CHAPTER

1

Targeting Members of the Epidermal Growth Factor Receptor Family to Improve Response to Chemotherapy

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Abstract

Decades of research on the four receptor tyrosine kinases (RTKs) that comprise the human epidermal growth factor receptor (HER) family have established these proteins as key oncogenes in many cancer types. The strong evidence supporting pro-tumorigenic roles of HER3, HER4, and especially epidermal growth factor receptor (EGFR) and HER2 in cancer has led to the generation and use of HER family-targeted agents that can inhibit one or more of these RTKs. Although these inhibitors are used as a single agent in some contexts, they are often used in combination with chemotherapeutic agents and have been shown to enhance the response to chemotherapy in many cancer types. In this chapter, we outline the basic biology of the HER family receptors before examining each of the four receptors, focusing on mutations and other alterations that occur in cancer and on preclinical and clinical studies in which HER family-targeted agents were used to delay and/or overcome chemoresistance. Throughout, we address open questions in the field and discuss current and future areas of research.

ABBREVIATIONS

ADCC	Antibody-dependent cell-mediated cytotoxicity
AREG	Amphiregulin
BTC	Betacellulin
CDK	Cyclin-dependent kinase
CDKN1A	Cyclin-dependent kinase inhibitor p21Cip1
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
EPGN	Epigen
Erα	Estrogen receptorα
EREG	Epiregulin
ERK	Extracellular signal-regulated kinase
FDA	Food and Drug Administration
HB-EGF	Heparin-binding epidermal growth factor-like growth factor

Targeting Cell Survival Pathways to Enhance Response to Chemotherapy. http://dx.doi.org/10.1016/B978-0-12-813753-6.00001-9

HER Human epidermal growth factor receptor
HNSCC Head and neck squamous cell carcinoma
JAK Janus kinase
LCC Large cell carcinoma
LUAD Lung adenocarcinoma
LUSC Lung squamous cell carcinoma
MEK Mitogen-activated protein kinase kinase
mTOR Mechanistic target of rapamycin
NRG Neuregulin
NSCLC Non-small cell lung cancer
PI3K Phosphoinositide 3-kinase
PTEN Phosphatase and tensin homolog deleted on chromosome ten
RTK Receptor tyrosine kinase
STAT Signal transducer and activator of transcription
TACE Tumor necrosis factor-α converting enzyme
TGF-α Transforming growth factor-α
TKI Tyrosine kinase inhibitor

1.1 INTRODUCTION

For many years, the backbone of nonsurgical cancer treatment comprised chemotherapy and radiation. Although effective in some cases, many tumors proved refractory to these interventions, and low survival rates in many cancer types reflected the inadequacy of available cancer treatments. As significant research efforts have led to the identification of oncogenic drivers and unique vulnerabilities in cancer cells, cancer treatment is increasingly moving toward precision medicine, in which alterations specific to each tumor guide the selection of treatment. A key component of precision medicine is the use of targeted agents that inhibit the oncogenic drivers upon which cancer cells depend for their survival and proliferation. Targeted agents can be used alone or in combination with chemotherapy and/or other treatment modalities and have demonstrated efficacy in many cancer types.

Among the oncogenic targets against which inhibitors have been developed are the four members of the human epidermal growth factor receptor (HER) family. These receptor tyrosine kinases (RTKs) are overexpressed and/or mutated in many cancer types and have been shown to promote tumorigenesis, tumor maintenance, and metastasis. Targeting HER family members is an attractive prospect not only because of their demonstrated role as oncogenic drivers, but also because they have been shown to mediate resistance to chemotherapy [1–8]. Although employed as a single agent for certain indications, HER family-targeted agents are often used in combination with chemotherapy, and the addition of these inhibitors to the treatment arsenal has improved outcomes for patients with many different cancer types.

In this chapter, we first provide a background on HER family members and the roles of each of the four receptors in cancer, then discuss examples of how HER family-targeted agents can improve response to chemotherapy and the mechanisms by which the enhanced responses are achieved. We also discuss current and future avenues of research that will inform efforts to more effectively harness the therapeutic potential of combining chemotherapy with HER family-targeted agents in the era of precision medicine.

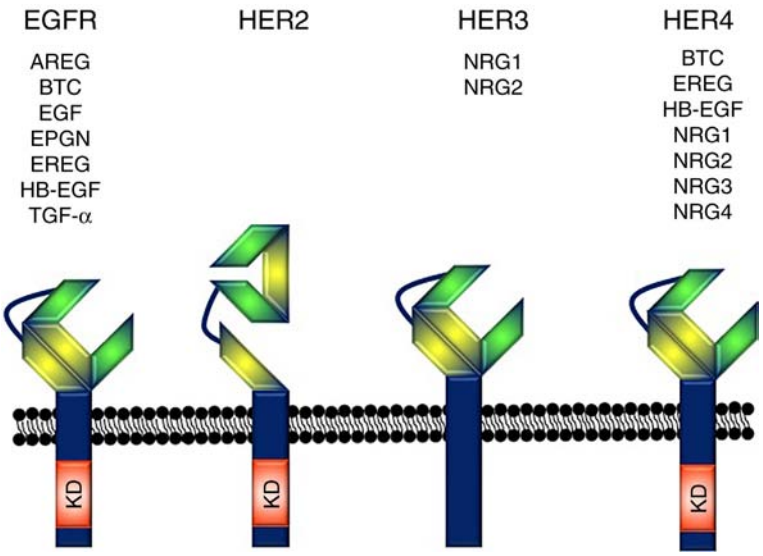

FIG. 1.1 HER family receptors. The four members of the HER family of RTKs are highly homologous in both sequence and structure, but they exhibit functional differences and are regulated by different ligands. The extracellular region of each HER family receptor contains two leucine-rich domains (green) and two cysteine-rich domains (yellow). Ligand binding to the leucine-rich domains of EGFR, HER3, and HER4 induces a conformational change that frees the N-terminal cysteine-rich domain to bind to that of another receptor molecule, facilitating homo- or heterodimerization. HER2 is constitutively available for dimerization, and no ligands have been identified for this receptor. EGFR, HER2, and HER4 each contain an active intracellular kinase domain (KD), while the catalytic activity of HER3 is greatly reduced compared to that of the other HER family receptors.

1.2 HER FAMILY RECEPTORS

The HER family of proteins, also called the epidermal growth factor (EGF) or ErbB family, comprises four RTKs: epidermal growth factor receptor (EGFR, HER1, ErbB1), HER2 (ErbB2, neu in rodents), HER3 (ErbB3), and HER4 (ErbB4) (Fig. 1.1). Residing primarily in the plasma membrane, the HER proteins recognize extracellular cues and transmit these signals to downstream signaling pathways that mediate a number of cellular processes [9–11]. In normal (nontransformed) cells, HER family receptors play a role in organ development and wound healing, among other physiological processes [12–15]. In cancer, HER family members are commonly implicated in tumorigenesis and tumor progression, and aberrant activation of one or more of these RTKs is often observed in cancer cells.

