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REVIEW

Personalizing therapies for gastric cancer: Molecular mechanisms and novel targeted therapies

Michael Luis, Ana Tavares, Liliana S Carvalho, Lúcio Lara-Santos, António Araújo, Ramon Andrade de Mello

Michael Luis, António Araújo, Ramon Andrade de Mello, Gastrointestinal Unit, Service of Medical Oncology, Portuguese Oncology Institute, 4200-072 Porto, Portugal
Michael Luis, Ana Tavares, Lúcio Lara-Santos, Gastrointestinal Unit, Experimental Pathology and Therapeutics Group, Research Center-Portuguese Oncology Institute, 4200-072 Porto, Portugal
Liliana S Carvalho, Department of Pharmacology and Therapeutics, Faculty of Medicine, University of Porto, 4200-319 Porto, Portugal
Liliana S Carvalho, Institute of Cellular and Molecular Biology, 4150-180 Porto, Portugal
Lúcio Lara-Santos, Gastrointestinal Unit, Section of Surgical Oncology, Portuguese Oncology Institute, 4200-072 Porto, Portugal
Ramon Andrade de Mello, Department of Medicine, Faculty of Medicine, University of Porto, 4200-319 Porto, Portugal
Author contributions: All authors contributed as the same for this manuscript preparation.
Correspondence to: Ramon Andrade de Mello, MD, PhD, Professor, Gastrointestinal Unit, Service of Medical Oncology, Portuguese Oncology Institute, Rua Dr. António Bernardino de Almeida, 4200-072 Porto,
Portugal. ramondemello@gmail.com
Telephone: +351-2250-84000-7323 Fax: +351-2250-84010
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Abstract

Globally, gastric cancer is the 4th most frequently diagnosed cancer and the 2nd leading cause of death from cancer, with an estimated 990000 new cases and 738000 deaths registered in 2008. In the advanced setting, standard chemotherapies protocols acquired an important role since last decades in prolong survival. Moreover, recent advances in molecular therapies provided a new interesting weapon to treat advanced gastric cancer through anti-human epidermal growth factor receptor 2 (HER2) therapies. Trastuzumab, an anti-HER2 monoclonal antibody, was the first target drug in the metastatic setting that showed benefit in overall survival when in association with platinum-5-fluorouracil based chemotherapy. Further, HER2 overexpression analysis acquired a main role in predict response for trastuzumab in this field. Thus, we conducted a review that will discuss the main points concerning trastuzumab and HER2 in gastric cancer, providing a comprehensive overview of molecular mechanisms and novel trials involved.

Key words: Gastric cancer; Human epidermal growth factor receptor 2; Biomarkers; Target therapies; Trastuzumab; Lapatinib; Pertuzumab; Trastuzumab-DM1; Afatinib

Core tip: Advanced gastric cancer is a very aggressive disease thought the standard chemotherapy protocols available. In 2010, Trastuzumab for Gastric Cancer trial showed that the combination of trastuzumab, could be considered a new standard option for patients with human epidermal growth factor receptor 2 (HER2) positive advanced gastric and gastro-esophageal junction cancer. Thus, a new era emerged for those patients due to the interesting possibility in prolong survival in a personalized setting (HER2 positive). Our manuscript will provide an overview of the molecular mechanisms involved and also promising targeted therapies in this field.

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INTRODUCTION

Gastric cancer has been described since 3000 BC in ancient Egypt. One of the first epidemiologic studies on cancer, with data spanning from 1760 to 1839, pointed gastric cancer as the most common and lethal. In modern times, it remains one of the most important forms of cancer, with different geographic, ethnic and socioeconomic distributions. Incidence is particularly high in Japan, China, South Korea, Chile and Costa Rica. The large regional variations in incidence possibly reflect different prevalences of *Helicobacter pylori* infection, which is responsible for more than 60% of gastric cancer globally. Globally, gastric cancer is the 4th most frequently diagnosed cancer and the 2nd leading cause of death from cancer, with an estimated 990000 new cases and 738000 deaths registered in 2008[1]. The human epidermal growth factor receptor 2 (HER2) protein, a 185 kDa protein (p^{185}) encoded by a gene located on chromosome 17q21 is a transmembrane tyrosine kinase receptor with an extracellular ligand-binding domain; a short transmembrane domain and an intracellular domain with kinase activity (Figure 1). It belongs to the epidermal growth factor receptor (EGFR) family of growth factors comprising four structurally related members, HER1 or ErbB1, also known as EGFR, HER2 or ErbB2, HER3 or ErbB3 and HER4 or ErbB4. Activation occurs through homo- or heterodimerization induced by ligands. HER2 is designated an orphan receptor which is believed to homodimerize independently of a ligand or to heterodimerize with another ligand-bound member of the EGFR family. Activation triggers a cascade of events that involves autophosphorilation and activation of the tyrosine kinase domain, Ras/Raf/mitogen-activated protein kinase pathway, phospholipase C-γ and phosphatidylinositol-3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) (Figure 2). HER2 receptors have also been found in nuclear localization, where they act as transcription factors for cycline D1 and p53[2,3]. Therefore, *HER2* (also known as *c-erbB-2/neu*) acts as an oncogene involved in the regulation of cell proliferation, differentiation, motility and apoptosis[4-8]. Heterodimers of HER2 with other members of the HER family, particularly with HER3, are the most mitogenic dimers and HER2 increases the affinity of EGFR, HER3 and HER4 to their ligands[9-12].

Analysing the molecular structure of HER2 allows new insights into approaching it as a potential therapeutic target (Figures 1 and 3). The extracellular domain of the receptor is subdivided into four subdomains. Whereas subdomains Ⅱ and Ⅳ are involved in the process of dimerization, subdomains Ⅰ and Ⅲ are the binding sites for pertuzumab and trastuzumab respectively, two of the most well studied HER2 inhibitors which will be discussed further on. The transmembrane domain of HER2 plays an important role in the process of dimerization and oncogenic mutations in this region were described. The intracellular domain contains the active enzyme site which activates different downstream pathways by phosphorylation[13-16].

