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Comprehensive Profiling of EGFR/HER Receptors for Personalized Treatment of Gynecologic Cancers

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Abstract

The primary gynecologic cancers include cancers of the endometrium, ovary, and cervix. Worldwide, cervical cancer is the most common gynecologic cancer, whereas endometrial cancer is the most common in the US. Ovarian cancer is the fifth most deadly cancer in women, with 5-year survival rates for advanced disease at only 27%. As such, there is an urgent need for reliable screening tools and novel targeted therapeutic regimens for these malignancies. The EGFR/HER family of receptors has been associated with the development and progression of many solid tumors. Despite clear roles for these receptors in other cancers, the expression of HER family members in gynecologic cancers and their relationship with disease stage, grade, and response to treatment remain controversial. In this review, we describe the existing evidence for the use of HER family members as diagnostic and prognostic indicators as well as their potential as therapeutic targets in gynecologic cancers.

1. Introduction

The human epidermal growth factor receptor (HER) family members are being explored in the clinic as diagnostic tools as well as therapeutic targets for a variety of solid tumors. Originally named for their homology to the erythroblastoma viral gene product, *v-erbB*, the HER family members have been studied for more than 40 years. In the mid-1990s, HER family members EGFR/HER1/ErbB1, HER2/ErbB2/neu, HER3/ErbB3, and HER4/ErbB4 and their ligands were established to play crucial roles in epithelial cell development since HER receptor knockout mice die at early stages of development. HER family members have also been shown to contribute to cancer development and progression, and multiple anticancer therapeutics targeting these receptors have been identified.

A comprehensive search of all original research and reviews published in MEDLINE and ClinicalTrials.gov through October 2013 was undertaken using the following key terms epidermal growth factor receptor; EGFR; HER2; HER3; HER4; endometrial cancer; ovarian cancer; and cervical cancer. Over 200 papers were evaluated, of which 119 are cited in this article. Reports were chosen for inclusion if data were collected from clinical specimens examined for an association with clinical parameters of disease severity or response to therapy. Select studies performed in cell models of gynecologic cancers are highlighted as potential future strategies that should be explored clinically.

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Conflict of Interest

The authors declare no competing interests.

2. HER biology and role in cancer

The four HER receptors recognize thirteen different but structurally related growth factors and contribute to development, homeostasis, and many pathologies. Each of the receptors is a type I transmembrane protein consisting of a heavily glycosylated ectodomain that contains a ligand binding site, a single transmembrane domain, an intracellular protein-tyrosine kinase catalytic domain (TKD) and a tyrosine-containing cytoplasmic tail (Figure 1).

In general, growth factor binding to regions I and III in the ectodomain induces a conformational change to convert the receptor ectodomain from a tethered, inactive conformation to an extended, active conformation. It should be noted that HER2 is considered an orphan receptor, with the ectodomain adopting an “extended” conformation in the absence of ligand. Upon ligand binding, the HER receptors form homo- or heterodimers. HER3 is widely considered a catalytically inactive receptor based on the conformation of its TKD, which only shares ~60% homology with the TKDs of other HER family members and has important amino acid substitutions in the catalytic core of the TKD [1]. HER2 and HER3 still contribute to signaling by heterodimerizing with other HER family members. Dimerization results in trans-phosphorylated on tyrosine residues in their cytoplasmic tails. Specific phosphotyrosine sites in the carboxyterminal tail serve as docking sites for the recruitment of cytoplasmic adaptor proteins and enzymes, initiating signaling cascades that control multiple cellular processes. Post receptor signaling by activated HERs includes four representative pathways: the Ras-Raf/MAPK kinase and STAT pathways that mainly regulate cell proliferation and differentiation, the PI3K/Akt cascade that is important for cell survival, and the phospholipase C γ (PLC γ) pathway that controls calcium-dependent events.

Mutations, gene amplification and protein overexpression of HER family members are all linked to carcinogenesis. Overexpression of EGFR and HER2 is well-documented in a variety of tumor types, with mutations in EGFR and, to a lesser extent HER2, detected in a subset of tumors. For example, in glioblastoma multiforme an EGFR splice variant, ErbB-1vIII, results in deletion of exon 3 and generation of a constitutively active receptor. Mutations within the tyrosine kinase domain (exons 18-21) of EGFR have also been documented in several tumor types, including endometrial cancer [2]. Many of these mutations, which render the receptor partially constitutively active, also augment sensitivity to TKIs [3-5]. Mutations in the HER2 ectodomain and TKD have also been documented in lung and breast cancers [6-8].

HER3 has received less attention as a cancer therapeutic target, principally because of early studies that established it as a catalytically inactive receptor. However, a seminal study in 2007 by Sergina et al., implicated HER3 in the escape of breast cancer cells from the small molecule tyrosine kinase inhibitor (TKI) gefitinib, which targets EGFR [9]. The authors proposed that HER3 phosphorylation is upregulated by “Akt-driven negative feedback signaling loops” and that this ultimately leads to resistance to TKI [9]. Six months later, HER3 was shown to be trans-activated by a distantly related receptor tyrosine kinase, MET, that is amplified in ~20% of gefitinib-resistant lung cancer cell lines [10]. In addition, a wealth of information on HER3 expression in primary tumors and cultured cancer cells has revealed that increased HER3 mRNA or protein levels correlate with poor prognosis in multiple human cancers, including cancers of the breast, ovary, prostate, gastrointestinal tract, head and neck, and lung [11].