Activation of HER family members is initiated when ligand binds to the extracellular domain of its cognate receptor [9,12,14,16]. Ligand binding triggers a conformational change that allows for homodimerization or heterodimerization with other HER family members or non-HER-family RTKs [9,12,14–24]. (An exception is HER2, which has no known ligands and is thought to be constitutively available for dimerization [1,9,14,15,19,20,23,25–29].) Dimerization facilitates allosteric activation of the kinase domains of the HER family receptors, promoting transphosphorylation of tyrosine residues in the intracellular domains (ICDs)

of their dimerization partners [9,14,16,17,19,20,23]. HER3 does not contain a catalytically active kinase, but is still able to allosterically activate its dimerization partners [1,14,16, 18–20,22,23,25,26,28–30].

The impact of phosphorylation on HER family receptor function depends on which sites are phosphorylated. Phosphorylation of certain residues promotes internalization and subsequent proteasomal degradation or endocytic recycling of the receptors, which ensures that activation of these receptors is transient and tightly regulated [14,31,32]. However, phosphorylation of most of the residues creates plasma membrane-proximal binding sites for downstream signaling molecules [14,17,26,29]. Binding of these molecules to HER family receptors leads to activation of a number of signaling pathways, including the phosphoinositide 3-kinase/Akt/mechanistic target of rapamycin (PI3K/Akt/mTOR), RAS/RAF/mitogen-activated protein kinase kinase/extracellular signal-regulated kinase (RAS/RAF/MEK/ERK), and Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathways [9,11,12,21,33,34]. The ability of HER family members to activate these signaling pathways is co-opted by cancer cells, particularly by those in which one or more of the genes encoding HER family members are amplified and/or mutated, to drive tumorigenesis, to promote tumor cell survival and metastasis, to enable immune evasion, and to mediate resistance to chemotherapy.

Although the precise mechanisms of resistance to chemotherapy depend on the chemotherapeutic agent(s) used, the cancer type, and other factors specific to a particular tumor, in general, resistance to chemotherapy arises when cancer cells are able to overcome the pro-death signaling induced by chemotherapeutic agents. HER family receptors are among the factors that have been shown to promote tumor cell survival in the presence of chemotherapy and are thereby implicated in chemoresistance [1–8]. More convincing evidence for a role for HER family RTKs in resistance to chemotherapy comes from reports of increased expression and/or activation of these RTKs in chemorefractory tumors and both preclinical and clinical data demonstrating that inhibiting one or more HER family receptors can overcome resistance to chemotherapy [1,2,7,8,35–37]. Thus, combining HER family-targeted inhibitors with chemotherapy is a rational approach to improving responses.

Other features that make HER family receptors attractive targets for drug development include their subcellular location and the presence of targetable kinase domains. HER family receptors reside primarily on the cell surface and are thus accessible to antibodies in the tumor microenvironment. Monoclonal antibody therapies that bind and inhibit HER family receptors have demonstrated efficacy in a multitude of cancer types. Small-molecule tyrosine kinase inhibitors (TKIs) that can inhibit EGFR, HER2, and/or HER4 have also proved effective in the clinic. (HER3 contains a catalytically impaired kinase domain; thus, kinase inhibition is not thought to be an effective approach to inhibit HER3 [1,15,16,26,30,38].) Some of these agents target more than one HER family member, while others are selective for a single receptor. Inhibitor selectivity and type (i.e., monoclonal antibody vs. TKI) can have important implications for treatment efficacy, as will be discussed in subsequent sections.

Although the four HER family receptors are highly homologous at both the sequence and structural levels and often cooperate to support the proliferation and/or survival of cancer cells, they also possess distinct, nonoverlapping functions. Moreover, different HER family receptors are implicated in different types of cancer, and the oncogenic alterations that occur

vary among cancer types. Thus, we will next cover each of the HER family receptors in turn, providing an overview of the specific alterations observed in each receptor and the cancer types in which they are implicated before describing methods to target these RTKs. Finally, we will, when possible, discuss clinical outcomes when HER family-targeted agents are combined with chemotherapy and preclinical studies elucidating the mechanisms by which these combinations elicit their antitumor effects.

1.2.1 EGFR

EGFR was the first member of the HER family of RTKs to be discovered and remains perhaps the most well-studied HER family member in cancer [9,39]. Evidence of a role for EGFR in cancer came from the seminal finding that the kinase domain of EGFR is homologous to the viral oncogene v-erb-B, which had previously been shown to induce transformation [9,40]. These and other discoveries, along with studies demonstrating that targeting EGFR can inhibit cancer cell proliferation and induce apoptosis in certain preclinical cancer models, established EGFR as a proto-oncogene and a potential drug target in cancer.

Activation of EGFR occurs when one of its ligands binds and elicits a conformational change that enables dimerization [12,33,34]. EGFR binds seven ligands: amphiregulin (AREG), betacellulin (BTC), EGF, epigen (EPGN), epiregulin (EREG), heparin-binding EGF-like growth factor (HB-EGF), and transforming growth factor-α (TGF-α) (Fig. 1.1) [20,25,29,34]. EGFR is able to both bind ligands and phosphorylate substrates and can thus form functional homodimers [5,13,24]. In addition, EGFR can dimerize with other HER family members or, possibly, with non-HER family RTKs, and the formation of dimers with different partners can modulate the outcome of EGFR activation [5,13,17]. Transphosphorylation of tyrosine residues in the EGFR ICD by its dimerization partners creates binding sites for downstream effectors, facilitating their activation [9,14,17]. Among the signaling pathways downstream of EGFR that have been shown to mediate EGFR-induced transformation are the RAS/RAF/MEK/ERK, PI3K/Akt/mTOR, and JAK/STAT pathways [11–13,33,34,41].

Aberrant activation of EGFR occurs in many cancer types, although the alterations that lead to this hyperactivation vary among cancer types. Some cancers exhibit overexpression and/or hyperactivation of wild-type EGFR, whereas activating mutations are observed in others [12,13,42]. Activating mutations, sometimes co-occurring with amplification of the *EGFR* gene, have been observed in non-small cell lung cancer (NSCLC) and glioblastoma [13,22,43,44]. In NSCLC, the most common mutations are exon 19 deletions and the L858R point mutation, both of which occur in the kinase domain and confer ligand-independent activation of EGFR [17,18,32,43,44]. In glioblastoma, mutations in the extracellular domain, including point mutations and in-frame deletions that lead to expression of a constitutively active truncated version of EGFR called EGFRvIII, are more common [43,45]. In other cancer types, activating mutations in *EGFR* are rarely observed, but wild-type EGFR serves as an oncogenic driver. For example, in head and neck squamous cell carcinoma (HNSCC), squamous cell carcinoma of the lung (LUSC), and breast cancer, amplification of wild-type *EGFR* occurs and often results in overexpression of the EGFR protein [12,13,15,17,42]. Transcriptional upregulation of *EGFR* in the absence of gene amplification has also been observed [13]. In addition, oncogenic fusion proteins containing the N-terminal domain of EGFR were recently identified in lung cancer [32].

