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C-erbB-2 in gastric cancer: present and perspectives for new therapeutic approaches

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ABSTRACT

Nowadays, gastric cancer is considered the second leading cause of cancer mortality wroldwide. In Romania, gastric cancer is a disease tardily diagnosed, when the surgical therapy needs a more complex approach and has higher complications, thus the survival rate reduces extensively. Contrary to its considerable incidence , the management in advanced gastric cancer has relatively little evolved. Recently, an increasing interest in the molecular biological prognostic and early diagnosis factors in human gastric carcinoma appeared, the role of c-erbB-2 gene abnormalities (i.e. amplification and overexpression) being investigated in aggressive stadia with poor outcomes. The present article is a review of the existent data refering to c-erb B-2 overexpression in human advanced gastric carcinoma as a prognostic factor, diagnosis marker, specific treatment target and long term results.

Keywords: *C-erbB-2, gastric cancer, HER2, prognostic factor, Trastuzumab.*

1. INTRODUCTION

Gastric cancer is the fourth most common cancer and the second cause of cancer-related death worldwide, accounting for nearly 160 000 deaths each year in China [1-3]. Barrett's esophagus and dysplasia are associated with the development of esophageal adenocarcinoma [4], while *Helicobacter pylori* infection, atrophic gastritis, intestinal metaplasia, and dysplasia are related with gastric adenocarcinoma [3].

Surgical resection is the mainstay of treatment and can cure patients diagnosed in early-stage phase. The survival rate of patients with advanced resectable gastric or gastroesophageal junction cancers, however, remains poor despite new treatment strategies, such as perioperative chemotherapy [5] or adjuvant chemoradiation [6]. However, most gastric cancer patients are diagnosed when the tumor is at an unresectable stage. For these patients, systemic chemotherapy is the main treatment option because it prolongs survival without quality of life compromise. Many single agents and combinations are active in the treatment of metastatic disease. Objective response rates range from 10% to 30% for single-agent therapy and 30% to 60% for combination regimens [7]. Platinum compounds, fluoropyrimidines, antracyclines, and, recently, taxanes and irinotecan are the most active drugs. Although a large number of chemotherapy regimens have been tested in randomized studies, there is no internationally accepted standard of care, and uncertainty remains regarding the choice of the chemotherapy regimen [8].

Survival of patients with advanced gastric cancer treated with palliative chemotherapy remains low. Therefore a better understanding of the molecular basis of cancer is needed for the development of rationally designed molecular targeted therapies, which interfere with the signalling cascades involved in cell differentiation, proliferation, and survival. Recently, an increasing interest in the molecular biological prognostic factors in human gastric carcinoma appeared. It has been shown that amplification and overexpression of C-erbB-2 gene in different human carcinomas is associated with increased tumor angiogenesis, chaotic proliferation, decreased cell apoptosis, tumor invasion and early lymph-nodes and distant metastasis [2,9].

C-erbB-2 proto-oncogene, HER2 (human epidermal growth factor receptor 2) or HER2/neu induces the expression of a 185-kDa transmembrane tyrosine kinase receptor protein called CD340 (cluster of differentiation 340).The epidermal growth factor receptors family are found on the surface of normal different cells (breast, colon,bladder, kidney, heart). The binding of different ligands to the extracellular domain initiates a signal transduction cascade that can influence many aspects of cell biology, including cell proliferation, apoptosis, adhesion, migration, and differentiation. Ligand binding induces homodimerization as well as heterodimerization with other types of HER proteins (HER1, HER3). HER2 does not bind to any known ligand, but it is the preferred heterodimerization partner for other members of the HER family. HER2 is encoded by a gene located on chromosome 17q21 [9-10]. The HER2 gene, located adjacent to the topoisomerase IIa genes, is related to the oncogene v-erbB of the avian erythroblastosis virus. In carcinomas, HER2 acts as an oncogene, mainly because high-level amplification of the gene induces protein overexpression in the cellular membrane and subsequent acquisition of advantageous properties for a malignant cell [11].

Recent studies indicate a role of HER2 in the development of numerous types of human cancer. HER2 overexpression and/or amplification have been detected in 10%-34% of invasive breast cancers and correlate with the worse clinical outcome, poor prognosis, constituting also a predictive factor of poor response to chemotherapy and endocrine therapy [12]. HER2 overexpression and/or amplification have also been observed in colon [13], bladder [14], ovarian [15], endometrial [16], lung [17], uterine cervix [18], head and neck [19], esophageal [20], and gastric carcinomas.

