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HER2 Expression Beyond Breast Cancer: Therapeutic Implications for Gynecologic Malignancies

Diana P. English, **Dana M. Roque**, and **Alessandro D. Santin**
Division of Gynecologic Oncology, Department of Obstetrics, Gynecology & Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA.

Abstract

HER2 or ErbB2 is a member of the epidermal growth factor family and is overexpressed in subsets of breast, ovarian, gastric, colorectal, pancreatic and endometrial cancers. HER2 regulates signaling through several pathways (Ras/Raf/mitogen-activated protein kinase and phosphatidylinositol-3 kinase/protein kinase-B/mammalian target of rapamycin pathways) associated with cell survival and proliferation. HER2 overexpressed and/or gene amplified tumors are generally regarded as biologically aggressive neoplasms. In breast, cervical, endometrial and ovarian cancer, there have been several studies linking the amplification of the c-erbB2 gene with chemo-resistance and overall poor survival. Tyrosine kinase inhibitors and immunotherapy with monoclonal antibodies targeting HER2 holds promise for patients harboring these aggressive neoplasms. Trastuzumab combined with cytotoxic chemotherapy agents or conjugated with radioactive isotopes is currently being investigated in clinical trials of several tumor types.

Introduction

Exome-wide analyses have recently greatly contributed to a better understanding of the biology of human neoplasms through the identification of mutations and copy number variations in genes crucial for the development of human tumors. More importantly, these studies have paved the way for a more rational drug design and the development/implementation of novel therapies specifically targeted against molecular aberrations present in a variety of human tumors.

The transmembrane epidermal growth factor type II receptor (i.e., HER2), represents the prototype of a stable molecular abnormality endowed with well-characterized functional consequences that is detectable in several of the most common human solid tumors including but not limited to breast, ovarian, endometrial, colon, non-small cell lung cancer, prostate and cervical cancer (1-4). Importantly, HER2 overexpression has been shown to correlate with a worse survival in both node-positive and node-negative breast cancer patients and to be of prognostic and potential therapeutic value in other solid tumor types including multiple gynecologic malignancies (5, 6).

The location of HER2 on the cell surface has contributed to its appeal as an immunotherapy target. Trastuzumab (human monoclonal anti-HER2 antibody) has provided a distinct therapeutic advantage in not only breast cancer but in other tumor types, for example HER2 positive advanced gastric or oesophagogastric junction adenocarcinoma. As such

Corresponding Author: Alessandro D. Santin MD, Division of Gynecologic Oncology, Department of Obstetrics, Gynecology & Reproductive Sciences, Yale University School of Medicine, 333 Cedar Street, LSOG 305, PO Box 208063, New Haven, CT 06520-8063, USA. alessandro.santin@yale.edu.

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Trastuzumab has received United States Food and Drug Administration (FDA) approval for the treatment of HER2 overexpressing breast and metastatic gastric cancer. The role of trastuzumab in gynecologic malignancies is still being explored with a Phase II trial underway in advanced stage uterine serous endometrial cancer.

Molecular Pathways

The human epidermal growth factor type II receptor HER2 (c-erbB2) gene product is a transmembrane receptor protein that includes a cysteine-rich extracellular ligand-binding domain, a hydrophobic membrane spanning region, and an intracellular tyrosine kinase domain. With no direct ligand identified to date, HER2 functions as a preferred partner for heterodimerization with other members of the epidermal growth factor receptor family (*i.e.*, HER1 or ErbB1, HER3 or ErbB3, and HER4 or ErbB4) and thus plays an important role in coordinating the complex ErbB signaling network that is responsible for regulating cell growth and differentiation (7).

In normal cells, there are two copies of the gene per cell. In the presence however of HER2 amplification there may be as many as 100 c-erbB2 genes per tumor cell (8-10). This gene amplification results in overexpression of HER2 at both the mRNA and protein levels. The over-expression of HER2 at such a high level results in the tyrosine kinases becoming constitutively activated. This is thought to be the result of crowding of adjacent HER2 receptors in the cell membrane (8-11). The transactivation of the tyrosine kinase part of the receptor subsequently activates gene transcription factors and other cell cycle regulatory molecules including downstream protein pathways such as Ras/Raf/mitogen-activated protein kinase (Ras/Raf/MAPK) and phosphatidylinositol-3 kinase/protein kinase-B/mammalian target of rapamycin (PI3K/AKT/mTOR) pathways (12). Alterations of cell cycle regulatory molecules may be critical for the formation and maintenance of the transformed phenotype resulting from HER2 gene amplification and protein overexpression (13). On the other hand, blockade of HER2 function may result in the reversal or prevention of these effects on cell cycle regulators.

Determination of HER2 Expression and Clinical Significance

Currently, HER2 expression status is routinely determined by immunohistochemistry (IHC), followed with additional fluorescent in situ hybridization (FISH) assays to verify equivocal IHC results. FISH identifies the number of HER2 gene copies in conjunction with the number of chromosome 17 centromere (CEP17) copies. It is a very sensitive and specific method which utilizes formalin-fixed, paraffin-embedded tissue (FFPE). FISH can also be applied to cell blocks or cytologic specimens. Generally, FISH scoring is considered more objective and quantitative than IHC scoring, however reproducibility is dependent on the thickness of tissue sections and the interpretation as well as recognition of the invasive component. FISH is not only more expensive than IHC but also is more time consuming requiring appropriate trained personal and the use of a fluorescence microscope (14).