In addition to alterations in *EGFR* itself, mutations in other genes involved in the regulation of EGFR can result in aberrant activation of this RTK. While the activation of EGFR is tightly regulated in normal (nontransformed) cells, these regulatory mechanisms can dissolve in cancer cells, leading to unchecked activation of the receptor and its downstream signaling molecules [46]. One mechanism for aberrant activation of EGFR is the loss of negative feedback pathways that, in normal cells, prevent sustained activation of these receptors and dampen signaling downstream. For example, hyperactivation of EGFR signaling can occur due to a loss of tumor suppressive phosphatases, such as phosphatase and tensin homolog deleted on chromosome 10 (PTEN), which inhibits EGFR signaling and can promote degradation of EGFR [45]. Increased autocrine and/or paracrine secretion of EGFR ligands by tumor cells and/or other cells in the tumor microenvironment is another mechanism by which EGFR can be aberrantly activated in cancer cells [34,47].

The importance of EGFR in cancer is demonstrated not only by the frequency of alterations that lead to increased expression of EGFR and/or activation of EGFR signaling, but also by the efficacy of EGFR-targeted therapies, both alone and in combination with chemotherapy and other treatment modalities, in several cancer types. The primary classes of EGFR inhibitors that have been employed in the clinic thus far are small-molecule TKIs and neutralizing monoclonal antibodies [48,49]. In general, EGFR-targeting TKIs are used to treat cancers harboring activating mutations in EGFR, whereas antibody-based therapies are more commonly used in the treatment of tumors that overexpress wild-type EGFR, although there are exceptions to this. Although EGFR-targeted therapies are used as a single agent for some indications, they are often combined with chemotherapy, surgery, and/or radiation.

A number of small-molecule TKIs have been approved by the United States Food and Drug Administration (FDA) for cancer treatment. Activating mutations in *EGFR* tend to confer sensitivity to EGFR-targeted TKIs; thus, these inhibitors are used primarily for the treatment of *EGFR*-mutant tumors (Table 1.1) [11,16,18,28,47,50]. Erlotinib (Tarceva) and gefitinib (Iressa) are reversible adenosine triphosphate (ATP)-competitive (type I) EGFR-specific inhibitors [18,32,51]. Afatinib (Gilotrif) is a second-generation, irreversible ATP-competitive TKI that targets HER2 and HER4 in addition to EGFR [9,18,32,51,52]. Third-generation TKIs that can overcome resistance to first- and second-generation TKIs are also being used in the clinic. Osimertinib (Tagrisso) is the only third-generation EGFR-targeted TKI that has been approved by the FDA thus far [12,51]. It has demonstrated efficacy against tumors bearing the T790M mutation in EGFR, the expression of which confers resistance to first- and second-generation inhibitors [12,51]. Osimertinib is selective for mutant EGFR (i.e., wild-type EGFR is largely spared) [51].

Three monoclonal antibodies targeting EGFR have been approved by the FDA for cancer treatment: cetuximab (Erbitux), panitumumab (Vectibix), and necitumumab (Portrazza) (Table 1.1) [12,33,53,54]. These monoclonal antibodies are often used in combination with chemotherapeutic agents and/or radiation. Although the efficacy of TKIs rests primarily on their ability to inhibit kinase activity, EGFR-targeted monoclonal antibodies work via a number of mechanisms, including outcompeting endogenous ligands, preventing dimerization, and inducing internalization and subsequent degradation of EGFR [12,13,33]. In addition, cetuximab and necitumumab, as IgG1-isotype antibodies, can induce antibody-dependent cell-mediated cytotoxicity (ADCC), in which immune cells expressing the FcγRIII receptor (primarily natural killer [NK] cells) recognize the Fc fragment of an antibody and release

TABLE 1.1 HER Family-Targeted Agents Approved by the FDA

Inhibitor	Trade name	Type of inhibitor	Target(s)	Cancer type(s)
Ado-trastuzumab emtansine	Kadcyla	Antibody-drug conjugate	HER2	HER2+ breast cancer
Afatinib	Gilotrif	TKI	EGFR, HER2, HER4	EGFR-mutant NSCLC
Cetuximab	Erbitux	mAb (IgG1 κ)	EGFR	HNSCC; KRAS wild-type, EGFR-expressing CRC
Erlotinib	Tarceva	TKI	EGFR	EGFR-mutant NSCLC; pancreatic cancer
Gefitinib	Iressa	TKI		EGFR-mutant NSCLC
Lapatinib	Tykerb	TKI	EGFR, HER2, HER4	HER2+ breast cancer
Necitumumab	Portrazza	mAb (IgG1 κ)	EGFR	LUSC
Osimertinib	Tagrisso	TKI	EGFR T790M	EGFR-mutant (T790M) NSCLC
Panitumumab	Vectibix	mAb (IgG2 κ)	EGFR	EGFR-expressing CRC
Pertuzumab	Perjeta	mAb (IgG1 κ)	HER2	HER2+ breast cancer
Trastuzumab	Herceptin	mAb (IgG1 κ)	HER2	HER2+ breast cancer; HER2+ gastric and GEJ adenocarcinoma

Abbreviations: TKI, tyrosine kinase inhibitor; mAb, monoclonal antibody; IgG, immunoglobulin G; NSCLC, non-small cell lung cancer; HNSCC, head and neck squamous cell carcinoma; LUSC, lung squamous cell carcinoma; CRC, colorectal cancer; GEJ, gastroesophageal junction.

cytolytic proteins that can kill tumor cells [12,13,53–57]. (It is thought that panitumumab, as an IgG2-isotype antibody, is less effective at inducing NK cell-mediated ADCC than cetuximab or necitumumab [33,49,53].) Induction of ADCC has been shown to contribute to the antitumor effects of these antibodies in preclinical studies [53].

EGFR alterations are commonly observed in NSCLC, with the frequency of alterations varying among the three NSCLC subtypes (adenocarcinoma [LUAD], LUSC, and large cell carcinoma [LCC] [12,17]). Approximately 10%–25% of lung adenocarcinomas bear activating mutations in *EGFR* [44,58]. As mentioned above, the most common of these activating mutations are exon 19 deletions and the L858R mutation, which occur in the kinase domain and result in constitutive activation of the kinase [17,18,32,44,51]. The current standard of care for first-line treatment of patients with *EGFR*-mutant NSCLC is an EGFR-targeted TKI used as a single agent [11]. The EGFR-targeted TKIs afatinib, erlotinib, gefitinib, and osimertinib have received approval from the FDA for the treatment of *EGFR*-mutant NSCLC [12,17,18,36,51,59,60].

Although these TKIs are primarily used as a single agent in *EGFR*-mutant NSCLC, they have been used in combination with chemotherapy in settings in which combining EGFR-targeted inhibitors with chemotherapeutic agents has demonstrated enhanced efficacy compared to monotherapy. For example, results of a recent clinical trial demonstrated that the addition of afatinib to the investigator's choice of chemotherapy improved the objective response rate and progression-free survival in NSCLC patients whose tumors had acquired resistance to first-generation EGFR TKIs (erlotinib and/or gefitinib) and afatinib [36]. However, results from another clinical trial in NSCLC in patients whose tumors had progressed on first-line gefinitib showed that continuing gefitinib treatment in combination with platinum-based doublet chemotherapy did not improve progression-free survival compared to chemotherapy alone [60]. These and other studies demonstrate that combining chemotherapy with EGFR-targeted TKIs may improve outcomes, but only for certain indications.

