2 expression and impact ductal carcinoma in situ. *Cancer* 118(17), 4354–4362 (2012).

237 Morse MA, Clay TM, Colling K *et al.* HER2 dendritic cell vaccines. *Clin. Breast Cancer* 3(Suppl. 4), S164–S172 (2003).

238 Li GB, Lu GX. A single low dose of dendritic cells modified with lentivirus containing a truncated neu gene can effectively suppress neu-overexpressing tumors. *J. Gene Med.* 12(7), 604–612 (2010).

239 Nabekura T, Nagasawa T, Nakauchi H, Onodera M. An immunotherapy approach with dendritic cells genetically modified to express the tumor-associated antigen, HER2. *Cancer Immunol. Immunother.* 57(5), 611–622 (2008).

240 Sakai Y, Morrison BJ, Burke JD *et al.* Vaccination by genetically modified dendritic cells expressing a truncated neu oncogene prevents development of breast cancer in transgenic mice. *Cancer Res.* 64(21), 8022–8028 (2004).

241 Tegerstedt K, Franzen A, Ramqvist T, Dalianis T. Dendritic cells loaded with polyomavirus VP1/VP2Her2 virus-like particles efficiently prevent outgrowth of a Her2/neu expressing tumor. *Cancer Immunol. Immunother.* 56(9), 1335–1344 (2007).

242 Felizardo TC, Wang JC, Mcgray RA *et al.* Differential immune responses mediated by adenovirus- and lentivirus-transduced DCs in a HER-2/neu overexpressing tumor model. *Gene Ther.* 18(10), 986–995 (2011).

243 Mossoba ME, Walia JS, Rasaiah VI *et al.* Tumor protection following vaccination with low doses of lentivirally transduced DCs expressing the self-antigen erbB2. *Mol. Ther.* 16(3), 607–617 (2008).

244 Karan D, Holzbeierlein JM, Van Veldhuizen P, Thrasher JB. Cancer immunotherapy: a paradigm shift for prostate cancer treatment. *Nat. Rev. Urol.* 9(7), 376–385 (2012).

245 Peethambaram PP, Melisko ME, Rinn KJ *et al.* A Phase I trial of immunotherapy with lapuleucel-T (APC8024) in patients with refractory metastatic tumors that express HER-2/neu. *Clin. Cancer Res.* 15(18), 5937–5944 (2009).

246 Conry RM, Lobuglio AF, Curiel DT. Polynucleotide-mediated immunization therapy of cancer. *Semin. Oncol.* 23(1), 135–147 (1996).

247 Jacob J, Radkevich O, Forni G *et al.* Activity of DNA vaccines encoding self or heterologous Her-2/neu in Her-2 or neu transgenic mice. *Cell. Immunol.* 240(2), 96–106 (2006).

248 Jacob JB, Quaglino E, Radkevich-Brown O *et al.* Combining human and rat sequences in HER-2 DNA vaccines blunts immune tolerance and drives antitumor immunity. *Cancer Res.* 70(1), 119–128 (2010).

249 Whittington PJ, Piechocki MP, Heng HH *et al.* DNA vaccination controls Her-2$^+$ tumors that are refractory to targeted therapies. *Cancer Res.* 68(18), 7502–7511 (2008).

250 Chen SA, Tsai MH, Wu FT *et al.* Induction of antitumor immunity with combination of HER2/neu DNA vaccine and interleukin 2 gene-modified tumor vaccine. *Clin. Cancer Res.* 6(11), 4381–4388 (2000).

251 Mukai K, Yasutomi Y, Watanabe M *et al.* HER2 peptide-specific CD8$^+$ T cells are proportionally detectable long after multiple DNA vaccinations. *Gene Ther.* 9(13), 879–888 (2002).

252 Norell H, Poschke I, Charo J *et al.* Vaccination with a plasmid DNA encoding HER-2/neu together with low doses of GM-CSF and IL-2 in patients with metastatic breast carcinoma: a pilot clinical trial. *J. Transl. Med.* 8, 53 (2010).

253 Pupa SM, Invernizzi AM, Forti S *et al.* Prevention of spontaneous neu-expressing mammary tumor development in mice transgenic for rat proto-neu by DNA vaccination. *Gene Ther.* 8(1), 75–79 (2001).

254 Tu CF, Lin CC, Chen MC *et al.* Autologous neu DNA vaccine can be as effective as xenogenic neu DNA vaccine by altering administration route. *Vaccine* 25(4), 719–728 (2007).