Chromogenic in situ hybridization (CISH) is another cost-effective and valid nucleic acid assay now being used which allows for the detection of the HER2 oncogene amplification using conventional enzymatic reactions and is applicable to FFPE tissues to which IHC was previously performed (13). Silver-enhanced in situ hybridization (SISH) like chromogenic in situ hybridization is a new bright-field technique that has been introduced for determining HER2 status. Both CISH and SISH combine features of IHC and fluorescent in situ hybridization. SISH is quicker to perform than FISH and has few requirements. SISH is a rapid fully automated assay providing permanently stained slides that are interpreted by conventional bright field microscopy which enables pathologists to evaluate slides within the context of tissue morphology and reduces error rates (14) (15). SISH is scored similarly

to CISH with 5 or fewer copies of the HER2 gene per nucleus in more than 50% of tumor cells defining no amplification and high amplification defined as more than 10 copies or large clusters or a mixture of multiple dots and large clusters of HER2 present in more than 50% of cancer cells (14). SISH has been shown to have high concordance rate with FISH in previous studies (15) (16).

Multiple studies have found the lack of a perfect correlation between gene amplification quantification and HER2 protein expression (11). Importantly, breast cancer studies have noted that HER2 protein product overexpression can also be found in the absence of gene amplification (17). Similar discrepancies between FISH and IHC results for HER2 have been noted in endometrial cancer. This underscores the need to consistently validate protein expression in c-erbB2 amplified tumors as protein expression may be discordant due to post-transcriptional modifications in mammalian cells (18). In the past some of the variation in HER2 overexpression and gene amplification has at least in part resulted from differences in the testing methods, interpretation, and scoring criteria used.

HER2 intensity determination by IHC previously was by FDA scoring criteria. The American Society of Clinical Oncology and the College of American Pathologists (ASCO/CAP) in 2007 published recommendations for optimal HER2 testing performance. This new testing algorithm relies on accurate and reproducible assay performance. Also included in the recommendations were methods to reduce assay variation such as specimen handling. The panel also suggested an algorithm for defining positive, negative and equivocal test results for both HER2 protein expression and gene amplification (19). The (ASCO/CAP) IHC score of protein expression is on a scale of 0 to 3+ and has generally been adopted widely. A score of 0-1+ represents a negative result and is assigned if there is no staining or weak, incomplete membrane staining in any proportion of tumor cells. An equivocal IHC results consists of specimens scored as 2+ and is described as complete membrane staining that is either non-uniform or weak in intensity but with obvious circumferential distribution in at least 10% cells or very rarely tumors that show complete membranes staining of 30% or fewer tumor cells. IHC positive result is 3+ cell surface expression defined as uniform intense membrane staining of >30% of invasive tumor cells (19).

HER2 protein overexpression has been found in several tumor types including breast, esophageal, lung, cervical, endometrial and ovarian cancer. Characterization of this increased expression has been exploited mostly in patients with breast cancer where it has a prognostic, predictive and therapeutic target value. Continued interest in the expression of this plasmalemmal glycoprotein in other malignancies has resulted in several papers describing the possible mechanism of HER tumorgenesis. The investigation of HER2 expression in gynecologic malignancies has produced strong evidence that overexpression is associated with increased tumor aggression. The mechanism of tumor aggressiveness includes resistance to immunologic mediators such as tumor necrosis factor (TNF-α), activated macrophages and lymphocyte-activated killer cells (20). HER2 overexpressed tumors have been found to have increased amounts of factors governing tumor invasiveness and angiogenesis. In breast, cervical, endometrial and ovarian cancer, there have been several studies linking the amplification of the c-erbB2 gene with chemo-resistance and overall poor survival (21-24). Also in breast cancer, discordant HER2 expression has been found between primary and metastatic sites. Interestingly, studies in gastric and epithelial ovarian cancer have shown that there is instead a corresponding level of HER2 expression in primary and metastatic sites (25-27).

HER2 Expression in Gynecologic Cancer

Ovarian Cancer

Epithelial ovarian cancer accounts for the majority of ovarian cancer cases and about 60% of all ovarian tumors. Surface epithelial ovarian tumors are generally subclassified based on cell type and malignant potential. Recent data has allowed the correlation of molecular pathologic information with conventional histopathologic diagnosis (28). Traditionally the 5 main subtypes of epithelial ovarian cancer are serous, mucinous, endometrioid, clear cell and transitional cell (Brenner type) tumors.

Serous ovarian carcinomas represent close to 50% of malignant tumors in this location (29). These tumors also account for 30% of genital tract malignancies and are responsible for the greatest number of deaths, as ovarian serous carcinomas commonly present with late stage disease. The 5-year survival for all stages is estimated to be between 20-38.1% (30, 31). Early detection of malignant lesions and identification of prognostic biomarkers are both paramount to improving the care of women with this malignancy.

The significance of HER2 immunoexpression in the serous ovarian tumors is controversial. Ovarian surface epithelium is weakly positive for HER2 and positive HER2 immunostaining may be found in benign/borderline ovarian tumors as well as in frankly malignant cases (32). Generally, the HER2 expression in ovarian serous carcinoma is far less than that observed in uterine serous carcinoma (29).

The structural similarity of EGFR and HER2 has led to the hypothesis that overexpression of both tyrosine kinase proteins are involved in signal transduction pathways that will finally result in ovarian cancer (30). A recent study analyzed EGFR, HER2 and Ki67 expression in 26 serous tumors of which 34.6% were malignant. Adenocarcinomas were HER2 positive in 55.5% of cases. Negative or weak EGFR/HER2 immunostain and low index of proliferation was associated with benign/borderline serous ovarian tumors. EGFR, HER2 and Ki67 correlated with tumor differentiation but this study as with several others did not find a correlation between HER2 surface expression and other clinicopathologic characteristics (33). Raspollini et al evaluated HER2 overexpression and amplification in FIGO stage IIIC, serous ovarian carcinomas from living patients. The authors compared tumors from patients with no evidence of disease 5 years after initial treatment to tumors of patients matched for stage, grade of differentiation, and treatment, who died of progressive disease within 2 years of primary treatment. HER2 status did not correlate with disease progression during first-line chemotherapy and was not statistically associated with clinical outcome in this group of patients (34). In a Finish study, a tissue microarray of 401 serous ovarian carcinomas was evaluated by CISH and IHC. Amplification was detected in 7% and low copy number increase (three-five fold) detected in 14% of these carcinomas. Although there was an overall low level of HER2 amplification, an increase in HER2 copy number was associated with a poor prognosis, a poor response to therapy and a shorter disease- free and overall survival (35).