The importance of addressing HER2 as a therapeutic target is underscored by a number of molecular and pathological findings. Amplified HER2 relates to processes of carcinogenesis and adverse pathologic features such as tumor size, invasion and metastastatic spread; the level of *HER2* gene expression is much higher in cancer cells than that in non-malignant adult cells[17]. HER2 overexpression has been reported in breast, lung, salivary gland, ovary, colon, prostate and pancreatic cancers[18,19].

About 10%-34% of invasive breast cancers present HER2 overexpression. Trastuzumab has shown survival advantage in early and metastatic disease and is now a part of standard care. HER2 overexpression stands as a poor prognosis marker for chemo- and endocrine therapy but at the same time as a positive predictive marker for treatment with trastuzumab. Furthermore, trastuzumab proved to be effective as adjuvant treatment in breast cancer with HER2 overexpression, with different chemotherapy regimens[20-26]. In gastric cancer, the prognostic role of HER2 overexpression remains controversial. The most important prognostic factor for gastric cancer is the tumor node metastasis (TNM) stage[20,27]. Initial works addressing the prognostic significance of HER2 overexpression reported a negative effect on overall survival (OS)[28,29]. However, conflicting results regarding the prognostic value of HER2 have been published more recently. Some studies found a negative effect of HER2 on prognosis with reduction in OS[17,20,29-36], others found no relationship[37-40] and a trend towards improved survival was found in one cohort[41]. A comprehensive review by Jørgensen *et al*[42] found that the majority of publications that fulfilled the selection criteria for the analysis, associated HER2-positive status with poor survival and clinicopathological characteristics such as serosal invasion, lymph node metastases, disease stage or distant metastases. Chua *et al*[43] recently reviewed 49 studies with data regarding the relation of HER2 with clinicopathological variables and survival and concluded that HER2 overexpression is associated with poor survival; results pertaining other variables were not conclusive. HER2 overexpression has also been suggested as a molecular abnormality in the development of intestinal type gastric cancer and HER2 expression increases with disease progression, leading to the suggestion that the initial timing of this event probably occurs in early stages. Barros-Silva *et al*[20] found overexpression and amplification in both components of mixed tumors (intestinal and diffuse components) and *HER2* amplification in early stages, supporting this idea of amplification in an early stage of carcinogenesis. Further support arises from the high levels of concordance between primary tumors and paired metastatic sites found by some authors, suggesting HER2 amplification as an early event and not acquired at a later moment by cells with metastatic potential[44]. Kataoka *et al*[45] found no HER2 positivity in the diffuse component of mixed type cases, but also found HER2 overexpression in early TNM T1a cases, pointing towards an early event[30,46]. Although these data tend to establish

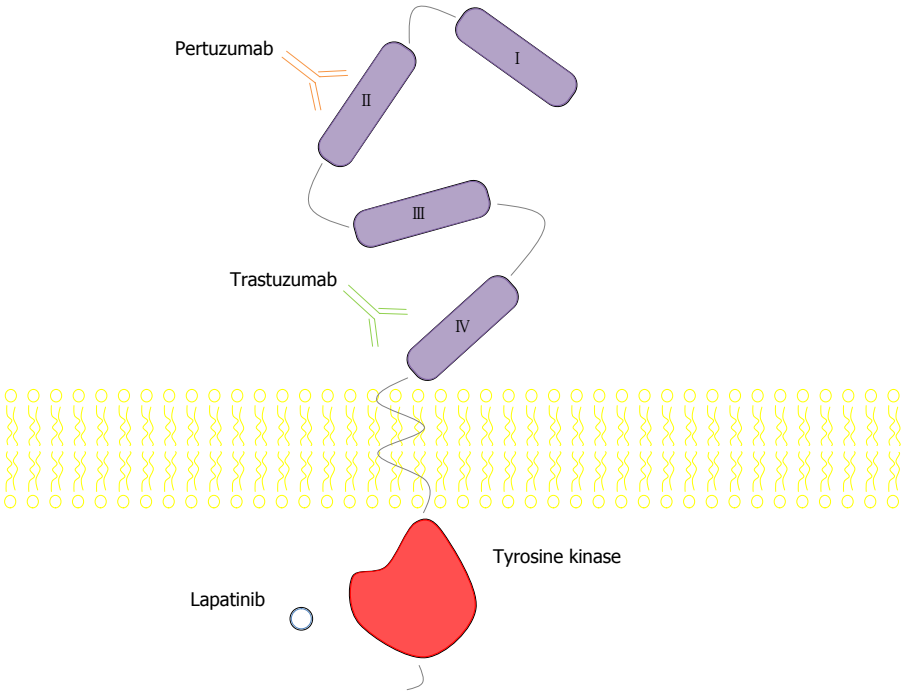

Figure 1 Human epidermal growth factor receptor 2 and binding sites. Ⅰ-Ⅳ: Epidermal growth factor receptor 1-4.

HER2 as a potential negative prognostic factor in gastric cancer, the relation seems not to be as consistent as in breast cancer[42]. In fact, more recent studies demonstrate no significant prognostic relationship. In a study involving 381 metastatic gastric cancer patients, Yanjigian *et al*[47] found that patients with HER2-positive gastric cancer had longer median OS compared with HER2-negative gastric cancer patients, but on multivariate analysis HER2 status was not an independent prognostic factor. Terashima *et al*[48] found no correlation with OS in 829 stage Ⅱ/Ⅲ resected gastric cancer patients. Hsu *et al*[49] investigated 1036 gastric cancer patients undergoing curative-intent resection. Although HER2 positivity emerged as a favourable prognostic factor for stage Ⅲ-Ⅳ gastric cancer on univariate analysis, it did not on multivariate adjustment.

Despite these conflicting results, it seems likely that HER2 is not associated with an adverse prognosis in gastric cancer in an extent similar to breast cancer; nevertheless, inhibition of the HER2 pathway in patients with HER2 amplification demonstrated clinical benefits. In this review, we will address the main advances in the treatment of advanced gastric cancer, focusing on the novel biomarkers and target therapies concerning HER2 signalling pathways.