255 Wang X, Wang JP, Rao XM, Price JE, Zhou HS, Lachman LB. Prime-boost vaccination with plasmid and adenovirus gene vaccines control HER2/neu+ metastatic breast cancer in mice. *Breast Cancer Res. BCR* 7(5), R580–R588 (2005).

256 Machiels JP, Reilly RT, Emens LA *et al.* Cyclophosphamide, doxorubicin, and paclitaxel enhance the antitumor immune response of granulocyte/macrophage-colony stimulating factor-secreting whole-cell vaccines in HER-2/neu tolerized mice. *Cancer Res.* 61(9), 3689–3697 (2001).

257 Gallo P, Dharmapuri S, Nuzzo M *et al.* Xenogeneic immunization in mice using HER2 DNA delivered by an adenoviral vector. *Int. J. Cancer* 113(1), 67–77 (2005).

258 Hartman ZC, Wei J, Osada T *et al.* An adenoviral vaccine encoding full-length inactivated human Her2 exhibits potent immunogenicty and enhanced therapeutic efficacy without oncogenicity. *Clin. Cancer Res.* 16(5), 1466–1477 (2010).

259 Gabitzsch ES, Xu Y, Balcaitis S, Balint JP Jr, Jones FR. An Ad5[E1-, E2b-]-HER2/neu vector induces immune responses and inhibits HER2/neu expressing tumor progression in Ad5 immune mice. *Cancer Gene Ther.* 18(5), 326–335 (2011).

260 Liu Y, Tuve S, Persson J *et al.* Adenovirus-mediated intratumoral expression of immunostimulatory proteins in combination with systemic Treg inactivation induces tumor-destructive immune responses in mouse models. *Cancer Gene Ther.* 18(6), 407–418 (2011).

261 Wang X, Wang JP, Maughan MF, Lachman LB. Alphavirus replicon particles containing the gene for HER2/neu inhibit breast cancer growth and tumorigenesis. *Breast Cancer Res.* 7(1), R145–R155 (2005).

262 Gao Y, Whitaker-Dowling P, Griffin JA, Bergman I. Treatment with targeted vesicular stomatitis virus generates therapeutic multifunctional anti-tumor memory CD4 T cells. *Cancer Gene Ther.* 19(4), 282–291 (2012).

263 Andreasson K, Tegerstedt K, Eriksson M *et al.* Murine pneumotropic virus chimeric Her2/neu virus-like particles as prophylactic and therapeutic vaccines against Her2/neu expressing tumors. *Int. J. Cancer* 124(1), 150–156 (2009).

264 Tegerstedt K, Lindencrona JA, Curcio C *et al.* A single vaccination with polyomavirus VP1/VP2Her2 virus-like particles prevents outgrowth of HER-2/neu-expressing tumors. *Cancer Res.* 65(13), 5953–5957 (2005).

265 Shahabi V, Seavey MM, Maciag PC, Rivera S, Wallecha A. Development of a live and highly attenuated Listeria monocytogenes-based vaccine for the treatment of Her2/neu-overexpressing cancers in human. *Cancer Gene Ther.* 18(1), 53–62 (2011).

266 Bernhard H, Neudorfer J, Gebhard K *et al.* Adoptive transfer of autologous, HER2-specific, cytotoxic T-lymphocytes for the treatment of HER2-overexpressing breast cancer. *Cancer Immunol. Immunother.* 57(2), 271–280 (2008).

267 Turtle CJ, Hudecek M, Jensen MC, Riddell SR. Engineered T cells for anti-cancer therapy. *Curr. Opin. Immunol.* 24(5), 633–639 (2012).

268 Shi H, Liu L, Wang Z. Improving the efficacy and safety of engineered T cell therapy for cancer. *Cancer Lett.* 328(2), 191–197 (2013).

269 Stancovski I, Schindler DG, Waks T, Yarden Y, Sela M, Eshhar Z. Targeting of T-lymphocytes to Neu/HER2-expressing cells using chimeric single chain Fv receptors. *J. Immunol.* 151(11), 6577–6582 (1993).

270 Altenschmidt U, Kahl R, Moritz D *et al.* Cytolysis of tumor cells expressing the Neu/erbB-2, erbB-3, and erbB-4 receptors by genetically targeted naive T-lymphocytes. *Clin. Cancer Res.* 2(6), 1001–1008 (1996).