Mucinous epithelial ovarian cancer (EOC) accounts for approximately 10% of epithelial ovarian cancers. Most mucinous tumors of the ovary are borderline tumors or stage 1 carcinomas. Early stage disease has an excellent prognosis but patients with disease spread beyond the ovary have an extremely poor prognosis. There is increasing evidence in support of HER2 targeted therapy in gastroesophageal cancers. Mucinous (EOC) resemble gastroesophageal adenocarcinoma and as such targeted molecular therapy may also be useful in the management of mucinous ovarian cancers (36). Generally, there were very few cases of mucinous tumors in previous series investigating the prognostic and therapeutic utility of HER2 in EOC (37-39). McAlpine et al reported in 2009, HER2 molecular results

on 33 mucinous carcinomas and 16 borderline mucinous tumors. HER2 overexpression/amplification was identified in 18% of both the borderline and malignant mucinous tumors. Also the use of trastuzumab resulted in objective responses in those patients with recurrent HER2 positive mucinous tumors (40). In a more recent study by the same research group, the presence of HER2 amplification/overexpression or KRAS mutation in mucinous carcinomas was associated with a decreased likelihood of disease recurrence or death (41). This study included a fairly large number of patients as there were 189 mucinous carcinoma cases with a total of 54 recurrences and 53 deaths from progressive disease (41).

The concept of more frequent HER2 amplification and protein expression occurring in mucinous carcinomas than any other type of ovarian epithelial malignancy has been supported in recent studies. A study from Singapore looked at a small cohort of 85 ovarian tumors, 17 of which were mucinous carcinomas. HER2 amplification and protein expression was evaluated by FISH, CISH and IHC. Mucinous carcinoma was the only subtype of ovarian cancer found to have HER2 amplification or protein expression within this cohort, with amplification noted in 35.3% of these tumors and protein expression in 29.4% (42).

Consistent with the preceding literature, McCaughan et al reported HER2 amplification and expression in all major epithelial ovarian cancer subtypes. Highest HER2 gene amplification was noted in mucinous carcinomas (25%) followed by mixed type carcinomas (11.9%), clear cell carcinomas (4%), serous papillary carcinomas (3%), and endometrioid carcinomas (2.1%) (43). The authors also noted intra-tumoral heterogeneity in HER2 expression by IHC in 20% of these tumors. Variable HER2 protein expression and intra-tumoral heterogeneity has also been previously reported in prior studies (44, 45) and may have a bearing on efficacy of HER2 targeted therapy.

Interest in the molecular genomic profile of ovarian clear cell carcinoma (OCCC), an aggressive and drug-resistant type of epithelial ovarian cancer, led recently to an important discovery related to HER2 gene amplification. Tan et al studied fifty pure primary ovarian clear cell carcinomas using high-resolution microarray-based comparative genomic hybridization. The authors suggested that HER2 may be a therapeutic target for this subgroup of epithelial ovarian carcinoma as gene amplification and protein expression was observed in 14% of OCCC. However, this conclusion is probably best suited for those clear cell carcinoma patients with definite HER2 overexpression (46). *In vitro* studies have found even higher levels of HER2 overexpression by IHC in ovarian clear cell carcinoma cell lines. The growth of these HER2 overexpressed clear cell carcinoma cell lines was also shown to be significantly and dose-dependently reduced by trastuzumab *in vitro* (47).

Ovarian endometrioid adenocarcinoma is considered to be a rare form of malignant transformation of ovarian endometriotic implants. EGFR and HER2 expression/amplification were evaluated by IHC and FISH respectively, in a series of intra-abdominal endometriotic implants and also in ovarian endometrioid adenocarcinoma. EGFR and HER2 were not overexpressed in endometriosis or in ovarian endometrioid adenocarcinoma. This study suggests that the EGF pathway may not be a potential target in these two disease processes (48).

As mentioned previously, some research in ovarian carcinoma has found HER2 expression to be an independent risk factor for decreased survival (35) (18). Conversely patients with negative HER2 have been noted to have better chemotherapy responses, higher rates of negative second-look laparotomy and also improved survival (23, 49).

In GOG160 – a phase II trial evaluating trastuzumab in patients with recurrent or refractory ovarian or primary peritoneal carcinoma, a low overall response rate of 7.3% in the 41 eligible patients with HER2 overexpression was found. An additional 16 patients or 39% of

the patients were found to a have a long stabilization of their disease. Trastuzumab was relatively well tolerated and several patients with responding or stable disease received therapy for over a year. Only 11.4% of tumors exhibited 2+ or 3+ expression and there was no relationship found between HER2 expression and clinical response or survival (37).

HER2 gene amplification and protein expression status in ovarian cancer was also examined in a large multicenter French study. In this trial, HER2 gene amplification was evaluated in 320 patients with advanced ovarian cancer, including 243 patients enrolled in a multicenter clinical trial of paclitaxel/carboplatin-based chemotherapy. The HER2 gene was overexpressed and amplified in 6.6% of tumors and the overall rate of HER2 protein expression (2+/3+) in this study was 13 %. There was no significant relationship found between HER2 status and other prognostic factors or with HER2 status and progression-free or overall survival. As HER2 was not identified to have any prognostic value, the authors proposed that paclitaxel may overcome the poor prognosis associated with HER2 overexpression and that there might be a possible interaction between HER2 expression and drug sensitivity (44).