The role of HER4 in cancer is also less understood as compared to EGFR and HER2, due in large part to the complicated nature of HER4 biology. Alternative splicing of the HER4

cytoplasmic domain results in expression of a PI3K binding site that mediates escape from apoptosis, though both isoforms can be expressed in cancer cells simultaneously [12]. Furthermore, HER4 can be proteolytically processed in response to ligand binding, similar to Notch and amyloid precursor protein [13]. Proteolysis results in generation of an intracellular domain fragment that has functions distinct from that of the intact receptor. Nuclear HER4 has been detected in several cancer types, including medulloblastoma and breast cancer, and shed HER4 ectodomain has been detected in serum from breast cancer patients [14]. In preclinical models, inhibition of HER4 proteolysis with a blocking antibody represses xenograft tumor growth in mice [14]. However, the importance of HER4 intracellular/ectodomain fragments as prognostic markers and drivers of disease remain poorly defined.

3. Diagnostic and prognostic use of HER family proteins in gynecologic cancers

The baseline expression of HER family of oncoproteins is increased in a variety of solid tumors compared to normal tissues. For example, EGFR is increased in bladder, head and neck, esophageal, ovarian and cervical carcinomas and has been associated with poor prognosis [15]. HER2 on the other hand is overexpressed in 10-30% of stomach, breast, endometrial, and ovarian cancers, and vulvar Paget's disease [16-18]. Of the four members of the HER family, HER3 and HER4 are less studied. Expression of HER3 has been reported in a range of adenocarcinomas that includes the breast, stomach, colon, prostate, kidney, lung, and uterus [19]. HER4 overexpression has been observed in medulloblastoma, thyroid, breast, and gastric malignancies, though it is reduced in prostate and pancreatic cancers [20, 21].

While analysis of EGFR expression in lung cancer and HER2 in breast cancer is relatively common practice, these receptors are not routinely assessed in gynecologic cancers. Typical methods to assess expression of HER receptors are immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH), an indicator of chromosomal amplification [22]. The expression analyses take into account the degree of overexpression using a scoring system, though there is not a standardized method [22]. Variability in receptor expression between tumor specimens can arise due to technical limitations rather than actual differences in expression. For example, the method of fixation, age of the tissue specimen, and differences in antibody sensitivity or specificity can all impact staining intensity with IHC, leading to either false positives or false negatives [23]. Internal controls are rarely incorporated into the IHC protocols, thereby limiting interpretation of comparative analyses across different studies [23]. In addition, expression levels of EGFR have been shown to vary among regions of the tumor, with higher EGFR on the tumor periphery as compared to the center of the tumor [24]. Despite these technical limitations, a comparison of HER2 analysis by IHC and FISH at several centers demonstrated a high degree of reproducibility, with greater consistency with FISH than IHC [25]. These findings underscore the variability attributed fixation methods. For studies that are cited herein, there was no standard method for determining expression. In most papers, receptor levels as determined by IHC were compared across samples. For some studies, FISH was used as an additional confirmatory step for receptor overexpression.

Analysis of receptor expression does not provide useful information regarding the function of the receptor in the tumor. Assessment of receptor activation using phospho site-specific antibodies is rarely performed except in translational research in clinical trials. Since mutations in EGFR or HER2 tyrosine kinase domain predict for a constitutively active receptor and enhanced sensitivity to targeted small molecule inhibitors, more recently

EGFR/HER2 mutational status has been examined as a predictive marker for response to EGFR/HER2 targeted therapies.

3.1 EGFR

3.1.1 Ovarian cancer—The epithelial lining of the ovary normally has weak expression of EGFR [26], while in epithelial ovarian carcinoma, overexpression is reported in 30-98% of cases [27]. In line with the wide range of cases with overexpression, EGFR expression has not been consistently shown to correlate with disease aggressiveness (Table 1). For example, in a study of 64 early vs. late stage epithelial ovarian cancer specimens, there was no difference in EGFR expression [28]. In spite of this, its expression has been reported to negatively correlate with disease free survival [29]. Similarly, EGFR overexpression is associated with poor prognosis and decreased therapeutic responsiveness in ovarian cancer patients [30, 31], which has driven the application of EGFR inhibitors as a targeted treatment.

3.1.2 Endometrial cancer—In endometrial cancer, the expression of EGFR has been noted in 43–67% of cases and is associated with a notable decrease in disease-free and overall survival [32], particularly for the more aggressive type II endometrial tumors [33]. Similarly, elevated serum EGFR (sEGFR) levels have been linked to better overall survival [34]. As with ovarian cancer, the correlation of EGFR expression with any specific clinical phenotype has been controversial, with some studies saying that there is no association [35] while others reporting otherwise. For example, a tissue microarray study of 279 endometrial tumors documented higher expression in the well-differentiated type I tumors (46% of tumors) compared to aggressive type II endometrial cancers (34% of tumors) [33].