In contrast to LUAD, overexpression of EGFR is more common than mutation in LUSC [12,17,54]. In this subtype, both TKIs and antibody-based therapies have demonstrated efficacy in the clinic [12,54]. However, the use of EGFR-targeted TKIs is largely limited to NSCLC tumors bearing activating mutations in *EGFR*, which are rare in LUSC [12,17]. In 2015, the EGFR-targeted monoclonal antibody necitumumab, in combination with gemcitabine and cisplatin, was approved by the FDA for first-line treatment of metastatic LUSC [12,54].

Activating mutations in EGFR are also rare in HNSCC [13]. However, EGFR overexpression occurs in up to 90% of HNSCC tumors, and EGFR has been established as an oncogenic driver in this disease [13,17,48]. Cetuximab is the only oncogene-targeted agent that has been approved by the FDA for treatment of HNSCC [12,48,61]. Although approved as a single agent for the treatment of patients with recurrent/metastatic HNSCC whose tumors have progressed on platinum-based chemotherapy, cetuximab is more commonly used in combination with chemotherapy and/or radiation [59,61]. FDA approval for cetuximab in combination with chemotherapy was granted based on results from a clinical trial in which 442 patients with recurrent/metastatic HNSCC were assigned to be treated with platinum-based chemotherapy plus fluorouracil alone or in combination with cetuximab [62]. The addition of cetuximab to this chemotherapy regimen improved overall survival compared to chemotherapy alone [62]. It should be noted that cetuximab has been shown to improve response in combination with chemotherapy in HNSCC, whereas other EGFR inhibitors have not fared as well in clinical trials in this disease, despite a number of studies demonstrating that erlotinib and panitumumab are effective in preclinical models of HNSCC [13,48,49,59,62]. It has been suggested, though not verified, that the enhanced clinical efficacy of cetuximab compared to other EGFR inhibitors tested in HNSCC may be explained, at least in part, by its ability to promote immune-mediated tumor clearance via induction of ADCC [49,59].

As in LUSC and HNSCC, *EGFR* is commonly overexpressed, but rarely mutated, in colorectal adenocarcinoma [24,47]. However, in contrast to HNSCC, both cetuximab and panitumumab are FDA approved for the treatment of metastatic colorectal cancer [12,24,33,61]. While initially approved broadly for the treatment of colorectal cancer on the basis of clinical trials in unselected patient populations, it was subsequently demonstrated that the clinical benefit of cetuximab in this cancer type is largely limited to patients with *KRAS* wild-type tumors, as activating mutations in *KRAS* maintain activation of oncogenic signaling pathways even in the presence of cetuximab [33,35,41,47]. As a result, EGFR-targeted antibodies are largely ineffective against *KRAS*-mutant colorectal tumors; thus, cetuximab is now approved

for the treatment of only *KRAS* wild-type tumors [33]. More recently, activating mutations in *BRAF* or *NRAS* were also shown to be negative predictive biomarkers for response to cetuximab and panitumumab in colorectal cancer [33,41]. Official recommendations from the FDA do not currently reflect these still-recent findings.

Nonetheless, cetuximab has demonstrated efficacy in combination with chemotherapy in some *KRAS*-mutant colorectal tumors [63]. Recently, Pozzi et al. [63] hypothesized that this effect may be due to cetuximab's ability to induce immune-mediated tumor clearance and set out to uncover the mechanism by which the combination of cetuximab and chemotherapy could produce an antitumor effect even in *KRAS*-mutant colorectal cancer cells. The authors reported in 2016 that the combination of cetuximab and the chemotherapy regimen FOLFIRI (leucovorin calcium, fluorouracil, and irinotecan hydrochloride) could trigger immunogenic cell death in colorectal cancer cells, thereby enhancing phagocytosis of these cells by dendritic cells and triggering an antitumor immune response mediated by $CD8^+$ T cells [63]. The induction of immunogenic cell death by the combination of cetuximab and FOLFIRI occurred in both *KRAS* wild-type and *KRAS*-mutant cells, although cells containing activating mutations in *BRAF* did not undergo immunogenic cell death when treated with this combination [63]. This study highlights the possibility that inhibition of EGFR (and perhaps other HER family receptors) in combination with chemotherapy may enhance the efficacy of chemotherapy by inducing immune-mediated tumor clearance.

These findings emphasize the need for more research to assess the impact of EGFR inhibition both alone and in combination with chemotherapy on tumor-immune cell interactions in order to identify approaches that can both inhibit tumor cell proliferation and survival and promote antitumor immune responses. Identifying such strategies will further the goal of improving outcomes for patients.

1.2.2 HER2

Following the discovery of EGFR and subsequent identification of its role as a proto-oncogene, researchers sought to add to the growing list of oncogenic drivers. HER2 was discovered in 1982, when the protein encoded by DNA that had been shown to induce transformation in mouse embryonic fibroblasts (NIH/3T3 cells) was found to be a 185-kDa phosphoprotein [64,65]. (This DNA had been isolated from carcinogen-induced rat neuroblastomas; thus, HER2 is referred to as *neu* in rodents [14,64,66,67].) HER2 was quickly implicated in human cancers, as it was shown to be overexpressed in breast tumors and correlated with poor prognosis in breast cancer [9,19,38,66,68]. Given that HER2 was initially discovered in transformed cells, it is unsurprising that the bulk of research on HER2 has focused on its roles in cancer and the development and use of HER2-targeted agents for cancer treatment.

HER2 is unique among the HER family receptors in that it does not require a ligand to be available for dimerization, as it is fixed in an open (untethered) confirmation (Fig. 1.1) [9,14–16]. Due to its constitutive availability for dimerization, HER2 is thought to be the preferred dimerization partner for the other HER family members, with HER2/HER3 heterodimers being particularly potent [1,8,9,14–16,19,20]. HER2/HER3 dimers have been implicated in tumorigenesis, tumor progression, and resistance to chemotherapy [1,8,14,25].

The *ERBB2* gene is altered in a number of cancer types. The most common alteration of *ERBB2* in cancer is gene amplification, which leads to overexpression of HER2 [14,15,69].

Particularly high frequencies of *ERBB2* amplification are observed in gastric/esophageal and breast cancers [1,3,9,14,69–75]. Activating mutations in HER2, many of which cluster in the kinase domain, have also been identified in breast cancer and lung adenocarcinoma and have been shown to promote tumor growth in preclinical models [9,16,22,28,50,74,76]. However, such mutations are uncommon [14,74].

