Symposium article

Targeting HER2 in other tumor types

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Summary
The human epidermal growth factor receptor-2 (HER2) is overexpressed/amplified in a range of tumor types including breast, ovarian, bladder, salivary gland, endometrial, pancreatic and non-small-cell lung cancer (NSCLC). HER2 is implicated in disease initiation and progression, associated with poor prognosis, and may also predict the response to chemotherapy and hormonal therapy. Anti-HER2 monoclonal antibodies (MAbs) have been designed to specifically antagonize the function of the HER2 receptor in HER2-positive tumors. Clinical phase II and III trials have demonstrated the efficacy of the humanized anti-HER2 MAb, trastuzumab (Herceptin), both as a single agent and in combination with chemotherapy in HER2-positive, metastatic breast cancer patients. However, the prevalence of HER2 overexpression/amplification in various tumor types raises the possibility of using anti-HER2 MAbs to antagonize the abnormal function of overexpressed HER2 receptors in HER2-positive tumors other than breast. Preliminary in vitro studies indicate that anti-HER2 MAbs suppress the proliferation of ovarian, gastric and NSCLC cell lines that overexpress the HER2 receptor. These results indicate that anti-HER2 MAbs may have important therapeutic significance in patients presenting with these or other human carcinomas. Clinical trials are either planned or underway to assess the therapeutic role of trastuzumab in NSCLC, bladder and ovarian cancer.

Key words: anti-HER2 monoclonal antibody, HER2, predictive marker, prognostic marker, therapeutic target

Introduction
The search for novel markers that may be of prognostic or predictive significance is of critical importance in the development of effective anti-cancer treatments. The human epidermal growth factor (HER) family is one of the most extensively investigated growth receptor families [1]. HER2 is a membrane-bound receptor with tyrosine-kinase activity [2, 3], and plays a pivotal role in oncogenic transformation and tumorigenesis by interacting with the other members of the HER family to potentiate intracellular signaling [4–6].

Overexpression/amplification of the HER2 receptor has been identified in a variety of cancer types, including carcinomas of the breast [7, 8], bladder [9–12], pancreas [13–15], non-small-cell lung cancer (NSCLC) [16–19], ovary [8, 20, 21], endometrium [22–24], colon [25], kidney [26], head and neck [27], stomach [28, 29], esophagus [30–32], and prostate [33, 34] (for a review, see [35]).

Examination of the continuously growing list of citations for HER2 overexpression reveals a range of incidences within individual tumor types. This disparity likely reflects a number of factors, including the series of cases analyzed, reagents, detection method and the scoring system employed. Another potential source of variability is contamination of the biopsy sample with surrounding stromal tissue. The high degree of variability in the level of HER2 overexpression in individual tumor types highlights the need to standardize diagnostic testing methods and employ large sample series.

Amplification of the HER2 gene is observed in a variety of tumor types and is the most common mechanism leading to HER2-protein overexpression in many tumor types [7, 8]. HER2-gene amplification appears to be an early event in the development of cancer [36]. In breast, ovarian and gastric cancers, the incidence of HER2-gene amplification correlates well with HER2 mRNA and protein overexpression [8, 20, 37]. For example, more than 90% of HER2-positive breast cancer tumors display amplification of the HER2 gene [20, 38–41]. In the small percentage of breast cancer tumors showing expression but no amplification, the role of HER2-receptor overexpression is unknown. It is proposed that, in these exceptional instances, HER2-protein overexpression may result from transcriptional or post-transcriptional dysregulation [8]. In cancers other than those in breast, ovary and stomach, the level of HER2-gene amplification is usually much lower than that of HER2 overexpression [18, 42, 43].

Slamon et al. first demonstrated that a HER2-positive status, which is detected in 20%–30% of breast cancer patients, independently predicts a reduced disease-free and overall survival [7]. The involvement of HER2 in the pathogenesis of breast cancer and the link between HER2-positive status and poor prognosis has been substantiated by a number of other studies [20, 39, 44–50]. The association between HER2-gene amplifica-
tion/receptor overexpression and poor clinical outcome is not confined to breast cancer. As discussed below, several lines of evidence exist to implicate HER2 amplification/overexpression in the pathogenesis of a number of cancer types [51].

**HER2 as a predictive factor**

The availability of gene markers to enable prediction of response to therapy would provide significant benefits in the treatment of a number of cancer types. A considerable amount of literature is available on the subject of HER2-gene amplification/receptor overexpression and its potential value in predicting response to chemotherapy and hormonal therapy. However, there is generally little concordance among results. In the case of breast cancer, patients whose tumors overexpress HER2 may develop resistance to certain hormonal and/or chemotherapeutic agents. Studies on the predictive value of HER2 status in determining response to anthracycline-based chemotherapy have yielded conflicting conclusions [52–57]. However, certain HER2-positive cell lines have been found to show greater resistance to tamoxifen and other hormonal treatments [58, 59], cisplatin [58] and taxanes [60]. The potential use of the HER2 receptor as a marker for resistance or sensitivity to chemotherapy or hormonal therapy in other cancers has also received some attention, as reviewed below, but has so far provided no definitive conclusions. It is also tempting to speculate that the HER2-related rapid proliferation may allow tumor cells to escape from treatment-induced apoptosis as well as drive a selection for resistant tumor cells.