Mutations in EGFR have gained considerable attention in recent years, in particular how these mutations affect response to therapy. A mutation in EGFR in exon 18, E690K, was identified in one patient enrolled on a Gynecologic Oncology Group (GOG) protocol 229D [2]. This patient, who had recurrent disease, demonstrated a progression-free survival of more than six months following treatment with the dual EGFR/HER2 inhibitor lapatinib. This is in line with data in lung cancer patients that mutations in the EGFR TKD increase sensitivity to EGFR small molecule inhibitors [3-5].

3.1.3 Cervical cancer—The normal surface epithelium of the cervix rarely displays EGFR expression, while in cervical cancer the range has been reported to be anywhere from 6% to 90% of cases [36]. The expression of EGFR appears to have a direct association with HPV infection as evidenced by its increasing expression as the grade of intraepithelial neoplasia increases [37]. In the setting of cervical dysplasia, EGFR expression is higher in the basal keratinocyte layer, with decreasing expression towards the more differentiated surface layer. By contrast, EGFR is expressed throughout the entire epithelial layer in the undifferentiated squamous cells in squamous metaplasia, raised condyloma, and cervical carcinoma *in situ* [36]. Staining intensity of EGFR has been noted to be stronger and more prevalent in squamous versus adenosquamous cervical carcinoma [37]. In a systematic review of cervical cancer patients that evaluated 82 biomarkers from 42 different studies, EGFR expression was associated with poor response to chemoradiation and indicated a poor prognosis [38]. Other studies have not found such an association of EGFR with poor prognosis [37].

3.2 HER2

3.2.1 Ovarian cancer—HER2 is mainly expressed in the surface epithelium of the ovary [26], with its amplification being rare in benign ovarian tumors, borderline neoplasms, and early stage malignancies [39]. HER2 is overexpressed in 25-30% of ovarian cancers [40].

One study found that HER2 overexpression is more frequent in familial ovarian carcinomas than sporadic cases [41], while another showed that there is an absence of high HER2 expression in familial cases [42]. In addition, ovarian cancer patients have detectable HER2 species in the serum, though serum HER2 levels do not distinguish malignant from benign ovarian tumors [43, 44].

Studies that evaluated the correlation between HER2 overexpression and aggressiveness and stage of disease yielded contradicting results. While some studies have shown no difference in protein expression of HER2 between early and advanced stage disease [28, 45], others have noted HER2 amplification in invasive epithelial ovarian cancers compared to borderline cancers and normal ovaries [46]. The vast majority of these studies have suggested that there is higher amplification and stronger staining for HER2 with advancing stage of ovarian [26, 47-49] and serous fallopian tube cancers [46, 50]. Although an argument can be made that HER2 amplification might be more pronounced in advanced stage disease, it indirectly implies that its use as a screening tool may not be as helpful for monitoring tumor activity and treatment response since the amplification of HER2 is more common in the later stages.

In terms of distinguishing between different epithelial subtypes of ovarian cancer (clear cell, serous, mucinous, and endometrioid), the studies have also been conflicting. A study of 107 patients with early stage ovarian cancer showed that HER2 expression is associated with serous and mucinous subtypes [31]. In addition, serous tumors stain for both HER2 and EGFR, while endometrioid and clear cell tumors are negative for these two proteins. Other studies, however, have not shown any significant correlation between the degree of amplification/overexpression of HER2 and cell type or grade [45, 46, 51]. HER2 expression has also been suggested as a potential tool for differentiating between histologically similar carcinomas with differing anatomic source and behavior. An example is between ovarian serous papillary carcinoma (OSPC) and uterine serous papillary carcinoma (USPC), where tumors of uterine origin display a more aggressive phenotype. Data from microarray analysis of OSPC vs. USPC identified HER2 as the most altered gene out of 116 surveyed genes [52]. HER2 levels also distinguish OSPC from the more aggressive primary peritoneal serous papillary carcinoma (PPSPC), which has higher HER2 expression than OSPC [53].

The use of HER2 in determining prognosis and treatment response in patients with ovarian cancer has also yielded mixed results. Two studies have shown statistically significant correlations between increased HER2 expression, worse prognosis and decreased survival [48, 54], particularly for patients in stage III and IV of the disease [55]. In a study of 73 ovarian cancer cases, 32% had high HER2 expression with significantly shorter survival (median, 15.7 months) compared to those with low HER2 expression (median, 32.8 months). These patients with high HER2 expression also had a lower rate of complete response to initial treatment and a higher rate of recurrence [56, 57]. Other studies have not found a correlation between HER2 expression and progression-free or overall survival or response to chemotherapy [49, 58-60]. HER2-negative familial cancers have a significantly improved 5-year survival rate (67%) as compared to sporadic cases (17%) [42]. Interestingly, a majority of these familial cases also had increased levels of the tumor suppressor p53, which may contribute to the improved prognosis.