Evidence cementing HER2 as an oncogenic driver in several cancer types has supported the development of HER2-targeted inhibitors. Both TKIs and monoclonal antibodies targeting HER2 have been approved by the FDA for the treatment of HER2-expressing cancers **(**Table 1.1**)**. The first FDA-approved HER2-targeted agent was trastuzumab (Herceptin), a humanized IgG1 monoclonal antibody [19,57,71,74]. The monoclonal antibody pertuzumab (Perjeta) has also been FDA approved for the treatment of HER2-positive breast cancer [9,71,74]. Although trastuzumab and pertuzumab share a target, they bind to different epitopes in the HER2 extracellular domain [9,19,77,78]. It has been shown that although pertuzumab can inhibit ligand-induced HER2/HER3 dimerization, trastuzumab primarily inhibits ligand-independent dimerization of these RTKs, conferring efficacy in HER2-overexpressing breast cancer cells in which HER2/HER3 dimers can form even in the absence of a ligand [15,19,38,74,78]. Thus, the combination of trastuzumab and pertuzumab can be more effective at inhibiting HER2 than either antibody alone [19,77]. The combination of trastuzumab, pertuzumab, and docetaxel has been shown to improve overall and progression-free survival compared to trastuzumab and docetaxel in patients with HER2-positive metastatic breast cancer [9,19,74,77]. HER2 is also overexpressed at a high frequency in gastric cancer, and trastuzumab has been approved by the FDA in combination with cisplatin and either capecitabine or 5-fluorouracil for the treatment of adenocarcinomas of the stomach and gastroesophageal junction [9,73]. In addition, the antibody-drug conjugate ado-trastuzumab emtansine (Kadcyla), which contains trastuzumab linked to the microtubule polymerization inhibitor emtansine (DM1), has been approved for the treatment of HER2-positive metastatic breast cancer that had been previously treated with trastuzumab and/or a taxane [9,74,75]. To date, lapatinib (Tykerb) is the only HER2-targeted TKI that has been approved by the FDA for cancer treatment [71,74]. Lapatinib is used in combination with letrozole or capecitabine for the treatment of HER2-positive breast cancer [8,9,72,74,79]. Although not currently approved for cancer treatment, the EGFR- and HER2-targeted TKI neratinib is currently being tested in clinical trials and has demonstrated efficacy in patients with HER2-positive breast cancer [9,18,71,72].

HER2-targeted agents have transformed the treatment of HER2-positive breast cancer. Before these agents became available, patients whose tumors expressed HER2 faced a poor prognosis compared to those with HER2-negative tumors [16,68,69]. When trastuzumab, the first FDA-approved HER2-targeted agent, was introduced, the trend reversed: patients with HER2-positive tumors treated with trastuzumab now achieved better outcomes than those with HER2-negative tumors, for whom trastuzumab was not a treatment option [69]. The fact that HER2 expression augured a poor prognosis prior to the development and use of HER2-targeted therapy suggests that HER2 may play a role in intrinsic resistance to chemotherapy, as chemotherapeutic agents, often in combination with surgery, were the backbone of breast cancer treatment prior to the introduction of targeted therapy.

Preclinical research has identified a number of mechanisms that may explain why HER2-positive tumors are less likely to respond to chemotherapy and how targeting HER2 may

enhance the efficacy of chemotherapy. A series of papers in the late 1990s and early 2000s demonstrated that HER2 can mediate resistance to the chemotherapeutic drug paclitaxel (Taxol) by inhibiting the cyclin-dependent kinase CDK1 (also referred to as Cdc2) [3,70,80]. Activation of CDK1 is required for entry into mitosis, and nonmitotic cells are largely protected from chemotherapeutic agents; thus, inhibition of CDK1 confers resistance to paclitaxel-induced apoptosis [3]. It has been reported that HER2 can phosphorylate (and thereby inactivate) CDK1 [3,80]. HER2 can also induce transcriptional upregulation of *CDKN1A*, which encodes the cyclin-dependent kinase inhibitor $p21^{Cip1}$ [2,3,70,80]. Activation of Src downstream of HER2 was shown to activate signal transducer and activator of transcription 3 (STAT3), a transcription factor that can induce expression of *CDKN1A* [80]. p21, in turn, can promote resistance to paclitaxel by inducing mitotic arrest via inhibition of the CDK1/cyclin B complex [2,70,80]. HER2 is also associated with expression of efflux pumps that can remove drugs from the cell, thereby promoting resistance to chemotherapeutic agents [2,81].

Just as HER2 inhibition has been shown to improve outcomes when added to chemotherapeutic regimens, chemotherapeutic agents can also enhance the efficacy of HER2-targeted agents. Like the EGFR-targeting antibody cetuximab, trastuzumab and pertuzumab are IgG1-isotype antibodies and have been shown to induce ADCC [9,19,55–57,78]. This is believed to contribute to the antitumor efficacy of these antibodies [55,56,73]. It has been shown that the chemotherapy drug paclitaxel can recruit NK cells to breast cancer tumors, thereby promoting trastuzumab-induced ADCC [56]. In addition, docetaxel can enhance trastuzumab-mediated ADCC by inducing both expression of NK cell-activating ligands on breast cancer cells and upregulation of their cognate receptor (NKG2D) on NK cells [57]. It should be noted, however, that others have reported that the ability of trastuzumab to induce ADCC is not affected by chemotherapy [55].

Thus far, HER2-targeted agents have only been approved by the FDA for the treatment of HER2-positive breast cancer and gastric adenocarcinoma [71,82]. However, HER2 has been shown to be overexpressed and/or mutated in a number of other cancer types, including ovarian and lung cancers [9,14,28]. Although HER2-targeted agents have not yet been approved for treatment of these cancer types, one might predict that HER2-targeted agents will soon be used in the treatment of patients with other cancer types in which HER2 is overexpressed and/or mutated, especially those for which preclinical studies suggest that using HER2 inhibitors, either alone or in combination with chemotherapy, is beneficial.

1.2.3 HER3

In contrast to EGFR and HER2, for which targeted agents have been in use for over a decade, HER3 has only recently been recognized as a promising drug target [15,25,29,38]. HER3 is increasingly being studied in the context of cancer, and roles for HER3 are being identified in a number of cancer types by basic and translational scientists. This coincides with the development and clinical testing of HER3-targeted agents that seek to capitalize on this research to improve outcomes for patients in whose tumors HER3 promotes tumor cell proliferation and survival and/or resistance to chemotherapy [15,25].

Amino acid substitutions in the HER3 kinase domain that were sustained following the gene duplication events that led to the generation of the four homologous HER family members greatly reduce the kinase activity of HER3 [23,26,38]. As a result, unlike the other HER family receptors, HER3 lacks appreciable kinase activity and is thus dependent on other RTKs

to phosphorylate the tyrosine residues in its C-terminal tail [14,15,18,20,22,23,29,30,38]. Because of this, HER3 homodimers are thought to be enzymatically inactive [14,30]. However, HER3 can form functional heterodimers with other RTKs upon binding of one of its ligands (neuregulin 1 [NRG1] and NRG2) (Fig. 1.1) [6,14,15,18,20,25,29]. As mentioned above, the mechanism of dimerization-induced activation of HER family members involves allosteric rather than kinase-dependent activation; thus, HER3 can promote phosphorylation by its dimerization partners even though it lacks inherent kinase activity [23,38]. HER2 is the preferred binding partner for HER3, but HER3 has also been shown to dimerize with EGFR [1,8,16,18,23,30,38,83].

Once phosphorylated, HER3 is a potent activator of the PI3K/Akt/mTOR signaling pathway, due to enrichment in PI3K-binding sites in its ICD [8,14]. Tyrosine-phosphorylated HER3 contains six PI3K docking sites and promotes PI3K activity [8,25,29,30,38]. The ability of HER3 to serve as a plasma membrane-adjacent binding partner for PI3K contributes to its role as an active participant in tumorigenesis and tumor progression in a number of cancer types, despite its lack of kinase activity [25,29].