Trastuzumab (Herceptin™) is a monoclonal antibody which specifically targets HER2 protein by directly binding the

extracellular domain of the receptor. Trastuzumab enhances survival rates in both primary and metastatic HER2-positive breast cancer patients [21]. The efficacy of trastuzumab in breast cancer patients has led to investigate its antitumor activity in patients with HER2-positive cancers, including gastric adenocarcinomas.

2. CAN BE CONSIDERED C-erbB-2 AMPLIFICATION A PROGNOSTIC FACTOR IN ADVANCED GASTRIC CANCER?

The most important prognostic factor for gastric cancer is the TNM stage. This classification system establishes the stage depending on the depth of invasion of gastric wall (T), the involvement of lymph nodes (N) and the presence of distant metastasis (M). Prognosis varies among patients in the same stage. Therefore, additional classification parameters need to be defined in addition to the TNM and the classic pathologic characteristics of the tumor in order to better identify the biologic subsets of this disease. Overexpression of C-erbB2 gene in gastric cancer, using immunohistochemistry (IHC), was first described in 1986 [22]. In 1990s, some studies reported a 9%-38% of HER2-positive tumors using polyclonal antibodies directed against different domains of C-erbB2 and restricting the evaluation to the staining of the cell membrane [23-24]. More recent studies, which determined HER2 overexpression by IHC using monoclonal antibody (HercepTest) and/or gene amplification by FISH (fluorescence in situ hybridization), have reported similar rates [25] (Table 1). The most specific and sensitive method of detecting C-erbB2 is PCR [26].

The role of HER2 as a prognostic factor in gastric cancer has been controversial because some of the initial studies failed to find an association with prognosis [31, 32]. A high correlation between HER2 expression and intestinal histological type was reported by several authors in 1990s [33-36].

This correlation has been confirmed in more recent studies (Table 2). In Gravalos *et al*. study it is observed a higher rate of HER2 overexpression in intestinal than in diffuse type (16% vs 7%) [37]. In the Finnish study, amplification of HER2 was strongly associated with poor carcinoma-specific survival, particularly evident in the subgroup of intestinal type of cancers (P = 0.0019) [38], which is usually considered to associate with more favorable prognosis than the diffuse type of gastric adenocarcinoma [39]. Intestinal-type cancers also exhibited higher rates of HER2 amplification than did diffuse-type cancers ($P < 0.05$) in the Korean study [29]. Finally, in the ToGA trial, HER2 positivity differed significantly by histological subtype (intestinal 34%, diffuse 6%, mixed 20%) [40].

Table 1. HER 2 expression in gastric cancer in different geographical areas.

Author	n	Geographic zone	Overexpression(%)	IHC	Amplification	Method
Yano *et al*. [25]	200	Japan	23	HercepTest	27%	FISH
Gravalos *et al*. [27]	166	Europe	13	HercepTest	If IHC 2+	FISH
Allgayer *et al*. [28]	203	Europe	91	MoAc+streptavidin-Biotin-elite kit	-	-
Park *et al*. [29]	182	Korea	16	HercepTest	Seven patients	FISH/CISH*
Lordick *et al*. [30]	1527	Europe, Asia, Latin America	22	HercepTest	-	FISH

*CISH, chromogenic in situ hybridization

Table 2. HER2 expression in gastric cancer.

Author	N	Histologic type				Localization		Method
		Intestinal(%)	Diffuse(%)	Mixed(%)	P	Gastric(%)	P	
Tanner *et al*. [37]	231	21,5	2	5	0,005	12	-	CISH
Gravalos et.al [27]	166	16	7	14	0,27	9,5	0,01	IHC,FISH
Lordick *et al*. [30]	1527	34	6	20	-	18	-	IHC,FISH

3. ANTI-HER2 TREATMENT: TRASTUZUMAB

Used first in breast cancer with C-erbB2 overexpression, Trastuzumab, a monoclonal anitibody that interferes with the HER2/neu receptor, is a target to be examined for the treatment of gastric cancer. Proposed mechanisms of trastuzumab actions include (1) inhibition of HER2 shedding, (2) inhibition of PI3K-AKT pathway, (3) attenuation of cell signalling, (4) antibody-dependent cellular cytotoxicity, and (5) inhibition of tumor angiogenesis [41]. Validated methods and scoring systems for

evaluating HER2 status exist in breast cancer, but not in gastric cancer. The aim was to establish a HER2 scoring system for gastric cancer to identify suitable patients for enrollment in a trial of trastuzumab (Herceptin) in advanced metastatic gastric cancer [42].