**HER2 as a therapeutic target**

The overexpression/amplification of the HER2 receptor and its apparent prognostic and predictive value in several types of cancer coupled with its extracellular location render this receptor a novel and important therapeutic target. One particular approach to achieving a therapeutic effect in HER2-positive cancers involves targeting the HER2 receptor using humanized anti-HER2 monoclonal antibodies (MAbs) directed against the extracellular domain of HER2. Numerous studies have investigated the potential of MAbs directed towards HER2 to activate the immune system in order to block the physiologic function of the HER2-signaling network [61]. Early research efforts focussed on the development of murine MAbs, one of which (muMab4D5) was subsequently selected for further clinical development and humanization to generate trastuzumab (Herceptin) [62, 63].

The efficacy of the humanized anti-HER2 MAb, trastuzumab has been reported in metastatic breast cancer patients [64, 65]. As first-line therapy in combination with chemotherapy [65], trastuzumab provides significant increases in survival in HER2-positive metastatic breast cancer patients. Based on the reported therapeutic efficacy of anti-HER2 monoclonal antibodies in breast cancer patients, and the increasing body of evidence to suggest that the HER2 gene plays a significant role in the pathogenesis of other cancers, it is proposed that anti-HER2 MAbs may also possess therapeutic efficacy in other tumor types.

**HER2 in NSCLC**

Although lung cancer remains a leading cause of death among all malignancies in men and women, medical and surgical intervention in the past 10 years has done relatively little to improve five-year survival rates in patients with this disease [66, 67]. These facts highlight the necessity of acquiring a more complete understanding of the molecular pathogenesis of lung cancer with a view to obtaining more effective measures of diagnosis, treatment and, ultimately, prevention.

Of the two major classes of lung cancer, small cell lung carcinoma (SCLC) and NSCLC, the latter is considered to be a possible target for anti-HER2 MAb therapy. The HER2 receptor is overexpressed in NSCLC biopsy specimens but not in normal bronchial epithelium, implying a link between HER2 overexpression and lung carcinogenesis [19]. Furthermore, approximately one-third of human NSCLC cell lines derived from human lung tumors show HER2-protein overexpression, which is present in all histologic subtypes including large-cell carcinomas, squamous-cell carcinomas and adenoscarcinomas [19]. Reports indicate that approximately 20% of patients presenting with NSCLC have HER2-positive tumors [16, 17]. The potential therapeutic value of anti-HER2 MAbs in NSCLC is increased by the reported association between HER2 overexpression and clinicopathologic features; HER2 overexpression is independently associated with diminished survival rates [16, 17].

As well as being of prognostic value in NSCLC, HER2 overexpression may represent an important predictive marker for response to treatment. Unlike SCLC, most NSCLCs are typically refractory to chemotherapy at the time of diagnosis [68], and high levels of HER2 are correlated with intrinsic chemoresistance [69]. The intrinsic multidrug resistance in HER2-positive NSCLC cell lines supports the potential role of HER2 as a marker for chemotherapeutic resistance in these carcinomas.

The substantial evidence to suggest an important functional role for HER2 overexpression in NSCLC prompted Kern et al. to culture HER2-positive NSCLC cell lines in the presence of the anti-HER2 muMab, 4D5 [70]. Significantly, 4D5 elicited a dose-dependent growth-inhibitory effect in HER2-positive human NSCLC cell lines and in a non-HER2-positive bronchial epithelial cell line transfected with the HER2 gene [70]. These findings were consistent with those from earlier *in vitro* studies of muMab4D5 in HER2-positive breast
cancer cell lines [71, 72]. The authors observed that inhibition of tyrosine-kinase function using the tyrosine-kinase inhibitor, Genistein, prevented the 4D5-induced growth inhibition [70], suggesting that growth suppression was likely due to tyrosine-kinase inhibition causing changes in HER2-phosphorylation status.

The involvement of the tyrosine-kinase activity of HER2 in mediating the inhibition of cell growth in vitro by anti-HER2 MAbs has been substantiated by Zhang and co-workers [73, 74]. Emodin, another agent known to suppress HER2 tyrosine-kinase activity, was growth inhibitory in HER2-positive, but not HER2-negative, human lung (and breast) cancer cells [73, 74]. Treatment of a HER2-positive NSCLC cell line with emodin also potentiated the cytotoxicity of cisplatin and doxorubicin in vitro (Figure 1) [74]. Tyrphostin AG825, a selective tyrosine-kinase inhibitor that preferentially inhibits HER2 kinase, also influences the chemosensitivity of NSCLC cell lines [75].