Uterine Cancer

Endometrial cancer is the most common gynecologic cancer in the Western world. Type 1 tumors are of the low-grade endometrioid subtype and type II tumors are grade 3 endometrioid tumors or the less common but more aggressive histological subtypes such as clear cell or uterine serous carcinoma. Rates of HER2 overexpression and amplification range from 4% to 69% in endometrial adenocarcinoma (50). Generally, HER2 expression and amplification is more common in higher grade and stage endometrial tumors. Investigation of HER2 status in a large number of endometrial cancer patients revealed that the lowest level of expression and amplification was seen in grade 1 endometrioid adenocarcinomas, with endometrial serous carcinomas having the highest rate of expression (51). HER2 expression/amplification in these tumors was associated with shorter overall survival. The results suggested that HER2 may play a role as a critical oncogene in high grade tumors but not in the low-grade type 1 endometrioid tumors (51). Several studies on endometrial adenocarcinoma have also demonstrated that HER2 gene amplification is of prognostic value and associated with a worse prognosis in histological types such as clear cell carcinoma and serous carcinoma. Amplification of HER2 has been documented in as many as 38% of clear cell endometrial carcinomas (52).

Over the last decade, there has been a strong interest in investigating HER2 receptor overexpression in uterine papillary serous carcinoma (USC). USC is the second most common endometrial cancer and accounts for approximately 10% of endometrial cancer. This subtype of endometrial cancer is highly biologically aggressive and accounts for a large number of endometrial cancer deaths (53-56). Alternative treatment approaches are always being investigated, as there is such a poor prognosis associated with this subtype of endometrial cancer. Histologically USC and ovarian serous tumors are indistinguishable. Molecular profiling studies have demonstrated c-erbB2 to be one of the most overexpressed genes in USC with the benefit of distinguishing USC from ovarian serous tumors (57).

Santin et al as well as multiple other research groups have shown that HER2 receptor is overexpressed in USC (scores 2+ and 3+ on IHC) in a large number of patients. They have reported expression rates from (18% to 80%) depending on the IHC technique used (58-60). Racial differences in HER2 expression have been noted in USC, with a disproportionately higher expression frequency and poorer clinical outcome in African-Americans compared to Caucasian patients (61).

Uterine carcinosarcomas are rare and extremely aggressive tumors and account for only 2-5% of all uterine cancers. Historically these tumors were called mixed mesodermal sarcoma or malignant mixed mullerian tumors (MMMT). Carcinosarcomas were considered as a subtype of uterine sarcomas but these tumors have over the last decade, been generally accepted to have a monoclonal origin and only few are truly collision tumors composed of epithelial and mesenchymal elements (62). Studies supporting the monoclonal origin of this tumor have found identical patterns of chromosomal inactivation as well as identical mutations of K-ras and p53 in the two components (63). The carcinomatous component is the “driving force” of these tumors and data has confirmed that the coexisting sarcomatous component is derived from the carcinoma or from a stem cell that has undergone divergent differentiation. As such, these tumors are best regarded as metaplastic carcinomas (62). These tumors are very difficult to treat and at present there are no established consensus guidelines for treatment. Many different therapeutic approaches have been explored but nevertheless an overall poor prognosis continues for patients diagnosed with this type of uterine cancer with an estimated recurrence rate of 40-60% (64).

Studies which have evaluated HER2 expression and gene amplification in endometrial carcinosarcoma have reported HER2 overexpression ranging from as low as 17% to as high as 43% (65). Notably, 2+ immunoreactivity was counted as positive HER2 expression in some of these studies. The difference in criteria for defining overexpression as well as a lack of a standard detection technique may explain the large variations of expression found in these previous studies. HER2 overexpression is noted to be higher in the carcinomatous component (56%) compared to the mesenchymal component (6%) of the endometrial carcinosarcomas (66). This overexpression of HER2 has not yet been successfully exploited for therapeutic effect, although encouraging preclinical data exists (67). Endometrial carcinosarcomas have generally showed a resistance to HER2 inhibitors. The mechanism for this resistance is poorly understood however it is hypothesized that even though receptor inhibition may occur in these tumors that the downstream signally persists in these cells.

Cervical Cancer

Cervical cancer is the third most common cancer worldwide with 80% cases occurring in the developing world. Cervical carcinoma has also been studied for HER2 protein expression. In a study by Hale et al, definite cervical cancer membrane staining was found in 38.7% of cases with stage 1B/IIA tumors (68). The proportion of positively staining cells was as high as 80% of neoplastic cells in some tumors and had a strong correlation with poor survival ($p<0.0001$). This study found that in patients with cervical adenocarcinomas, there was significantly more HER2 staining in tumors of patients with lymph node metastasis identified. Squamous and adenosquamous carcinomas however had the association of positive HER2 staining with poor prognosis most apparent in those patients without lymph node metastases. HER2 protein expression may therefore help to identify the subset of patients without lymph node metastasis who may be at a higher overall risk of death and may benefit from early adjuvant therapy (68).