271 Haynes NM, Smyth MJ, Kershaw MH, Trapani JA, Darcy PK. Fas-ligand-mediated lysis of erbB-2-expressing tumour cells by redirected cytotoxic T-lymphocytes. *Cancer Immunol. Immunother.* 47(5), 278–286 (1999).

272 Uherek C, Tonn T, Uherek B *et al.* Retargeting of natural killer-cell cytolytic activity to ErbB2-expressing cancer cells results in efficient and selective tumor cell destruction. *Blood* 100(4), 1265–1273 (2002).

273 Turatti F, Figini M, Alberti P, Willemsen RA, Canevari S, Mezzanzanica D. Highly efficient redirected anti-tumor activity of human lymphocytes transduced with a completely human chimeric immune receptor. *J. Gene Med.* 7(2), 158–170 (2005).

274 Ahmed N, Ratnayake M, Savoldo B *et al.* Regression of experimental medulloblastoma following transfer of HER2-specific T cells. *Cancer Res.* 67(12), 5957–5964 (2007).

275 Turatti F, Figini M, Balladore E *et al.* Redirected activity of human antitumor chimeric immune receptors is governed by antigen and receptor expression levels and affinity of interaction. *J. Immunother.* 30(7), 684–693 (2007).

276 Li S, Yang J, Urban FA *et al.* Genetically engineered T cells expressing a HER2-specific chimeric receptor mediate antigen-specific tumor regression. *Cancer Gene Ther.* 15(6), 382–392 (2008).

277 Daldrup-Link HE, Meier R, Rudelius M *et al. In vivo* tracking of genetically engineered, anti-HER2/neu directed natural killer cells to HER2/neu positive mammary tumors with magnetic resonance imaging. *Eur. Radiol.* 15(1), 4–13 (2005).

278 Demirtzoglou FJ, Papadopoulos S, Zografos G. Cytolytic and cytotoxic activity of a human natural killer cell line genetically modified to specifically recognize HER-2/neu overexpressing tumor cells. *Immunopharmacol. Immunotoxicol.* 28(4), 571–590 (2006).

279 Kruschinski A, Moosmann A, Poschke I *et al.* Engineering antigen-specific primary human NK cells against HER-2 positive carcinomas. *Proc. Natl Acad. Sci. USA* 105(45), 17481–17486 (2008).

280 Pegram HJ, Jackson JT, Smyth MJ, Kershaw MH, Darcy PK. Adoptive transfer of gene-modified primary NK cells can specifically inhibit tumor progression *in vivo*. *J. Immunol.* 181(5), 3449–3455 (2008).

281 Liang X, Weigand LU, Schuster IG *et al.* A single TCR alpha-chain with dominant peptide recognition in the allorestricted HER2/neu-specific T cell repertoire. *J. Immunol.* 184(3), 1617–1629 (2010).

282 Biburger M, Weth R, Wels WS. A novel bispecific tetravalent antibody fusion protein to target costimulatory activity for T-cell activation to tumor cells overexpressing ErbB2/HER2. *J. Mol. Biol.* 346(5), 1299–1311 (2005).

283 Muniappan A, Banapour B, Lebkowski J, Talib S. Ligand-mediated cytolysis of tumor cells: use of heregulin-zeta chimeras to redirect cytotoxic T-lymphocytes. *Cancer Gene Ther.* 7(1), 128–134 (2000).

284 Nakazawa Y, Huye LE, Salsman VS *et al.* PiggyBac-mediated cancer immunotherapy using EBV-specific cytotoxic T-cells expressing HER2-specific chimeric antigen receptor. *Mol. Ther.* 19(12), 2133–2143 (2011).

285 Marcus A, Waks T, Eshhar Z. Redirected tumor-specific allogeneic T cells for universal treatment of cancer. *Blood* 118(4), 975–983 (2011).

286 Morgan RA, Dudley ME, Rosenberg SA. Adoptive cell therapy: genetic modification to redirect effector cell specificity. *Cancer J.* 16(4), 336–341 (2010).

287 Curran KJ, Pegram HJ, Brentjens RJ. Chimeric antigen receptors for T cell immunotherapy: current understanding and future directions. *J. Gene Med.* 14(6), 405–415 (2012).

288 Gilham DE, Debets R, Pule M, Hawkins RE, Abken H. CAR-T cells and solid tumors: tuning T cells to challenge an inveterate foe. *Trends Mol. Med.* 18(7), 377–384 (2012).