HER2 TESTING IN GASTRIC CANCER

A precise analysis is necessary in order to address the status of HER2 expression in gastric cancer, which constitutes an essential step to select the patients feasible to treatment with HER2 target therapies. Techniques include primarily immunohistochemistry (ICH) and *in situ* hybridization (ISH), which constitute standard techniques also used in the current practice of HER2-status determination in breast cancer. Current evidence suggests the need to adopt the methods used in breast cancer in order to address HER2 expression in gastric cancer[50]. Considering the different biologic origin of the tissue, the high density of glandular structures needs to be understood in its context. In gastric tissue, ICH staining for HER2 occurs typically on the basolateral membrane and less so on the luminal aspect of the cell, conferring an U-shaped appearance to the staining whereas completeness of the membrane staining is the rule for higher scores in breast cancer. Another difference concerns the heterogeneity of immunostaining which is rare in breast, but frequent in gastric tumors. ICH should be used as primary test; cases with ICH score 3+ are candidates for HER2 directed therapy, 2+ scoring cases should be re-tested using ISH; in the case of ISH positivity patients are eligible for these therapeutic modality[51].

HER2-DIRECTED THERAPIES IN GASTRIC CANCER

In January 2010, the European Medicines Agency granted approval to trastuzumab plus chemotherapy in the treatment of with IHC 3+ or 2+/metastatic adenocarcinoma of the stomach or gastro-esophageal junction (GEJ)[52]. The United States Food and Drug Administration approved trastuzumab for HER2 overexpressing patients, without further specification[53].

Trastuzumab is a fully humanized monoclonal antibody that binds to the extracellular domain of the receptor, acting by blockage of the HER2 receptor cleavage, inhibition of dimerization, as well as by the induction

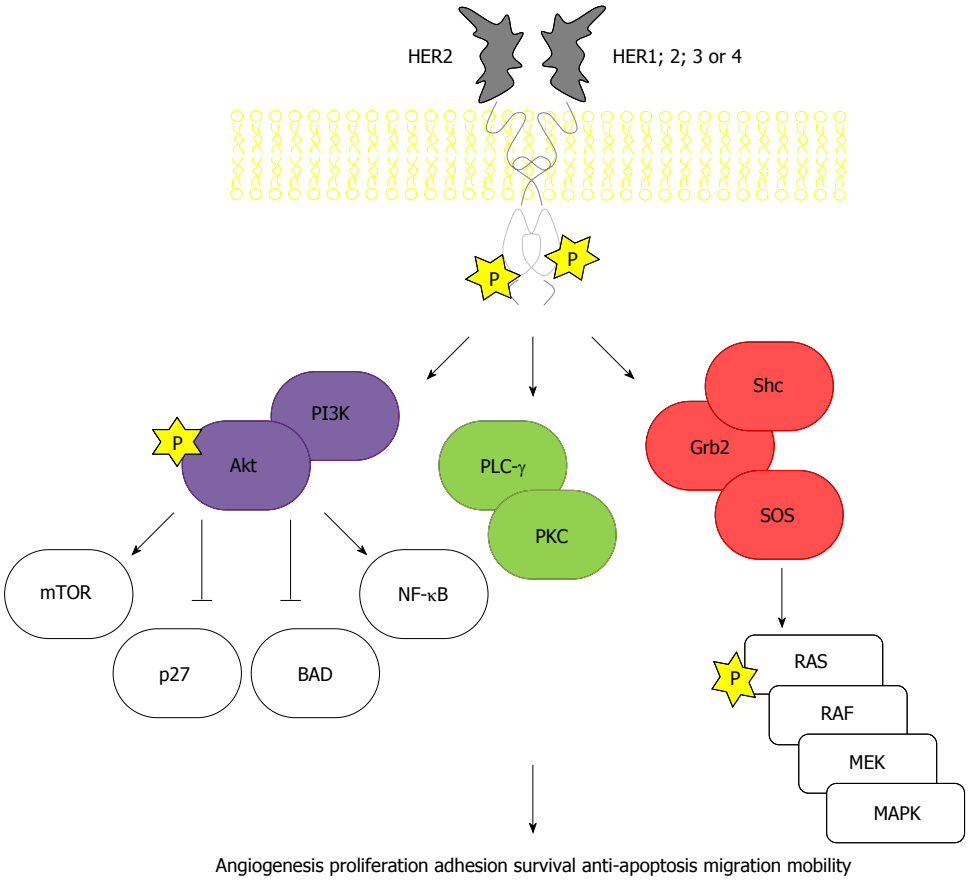

Figure 2 Human epidermal growth factor receptor 2 signalling pathways. HER: Human epidermal growth factor receptor; PI3K: Phosphoinositide 3-kinase; BAD: Bcl-2-associated death promoter protein; NF-κB: Nuclear factor κB; PLC-γ: Phospholipase C gamma 1; PKC: Protein kinase C; Grb2: Growth factor receptor-bound protein 2; SOS: Son of Sevenless; MEK: Mitogen-activated protein kinase kinase 1; MAPK: Mitogen-activated protein kinase; mTOR: Mammalian target of rapamycin; RAS: Rat sarcoma viral oncogene; RAF: Rapidly accelerated fibrosarcoma.

of antibody-dependent cellular cytotoxicity (ADCC), increasing endocytosis of the receptor and possibly through anti-angiogenic effects[54-56]. It was developed in the 1990s, after murine monoclonal antibodies directed to the extracellular domain of HER2 were produced and evaluated in cell lines and xenografts[57-59].