More recently Bellone et al reported on HER2 expression in early stage cervical cancer and the sensitivity of primary cervical cell lines to trastuzumab (Herceptin®) (69). The research group found that 94% (17 out of 18) fresh primary as well as established cervical cancer cell lines expressed HER2 by flow cytometry. In this study all the primary cervical cancer cell lines established in the laboratory and found to have HER2 overexpression were from primary tumor biopsies reported as negative previously (69). IHC studies on biopsies from some recurrent lesions demonstrated a strong (3+) expression for HER2 despite the fact that biopsies from the primary tumors at time of the initial cancer diagnosis was HER2 negative. Berchuck et al also described strong staining for HER2 by metastatic tumors at the time of

recurrent disease (70). These observations suggest that HER2 positive cells may have an *in vitro* and *in vivo* selective growth advantage over HER2 negative cells.

The HER2 overexpressed cervical cell lines in this study by Bellone et al were significantly sensitive to Herceptin-mediated antibody-dependent cellular cytotoxicity (ADCC) and to Herceptin-mediated inhibition of tumor growth. The authors concluded that therapy which targets HER2 may be more effective in patients with cervical cancer than indicated by the common low HER2 expression noted on primary cervical tumors (69).

Vulvar Paget's Disease

Vulvar Paget's disease is the most common form of extramammary Paget's disease. The status of HER2 expression in vulvar Paget's disease has been evaluated by several studies. Reports of HER2 overexpression range from 5-80% of vulvar Paget's disease cases. The recurrence rate in the first eight years after surgical excision is approximately 30% (71). As such, interest continues to grow in developing new therapeutic approaches for this disease. In the Yale experience study, over 50% of patients with vulvar Paget's disease overexpressed HER2 (72). This data prompted the Phase II trial which is now underway for the evaluation of Trastuzumab in patients with HER2 positive persistent or recurrent vulvar Paget's disease. (Table I)

HER2, Other Prognostic Markers and Therapeutic Targets

Several studies have sought to evaluate potential biomarkers useful for predicting drug responsiveness and disease course. In breast cancer HER2 overexpression has been associated with both resistance to chemotherapeutic agents and also is predictive of a favorable response to other agents (73, 74). In a recent study of metastatic breast cancer patients treated with trastuzumab and paclitaxel, Jung and colleagues found that high HER2 amplification index and high class III β-tubulin expression were predictive of a good response to therapy (74).

The utility of molecular markers including HER2, Ki67, ER and PR were evaluated in 72 patients with advanced ovarian cancer. None of these markers were associated with progression free survival, however Ki67 nuclear expression (a marker of proliferation) was associated with a higher rate of complete response ($p=0.05$). HER2 overexpression was only 5% in this study and as such was not found to have a predictive or prognostic role in this study (75). Some larger studies in ovarian cancer patients have shown however the potential predictive role of HER2.

The status and role of HER2 and class III β-tubulin was also evaluated in metastatic extramammary Paget's disease (76). In one study 63% of extramammary Paget's disease tumors overexpressed HER2 and positive class III β-tubulin reactivity was observed in 22% of these tumors. Class III β-tubulin expression is associated with taxane resistance. The authors suggested that HER2-targeted immunotherapy combined with taxane derivatives is a therapeutic option for metastatic extramammary Paget's disease based on high HER2 and low class III β-tubulin expression results. Both HER2 and class III β-tubulin may be appropriate biomarkers for guiding treatment of this disease (76).

Immunotherapy

Although multiple mechanisms of action have been attributed to anti-HER2 monoclonal antibodies including inhibition of tumor proliferation and/or promotion of cell cycle arrest (secondary to decrease HER2 receptor dimerization) and induction of apoptosis, strong experimental evidence suggests that engagement of Fc receptors on effector cells (i.e.,

mainly natural killer (NK) cells) represents the dominant component of the *in vivo* activity of these antibodies against tumors. Humanized monoclonal antibodies (mAb) against HER2 are known to induce tumor lysis through antibody-dependent cellular cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC), and consistent with this view, the efficacy of these mAb in mice defective for Fc has been previously demonstrated to be only about 30% of the activity shown by the intact antibody when it is able to engage the Fc receptors on NK cells (77). This theory is further supported by experimental studies comparing the *in vitro* and *in vivo* activities of IgG and $F(ab')_2$ fragments in a mixture of three monoclonal anti-HER2 antibodies. In these reports, the *in vitro* anti-proliferative and pro-apoptotic effects of IgG and $F(ab')_2$ were similar but only the IgGs had significant antitumor activity *in vivo.* The importance of the Fc portion of trastuzumab for effective *in vivo* activity is supported by studies demonstrating that even a mixture of three anti-HER2 monoclonal antibodies which were highly effective at inducing cell death *in vitro* still required Fc-mediated effector function for optimal functioning *in vivo* (78).

Clinical results have also shown that there is a improved response to trastuzumab in patients with a particular Fc polymorphism resulting in a higher NK affinity to IgG1, again lending support that ADCC inclusive of its mediators is critical for the *in vivo* efficacy of trastuzumab (79, 80). Moreover other clinical data has demonstrated that patients responding to neoadjuvant trastuzumab showed a four-fold increase in antibody-dependent lytic activity from isolated peripheral blood mononuclear cells in contrast to patients without a response (81) .

Trastuzumab and Pertuzumab

As mentioned above, trastuzumab (Herceptin®, Genentech, CA, USA) is a humanized monoclonal IgG1 antibody that works both through recruitment of NK cells and initiation of ADCC as well as abrogation of downstream effectors (77, 81, 82). It is FDA-approved as an adjunct to cyclophosphamide, paclitaxel and doxorubicin in the treatment of early-stage HER2 positive, node-positive breast cancer and as a single agent for adjuvant treatment of early-stage, HER2 positive, high-risk ER/PR-negative breast cancers following multi-modality anthracycline-based therapy (83). Based on sound biologic plausibility, there is also considerable interest in applications for HER2 overexpressing gynecologic malignancies. As assessed by standard ^{51} chromium release assays, trastuzumab results in ADCC in the range of 25–60% against USC that overexpress HER2 (84).