289 Shirasu N, Kuroki M. Functional design of chimeric T-cell antigen receptors for adoptive immunotherapy of cancer: architecture and outcomes. *Anticancer Res.* 32(6), 2377–2383 (2012).

290 Wu R, Forget MA, Chacon J *et al.* Adoptive T-cell therapy using autologous tumor-infiltrating lymphocytes for metastatic melanoma: current status and future outlook. *Cancer J.* 18(2), 160–175 (2012).

291 Porter DL, Levine BL, Kalos M, Bagg A, June CH. Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. *N. Engl. J. Med.* 365(8), 725–733 (2011).

292 Morgan RA, Yang JC, Kitano M, Dudley ME, Laurencot CM, Rosenberg SA. Case report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognizing ERBB2. *Mol. Ther.* 18(4), 843–851 (2010).

293 Chandarlapaty S, Sawai A, Ye Q *et al.* SNX2112, a synthetic heat shock protein 90 inhibitor, has potent antitumor activity against HER kinase-dependent cancers. *Clin. Cancer Res.* 14(1), 240–248 (2008).

294 Chiosis G, Lucas B, Shtil A, Huezo H, Rosen N. Development of a purine-scaffold novel class of Hsp90 binders that inhibit the proliferation of cancer cells and induce the degradation of Her2 tyrosine kinase. *Bioorg. Med. Chem.* 10(11), 3555–3564 (2002).

295 Chiosis G, Timaul MN, Lucas B *et al.* A small molecule designed to bind to the adenine nucleotide pocket of Hsp90 causes Her2 degradation and the growth arrest and differentiation of breast cancer cells. *Chem. Biol.* 8(3), 289–299 (2001).

296 Ge J, Normant E, Porter JR *et al.* Design, synthesis, and biological evaluation of hydroquinone derivatives of 17-amino-17-demethoxygeldanamycin as potent, water-soluble inhibitors of Hsp90. *J. Med. Chem.* 49(15), 4606–4615 (2006).

297 Le Bras G, Radanyi C, Peyrat JF *et al.* New novobiocin analogues as antiproliferative agents in breast cancer cells and potential inhibitors of heat shock protein 90. *J. Med. Chem.* 50(24), 6189–6200 (2007).

298 Neckers L. Effects of geldanamycin and other naturally occurring small molecule antagonists of heat shock protein 90 on HER2 protein expression. *Breast Dis.* 11, 49–59 (2000).

299 Neckers L. Development of small molecule Hsp90 inhibitors: utilizing both forward and reverse chemical genomics for drug identification. *Curr. Med. Chem.* 10(9), 733–739 (2003).

300 Park H, Kim YJ, Hahn JS. A novel class of Hsp90 inhibitors isolated by structure-based virtual screening. *Bioorg. Med. Chem. Letters* 17(22), 6345–6349 (2007).

301 Porter JR, Fritz CC, Depew KM. Discovery and development of Hsp90 inhibitors: a promising pathway for cancer therapy. *Curr. Opin. Chem. Biol.* 14(3), 412–420 (2010).

302 Soga S, Akinaga S, Shiotsu Y. Hsp90 inhibitors as anti-cancer agents, from basic discoveries to clinical development. *Curr. Pharm. Des.* 19(3), 366–376 (2013).

303 Yi F, Regan L. A novel class of small molecule inhibitors of Hsp90. *ACS Chem. Biol.* 3(10), 645–654 (2008).

304 Yu XM, Shen G, Neckers L *et al.* Hsp90 inhibitors identified from a library of novobiocin analogues. *J. Am. Chem. Soc.* 127(37), 12778–12779 (2005).

305 Chandarlapaty S, Scaltriti M, Angelini P *et al.* Inhibitors of HSP90 block p95-HER2 signaling in Trastuzumab-resistant tumors and suppress their growth. *Oncogene* 29(3), 325–334 (2010).

306 Wong C, Chen S. Heat shock protein 90 inhibitors: new mode of therapy to overcome endocrine resistance. *Cancer Res.* 69(22), 8670–8677 (2009).

307 Raja SM, Clubb RJ, Bhattacharyya M *et al.* A combination of Trastuzumab and 17-AAG induces enhanced ubiquitinylation and lysosomal pathway-dependent ErbB2 degradation and cytotoxicity in ErbB2-overexpressing breast cancer cells. *Cancer Biol. Ther.* 7(10), 1630–1640 (2008).