3.2.2 Endometrial cancer—The majority of the literature has suggested that HER2 has potential use as a biomarker in endometrial cancer to predict disease stage, tumor aggressiveness, prognosis, and response to treatment. Immunohistochemical staining demonstrates mild baseline HER2 expression in the normal endometrium regardless of the phase of the menstrual cycle [61, 62]. Among endometrial cancer specimens, 13-45.4% will

exhibit HER2 overexpression [35, 63, 64], with elevated HER2 in 9-14% of endometrial adenocarcinomas [61, 62].

The intensity of HER2 immunohistochemical staining directly correlates with disease stage, grade, and non-endometrioid tumor subtype [35, 65]. For example, 14.3% of well-differentiated and moderately differentiated endometrioid carcinomas have HER2 expression, while 45.4% of the more aggressive USPC subtypes express high HER2 [64]. For the subpopulation of patients with USPC, the increased expression of HER2 denotes a more advanced surgical stage and a lower overall and disease-free survival [66-68]. However, other studies failed to detect a correlation between HER2 expression and clinical prognosis [69], and HER2 expression does not predict for recurrence [70].

Interestingly, several studies have reported correlations between HER2 and other biomarkers. In a study of 34 endometrial cancer patients, 24% demonstrated positive staining for both EGFR and HER2, which was associated with a poorly differentiated cancer phenotype [71]. Tumors with high HER2 expression have an inverse relationship with estrogen receptor (ER) and progesterone receptor (PR) [61, 62]. The absence of HER2 in association with positive ER expression portends a longer disease-free and overall survival [72]. If HER2 overexpression is a marker of aggressive disease, these studies support the idea that the subgroup of endometrial cancers that lack HER2 may be hormone sensitive and potentially responsive to hormonal treatment.

Another interesting relationship that has been observed is between HER2 immunostaining and p53. In a study of 114 endometrial cancer specimens, tumors with dual staining for nuclear p53 and membrane HER2 had a higher chance of recurrence [63]. High p53 immunostaining in specimens that are chemo-naïve denotes a mutant p53 rather than wild-type p53 [73]. Though HER2 is not as strong of a prognostic marker as p53, HER2 overexpression appears to amplify the effects of p53 [63].

3.2.3 Cervical cancer—Like EGFR, HER2 is also rarely expressed in the normal surface epithelia of the lower genital tract, with higher HER2 expression in the basal layer as compared to the surface layer [36]. The degree and frequency of HER2 overexpression in cervical lesions has been directly correlated with the grade of dysplasia or stage of cancer [74, 75]. However, a study involving tissues collected from 150 cervical carcinoma patients did not detect a correlation between HER2 overexpression and tumor grade, survival, or recurrence despite expression of HER2 in 22% of tumors [76]. Nevertheless, the utility of HER2 as a biomarker in the setting of disease monitoring may be worthy of further investigation. This idea is based on findings that patients who respond to chemotherapy have an increase in serum HER2 levels, while those that did not had the same or decreased levels [77]. The interpretation of these data is that, as the tumor cells die, HER2 molecules are released and can be detected in the serum. Thus, serum levels of HER2 may serve as a surrogate marker for treatment response.

3.3 HER3

3.3.1 Ovarian cancer—Overexpression of HER3 has been reported in epithelial ovarian cancers [30]. Its expression appears to correlate with poorer overall prognosis [78]. In vitro studies demonstrate that HER3 depletion with an anti-HER3 monoclonal antibody promotes anti-proliferative effects in a subset of ovarian cancer cells and prolonged survival in a mouse model [79]. Moreover, in preclinical models Akt inhibition results in increased expression and phosphorylation of HER3 [80], which could explain development of resistance to PI3K/Akt pathway inhibitors. In addition, HER3 may also have a role in resistance to targeted HER2 and EGFR treatments as has been shown by Sergina et al, who made the landmark discovery that prolonged treatment of breast cancer cells with the EGFR

inhibitor gefitinib results in increased activation of HER3 [9]. Thus, the observed treatment resistance in those who overexpress HER3 may explain its association with poorer prognosis.

3.3.2 Endometrial cancer—HER3 expression is weakly positive in both phases of the menstrual cycle [81]. In contrast, HER3 is overexpressed in 30% of endometrial adenocarcinomas, with a notably higher protein expression in well differentiated tumors compared to poorly differentiated subgroups [81]. HER3 overexpression also correlates with response to lapatinib, the dual EGFR/HER2 inhibitor [32]. These data support the known role of HER3 as a heterodimerization partner for HER2 and EGFR.

3.3.3. Cervical cancer—There is a paucity of studies relating to HER3 and cervical cancer. In a study of 78 patients with squamous cell carcinomas of the cervix, overexpression of HER3 was detected in 74.4% of cases as determined by immunohistochemistry and was correlated with poor prognosis [82]. HER3 overexpression has also been linked with a squamous cell histologic subtype [83]. A meta-analysis of several solid tumors, including the above study for cervical cancer, showed that HER3 overexpression correlated with a worse three- and five-year overall survival [84].