The therapeutic potential of anti-HER2 immunotherapy *in vitro* against HER2 expressing carcinosarcomas of the female genital tract has recently been investigated (67). Our research group described HER2 gene and protein expression in primary carcinosarcoma cell lines and also for the first time demonstrated *in vitro* trastuzumab-mediated ADCC against uterine and ovarian carcinosarcoma cell lines. Although only a minority of carcinosarcoma demonstrated HER2 overexpression, few would argue against the addition of trastuzumab to the armamentarium of agents for treating HER2 positive carcinosarcoma patients in view of the high rate of recurrence and the generally poor responses to chemotherapy (67).

Several case reports exist in the literature of trastuzumab clinical activity in heavily pretreated advanced stage endometrial carcinoma patients with HER2 overexpression by IHC. The histological subtypes reported on were uterine serous and high-grade endometrioid tumors. The responses varied from complete response to stable disease with the longest period of stable disease being 11 months (85-87). Despite encouraging case reports (85-87), when evaluated as a single-agent, trastuzumab 4 mg/kg in week 1 then 2 mg/kg weekly until disease progression in stage III/IV or in recurrent endometrial cancers at the Phase II level in GOG-181B failed to demonstrate significant activity (52). Since combination therapy with trastuzumab and chemotherapy is generally more effective than single agent trastuzumab in

HER2 positive breast and gastric cancer, a multi-institutional Phase II trial is underway to investigate whether the addition of trastuzumab to paclitaxel and carboplatin chemotherapy improves progression free survival (PFS) when compared to paclitaxel and carboplatin alone in stages III/IV and recurrent USC patients overexpressing HER2 at 3+ level by IHC or positive by FISH (88).

Pertuzumab (Omnitarg®, Genentech, South San Francisco, CA, USA) is a humanized IgG1 mAb HER heterodimerization inhibitor that binds domain II of the erbB2 receptor. Compared to trastuzumab, pertuzumab inhibits a broader array of downstream signal transduction pathways through abrogation of lateral signal transduction (89-93). El-Sawhi and colleagues (84) investigated the sensitivity of USC cell lines to heterologous peripheral blood lymphocytes (PBLs) in the presence of pertuzumab (2.5 μg/mL), trastuzumab (2.5 μg/ml), and the combination of both in cell lines expressing high levels of HER2. Significant cytotoxicity was achieved with pertuzumab (mean ± SD: 61 ± 25.6%; range, 32.5-67.3%; p=0.0001) and trastuzumab (mean ± SD: 56.3 ± 14.2%; range, 32.5-77.1%; p=0.0001) compared with control, (i.e., PBLs alone [mean ± SD: 3.2±5.6%; range, 0-19%] or PBLs and rituximab (anti-CD20) [mean ± SD: 2.5 ± 3.8%; range, 0-13%]). Combination of the two antibodies significantly increased ADCC in low HER2 expressing USC cell lines (84).

Trastuzumab Emtansine (TDM-1)

Trastuzumab emtansine (T-DM1, Genentech/Roche) is a novel antibody-drug conjugate that combines trastuzumab with targeted delivery of the antimicrotubule agent DM1. DM1 belongs to the maytansine class of chemotherapeutic agents. On average 3-4 molecules of DM1 bind to each trastuzumab molecule. TDM-1 has demonstrated robust clinical activity in heavily-pretreated patients with trastuzumab-refractory HER2 positive breast cancer with a 43.6% objective response rate and median PFS of 9.6 months (94). The common side effects reported with this new drug are fatigue, thrombocytopenia and epistaxis. Similarly promising anti-tumor activity has been noted in HER2 positive gastric tumors with resistance to trastuzumab (95). However no clinical trials have yet been conducted in gynecologic cancer. Clinical trials exploring T-DM1 therapy in HER2 positive advanced/recurrent and/or refractory USC are warranted.

Augmenting Targeted Immunotherapy

Faulty immunosurveillance represents an important contributor in cancer development and progression. Even though immune responses such as those mediated by natural killer cells are thought to play an important role to keep tumor growth in check, eventually these mechanisms become overwhelmed and immunologic escape occurs, at which time disease becomes apparent (96-98). The cytokine interleukin (IL)-2 controls the growth and differentiation of a number of lymphocyte subsets, notably CD8+ cytotoxic T cells (T_c), NK cells, and CD4+ helper T regulatory cells (T_{regs}). High-dose recombinant IL-2 (aldesleukin, Proleukin®, Novartis, Switzerland) was approved by the Federal Drug Administration (FDA) as early as 1998 for treatment in metastatic melanoma and renal cell carcinoma (99). IL-2 therapy is plagued by significant pulmonary and hepatic toxicities and may lead to parallel expansion of T_{regs} able to blunt antitumor immune responses, thereby limiting the beneficial effects of expanding tumor-reactive CD8+ T_c (100, 101).

Augmenting targeted immunotherapy in particular natural killer cell function with IL-2 or with agonist monoclonal antibody for costimulatory receptors on NK cells is a promising strategy (102). El-Sawhi and colleagues demonstrated enhanced cytotoxicity when pertuzumab was combined with low doses of IL-2 (mean ± SD: 46.9 ± 11%; range, 22.4-74.2%) versus pertuzumab alone (mean ± SD: 41.5±12.8%; range, 15.8-71.9%) (p=0.04) in preclinical experiments. Similarly, pertuzumab in combination with IL-2

induced significantly higher cytotoxicity when compared with PBLs treated with IL-2 (p= 0.03) or PBLs treated with IL-2 in the presence of rituximab (p=0.03). In contrast, no significant increase in cytotoxicity was detected after 5 hours of IL-2 treatment in the absence of pertuzumab or in the presence of rituximab (84).