Preclinical data

Overexpression of HER2 in gastric cancer cells was first reported in 1986 by Sakai *et al*[60] and Fukushige *et al*[61]. Preclinical models of gastric cancer were successful in demonstrating the inhibitory effect of trastuzumab on human gastric cancer cell lines *in vitro* and in mice xenografts *in vivo*, with additive and synergistic antineoplastic effects in combination with chemotherapy[59,62-65]. A study by Tanner *et al*[17] points out a gastric cancer cell line that was as sensitive to trastuzumab as a breast cancer cell line, both of them with amplified *HER2*, while Matsui *et al*[63] reported suppression of tumor growth in a xenograft model. Enhanced antineoplastic effects were observed with capecitabine, cisplatin, docetaxel, paclitaxel and irinotecan[62], and a further synergistic effect with cisplatin has been found by Kim *et al*[64].

Clinical data

Although information on the specific pathways involved is scarce, *HER2* has been shown to be amplified in gastric cancer and HER2 is progressively regarded as an important biomarker and driver of cancerization in gastric cancer, with studies pointing out amplification or overexpression in 7%-34% of tumors, mainly in the intestinal type and in GEJ and proximal tumors[17,27,66].

Cortés-Funes *et al*[67] presented preliminary results of a phase Ⅱ study involving 21 chemothreapy-naïve patients with HER2 overexpressing locally advanced or metastatic gastric cancer. Trastuzumab at a loading dose of 8 mg/kg and maintenance dose of 6 mg/kg and cisplatin 75 mg/m^2 were administered every 21 d until progression, unacceptable toxicity or withdrawal of consent. Response rate was of 35%, with 17% of patients achieving stabilization. The tolerability profile was favourable; no grade 4 toxicity was observed and most the frequent grade 3 events were asthenia, nausea or vomiting, diarrhea, hiporexia and neutropenia. Data from another preliminary phase Ⅱ study involving 16 gastric cancer patients were presented by Egamberdiev *et al*[68]. Trastuzumab 6 mg/kg was administered once in addition to cisplatin 100 mg/m^2 during 3 d + fluorouracil (5-FU) 1000 mg/m^2 3 d + leicovirin 100 mg/m^2 3 d, every 3 wk. Authors reported an objective response rate of 54.5% in the combined therapy group *vs* 33.3% in the chemotherapy-only group and a median remission duration of 8.3 mo *vs* 5.2 mo. In a recent phase

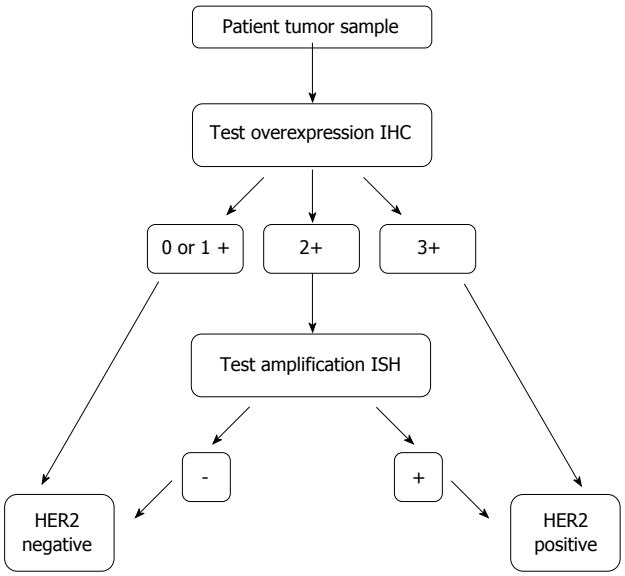

Figure 3 Human epidermal growth factor receptor 2 testing algorithm. IHC: Imunohistochemistry; HER2: Human epidermal growth factor receptor 2; ISH: *In situ* hybridization.

Ⅱ study carried out by Grávalos *et al*[69], chemo-naïve patients with non-resectable advanced or metastatic gastric or GEJ adenocarcinoma overexpressing HER2 were treated with trastuzumab 8 mg/kg as loading dose and 6 mg/kg in subsequent cycles + cisplatin 75 mg/m^2 every 3 wk. Twenty-two out of 228 patients (9.6%) enrolled had HER2 overexpression. An overall response rate of 32% was found, with disease control achieved in 64% of patients; median time to progression was 5.1 mo. No grade 4 toxicities occurred, whereas most frequent grade 3 adverse events were asthenia, neutropenia, anorexia, diarrhoea and abdominal pain. Interestingly, higher baseline HER2 extracellular domain levels associated with better response to therapy.

In more recent studies, HER2 overexpression was found to be lower than previously reported, especially in distant gastric cancers[70]. Resectable gastric cancer has reported HER2-positive ratios of 8.1% and 11.7%, suggesting that in resectable gastric cancer HER2 positive status might be less frequent than in advanced gastric cancer[71,72].

The phase Ⅲ ToGA trial constitutes a milestone, establishing trastuzumab as the first biological therapy that demonstrated survival benefits in gastric cancer[62,63]. ToGA was a multicenter, international trial, undertaken in 24 countries[73]. It evaluated the combination of trastuzumab with standard chemotherapy (cisplatin + either capecitabine or 5-FU) in advanced (inoperable locally advanced, recurrent or metastatic) HER2-positive gastric cancer as a first-line therapy *vs* chemotherapy alone. Patients were treated with six cycles of chemotherapy in both treatment arms, with patients in the experimental arm continuing to be treated with trastuzumab until disease progression. Cisplatin 80 mg/m^2 was given on day 1 by intravenous infusion. Capecitabine 1000 mg/m^2 was given orally twice a day for 2 wk followed by a 1-wk rest or 5-FU 800 mg/m^2 per day was given by continuous infusion on days 1-5 of each cycle. Trastuzumab was given intravenously at a loading dose of 8 mg/kg on day 1 of the first cycle, followed by 6 mg/kg afterwards.

The primary objective of the study was to compare OS in both arms, and the secondary objectives were to compare progression-free survival (PFS), time to progression, overall response rate, disease control, duration of response and quality of life between the two treatment arms. Among 3665 tumor tissue specimens screened for HER2 positivity, 22% were HER2 positive (34% of the intestinal type *vs* 6% of diffuse and 20% of mixed types). Assessment was done with IHC and fluorescence ISH (FISH), according to Figure 3. The highest rate was observed in 34% of GEJ cancer and 20% of gastric cancer samples[74], which is in conformity with other studies where positivity rates for the GEJ are between 24%-35% and in gastric cancer samples comprise 9.5%-21.0%[17,27,75-77].