3.4 HER4

3.4.1 Ovarian cancer—In a study of 53 ovarian cancer specimens, immunohistochemistry showed expression of HER4 in 89-93% of tumors, and HER4 expression is higher in serous as compared to endometrioid tumors [21]. Interestingly, HER4 is not an independent indicator of survival, but co-expression of HER4 with HER2 is inversely related to long-term survival among stage III serous tumors [21]. Studies in cell lines support the clinical data by demonstrating that high co-expression of HER4 and HER2 predicts for improved survival. Those preclinical studies also suggested that HER3 signaling through HER4 might inhibit rather than promote growth. Finally, overexpression of HER4 in ovarian cancer cells denotes cisplatin resistance [21], though no corresponding clinical data have been reported.

3.4.2 Endometrial cancer—Endometrial glands and stroma normally have weak to moderate expression of HER4. HER4 and its ligands have also been noted to have higher expression during the secretory phase versus the proliferative phase of the menstrual cycle, which suggests a possible role in maturation and anti-proliferative effects in the endometrium [81]. HER4 is expressed at a modest level in endometrial cancer, with a reported overexpression in about 15% of cases [81].

3.4.3. Cervical cancer—HER4 has been shown to be overexpressed in 79.5% of squamous cell cervical tumors [82]. HER4 overexpression has been shown to correlate with elevated levels of EGFR, HER2, and HER3 [82, 83]. Interestingly, tumors with elevated HER2 and HER4 were diagnosed at a lower stage [83], and there was no association between HER4 expression and overall survival [82].

4. Use of HER-targeted inhibitors in gynecologic cancers

Currently the standard treatment for advanced ovarian or endometrial cancers includes platinum and taxane combination chemotherapy after cytoreductive surgery. The initial response rate in these patients is high, ranging from 70-80%. Unfortunately, most of these patients will recur, leaving an overall 5-year survival of 20-30%. Several targeted therapies have been examined, and the EGFR family of receptors has been no exception. These

receptors have been targeted by two types of inhibitors: small molecule TKIs and monoclonal antibodies (mAb).

The TKIs target the active kinase domain and typically compete with ATP binding (Figure 2). The monoclonal antibodies target the extracellular region of the receptors to 1) block ligand binding and induce receptor internalization without activating the receptor; or 2) interfere with receptor dimerization by binding to the dimerization loop or other regions within the ectodomain (Figure 2). To date, several of these reagents have been FDA-approved for the treatment of various types of cancers (Table 2). No EGFR/HER2 inhibitors have been FDA-approved for the treatment of gynecologic cancers, though several have been explored clinically and are described in this section (Table 3).

4.1 Small molecule tyrosine kinase inhibitors as single agents

The most common small molecule inhibitors of HER family proteins that have been evaluated clinically include EGFR inhibitors gefitinib and erlotinib, dual EGFR/HER2 inhibitor lapatinib, pan-ErbB inhibitor canertinib, EGFR/VEGFR/RET inhibitor vandetanib and partial EGFR antagonist leflunomide. Gefitinib was not found to be active in endometrial and ovarian cancers [34, 85], though one endometrial cancer patient on GOG protocol 229C demonstrated a complete response [34]. Interestingly, this patient did not have an identifiable EGFR mutation that could explain sensitivity to gefitinib. Lapatinib also had limited efficacy in unselected endometrial cancer patients [2, 86], but it achieved a complete response in a patient with a previously unreported EGFR mutation at E690K in exon 18, which is in the tyrosine kinase domain [2]. While the response of the patient with an EGFR mutation explains the response to lapatinib, the response to gefitinib sets the stage for understanding more clearly the molecular mechanisms of response [34].

Though clinical trials of TKIs in gynecologic cancers to date have not pre-selected patients based in EGFR/HER2 expression, preclinical studies have examined how EGFR expression relates to response to targeted EGFR agents. For example, studies in an endometrial cancer cell line revealed that targeted EGFR inhibition produces better cell growth inhibition in cells with high expression of EGFR and its ligand TGF- α [87].

4.2 Monoclonal antibodies as single agents

The monoclonal antibodies include EGFR inhibitors cetuximab, matuzumab, and panitumumab and HER2 inhibitors trastuzumab and pertuzumab. As compared to the TKI trials, many of the anti-EGFR/HER2 antibody trials pre-selected for patients with expression of the target receptor. While an early trial of trastuzumab in seven preselected advanced ovarian cancer patients with HER2-positive tumors suggested activity (two complete responses, three partial responses) [88], a subsequent trial in 41 patients only demonstrated a complete response for one patient [89]. Other trials in which the patients were pre-selected for receptor positivity (i.e., matuzumab in EGFR-positive ovarian cancers [92]; trastuzumab in HER2-positive endometrial cancers [90]) yielded similarly disappointing results.