ToGA (Trastuzumab for Gastric Cancer) was an open-label, international, phase 3, randomised controlled trial undertaken in 122 centres in 24 countries. Patients with gastric or gastro-esophageal junction cancer were eligible for inclusion if their tumours showed overexpression of HER2 protein by immunohistochemistry or gene amplification by fluorescence in-situ hybridisation [42]. Thus, it is current practice to test all new diagnoses of gastric cancer for HER2 by IHC [43]. Tumors can be classified by IHC as IHC 0/1+, negative resulted; IHC2+, equivocal resulted and it is recommended FISH testing, and IHC3+, positive resulted [42, 44]. In the mentioned study, patients with gastric cancer or gastroesophageal junction cancer that showed HER2 overexpression were eligible for the analysis and randomized in two arms. To one arm standard chemotherapy alone (5-FU/capecitabine plus cisplatin) was administered while to the other arm it was administered chemotherapy plus transtuzumab. Median overall survival was 13.8 mo in those assigned to trastuzumab plus chemotherapy compared with 11.1 mo in those assigned to chemotherapy alone [45, 46]. The median of progression-free survival (PFS) was increased with the addition of transtuzumab to standard chemotherapy: 6.7 mo in the transtuzumab arm and 5.5 mo in the chemotherapy alone arm. The overall response rate was 47.3% *vs* 34.5% in transtuzumab plus chemotherapy and chemotherapy, respectively. The toxicity did not increased substantially with transtuzumab addition; however, the most common grade 3/4 adverse reactions associated with trastuzumab in metastatic GC were neutropenia, diarrhea, fatigue, anemia, stomatitis, weight loss, upper respiratory tract infections, fever, thrombocytopenia, mucosal inflammation, nasopharyngitis and dysgeusia. Thus, the ToGA trial showed that trastuzumab in combination with chemotherapy can be considered as a new standard option for patients with HER2-positive advanced gastric or gastroesophageal junction cancer. So, trastuzumab was approved by the Food and Drug Administration and the European Medicines Agency (EMA) for patients with HER2-positive metastatic GC or GEJ who have not received previous anticancer therapy for metastatic disease [47].

Trastuzumab has established efficacy against breast cancer with overexpression or amplification of the HER2 oncogene. The standard of care is 1 year of adjuvant trastuzumab, but the optimum duration of treatment is unknown. There were compared 2 years of treatment with trastuzumab with 1 year of treatment, and it was updated the comparison of 1 year of trastuzumab versus observation at a median follow-up of 8 years, for patients enrolled in the HERceptin Adjuvant (HERA) trial [48]. To expand these breast cancer findings of trastuzumab monotherapy into the setting of gastric cancer, a pilot study was conducted in which patients who progressed while on chemotherapy for metastatic or locally advanced HER2-positive gastric cancer were treated with trastuzumab monotherapy [49]. However, the study only involved four patients; therefore, additional studies are needed to confirm the potential of trastuzumab monotherapy. The ToGA trial indicated that starting trastuzumab in combination with chemotherapy and then continuing trastuzumab monotherapy until disease progression extended overall survival in patients with gastric cancer [40].

An important clinical issue is whether or not to continue molecular-targeted drugs upon disease progression. Currently, anticancer drugs, particularly cytotoxic drugs, are generally discontinued upon disease progression, and the patients started on subsequent aggressive treatment. Is there any evidence to support the validity of this approach, or should the molecular-targeted drug be continued [50]? No studies have examined this approach in the context of gastric cancer.