In view of the estimated 13%-55% incidence of HER2 overexpression in NSCLC, these observations reinforce the potential therapeutic advantages of targeting the HER2 receptor in NSCLC and provide a rationale for clinical evaluation of trastuzumab. Investigation of the therapeutic role of trastuzumab in NSCLC is already underway; two clinical trials of trastuzumab in combination with anti-cancer agents are ongoing or planned in NSCLC. These trials aim to evaluate the efficacy of gemcitabine plus cisplatin ± trastuzumab, and paclitaxel plus carboplatin ± trastuzumab.

HER2 in ovarian cancer

Ovarian cancer is the most lethal of the gynecologic cancers and, unlike breast cancer, it has few prognostic indicators of clinical outcome. The most important prognostic parameters for ovarian carcinomas are tumor stage, histologic subtype, degree of malignancy and residual tumor after surgical treatment [76]. These, unfortunately, present an incomplete picture of the tumor biology of ovarian cancer, underscoring the particular need for novel markers that may be of prognostic or predictive significance in this cancer type. While HER2 expression has been reported in the majority of normal ovaries [77], a HER2 overexpression rate of 18%-43% above normal can be identified in ovarian tumor tissue [8, 20, 21, 37]. As in breast cancer, the level of HER2 overexpression reflects that of HER2 amplification [8].

The prognostic significance of HER2 overexpression in advanced ovarian epithelial cancer remains somewhat controversial. Slamon and co-workers reported an approximately 20%-30% HER2-overexpression rate in ovarian carcinomas and found that HER2-gene amplification, as determined by Southern blotting, and HER2 overexpression, as determined by immunohistochemistry (IHC) significantly correlated with clinical outcome [8]. The association of HER2 positivity with a worse prognosis has been confirmed by others (Figure 2) [20, 21, 37, 77, 78]. However, there is also evidence in support of HER2 overexpression having no adverse prognostic significance in ovarian cancer [79, 80].

One study has shown that HER2 overexpression may be a useful marker to identify ovarian cancer patients who are most likely to benefit from high-dose chemotherapy [78]. Meden et al. demonstrated that platinum-based chemotherapy resulted in significantly longer survival in HER2-negative, but not HER2-positive, ovarian cancer patients. The authors therefore concluded that overexpression of HER2 may be a marker of relative resistance to normal-dose chemotherapy, but at higher doses the chemoresistance may be overcome. Thus, patients presenting with HER2-positive ovarian tumors may benefit from high-dose chemotherapy. Additional studies are, however, warranted to ascertain the value of HER2 as a predictor of ovarian tumors that are resistant or responsive to specific chemotherapeutic agents or high-dose chemotherapy.

As a result of the similarities in the pathobiology of the HER2 oncogene between breast and ovarian cancer, many of the preclinical studies to evaluate the efficacy of anti-HER2 MAbs in breast cancer examined ovarian cancer in parallel. In vitro and in vivo studies revealed that anti-HER2 MAbs potentiate the cytotoxicity of
cisplatin and carboplatin in ovarian cells [81–84]. The potent tumor-suppressive activity of trastuzumab has also been confirmed in ovarian tumor xenografts in mice [85]. Significantly, there is a clear synergy between anti-HER2 MAbs and cisplatin, including the reversal of cisplatin resistance in ovarian cancer cells [83]. The increased killing of HER2-positive ovarian cancer cells cannot be attributed simply to the additive effect of the two drugs acting independently. The mechanism underlying the synergy with platinum-derived agents is postulated to involve the reduction in unscheduled DNA synthesis resulting from administration of cisplatin [83]. This synergistic therapeutic activity, termed receptor-enhanced chemosensitivity, presents a rationale for further clinical investigation of anti-HER2 MAbs in women with HER2-positive ovarian tumors. A potential therapeutic role for trastuzumab may be as an initial combination option with platinum for ovarian cancer. Indeed, clinical trials are already planned for this indication, including a randomized, phase II trial examining paclitaxel plus cisplatin/carboplatin ± trastuzumab, which is expected to start in April 2000.

HER2 in pancreatic cancer

Studies indicate that the HER2-receptor protein is synthesized in the normal exocrine pancreas and is frequently overexpressed in well-differentiated adenocarcinomas of the pancreas [14]. Furthermore, IHC analysis using anti-HER2 MAbs suggested that increased HER2 levels may be a useful prognostic marker [13, 15]. A total of 48% of 21 pancreatic cancers of ductal origin displayed HER2 overexpression, which was closely and inversely related to survival (HER2 positive 19.1 vs. HER2 negative 7.3 months, P < 0.01) [13].