An obvious question is why, despite positive staining for target expression, these agents failed to demonstrate clinical activity for the majority of patients. One possibility is that inhibition of the receptor is cytostatic rather than cytotoxic. Indeed, recent studies in breast cancer suggest that HER2 is stabilized by chaperone proteins, which would prevent receptor degradation [91]. Alternatively, the pre-selected tumors may have varying levels of EGFR/HER2 receptor expression, which would be reflected by differential sensitivity to targeted agents. Another explanation could be due to a constitutively active receptor that does not contain binding sites for the antibody, such as truncations of HER2 [91]. Next, other mutations or overexpression of other non-targeted HER family receptors may drive the

tumor phenotype. For the patients that did respond, those tumors may be dependent upon EGFR/HER2 signaling for growth, as has been demonstrated in breast cancer [91, 92]. Clearly an in-depth analysis of the EGFR/HER2 downstream signaling pathways in pre-treatment tumor specimens is necessary to understand why these targeted agents failed.

4.3. HER inhibitors in combination with chemotherapy

In addition to single-agent studies, use of EGFR family inhibitors has been explored in combination with chemotherapy. Erlotinib has been the target of some optimism in the treatment of ovarian cancer based on a phase II study of daily erlotinib in combination with paclitaxel and carboplatin [93]. However, this trial was not randomized, and the results should thus be interpreted with caution since most ovarian cancers are initially responsive to chemotherapy. This is reinforced by trials combining carboplatin with either erlotinib or cetuximab. In these studies, the response to dual therapy was similar to reported findings for single agent carboplatin in a similar setting [94-96]. In the trial of cetuximab and carboplatin, expression of EGFR in pre-treatment tumor had no correlation with response.

The above trials suggest that the addition of EGFR/HER2 inhibitors to the standard chemotherapy regimen in unselected populations does not significantly enhance response. By contrast, many of the FDA-approved EGFR/HER2 inhibitors for tumors at other sites are directed at patients with a specific molecular fingerprint. For example, cetuximab in combination with chemotherapy is indicated for EGFR positive, K-ras wild-type colorectal cancer patients (Table 2). The ongoing trial of carboplatin/paclitaxel with and without trastuzumab in serous endometrial cancer (NCT01367002) has the potential to be the first endometrial cancer trial with sufficient power to determine the utility of combining trastuzumab with chemotherapy for HER2-overexpressing cancers. Enrollment criteria for this study include HER2 overexpression at the 3+ level by IHC or HER2 positivity by FISH.

The failure of EGFR/HER2 inhibitors as single agents in pre-selected patients does not necessarily preclude their activity in combination with chemotherapy. Trials of molecular agents in combination with chemotherapy were designed to determine whether targeted agents can produce an additive increase in the efficacy of chemotherapy. These trials did not take into consideration that specific molecular alterations can produce synergy. Such synergistic cell death in combination with chemotherapy encompasses the idea of synthetic lethality.

Work from our group has pioneered the implementation of synthetic lethality into treatment for endometrial cancer. Using p53-null endometrial tumors as a model system, we found that gefitinib produces a synergistic enhancement of the inhibitory action of paclitaxel in p53-inactivated endometrial cancer cells [97]. In p53-null cells, the G2/M checkpoint is maintained through an alternate pathway that includes activation of p38 MAPK/MK2, which results in inactivating phosphorylation of cdc2. Mechanistic studies revealed that treatment with gefitinib in combination with paclitaxel overcomes this checkpoint to reduce cdc2 phosphorylation [97]. All cells are shifted into M phase, where they are sensitive to paclitaxel and undergo mitotic catastrophe. These data highlight how selection of molecular inhibitors based on the driver mutations can be used to enhance sensitivity to chemotherapy and set a standard for the future design of clinical trials for gynecologic cancers.

More recently, we have confirmed that rational use of a molecular inhibitor can overcome resistance to paclitaxel [98]. To test the effect of gefitinib on sensitivity to paclitaxel, viability was tested in the p53-null parental Hec50 and paclitaxel-resistant sublines Hec50A and Hec50E. Despite higher baseline paclitaxel resistance (Figure 3a), all cells remained exquisitely sensitive the combination of paclitaxel and gefitinib as demonstrated by an increase in the percentage of mitotic cells and the number of cells in G2/M (Figure 3b, c).

These findings indicate that gefitinib can create synthetic lethality to paclitaxel in p53-null endometrial tumors. Such strategies may be broadly applicable to tumors with other driver mutations that control sensitivity to chemotherapy. Indeed, while these studies were conducted in endometrial cancer cells, the findings are likely applicable to ovarian cancer given that nearly all ovarian tumors have a mutation in p53 that alters its function.