HER3 overexpression has been observed in many cancer types, including colorectal, breast, lung, ovarian, and pancreatic cancers; melanoma; and human papillomavirus-positive (HPV^+) HNSCC, and has been associated with poor prognosis [25,38,84–87]. In addition, point mutations in HER3, many of which are thought to promote its activation, have been identified [15,25,29,30]. In contrast to HER2, in which most of the activating mutations identified occur in the kinase domain, activating mutations in HER3 largely cluster in the extracellular domain and are hypothesized to promote adoption of the untethered (open) conformation, thereby fostering dimerization (although this hypothesis has yet to be rigorously tested) [15,29]. However, mutations in the kinase domain of HER3 have also been identified, although the functional significance of these mutations has not yet been definitively determined [15,29]. HER3 can also be aberrantly activated in cancer due to autocrine and/or paracrine secretion of its ligand neuregulin 1 (NRG1) [7,25,83,87]. In addition, HER3 can cooperate with its dimerization partners to promote activation of oncogenic signaling pathways, particularly the PI3K/Akt/mTOR pathway. This is especially evident in HER2-expressing tumors, in which HER3, as a dimerization partner for HER2, has been shown not only to drive tumorigenesis, but also to play a role in both intrinsic and acquired resistance to chemotherapy [1,2,4,8].

To date, no drugs designed to specifically target HER3 have been approved by the FDA for cancer treatment. However, several HER3 inhibitors are in advanced clinical development. Because HER3 lacks apparent kinase activity, it is thought that using TKIs is not an effective method of HER3 inhibition [38]. However, HER3 is susceptible to inhibition by extracellular neutralizing antibodies [25,29,38]. Several such antibodies have been developed in recent years, and some of these are now being tested in clinical trials. The HER3-targeted antibody CDX-3379 (formerly KTN3379) has demonstrated efficacy in preclinical cancer models and has been tested in clinical trials in HNSCC and thyroid cancer (NCT02473731 and NCT02456701, respectively) [86]. Another anti-HER3 monoclonal antibody, patritumab, is also slated to be tested in upcoming clinical trials, including the I-SPY 2 trial in breast cancer (NCT01042379). It should be noted that although specifically inhibiting HER3 is one method to target HER3-mediated signaling, targeting RTKs with which HER3 dimerizes can also result in inhibition of HER3-mediated signaling [18,19,29,71]. For example, treating gastrointestinal carcinoma

cell lines or patient-derived xenografts with the TKI afatinib or a combination of the HER2 inhibitors lapatinib and trastuzumab can reduce HER3 phosphorylation [71].

Although targeting HER3 in cancer types in which it has been shown to be an oncogenic driver is one potential use of HER3 inhibitors, preclinical data also support a role for HER3 targeting to overcome resistance to chemotherapy. HER3 was shown to mediate resistance to paclitaxel in HER2-overexpressing breast cancer cells by promoting expression of survivin, a protein that prevents apoptosis [1,2]. PI3K/Akt signaling downstream of HER3 has also been shown to promote tumor cell survival in the presence of chemotherapeutic agents [4,8]. A 2003 study assessing the role of HER family members in resistance to chemotherapy identified HER2/HER3-mediated activation of the PI3K/Akt signaling pathway as a mechanism of resistance to a number of chemotherapeutic agents [4]. In this study, exogenous overexpression of HER2 in the HER3-expressing breast cancer cell line MCF7, which expresses a low level of HER2 at baseline, induced PI3K/Akt signaling [4]. HER2 overexpression promoted resistance to the chemotherapeutic agents camptothecin, doxorubicin, etoposide, 5-fluorouracil, and paclitaxel via activation of the PI3K/Akt pathway [4]. In 2012, Bezler et al. [8] reported that treatment of ovarian cancer cells with either doxorubicin or cisplatin induced expression of HER3 ligands, leading to activation of HER3/PI3K/Akt signaling in a HER2-dependent manner. Upregulation of this signaling axis reduced doxorubicin- and cisplatin-induced apoptosis [8]. Collectively, these findings support a role for the HER2/HER3 heterodimer in chemoresistance and suggest that targeting HER3 may delay and/or overcome resistance to chemotherapy.

These and other preclinical studies and the observation of HER3 overexpression in a number of cancer types have established HER3 as an emerging therapeutic target [15,38,82]. As HER3-targeted agents are being tested in clinical trials and becoming more readily available for preclinical research, additional roles for HER3 in chemoresistance may be identified and may lead to the use of HER3-targeted agents in combination with chemotherapeutic agents in the coming years.

1.2.4 HER4

HER4 was the fourth member of the HER family to be discovered (Fig. 1.1); the gene encoding HER4 (*ERBB4*) was cloned in 1993. HER4 has been well studied in the context of development of the brain, heart, and other organs, and as an essential participant in a number of physiological processes, including lactation (which is particularly relevant to studies on the roles of HER4 in breast cancer) [20,26,88–91]. However, the role of HER4 in cancer is not as well established as for the other three HER family members, in part due to conflicting reports on the prognostic implications of HER4 expression [10,15,21,26,89,92–94]. Most studies on HER4 in cancer report a tumor-suppressive role for this RTK, as it can promote differentiation and apoptosis of cancer cells, and several studies have reported reduced HER4 expression in tumors compared to tumor-adjacent normal tissue [21,82,95]. On the other hand, overexpression of and activating mutations in HER4 have also been identified [15,16,21,38,52,92,93,96,97], and HER4 has been shown to induce transformation and proliferation in some preclinical studies [10,21,52,93]. These discrepancies highlight the need for more research on the role of HER4 in cancer and the importance of considering the context in which the roles of HER4 are assessed (including the cancer type, HER4 isoform(s)

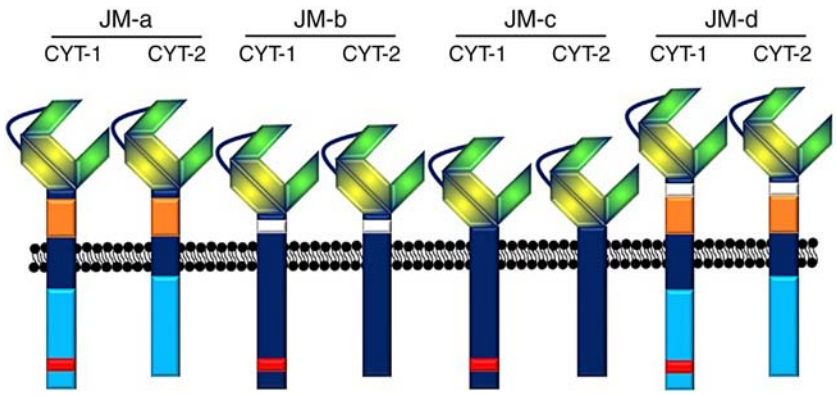

FIG. 1.2 HER4 isoforms. Alternative splicing leads to the generation of HER4 isoforms that vary in the juxtamembrane (JM) and cytosolic (CYT) domains. The four juxtamembrane variants (JM-a, JM-b, JM-c, and JM-d) can pair with either of the two cytosolic variants (CYT-1 and CYT-2). The JM-c isoform lacks both the JM-a and JM-b sequences (orange and white rectangles, respectively), whereas JM-d isoforms contain both these sequences. HER4 receptors containing JM-a (i.e., the JM-a and JM-d isoforms) are susceptible to proteolytic cleavage by TACE and subsequent cleavage of the receptor by γ-secretase. This leads to the release of the HER4 ICD (light blue rectangles). CYT-1 and CYT-2 differ in that CYT-1 is generated by inclusion of an additional exon that encodes a PI3K-binding site (red rectangle).

expressed, and coexpression of other proteins) in order to understand the functions of HER4 and the potential impacts of targeting this RTK in cancer.