The combination of trastuzumab with chemotherapy in advanced HER2-positive cancer patients led to significantly better OS compared to the same chemo-therapeutic regimen alone (median OS in the combination therapy group was 13.8 mo against 11.1 mo in the chemotherapy-alone group). This effect was observed in patients with intestinal type gastric cancer but not in those with diffuse type gastric cancer[73,78]. Median PFS (6.7 mo *vs* 5.5 mo) and radiological response rate (47% *vs* 35%,) also improved with trastuzumab therapy. Exploring these data further, a sub-group analysis of the ToGA study which excluded patients with IHC 0-1+ FISH+ disease, found a main gain in median survival of 4.2 mo, comparable to the figures in breast cancer[28]. In fact, patients with strongest HER2 expression (IHC 3+ FISH+) gained the greatest benefit, with a median survival of 17.9 mo in patients treated with trastuzumab *vs* 12.3 mo with chemotherapy alone.

A summary of selected clinical trials of trastuzumab in gastro-esophageal cancer can be found in Table 1.

Adjuvant treatment

In this behalf, it is important to consider the possible benefits of trastuzumab in the adjuvant setting for earlier stages of the disease; however activity of targeted therapeutics in advanced disease should not automatically be extrapolated into the adjuvant setting, as results may be misleading[79]. Trials have been initiated which intend to investigate anti-HER2 therapeutics in this setting[80,81]. Early onset gastric cancer (presenting at or under the age of 45) seems to have lower HER2 overexpression than in late onset cases, with possible different molecular genetic pathways[82-84]. Ongoing clinical trials with trastuzumab can be found in Table 2.

Maintenance therapy

From a clinical perspective, data known from breast cancer suggest that trastuzumab administration after disease progression might have benefits in OS[23,85]. In an observational of study of 623 patients, median time to progression was longer in patients who continued trastu-

Table 1 Selected clinical trials of trastuzumab in gastro-esophageal cancer

Ref.	Phase	Treatment	*n*	OS (mo)	PFS (mo)	RR	CR	PR
Bang *et al*[73]	Ⅲ	5-FU + cisplatin or capecitabine + cisplatin	290	11.1	5.5	34.50%	NA	NA
		Trastuzumab + 5-FU + cisplatin or trastuzumab + capecitabine + cisplatin	294	13.8	6.7	47.30%	NA	NA
Cortés-Funes *et al*[67]	Ⅱ	Trastuzumab + cisplatin	21	NA	NA	41.10%	5.80%	35.00%
Egamberdiev *et al*[68]	Ⅱ	Trastuzumab + leucovirin + cisplatin + 5-FU	16	NA	8.3	54.50%	NA	NA
		Leucovirin + cisplatin + 5-FU	18	NA	5.2	33.30%	NA	NA
Grávalos *et al*[69]	Ⅱ	Trastuzumab + cisplatin	22	NA	5.1	32.00%	NA	NA

OS: Overall survival; PFS: Progression-free survival; 5-FU: 5-fluorouracil; RR: Response rate; CR: Complete response; PR: Partial response; NA: Not available.

Table 2 Clinical trials with trastuzumab-based combination therapies

Setting, therapy line	ID	Phase	*n*	Treatment combined with trastuzumab	Primary EP	Status
Operable disease	NCT01196390	Ⅲ	480	Carboplatin, paclitaxel, radical radiotherapy	PFS	Recruiting
	NCT01472029	Ⅱ	53	5-FU, leucovorin, docetaxel, oxaliplatin	Rate of CR	Recruiting
	NCT01130337	Ⅱ	45	Oxaliplatin, capecitabine	PFS	Active, not recruiting
Advanced first line	NCT01450696	Ⅲ	400	Cisplatin, capecitabine	OS	Recruiting
	NCT01503983	Ⅱ	51	Oxaliplatin, capecitabine	OS	Recruiting
	NCT01461057	Ⅱ	30	Cisplatin, capecitabine, pertuzumab	Safety	Active, not recruiting
	NCT01396707	Ⅱ	56	Oxaliplatin, capecitabine	RR	Recruiting
	NCT01364493	Ⅱ	51	Oxaliplatin, capecitabine	RR	Recruiting
	NCT01359397	Ⅱ	80	Docetaxel, oxaliplatin, capecitabine, bevacizumab	PFS	Recruiting
	NCT01228045	Ⅱ	30	Cisplatin, S-1	RR	Unknown
	NCT01191697	Ⅱ	36	Oxaliplatin, capecitabine, bevacizumab	RR	Recruiting
Advanced second line	NCT01402401	Ⅱ	48	AUY922	RR	Terminated

ID, NCT identification (information available at http://clinicaltrials.gov, as accessed June 28, 2013). OS: Overall survival; PFS: Progression-free survival; 5-FU: 5-fluorouracil; RR: Response rate; CR: Complete response; PR: Partial response; NA: Not available.

zumab beyond progression than in those who stopped (10.2 mo *vs* 7.1 mo)[86]. Data from an interventional study involving 156 patients revealed OS rates of 20.4 mo *vs* 25.5 mo and response rates of 27.0% *vs* 48.1% in patients who stopped and continued trastuzumab beyond progression, respectively. Continuation of trastuzumab beyond progression was not associated with increased toxicity[87]. However, the issue is still a matter of debate, as increasing therapeutic options pose a challenge on the best possible sequencing and combinations of these interventions[88-90].

Perioperative treatment

Perioperative chemotherapy regimens have shown promising results in gastric cancer. The MAGIC trial randomized over 500 patients to either surgery alone or perioperative chemotherapy consisting of epirubicin, cisplatin and fluorouracil (3 cycles before and 3 cycles after surgery). This triplet therapy demonstrated a decrease in tumor size and improved PFS and OS in comparison with surgery alone[91,92]. In addition, some data indicate that response to neoadjuvant treatment is a major predictive factor of survival after curative surgical resection[93].