There are certain situations in which targeting EGFR or HER2 may not be sufficient to overcome the effect of the driver mutation(s) and restore chemosensitivity. For example, the G2/M checkpoint pathway can be co-opted by oncogenic alterations including p53 gain-of-function (oncogenic) mutants and activated Ras mutants [99, 100]. Constitutive activation of p38 MAPK and downstream MK2 leads to an inhibitory phosphorylation event on the phosphatase Cdc25C, inhibition of cdc2, G2/M checkpoint maintenance, and resistance to paclitaxel. Mutations in the genes encoding PTEN, PI3K, and FGFR2, which are common in endometrial cancer, have the potential to induce the same downstream resistance pathway via constitutive activity of AKT and/or ERK in the cytoplasm and upregulation of PLK1 in the nucleus [101-109]. Thus, targeting EGFR or HER2 in these cells would have no effect because the activating mutation is downstream of receptor blockade.

4.4 Immunotherapeutics targeting HER family members

While monoclonal antibodies have proven effective the treatment of some solid tumors, passive immunity poses some limitations in their use. An emerging alternative is the use of vaccines to induce active immunity against tyrosine kinase receptors such as the HER receptors. Cancer vaccine design varies widely and may be targeted against specific tumor antigens, protein components of the receptor extracellular domain, DNA sequences, or epitope chimeras [110]. In a clinical trial of two antibodies against HER2 B-cell epitopes combined with a T-cell immunogenic epitope, six of 24 patients with solid tumors had a clinical response, including one endometrial and two ovarian cancer patients [111]. Other HER-targeted peptide vaccines that are in the pipeline may have efficacy in gynecologic cancers, such as a phase I trial that utilizes a vaccine against trastuzumab-like and pertuzumab-like binding sites (NCT01376505). The potential use of vaccines in gynecologic cancer in combination with other treatment modalities is also being explored. Examples include trials in ovarian cancer patients that are combining a HER2 vaccine with colony-stimulating factors, with the goal of boosting the quantity of immune cells and thereby improving the immunogenic response (NCT00436254, NCT00003002). Combination treatment of vaccines with monoclonal antibodies and chemotherapeutic agents may also have potential clinical benefit but are currently not being explored in gynecologic malignancies.

5. Conclusions and future directions

In summary, a growing body of literature suggests that, while HER family members have limited prognostic value in gynecologic cancers, they still represent potential therapeutic targets. There exists a large chasm between the promising *in vitro* results and those from the clinical trials of both single-agent inhibitors as well as combinatorial regimens of HER family inhibitors and chemotherapy.

Several observations from these trials can be made. First, most trials are conducted on heavily pretreated patients. It is likely that response to any agent will be low in this population. Heavily pretreated patients are also more likely to be immunocompromised, and the reliance on cellular cytotoxic immunity in the use of the antibody-based agents is not known. Second, many of the clinical trials have been conducted on unselected patients, and few investigated HER family expression or specific mutations as translational endpoints. The unimpressive efficacy of these agents in clinical trials to this point may not be the fault

of the agent, but rather in ineffective trial design. In order to accurately identify the subset of patients who will respond to these agents, clinical trials must begin to include molecular genetics in their trial design.

While tumors with EGFR/HER2 mutations may be more sensitive to EGFR/HER2-targeted therapies, the proportion of patients with such mutations is very low compared to the total population of patients with gynecologic cancers. In addition, other mutations that affect HER signaling may contribute to a lack of response. For example, upregulation of Akt via PI3K/PTEN mutations and ERK via K-ras mutations drives survival and growth. Given that Akt/ERK signaling is downstream of HER family members, targeted EGFR/HER2 inhibitors likely will not be effective in these cancers. Thus, we should take lessons from the treatment of colorectal cancer patients [112] and select patients based on EGFR/HER2 expression and screen for mutations in genes downstream of EGFR/HER2 that would negatively affect response to the targeted therapy.

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Fig. 1. Key structural features in HER family proteins

All proteins contain an extracellular ectodomain, a single α -helical transmembrane domain, and an intracellular tyrosine kinase domain (TKD). The ectodomain can be divided into four regions, with regions I and III contributing to ligand binding and region II mediating homo- or heterodimerization. HER2 is an orphan receptor, with the orientation of the ectodomain in a ligand-bound form. HER3 is catalytically inactive and must heterodimerize with other family members.

Figure 2. Mode of action of HER family inhibitors

Monoclonal antibodies (mAb) bind to the ectodomain region to either inhibit dimerization or to inhibit ligand binding and induce receptor internalization and degradation. Tyrosine kinase inhibitors (TKI) bind to the ATP binding pocket of the TKD. Table 2 contains a list of FDA-approved EGFR/HER2 mAbs and TKIs. Table 3 summarizes clinical trials of EGFR/HER2 inhibitors in gynecologic cancers.

Figure 3. EGFR inhibitor gefitinib creates synthetic lethality to paclitaxel in p53-null endometrial cancer cells

(A) Effect of paclitaxel on viability of parental Hec50 and paclitaxel resistant Hec50-A and Hec50-E cells after 72 hrs. (B) Percentage of mitotic cells after treatment with gefitinib (10 μ M), paclitaxel (10 nM), or the combination for 24 hrs. (C) Cell cycle distribution as measured by flow cytometry 24 hrs after treatment.

Table 1

Expression of HER proteins as indicators of prognosis, response to therapy, and tumor aggressiveness.