HER4, like EGFR, can both bind ligands and phosphorylate substrates. Upon binding of one of its seven ligands, HER4 can form functional homodimers or heterodimerize with other RTKs, especially HER2 [22,26,82,97,98]. HER4 is activated by BTC, ERG, HB-EGF, and NRG1-4 (Fig. 1.1) [6,20,25,29]. In addition to the traditional ligand binding/dimerization mechanism of activation, HER4 is further regulated by alternative splicing and proteolytic cleavage [6,10,21,26,82,89,92,98–100]. Alternative splicing leads to the expression of several HER4 isoforms that vary in the juxtamembrane and cytosolic domains (Fig. 1.2) [6,10,21,26,82,89,92,99,101]. It is important to consider the isoform(s) expressed when assessing the functions of HER4, as these isoforms exhibit functional differences [6,21,26,93].

The four different splice variants of the (extracellular) juxtamembrane domain are referred to as JM-a, JM-b, JM-c, and JM-d [6,26,101]. (Often, only the JM-a and JM-b variants are mentioned.) JM-c-containing isoforms lack the juxtamembrane domain [6,101]. JM-a- and JM-d-containing isoforms possess longer juxtamembrane domains than JM-b-containing isoforms and are susceptible to proteolytic cleavage by tumor necrosis factor α-converting enzyme (TACE, also called ADAM17) upon the binding of certain ligands [10,20,21,26,27,89–91,93,94,99–101]. TACE-mediated cleavage of JM-a- and JM-d-containing isoforms leads to shedding of the HER4 ectodomain and subsequent cleavage of the remaining membrane-associated receptor by γ-secretase to generate a soluble form of the HER4 ICD [10,20,21,26,27,82,89–94,97,100]. The ICD contains a constitutively active kinase domain and a nuclear localization sequence and has been shown to perform a number of functions (Fig. 1.3) [26,27,91,94]. The ICD can localize to mitochondria and act as a BH3-only (pro-apoptotic) protein [26,90,94,102]. The

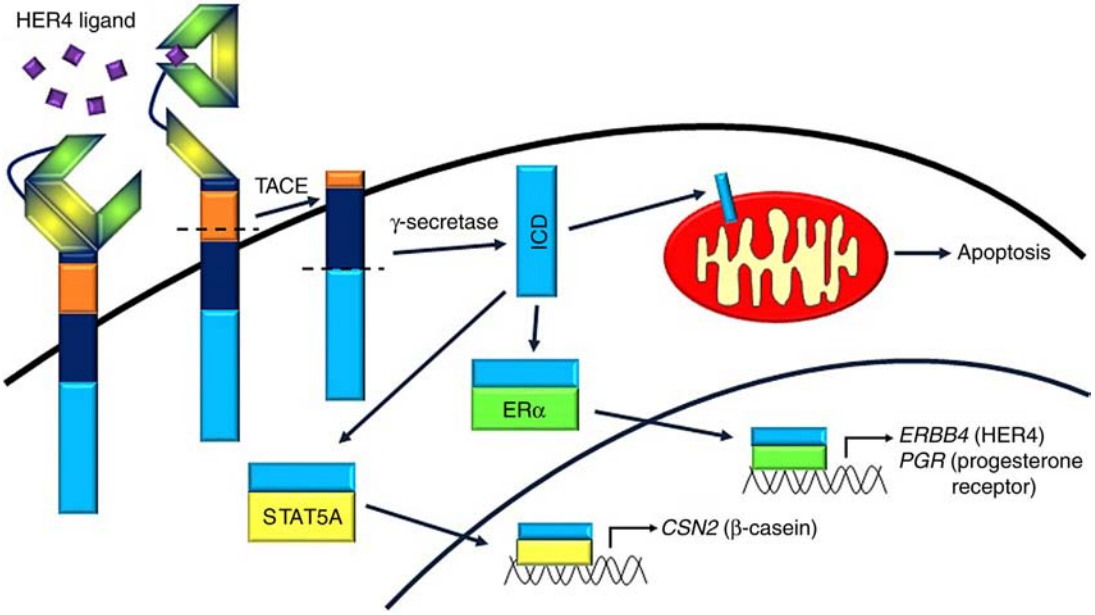

FIG. 1.3 Generation and functions of the HER4 ICD. Ligand binding to HER4 JM-a-containing isoforms induces TACE-mediated cleavage of the HER4 ectodomain. The remaining HER4 molecule can then be cleaved by γ-secretase to release the ICD, an 80-kDa molecule that performs a number of functions. The ICD contains a nuclear localization sequence (NLS) and can facilitate nuclear translocation of signal transducer and activator of transcription 5A (STAT5A). The ICD/STAT5A complex binds to the promoter of the gene *CSN2*, which encodes β-casein, stimulating its transcription. The ICD also serves as a co-activator of estrogen receptor α (ERα)-induced transcription, leading to the expression of genes such as *PGR* (progesterone receptor) and *ERBB4* (HER4). In addition, the ICD can migrate to mitochondria and promote activation of the pro-apoptotic protein BAK, a member of the Bcl-2 protein family (not shown). This results in cytochrome c release and induction of apoptosis.

ICD can also bind to the transcription factor signal transducer and activator of transcription 5A (STAT5A) and can promote STAT5A-induced gene expression [20,26,90,91,93]. In addition, the ICD has been shown to serve as a coactivator of estrogen receptor alpha (ERα)-mediated transcription, promoting expression of genes such as *PGR* (which encodes the progesterone receptor) and *ERBB4* (HER4). Overexpression of the JM-a CYT-2 isoform of HER4 (which can be cleaved to generate the ICD) was shown to promote proliferation of the ER-positive breast cancer cell line MCF7 [10,90,93,94,102]. Thus, the HER4 ICD has been shown to play both tumor-suppressive and pro-tumorigenic roles.

Alternative splicing affecting the cytosolic region of HER4 supplies an additional mechanism of regulation. CYT-1 and CYT-2 are variants in the cytosolic domain of HER4 [10,82,92–94]. Although similar, CYT-1 is generated by inclusion of an additional exon that encodes a binding site for PI3K [6,10,26,82,89,92–94]. In preclinical models of breast cancer, shifting expression in favor of CYT-2-containing isoforms reduced proliferation of breast cancer cells [92]. However, the CYT-1 isoform itself has been shown to inhibit proliferation and promote differentiation in mammary epithelial cells [92–94]. Thus, a blanket

statement regarding the implications of CYT-1 versus CYT-2 expression would not be supported by the literature at this point.