Although there is no trial so far reporting results on the role of trastuzumab in the neoadjuvant setting, a number of case reports with trastuzumab-containing neoadjuvant chemotherapy regimens have been published, with interesting outcomes; complete pathological responses were attained in 2 cases and a partial response with tumor mass reduction allowing for an extensive surgery in another case[94-96].

Pharmacokinetics and pharmacodynamics

Most data regarding the pharmacokinetic and pharmacodynamic profiles of trastuzumab stem from studies in breast cancer. A low systemic clearance (5.15 ± 2.45 mL/kg per day) and volume of distribution (44 mL/kg) have been described. Serum minimum concentrations of 10 μg/mL are needed to attain anti-proliferative effects and ADCC. With the usual loading dose of 4 mg/kg followed by 2 mg/kg per week, trastuzumab achieves and maintains serum minimum concentrations greater than 20 μg/mL. Recent results demonstrate that trastuzumab 6 mg/kg every 3 wk lead to the same plasma trough levels as trastuzumab 2 mg/kg weekly. Trastuzumab has been found not to exhibit dose-related nonlinear pharmacokinetics and the value of half-life of trastuzumab has an estimated value of 28.5 d[97,98]. No relevant drug interactions have been reported to date and elimination pathways remain largely unknown[99]. An extensive review about the pharmacodynamic and pharmacokinetic profiles, tolerability and dosage of trastuzumab in gastric cancer has been elaborated by Croxtall *et al*[100]. Targeted delivery systems involving anti-HER2 antibody mediated nano-scaled systems, drug conjugates, and fusion proteins are under active investigation[4,101,102].

Safety

The most commonly described adverse events with trastuzumab are infusion-related, described as fever, rigors, chills, nausea, dyspnea, and hypotension, and are present in about 40% of patients after the first administration and in 5% with subsequent treatment[103]. Trastuzumab has been extensively evaluated in breast cancer with a wide range of chemotherapeutic agents showing no significant overlapping toxicity, with one important exception, regarding an increased risk of cardiotoxicity. Trastuzumab-related cardiac dysfunction is largely reversible on withdrawal of the antibody. However, significant cardiopathy such as valvular heart disease, angina pectoris, previous transmiocardial infarction and heart failure with left ventricular ejection fraction (LVEF) < 50% are generally regarded as counter-indications for trastuzumab use[28]. With the chemotherapy doublet regimen evaluated in the ToGA trial, trastuzumab contributed with little added toxicity; no increase in chemotherapy related grade 3-4 toxicities (68% both arms) or cardiac events (6% both arms) were found. Nonetheless the number of patients with cardiac dysfunction (considered a ⩾ 10% drop in LVEF to an absolute value < 50%) was low in both arms (5% trastuzumab + chemotherapy *vs* 1% chemotherapy alone). The European Society for Medical Oncology[104], issued a statement regarding the cardiac monitoring of patients receiving trastuzumab. Clinical evaluation and assessment of cardiovascular risk factors and comorbidities should be performed in every patient proposed for treatment with trastuzumab[105]. While screening algorithms for trastuzumab-induced cardiomyopathy provide guidance, patient-based strategies of surveillance remain important. Many clinical trials involving patients with metastatic breast cancer include a screening study to document the baseline LVEF, followed by serial monitoring at 8- to-16-wk intervals[106].

In the ToGA trial, serious adverse events were reported in 32% of patients treated with trastuzumab + chemotherapy and 28% in the chemotherapy group; with treatment-related mortality of 3% and 1% respectively. The adverse events were similar between both groups, with no difference in the overall rate of adverse events. Nausea, neutropenia, vomiting, and anorexia were the most frequently reported adverse events. Patients treated with trastuzumab + chemotherapy had slightly higher rates of diarrhoea, stomatitis, anemia, thrombocytopenia, fatigue, chills, weight loss, pyrexia, mucosal inflammation, and nasopharyngitis[73]. In a phase Ⅱ study with trastuzumab and cisplatin as first-line therapy in GEJ and gastric cancer, trastuzumab showed a favourable toxicity profile[69].

Resistance to trastuzumab

Whilst data regarding mechanisms of resistance to trastuzumab in gastroesophageal cancer is scarce, important information can be retrieved from previous knowledge in the treatment of breast cancer. Primary resistance to single-agent trastuzumab in HER2-overexpressing metastatic breast carcinomas is described in 66%-88% of cases, with resistance eventually ensuing after a relatively short treatment period; in fact, the majority of patients who achieve an initial response to trastuzumab-based regimens develop resistance within 1 year (PFS between 6.7 and 7.4 mo)[85,107-109].

Proposed resistance mechanisms include aberrations in the PI3K/AKT/mTOR pathway with or without loss of the phosphatase and tensin homologue protein (PTEN) tumor suppressor gene, accumulation of truncated forms of the HER2 receptor that lack the extracellular trastuzumab-binding domain (collectively known as p95HER2), loss of phosphatase, activation of other tyrosine kinase receptors including the insulin-like growth factor receptor (IGF-1R), increased expression of membrane-associated glycoprotein (MUC1 and MUC4) and cyclin E overexpression[85,109-111].

PTEN inhibits PI3K, thereby inhibiting the PI3K/AKT/mTOR pathway. Loss of this tumor suppressor gene leads to at least partial resistance to trastuzumab. Indeed, both PIK3 mutations and PTEN loss were associated with inferior PFS and OS in a retrospective study of 256 HER2-positive metastatic breast cancer patients treated with trastuzumab[112]. A potential role for PI3K, AKT or mTOR inhibitors seems to exist, since these agents preclude the constitutive activation of this pathway, reversing PTEN loss-induced trastuzumab resistance[113-116].