Receptor	Site	Predictive?	References
Theragnostic Indicator			
EGFR	Ovary	Conflicting	Yes: [29-31] No: [30]
	Endometrium	Yes	[33, 34]
	Cervix	Conflicting	Yes: [37, 38] No: [37]
HER2	Ovary	Conflicting	Yes: [42, 54-57] No: [48, 49, 58-60]
	Endometrium	Conflicting	Yes: [63, 66-68, 72] No: [63, 69, 70]
	Cervix	No	[76]
HER3	Ovary	Yes	[78]
	Endometrium	Yes	[81]
	Cervix	Yes	[82, 84]
HER4	Ovary	Yes	[21]
	Endometrium	Yes	[81]
	Cervix	No	[82]
Predictor of Aggressive Disease			
EGFR	Ovary	No	[28]
	Endometrium	Conflicting	Yes: [33] No: [35]
	Cervix	Conflicting	Yes: [36, 37] No: [37]
HER2	Ovary	Conflicting	Yes: [26, 31, 39, 47-49, 52, 53] No: [43, 45, 46, 51]
	Endometrium	Conflicting	Yes: [35, 64-68] No: [71]
	Cervix	Conflicting	Yes: [74, 75] No: [76]
HER3	Ovary	Not determined	
	Endometrium	Not determined	
	Cervix	Not determined	
HER4	Ovary	Yes	[21]
	Endometrium	Not determined	
	Cervix	Not determined	

Table 2

FDA-approved HER-targeted therapies

Drug name	Type of inhibitor	Indication
EGFR Targeted Therapies		
Panitumumab	mAb	EGFR+ metastatic CRC with disease progression or following fluoropyrimidine, oxaliplatin, or irinotecan.
Cetuximab	mAb	CRC: 1) First-line in EGFR+/K-Ras WT metastatic cancer in combination with FOLFIRI (irinotecan, 5-FU, leucovorin). 2) Irinotecan- refractory disease in combination with irinotecan. 3) Single-agent in recurrent metastatic EGFR+ disease intolerant to irinotecan. Recurrent or metastatic SCCHN : 1) First-line treatment in combination with platinum-based therapy and 5-FU. 2) In combination with radiotherapy after failure of platinum-based therapy.
Erlotinib	Reversible TKI	Locally advanced or metastatic NSCLC: 1) After failure of one prior chemotherapy regimen. 2) Maintenance treatment after 4 cycles of platinum-based first-line chemotherapy. Locally advanced, unresectable, or metastatic pancreatic cancer in combination with gemcitabine.
Gefitinib	Reversible TKI	NSCLC in patients that have previously or are currently benefited from single agent treatment.
HER2 Targeted Therapies		
Trastuzumab	Monoclonal antibody	Metastatic HER2+ breast cancer: 1) First-line either as a single agent or in combination with endocrine therapy or chemotherapy (doxorubicin, cyclophosphamide, paclitaxel). 2) Adjuvant therapy: HER2+ metastatic gastric or gastroesophageal cancer in combination with cisplatin and capecitabine or 5-FU.
Pertuzumab	mAb	HER2+ metastatic breast cancer without prior anti-HER2 therapy or chemotherapy for metastatic disease in combination with trastuzumab and docetaxel.
Dual EGFR/HER2 Targeted Therapy		
Lapatinib	Reversible TKI	Advanced HER2+ breast cancer: 1) In combination with capecitabine after failure of chemotherapy and trastuzumab. 2) In combination with letrozole in patients indicated for hormone therapy.

Abbreviations: mAb: humanized monoclonal antibody; 5-FU: 5-fluorouracil; CRC: colorectal cancer; SCCHN: squamous cell carcinoma of the head and neck; NSCLC: non-small cell lung carcinoma

Table 3

EGFR-targeted therapies in Gynecologic Cancers

Agent	Phase	Response Rate	Further Studies	References
Cervix				
Lapatinib	II, single agent	5%		[113]
Cetuximab	II, single agent II, combination (cisplatin)	0% 9-14%	GOG76DD, 227E, 9918	[114] [115]
Gefitinib	II, single agent	0% (20% with SD)		[116]
Endometrium				
Lapatinib	II, single agent	3.3% PR (23% with SD)		[2]
Erlotinib	II, single agent	12.5%		[117]
Gefitinib	II, single agent	3.8% (27% with SD)		[34]
Trastuzumab	II, single agent	0% (36% with SD)		[90]
Ovary				
Lapatinib	II, single agent II, combination (topotecan)	0% 1/18 PR	GOG 170D	[86] [118]
Erlotinib	II, combination (carboplatin/paclitaxel) II, combination (carboplatin)	11, 29% (did not meet efficacy endpoints) 7, 57% (Pt refractory vs. sensitive)		[93] [95]
Pertuzumab	II, combination (gemcitabine)	13.8%		[119]
Trastuzumab	II, single agent in HER2-positive tumors			[89]
Matuzumab	II, single agent in EGFR-positive tumors			(93)

Abbreviations: SD: stable disease; PR: partial response