Another clinical issue reffers to the potential using trastuzumab as perioperative chemotherapy in gastric cancer. Perioperative chemotherapy is increasingly being considered as part of the treatment of various cancers, as it should allow earlier delivery of systemic treatment to the target lesion [50]. In terms of gastric cancer, Cunningham *et al*. [51] reported that a perioperative regimen consisting of epirubicin, cisplatin, and infused fluorouracil decreased tumor size and stage and improved progression-free and overall survival compared with surgery alone in patients with gastric cancer. Several case reports have documented favorable outcomes of trastuzumab as part of perioperative chemotherapy for gastric cancer [52, 53]. Both of these patients had complete pathological response after trastuzumab-based chemotherapy. Postmarketing clinical trials are now underway in Spain and Germany to examine the efficacy of perioperative adjuvant chemotherapy with trastuzumab in patients with HER2-positive gastric cancer [54]. The results of these studies should support an indication for trastuzumab as part of a perioperative chemotherapeutic regimen for treating HER2-positive gastric cancer [50].

4. NOVEL COMBINATIONS IN C-erbB2 POSITIVE GASTRIC CANCER

Novel combinations include pertuzumab (a HER2 dimerization inhibitor), lapatinib (a HER1/HER2 tyrosine kinase inhibitor), bevacizumab (an antiangiogenic agent), tanespimycin (a heat shock protein inhibitor), antiestrogen therapies, and an antibody-drug conjugate (trastuzumab-DM1).

Lapatinib is an orally active synthetic drug [55, 56] that is approved in Japan for HER2-positive breast cancer in combination with capecitabine [57]. Lapatinib inhibits HER2 signaling by blocking tyrosine kinase activity. In the Lapatinib (Tykerb) with Paclitaxel (Taxol) in Asian ErbB2+ (HER2+) Gastric Cancer Study (TYTAN), for example, patients across five Asian countries are to be randomly assigned to lapatinib (1,500 mg daily) plus paclitaxel (80 mg/m^{2}weekly) or paclitaxel alone. The primary endpoint of the study is overall survival. This study did not show an improvement in the primary endpoint. However, the efficacy of lapatinib was strongly suggested in the IHC+3 subset. These results indicate that the definition of HER2-positive gastric cancer is very important for the development of new anti-HER2 drugs [58].

T-DM1 is an antibody-drug conjugate in which trastuzumab is conjugated to a cytotoxic compound, emtansine (DM1) [59]. T-DM1 combines the mode of action of trastuzumab with the targeted delivery of a potent cytotoxic. Upon binding of the trastuzumab moiety to HER2, T-DM1 is internalized into the tumor cell, releasing the DM1 moiety, which inhibits microtubules. A trial is now underway to examine the efficacy and safety of T-DM1 compared with standard taxane therapy in patients with HER2-positive gastric cancer . In this study, patients will be randomized to one of three groups, 3.6 mg/kg T-DM1 every 3 weeks, 2.4 mg/kg T-DM1 every week, or standard taxane therapy, for at least four cycles (12 weeks). Planned endpoints include overall survival, progression-free survival, duration of response, and time to gastric cancer symptom progression, as well as safety [60].

Pertuzumab is a monoclonal antibody that prevents dimerization of HER2 with other HER receptors [61]. Its efficacy in combination with trastuzumab in patients with HER2-positive metastatic breast cancer has been demonstrated in a phase III clinical trial [62].

Another important future treatment perspective is NeuVax (Nelipepimut-S). This vaccine peptide is an immunodominant nonapeptide derived from the extracellular domain of the HER2 protein, a well established target for therapeutic intervention in breast carcinoma. The nelipepimut sequence stimulates specific CD8+ cytotoxic T lymphocytes following binding to HLA-A2/A3 molecules on antigen presenting cells. These activated specific cells recognize, neutralize and destroy through cell lysis HER2 expressing cancer cells, including occult cancer cells and micrometastatic foci [63-64]. The PRESENT phase 3 study wants to attest the vaccine′s mechanism in women breast cancer. Based on the molecular point of view it can also be a future study thema in gastric cancer HER2 positive.

5. CONCLUSIONS

C-erb B2 gene has served as a prognostic and predictive biomarker in breast and gastric/gastroesophageal cancers. Multiple therapies against HER2 appeared in the treatment of C-erb B2 overexpressing breast and gastric cancers improving the clinical outcome. Various novel HER2 directed agents alone or in combination are under investigation and in near future we will be expecting more varied implications of HER2 directed therapies. Till more robust data on the prognostic significance of HER2 in other cancers is available, HER2 testing and HER2 directed therapies are recommended in breast and gastric/gastroesophageal cancers.

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