HER4 alterations observed in cancer include those that promote expression and/or activation of HER4 and those that lead to reduced HER4 levels in tumors. Activating mutations in HER4 have been reported in melanoma, breast cancer, NSCLC, and HNSCC [16,22,38,52,92,93,96,97]. Some of these activating mutations occur in the kinase domain and promote activation of downstream signaling molecules and/or HER4 cleavage. On the other hand, expression of both HER4 and one of its ligands, neuregulin 4 (NRG4), was found to be decreased in gastric cancer tumors compared to adjacent normal tissue [82]. Moreover, hypermethylation of the *ERBB4* promoter, resulting in reduced expression of HER4, has been observed in breast cancer [95]. Collectively, these studies suggest that while HER4 acts as an oncogene in certain settings, it plays a tumor-suppressive role in other contexts.

In some cancers, such as colorectal cancer and gastrointestinal stromal tumors originating from the stomach, expression of HER4 has been shown to be a negative prognostic marker, whereas in bladder cancer, HER4 expression has been associated with a favorable outcome [103,104]. Even within a particular cancer type, the role of HER4 is somewhat controversial, as expression of HER4 has been reported to be both a negative and a positive prognostic marker in breast cancer [26,105]. For example, expression of HER4 on the cell surface was associated with increased survival in patients with ER-positive breast cancer, but not in those with ER-negative breast tumors [10,90]. In the same study [10], it was reported that nuclear localization of the HER4 ICD, as opposed to HER4 expression on the cell membrane, was associated with decreased survival in both ER-positive and -negative breast cancer [10].

Several factors may explain the apparent discrepancies in studies investigating HER4 function. The unique functions of the different HER4 isoforms likely contribute to the confusion; however, because many studies do not assess which isoform is expressed, it is not possible to definitively determine whether differential isoform expression can explain the discrepancies [10,21,26,89,92–94]. The subcellular localization of HER4 also has important implications for its function and its ability to serve as a predictive prognostic marker [10,89,92,94]. Moreover, it would be prudent to take into account the expression of proteins that are known to interact with HER4, either directly or as components of a common signaling pathway, when assessing the function of HER4 [92]. This is exemplified in the differences in the prognostic implications of HER4 expression in ER-positive and -negative breast cancer, but could also apply to coexpression with other molecules [10]. For example, heterodimerization with other HER family members may influence HER4 function and the effect of HER4 expression on prognosis. In addition, Chuu et al. [26] have suggested that the medical history of breast cancer patients (and of the patients from whom preclinical models were derived) should be considered when studying HER4, as the functions of HER4 can be affected by such factors as pregnancy, lactation, and hormone treatments.

Perhaps because expression of HER4 has been shown to correlate with opposing outcomes (e.g., increased vs. decreased overall survival), the development of HER4-targeting agents lags behind that of other three HER family members [93]. However, targeted agents that can inhibit HER4 have been used in the clinic. Although the primary targets of the TKIs lapatinib, afatinib, and neratinib are EGFR and HER2, HER4 is also inhibited by these agents [9,18,52,72,96]. Naturally, it is not possible to determine if or to what degree HER4 inhibition contributes to the efficacy of these drugs in patients, as the relative influence of EGFR,

HER2, and HER4 inhibition cannot be dissected experimentally in this context. Thus, a role for HER4 inhibition in the efficacy of lapatinib, afatinib, and neratinib cannot be definitively determined.

Some preclinical data suggest that inhibition of HER4 may enhance response to chemotherapy. In 2013, Hegde et al. [7] reported that tumor cells that persisted after chemotherapy in a $Kras^{G12D}$-expressing genetically engineered mouse model of NSCLC and in human NSCLC cell line xenografts expressed increased levels of HER3, HER4, and neuregulin 1 (NRG1, a ligand for HER3 and HER4) compared to treatment-naïve tumors. The authors also found that inhibition of HER4 could delay or prevent tumor relapse following chemotherapy [7]. Notably, the tumors that grew in the genetically engineered mouse model used in this study were engineered to express $Kras^{G12D}$, not HER4 or another HER family member, suggesting that inhibition of HER4 could be a strategy to delay and/or overcome chemoresistance even in tumors in which HER4 is not thought to be the primary oncogenic driver. This warrants future investigation.

Continued research on the functions of HER4 in different contexts and the patterns of expression of HER4 isoforms in tumors will likely improve the ability of physicians and researchers to use HER4 expression as a prognostic indicator and could help to identify cancer types in which a HER4-targeted agent (either one that specifically targets HER4 or one that can inhibit multiple HER family members, such as afatinib and lapatinib) may demonstrate efficacy [52,96]. On the other hand, the identification of antitumorigenic roles for HER4 in several cancer types also raises the question of whether HER4 inhibition by HER family-targeted TKIs currently in use in the clinic might be hampering the efficacy of these drugs. This should be considered when using TKIs that can target HER4, as the use of such an inhibitor for the treatment of a tumor in which HER4 is playing a tumor suppressive role might not be advisable. Again, however, more research is needed on this topic.

1.3 CONCLUSIONS

EGFR, HER2, HER3, and HER4 have, to varying degrees, been implicated in tumorigenesis and tumor maintenance. Monoclonal antibodies and TKIs targeting EGFR, HER2, and, more recently, HER3, have been developed and used in the treatment of cancer types in which these RTKs have been shown to play an oncogenic role. Encouragingly, the use of HER family inhibitors has resulted in improved outcomes for patients, and HER family-targeted agents continue to be developed and tested in clinical settings, expanding the number of indications for which HER family-targeted agents are approved by the FDA. In addition to their roles in tumor cell proliferation and survival, HER family receptors have been shown to mediate resistance to chemotherapy, making inhibition of these RTKs a rational cotargeting strategy in the cancer types in which HER family members are expressed. However, even combining HER family inhibitors with chemotherapy does not necessarily lead to durable or even short-term response to treatment, highlighting the need for more basic and translational research on HER family receptors and HER family-targeted agents, both alone and in combination with chemotherapy, to identify more effective treatment strategies.

Given the many roles of HER family members and their downstream effectors in cancer, there are many potential future avenues of research that may further this goal. Of these, it will

be particularly interesting and timely to assess the effects of HER family inhibitors on antitumor immune responses and to identify drug combinations that enhance immune-mediated tumor clearance. Although not discussed extensively in this chapter, resistance to HER family-targeted agents remains a major obstacle in cancer treatment, and devising methods to overcome resistance will improve patient outcomes. In addition, continued research on the functions of HER4 could help to identify cancer types in which HER4 targeting may be effective and avoid the potential consequences of utilizing agents that can target HER4 for the treatment of tumors in which HER4 is acting as a tumor suppressor. Finally, as advances in DNA sequencing and other methods to characterize patients' tumors are making these analyses more economically feasible and more common, there may be opportunities to uncover additional cancer types in which HER family members are playing an oncogenic role. Using this information to identify and treat patients who might otherwise not be considered candidates for treatment with HER family-targeting agents could improve outcomes, thus realizing the goal of precision medicine.

Acknowledgements

This work was supported by the National Institutes of Health grants R01 DE024728 (D.E.J.), P50CA097190 (D.E.J. and J.R.G.), and R01 DE023685 (J.R.G.).

Conflict of Interest: No potential conflicts of interest were disclosed.

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