Membrane Complement Regulatory Proteins

One mechanism by which tumors escape immune surveillance is through antigen evolution and upregulation of membrane complement regulatory proteins (mCRPs) that may hinder complement-dependent pathways (103). The mCRPs CD46 (membrane cofactor protein), CD55 (decay-accelerating factor) and CD59 (protectin) have been shown to be upregulated in colorectal, cervical, prostate and renal cell carcinomas (104-106). mCRPs upregulation lead to inactivation of C4b/C3b, dissociation of C3/C5-convertases and prevention of membrane-attack complex assembly (107). Recently, our research group has shown that USC overexpress CD46, 55 and 59 relative to normal endometrial cells; knockdown via siRNAs of CD55 and CD59 but not CD46 significantly sensitized USC to CDC (from 6.8 to 11%) and ADCC (from 48 to >60%) (108). Inhibition of mCRPs on type II endometrial cancers harboring c-erbB2 gene amplification may prove to be a useful strategy to improve the response of these aggressive tumors to trastuzumab-mediated CDC and ADCC (108).

mTOR inhibitors, Tyrosine kinase inhibitors and Anti-HER2 Therapy

Blocking mTOR complex-1 induces upregulation of HER2 expression mediated by mTOR complex-2 in preclinical models (109). Clinical benefits were seen in patients with trastuzumab-resistant HER2 positive breast cancer treated with everolimus (mTOR inhibitor) in phase I/II trials (110, 111). Thus, evaluation of mTOR inhibitors in combination with HER2 targeted therapy should be investigated in gynecologic cancer.

Dual tyrosine kinase inhibitors (TKI) such as lapatinib have shown efficacy in HER2 positive trastuzumab-resistant breast cancers, esophageal and gastric adenocarcinomas. Lapatinib's additive or synergistic effects with chemotherapy have also been demonstrated in these tumor types (109, 112). Lapatinib is a reversible dual inhibitor of both HER2 and EGFR and in preclinical models has shown effectiveness in restoring trastuzumab sensitivity (113). Accumulation of the truncated form of HER2, p95-HER2, which lacks the trastuzumab binding site but is able to maintain tyrosine kinase activity is one mechanism of trastuzumab resistance. Lapatinib is able to inhibit p95-HER2 phosphorylation and as a result reduce growth of HER2 driven malignancies (114). Pan-HER TKIs which inhibit epidermal growth factor family receptors and their downstream pathways have also proved beneficial in solid tumor clinical trials (115). Also new in therapeutic approaches for solid tumors is the targeting of heat shock protein 90 (hsp90) molecular chaperone. Hsp90 is the overseer for several oncogenic proteins including HER2, AKT and others involved in signal transduction and cell cycle regulation (116, 117). Hsp90 inhibitors have shown encouraging clinical activity in HER2 positive metastatic breast cancer. Considering that most solid tumors, including gynecologic tumors, are complex and have several genetic abnormalities, a single targeted agent is unlikely to be effective over a long period of time and combination therapy is crucial. Hsp90 inhibitors are thus currently under evaluation in early phase clinical trials as single agent therapy or in combination with trastuzumab (118, 119). (Table II)

Radioimmunotherapy

Radioimmunotherapy has been explored in the management of colon, metastatic renal and prostate cancer with encouraging results (120-122). Preclinical studies have recently shown the safe applicability of radioimmunotherapy in gynecologic cancer and other tumor types (123, 124). A phase I trial evaluating lead-212 (^{212}Pb)-trastuzumab in patients with HER2

positive ovarian, pancreatic, colon, gastric, endometrial or breast cancer patients documented to have peritoneal studding or positive washings (intraperitoneal disease) is ongoing. This represents a potential treatment approach for patients with metastatic disease utilizing this lead isotope with a short path length specifically targeted to malignant cells by the trastuzumab antibody (125).

Anti-HER2 vaccine

While trastuzumab is an effective immunotherapeutic agent against a variety of tumors overexpressing HER2, it potentially has limitations of eventual drug resistance and risk of cardiotoxicity, especially in patients on previous anthracycline based regimens. Therefore interest naturally surfaced overtime in anti-HER2 vaccines as experience with trastuzumab grew. The use of a vaccine that induces or stimulates a preexisting anti-HER2 immune response has several advantages including fewer injections for patients but the most important being the possibility of establishing a memory immune response capable of preventing disease recurrence. Consistent with this view, several clinical trials are underway in patients with stage II, III or stage IV breast, ovary, colon or non-small cell lung cancers with HER2 expression. A phase I/II randomized trial of HER2 peptide-loaded DC vaccination with or without cyclophosphamide for consolidation therapy of advanced ovarian cancer was reported in 2011. This study revealed that HER2 peptide-loaded DC vaccination elicited modest immune response. Of 11 patients receiving the vaccine, 2 recurred during vaccination, 3 patients recurred at 6, 17 and 26 months and 6 patients had no evidence of disease at 36 months. This study also showed that a single intravenous dose of cyclophosphamide ($300mg/m^2$) has no effect on the number of circulating T_{regs} despite previous evidence reported of cyclophosphamide enhancing tumor immunotherapy by eliminating T_{regs} (126-128). Immunological tolerance against HER2 is a significant obstacle to effective vaccination against this oncoprotein (129).

Generally toxicities reported with the HER2 vaccine have been commonly local reactions of erythema, induration, pruritus and inflammation. The most frequent systemic toxicities were fatigue, headache, arthralgias, myalgias, chills, bone and back pain. No additive cardiotoxicity or autoimmunity has been confirmed as a result of this vaccine therapy.