HER2 in gastric cancer

Surgical resection is the only potential cure for patients presenting with gastric cancer [86]. The prognosis of gastric cancer following surgical resection is influenced by a variety of factors including tumor stage [87] and number of metastatic lymph nodes [87, 88]. The HER2 receptor is overexpressed in at least 20% of intestinal-type gastric cancers and HER2-positive tumors are known to have a poor clinical course [29, 89, 90]. Examination of HER2-receptor status may therefore facilitate identification of patients who are at increased risk for shorter survival. Using an in vitro animal model of human gastric cancer, a combination of anti-HER2 muMAbs was found to exhibit anti-proliferative activity [91]. Subsequently, a single i.v. dose of trastuzumab was shown to inhibit tumor growth by 50% in a mouse xenograft model of human gastric cancer overexpressing the HER2 receptor protein [92].

HER2 in bladder cancer

Bladder cancer patients presenting with HER2-positive tumors have a significantly lower survival rate than those with HER2-negative tumors [10, 12]. In a study of 88 patients, 26% were found to have elevated HER2 expression that correlated with advancement of tumor grade and stage. Remarkably, the five-year disease-free survival rate was 48.5% for patients with HER2-negative tumors compared with only 9.7% for HER2-positive patients [10]. Thus, the HER2 gene product appears to be useful in independently predicting a poor prognostic subgroup of patients with bladder cancer. The implication of the HER2 receptor in the pathogenesis of this disease supports clinical assessment of anti-HER2 MAbs in this cancer. A randomized, phase II trial, due to commence in June 2000, will examine gemcitabine plus cisplatin ± trastuzumab.

HER2 in head and neck squamous cell cancer

HER2 overexpression occurs in a subset of head and neck squamous cell carcinoma (HNSCC) tumors and cell lines [27]. Analysis of 11 HNSCC cell lines revealed high HER2 overexpression in 16% of samples, and moderate and low HER2 expression in 31% and 35% of samples, respectively, implying that HER2 overexpression levels are considerably variable in HNSCC cell lines. It is unclear whether HER2 amplification accompanies HER2 expression, and no significant correlation has been found between HER2 overexpression and patient survival [93, 94]. Further investigations are indicated to determine the biologic role of HER2 expression in the clinical progression of these lesions, and the potential therapeutic suitability of anti-HER2 MAbs in HNSCC.

HER2 in other solid tumors

In addition to the cancers discussed above, overexpression and the potential prognostic value of the HER2 oncogene has been reported in many other cancer types, including endometrial [95–97], osteosarcoma [98] and prostate [34]. A correlation of HER2 expression with histologic response to preoperative chemotherapy and event-free survival suggests that clinical trials of antibodies that target this receptor, such as trastuzumab, should also be considered for the treatment of osteosarcoma [98]. Similarly, HER2-gene amplification has been found to correlate with local progression in endometrial adenocarcinoma [97, 99]. Overamplification of the HER2 gene to at least five copies was significantly correlated with the histologic grade of endometrial carcinoma and vascular or lymphatic invasion, but not with overall patient survival [96]. A recent publication has reported an 11% incidence of HER2 overexpression in patients with poorly differentiated carcinoma/adenocarcinoma of unknown primary site, the majority of whom had
The authors have not reported any financial relationships with companies whose products are mentioned in the text.

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Summary and conclusion

In summary, overexpression of the HER2 receptor has been identified in a broad spectrum of human cancers. However, non-standardization of routine testing or population selection bias has generated considerable variation among the incidences of HER2 expression within individual tumor types. Breast cancer patients whose tumors overexpress HER2 are recognized as having a more aggressive disease behavior and an inferior clinical outcome compared with those who are HER2 negative. Despite the poor prognosis of these patients, trastuzumab, an anti-HER2 monoclonal antibody developed to specifically target HER2-positive tumors, has shown to be capable of signiﬁcant increases in overall survival. It is postulated that the potential for HER2-positive breast cancer patients to adopt anti-HER2 MAb therapy may be reproduced in other HER2-positive tumors in which HER2 has been implicated in pathogenesis. Although HER2 overexpression is frequently associated with poor outcomes in a number of tumor types, the value of HER2 in evaluating the prognosis of patients requires further investigation. Clinical trials already started or planned to assess the potential role for trastuzumab alone or in combination with chemotherapy in patients presenting with HER2-negative NSCLC, ovarian and bladder carcinomas.

References

5. Graus-Porta D, Behri RR, Daly JM et al. ErbB-2, the preferred heterodimerization partner of all ErbB receptors, is a mediator of lateral signaling. EMBO J 1997; 16: 1647–55.


71. Hudziak RM, Lewis GD, Winget M et al. p185HER2 monoclo-


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