Truncated forms of HER2 which arise through the proteolytic shedding of the extracellular domain of full-length HER2 or by alternative translation initiation from two methionine residues are the predominant HER2 forms in some tumors. The biological function of p95HER2 has not been fully characterized, though overexpression of p95HER2 has been shown to lead to growth of tumor xenografts in nude mice. The p95HER2 protein has kinase activity, and this activity is required for tumor growth; however, the mechanisms involved and its possible relationship with those used by full-length HER2 are still unknown. Importantly, since p95HER2 lacks the binding site for trastuzumab, it conveys resistance to this antibody. p95HER2 is expressed in approximately 30% of HER2-positive breast tumors and is correlated with poor disease-free survival and increased nodal metastasis when compared with patients that express full-length HER2[110,117]. p95HER2 can therefore be seen as a prognostic and predictive biomarker in breast cancer. In one study analysing 93 metastatic breast tumors, patients overexpressing p95HER2 were found to have a higher incidence of lung metastases and had significantly shorter PFS and OS with trastuzumab treatment in comparison with patients expressing only the full-length receptor[118]. Tumors that express p95HER2 may be resistant to trastuzumab but sensitive to the inhibitory effects of lapatinib, a low-molecular-weight dual tyrosine kinase inhibitor (TKI) of HER1/2 that has activity in patients with HER2-expressing tumors that are resistant to trastuzumab. Combination of trastuzumab

with lapatinib has been evaluated in women with HER2-positive, trastuzumab-refractory metastatic breast cancer. Lapatinib with trastuzumab was superior to lapatinib alone in clinical benefit: complete response, partial response, and stable disease for ⩾ 24 wk was observed in 24.7% of patients in the combination arm *vs* 12.4% in the monotherapy arm[119,120]. According to some authors this combination could provide a chemotherapy-free option after first line chemotherapy + trastuzumab[109].

Increased signalling through other receptor TKIs including EGFR, HER3, MET and IGF-1R has been found in cells resistant to HER2-targeting treatments[109]. PI3K/AKT/mTOR pathway activation through up-regulation of HER3 signalling was demonstrated after exposure of breast cancer cells to HER TKIs[121]. On the other hand, pertuzumab, a HER2-HER3 dimerization inhibitor has demonstrated activity against trastuzumab resistant breast cancer cells[122]. Taking this findings into account, HER3 seems to play an important role in the mechanism of trastuzumab resistance.

In preclinical studies, co-expression of HER2 and IGF-1R in breast cancer cells resulted in loss of sensitivity to trastuzumab, conversely, inhibiting ligand-mediated activation of IGF-1R restored sensitivity to trastuzumab, therefore pointing towards a possible strategy to reduce or delay trastuzumab resistance[123,124].

Overcoming resistance to trastuzumab

Strategies to overcome trastuzumab resistance imply the important fact that many HER2-positive gastric tumors retain dependency on downstream signalling *via* the HER2 pathway. Therefore, besides other anti-HER2 agents (described in the following section), a focus on targeting these downstream signalling molecules has emerged[125,126]. Implied targets include mTOR inhibitors, HSP90 inhibitors and MET inhibitors; particularly interesting data exists concerning the possibility to combine some of these agents with anti-HER2 agents on which a patient has progressed, as the potential to reverse resistance to trastuzumab has been demonstrated[127-129].

OTHER ANTI-HER2 AGENTS

Lapatinib

Lapatinib is a dual TKI active on both EGFR and HER2, with known activity in trastuzumab resistant advanced breast cancer; data suggests that there is no cross-resistance with trastuzumab and lapatinib restored trastuzumab sensitivity in preclinical models[28,130,131]. Wainberg *et al*[132] evaluated the effect of lapatinib in HER2-amplified cell lines and xenograft models, concluding that lapatinib inhibits the growth of HER2-amplified cancer cell lines, induces cell cycle arrest and apoptosis and acts synergistically with trastuzumab.

It is approved as combination therapy with capecitabine for patients with HER2-overexpressing breast cancer with prior progression on trastuzumab, an anthracycline and a taxane[133]. In a phase Ⅱ trial conducted by Galsky *et al*[134], patients with HER2 amplified gastro-esophageal, bladder, ovarian, or uterine tumors were enrolled into a double-blinded randomized discontinuation study of lapatinib 1500 mg *per os* a day. Of a total of 141 patients screened, 32 patients with HER2 amplified tumors were enrolled in the study. At 3 mo, 1 (3%) patient had a complete response (CR), 9 (28%) had stable disease, 20 (63%) had progressive disease, and 2 (6%) were unknown. Unfortunately, due to low response rate and slow enrolment, the study had to be closed early. Concerning gastro-esophageal cancer, a modest CR rate of 6.25% was reported. A phase Ⅱ study with lapatinib as first-line therapy in 47 patients with advanced gastric cancer showed modest single-agent activity, with 12% response rate, 20% disease stabilization, 7% of patients experiencing partial response and a median OS of 5 mo, less than that seen with conventional cytotoxic chemotherapy[135]. Another phase Ⅱ study of lapatinib monotherapy in patients with HER2-overexpressing GEJ or esophageal cancer reported limited single-agent activity, with no objective responses and stable disease in 8% of pacients[136]. Lapatinib in conjunction with capecitabine in the first line treatment of HER2 positive metastatic gastric cancer setting was addressed in a multicenter phase Ⅱ trial, reporting a response rate of 22% and stable disease rate of 45%[137]. In another phase Ⅱ trial, partial response of 24% and stable disease in 34% of patients was reported with lapatinib + capecitabine. Most frequent grade 3 and 4 side effects were anorexia, hand-foot syndrome, anemia and nausea; no significant cardiotoxicity was reported[138]. Two phase Ⅲ studies evaluating the role of lapatinib in combination with chemotherapy in advanced esophagogastric cancer are currently being conducted, the LOGIC trial[139,140] (combination of lapatinib with oxaliplatin and capecitabine as first-line treatment) and the TYTAN trial[141,142] (lapatinib in combination with weekly paclitaxel in second-line setting). OS results from the LOGIC trial are expected in 2014. Data from the TYTAN trial were presented at ASCO GI 2013. 430 patients were randomized, with an OS of 11 mo for the experimental arm *vs* 8.9 mo for the paclitaxel-alone arm; the subgroup of patients with HER2 3+ expression score attained an OS of 14 mo.