Conclusions and Future Directions

HER2 protein expression has a potential key role as both a prognostic marker and as therapeutic option in gynecologic tumors. The elucidation of genetic mutations which may modify tumor response to targeted agents and the correlation with HER2 expression is expected to provide important information for future cancer treatment. The advent of pharmaco-genomics and targeted therapy has provided the possibility for tailored tumor treatment and with molecular profiling of gynecologic tumor types comes the added potential for discovering novel and/or improved therapeutic strategies aimed at a particular gene product. Enhanced understanding of tumor heterogeneity will further facilitate a multifaceted approach to cancer treatment from the point of diagnosis with the hope of achieving a durable response.

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Table I

HER2 Targeted Clinical Trials Ongoing in Gynecologic Cancer

Intervention	Clinical Setting	Tumor Type	Phase	ClinicalTrials.gov Identifier
MGAH22 (Fc-Optimized Chimeric Anti-HER2 Monoclonal Antibody)	Progressive Disease	Non-Small Cell Lung Neoplasms Prostate Neoplasms Bladder Neoplasms Ovarian Neoplasms Breast Neoplasms	1	NCT01195935
INK128 (mTOR inhibitor-blockade of TORC1/2 signaling), paclitaxel, trastuzumab	Advanced or Metastatic Solid Tumors	Advanced Solid Malignances	1	NCT01351350
HER2 Vaccine (B cell peptide vaccine)	Metastatic Solid Tumors	Malignant Solid Tumour Breast Cancer Malignant Tumor of Colon GIST Ovarian Cancer	1	NCT01376505
Lapatinib (Dual Kinase Inhibitor) and bortezomib (Proteasomal Inhibitor)	Advanced or metastatic Solid Tumors	Advanced Solid Tumors	1	NCT01497626
Ertumaxomab (Bi-specific antibody Anti-HER2 × Anti-CD3 Antibody)	Progressive Disease	Advanced Solid Tumors	1/2	NCT01569412
TrasGEX™ (Glycooptimized trastuzumab)	Advanced or Metastatic Solid Tumors	Solid tumors	1	NCT01409343
Trastuzumab (Humanized HER2 monoclonal antibody)	Recurrent or Persistent Disease	Paget's Disease of the Vulva	2	NCT01427244
Combinations of pazopanib (vascular epidermal growth factor tyrosine kinase inhibitor) and either lapatinib (tyrosine kinase inhibitor) or trastuzumab	Refractory or Recurrent Solid Tumors	Advanced solid tumors	1	NCT01454804
^{212}Pb-TCMC-Trastuzumab (alpha particle radio immunotherapy targeted by trastuzumab antibody)	Unresponsive Disease	Breast Neoplasms Peritoneal Neoplasms Ovarian Neoplasms Pancreatic Neoplasms Stomach Neoplasms	1	NCT01384253
Carboplatin/Paclitaxel with or without Trastuzumab	Advanced or Recurrent Disease	Endometrial Cancer (USC)	2	NCT01367002
MM-111 in Combination With Multiple Treatment Regimens (bispecific antibody targeting the ErbB2/ErbB3 heterodimer)	Progressive or Recurrent Disease	Advanced Solid Tumors	1	NCT01304784
MK-2206 (AKT Inhibitor) in Combination With Weekly Paclitaxel With or Without Trastuzumab	Advanced or Recurrent Disease	Advanced Solid Tumors	1	NCT01235897

Table II

HER2 Targeting Agents and Mechanisms of Resistance

Agent	Target	Mechanism of Resistance	Factors Involved
Monoclonal Antibodies		Alterations in binding sites or tyrosine kinase domain Overexpression of alternative ErbB ligands and dimerization receptors Loss of downstream controllers Dimerization and interaction with other structurally unrelated receptors	p95HER2, MUC4 EGFR, ErbB ligands (TGFα, EGF, HB) PTEN IGF1R, MET
Trastuzumab Pertuzumab T-DM1	HER2 HER2 HER2		
Tyrosine Kinase Inhibitors		Alterations in tyrosine kinase receptor domain Acquisition of new receptor mutations or molecular abnormalities in receptor signaling pathways Activation of further downstream signaling pathways	Extracellular mutations in the tyrosine kinase domain KIT and PDGFRA receptor signaling pathway PI3K-AKT, mTOR
Lapatinib (reversible) Neratinib (irreversible) Afatinib (irreversible) Canertinib (irreversible)	HER1, HER2 HER1, HER2, HER4 HER1, HER2, HER4 HER1, HER2, HER4		
Inhibitors of Downstream Targets		Activation of further downstream signaling pathways	PI3K-AKT, mTOR, MEK, MAPK
Everolimus BKM120 BEZ-235 GS-1101 NVP-BKM120 GDC-0941 GSK458 GDC-0980 PI-103	mTOR PI3K/AKT PI3K/AKT/mTOR PI3K PI3K PI3K PI3K/mTOR PI3K/mTOR PI3K/mTOR		
HSP90 Inhibitors		Up-regulation of alternative pathways	NF-κB, MAPK
Tanespimycin Retaspimycin AUY922	hsp90 hsp90 hsp90		

Abbreviations: MUC4= mucin 4 cell surface associated glycoprotein, EGFR= epidermal growth factor receptor, TGF= transforming growth factor, HB= heparin binding, IGF1R= insulin-like growth factor-1 receptor, PDGFRA= platelet-derived growth factor receptor alpha polypeptide, mTOR= mammalian target of rapamycin, PI3K= phosphoinositide-3-kinase, AKT=protein kinase B, MAPK= mitogen activated protein kinase, HSP90=heat shock protein 90, HER=human epidermal growth factor receptor, PTEN= phosphatase and tensin homolog, T-DM1=trastuzumab-DM1, NF- κB= nuclear factor-kappaB