308 Wainberg ZA, Anghel A, Rogers AM *et al.* Inhibition of HSP90 with AUY922 induces synergy in HER2 amplified trastuzumab resistant breast and gastric cancer. *Mol. Cancer Ther.* 12(4), 509–519 (2013).

309 Leow CC, Chesebrough J, Coffman KT *et al.* Antitumor efficacy of IPI-504, a selective heat shock protein 90 inhibitor against human epidermal growth factor receptor 2-positive human xenograft models as a single agent and in combination with trastuzumab or lapatinib. *Mol. Cancer Ther.* 8(8), 2131–2141 (2009).

310 Oude Munnink TH, De Vries EG, Vedelaar SR *et al.* Lapatinib and 17AAG reduce 89Zr-trastuzumab-F(ab')2 uptake in SKBR3 tumor xenografts. *Mol. Pharm.* 9(11), 2995–3002 (2012).

311 Jhaveri K, Miller K, Rosen L *et al.* A Phase I dose-escalation trial of trastuzumab and alvespimycin hydrochloride (KOS-1022; 17 DMAG) in the treatment of advanced solid tumors. *Clin. Cancer Res.* 18(18), 5090–5098 (2012).

312 Modi S, Stopeck A, Linden H *et al.* HSP90 inhibition is effective in breast cancer: a Phase II trial of tanespimycin (17-AAG) plus trastuzumab in patients with HER2-positive metastatic breast cancer progressing on trastuzumab. *Clin. Cancer Res.* 17(15), 5132–5139 (2011).

313 Gartner EM, Silverman P, Simon M *et al.* A Phase II study of 17-allylamino-17-demethoxygeldanamycin in metastatic or locally advanced, unresectable breast cancer. *Breast Cancer Res. Treat.* 131(3), 933–937 (2012).

314 Choi S, Choi Y, Dat NT, Hwangbo C, Lee JJ, Lee JH. Tephrosin induces internalization and degradation of EGFR and ErbB2 in HT-29 human colon cancer cells. *Cancer Lett.* 293(1), 23–30 (2010).

315 Hong RL, Spohn WH, Hung MC. Curcumin inhibits tyrosine kinase activity of p185neu and also depletes p185neu. *Clin. Cancer Res.* 5(7), 1884–1891 (1999).

316 Jeong JH, An JY, Kwon YT, Li LY, Lee YJ. Quercetin-induced ubiquitination and down-regulation of Her-2/neu. *J. Cell. Biochem.* 105(2), 585–595 (2008).

317 Zhou NN, Tang J, Chen WD *et al.* Houttuyninum, an active constituent of Chinese herbal medicine, inhibits phosphorylation of HER2/neu receptor tyrosine kinase and the tumor growth of HER2/neu-overexpressing cancer cells. *Life Sci.* 90(19–20), 770–775 (2012).

318 Palmer K, Sharan N, Emtage P, Gauldie J, Muller WJ, Wan Y. Intratumoral administration of an adenovirus expressing a kinase dead form of ErbB-2 inhibits tumor growth. *Gene Ther.* 9(13), 898–905 (2002).

319 Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat. Rev. Cancer* 12(4), 252–264 (2012).

320 Pardoll DM. Immunology beats cancer: a blueprint for successful translation. *Nat. Immunol.* 13(12), 1129–1132 (2012).

321 Maes H, Rubio N, Garg AD, Agostinis P. Autophagy: shaping the tumor microenvironment and therapeutic response. *Trends Mol. Med.* 19(7), 428–446 (2013).

322 Fang H, Declerck YA. Targeting the tumor microenvironment: from understanding pathways to effective clinical trials. *Cancer Res.* 73(16), 4965–4977 (2013).

323 Altieri DC. Mitochondrial HSP90s and tumor cell metabolism. *Autophagy* 9(2), 244–245 (2013).

324 Junttila MR, De Sauvage FJ. Influence of tumour microenvironment heterogeneity on therapeutic response. *Nature* 501(7467), 346–354 (2013).

325 Reisfeld RA. The tumor microenvironment: a target for combination therapy of breast cancer. *Crit. Rev. Oncog.* 18(1–2), 115–133 (2013).

326 Korkaya H, Wicha MS. HER2 and breast cancer stem cells: more than meets the eye. *Cancer Res.* 73(12), 3489–3493 (2013).