As previously stated, dual blockade with lapatinib and trastuzumab in metastatic breast cancer patients that progressed on trastuzumab-containing regimens improved PFS and clinical response rate[120]; a clinical case reported durable stable disease in a patient treated with this strategy despite progression during prior chemotherapy with trastuzumab[143].

Pertuzumab

Pertuzumab is a monoclonal antibody targeting HER2 in domain Ⅱ (Figure 1), preventing formation of the highly mitogenic HER2/HER3 dimer. Available data stem mostly from breast cancer. As with trastuzumab, the antibody is not effective in patients without amplification of HER2[144]. In the phase Ⅲ CLEOPATRA

Table 3 Clinical trials with other anti-human epidermal growth factor receptor 2 agents

Setting, therapy line	ID	Phase	*n*	Treatment	Primary EP	Status
Operable disease	NCT00450203	Ⅲ	370	Lapatinib, epirubicin, cisplatin, capecitabine	OS	Recruiting
Advanced first line	NCT00680901 LOGiC	Ⅲ	535	Lapatinib, oxaliplatin, capecitabine	OS	Active, not recruiting
	NCT01395537	Ⅱ	43	Lapatinib, carboplatin, paclitaxel	Safety, RR	Active, not recruiting
	NCT01123473	Ⅱ	192	Lapatinib, epirubicin, cisplatin, capecitabine, 5-FU	PFS	Unknown
	NCT00526669	Ⅱ	67	Lapatinib, capecitabine	RR	Active, not recruiting
Advanced second line	NCT00486954 TYTAN	Ⅲ	273	Lapatinib, paclitaxel	OS	Completed
	NCT01522768	Ⅱ	27	Afatinib	RR	Recruiting
	NCT01152853	Ⅱ	28	Dacomitinib	PFS	Unknown
	NCT01145404	Ⅱ	76	Lapatinib, capecitabine	RR	Active, not recruiting

OS: Overall survival; PFS: Progression-free survival; 5-FU: 5-fluorouracil; RR: Response rate; CR: Complete response; PR: Partial response; NA: Not available.

study, 808 patients with HER2-positive metastatic breast cancer received placebo + trastuzumab + docetaxel (control group) or pertuzumab + trastuzumab + docetaxel (pertuzumab group). Median PFS was 12.4 mo in the control group *vs* 18.5 mo in the pertuzumab group. The hazard ratio for the addition of pertuzumab to docetaxel + trastuzumab for PFS was 0.62, with moderate toxicity added by the second antibody[145]. Pre-clinical results show potentiation of trastuzumab antitumour activity when combined with pertuzumab[146]. Pertuzumab is currently under investigation in a phase Ⅱ study, in the first line gastric setting in combination with trastuzumab and platinum-fluoropyrimidine based chemotherapy[147].

Trastuzumab emtansine

Trastuzumab emtansine (T-DM1) is an antibody-drug conjugate, which combines trastuzumab with the targeted delivery of the cytotoxic agent DM1, a derivative of maytansine, and a potent antimicrotubule agent. As systemic therapy, gastrointestinal toxicity limits the therapeutic usefulness of the agent[109]. In xenograft models, T-DM1 was found more effective than trastuzumab alone, with positive results independent of the tumor burden at therapy initiation, or preceding treatment with trastuzumab[101]. In a phase Ⅱ study by Burris *et al*[148], T-DM1 had robust single-agent activity in patients with heavily pre-treated, HER2-positive metastatic breast cancer, with a favourable toxicity profile. In breast cancer, the EMILIA trial assigned patients with HER2-positive advanced breast cancer, previously treated with trastuzumab and a taxane, to T-DM1 or lapatinib + capecitabine. Median PFS was 9.6 mo with T-DM1 *vs* 6.4 mo with lapatinib plus capecitabine; with an objective response rate of 43.6% for T-DM1[149]. Taken together, results from preclinical studies and breast cancer clinical trials point out T-DM1 as a promising agent to be evaluated in gastro-esophageal cancer. Currently, a phase Ⅱ-Ⅲ study is ongoing to evaluate T-DM1 *vs* taxane in patients with previously treated locally advanced or metastatic HER2+ gastric and GEJ cancer.

Pan-HER TKIs

Irreversible small molecule pan-HER TKIs causes tumor regression in HER2-overexpressing human gastric cancer xenograft models. They act by inhibition of HER family receptor phosphorylation and blocking of hetero-dimerization among them. Pre-clinical data reveal a synergistic effect with other molecular targeted agents (including trastuzumab) and chemotherapeutic agents. Currently investigated pan-HER TKIs include dacomitinib and afatinib[150,151].

Selected ongoing clinical trials exploring other anti-HER2 agents can be found in Table 3.

OTHER HER2-DIRECTED STRATEGIES

HER2 vaccines, both DNA and peptide-based, are actively researched in the field of breast cancer and results indicate a possible future role for this modality in combination with other HER2 targeted therapies. A phase I study carried out by Hamilton *et al*[152] combined HER2 immunization with lapatinib found this combination to be safe and immunogenic, however, the anticancer activity of immunization-induced antibodies is still not well characterized. Successful repression of the HER2 gene by the means of adenovirus constructs rises expectations for possible applications in cancer treatment[153]. Radioimmunotherapy is another possible application of HER2 directed homing, with authors currently evaluating 212Pb immunoconjugates with trastuzumab in intraperitoneal application after preclinical studies showed interesting results[154,155].

CONCLUSION

Now, times are changing. New strategies had been developed and implemented for advanced gastric cancer treatment. HER2 acquired a main role in gastric cancer management and current is also mandatory in order to predict trastuzumab response in association with standard platinum-based chemotherapy. Furthermore, others drugs are in developing to overcome resistance to trastuzumab, serious treatment-related toxicities and also help oncologists to improve treatments approaches. In future, genomic profiles will probably be part of clinical routines

for personalizing therapies and improve outcomes of those patients.

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