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TRASTUZUMAB IN ADVANCED GASTRIC CANCER: PROPORTION OF ELIGIBLE CASES IN A SINGLE CENTRE

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- Artigo de revisão: Michael Luis, Ana Tavares, Luis-Silva Carvalho, Lucio Lara-Santos, António Araújo, Ramon A de Mello. Personalizing therapies in advanced gastric cancer – molecular mechanisms, current biomarkers and novel anti-HER2 therapies World J Gastroenterol. 2013 October 14; 19(38): 6383–6397
- Capítulo de livro: Michael Luis, Ramon A de Mello. HER2 Over-Expression and Gastric Cancer: Molecular Mechanisms and Target Therapies. In: Atta-ur-Rahman and M. Iqbal Choudhary (eds.), Frontiers in Anti-Cancer Drug Discovery, Volume (2), Bentham Science Publishers, 2013. ISBN: 978-1-60805-809-9

ABSTRACT

Gastric cancer is the fourth most commonly diagnosed cancer and the second leading cause of cancer death worldwide. *HER2* (human epidermal growth factor receptor 2), an oncogene of the HER family of growth factors has been shown to be overexpressed in gastric cancer. Trastuzumab is a monoclonal antibody targeting HER2, which demonstrated to significantly prolong overall survival in association with standard chemotherapy in this patients in the ToGA (Trastuzumab for GAstric Cancer) trial. These data contributed for the approval of trastuzumab in combination therapy in the treatment of metastatic adenocarcinoma of the stomach or gastro-esophageal junction by various international authorities. Aiming to address the particular issues of the patients diagnosed and treated at Instituto Português de Oncologia do Porto (IPOP), a retrospective cohort study was designed in order to estimate the proportion of patients eligible for trastuzumab therapy at this particular centre.

Ninety-eight patients admitted during 2005 and 2006 at IPOP with metastatic or locally advanced gastric or GEJ intestinal-type adenocarcinoma were included and characterized according to clinicopathological variables. HER2-status was determined by immunohistochemistry. Ambiguous cases were analyzed by silver in-situ hybridization.

Overall survival in the study sample was 36 months [24-48], with a disease-free survival of 12 months [8-16]. Overall positivity for HER2 was 16.3%. HER2 correlated significantly with pathological stage, N-status and N-ratio *($p < 0.05$).* Overall survival in HER2- patients was 43 vs. 19 months in HER2 positive patients *($p=0.032$)*.

Our data suggest an important role for HER2 as prognostic factor. The methods used for HER2 assessment proved to be efficient. Anti-HER2 therapeutics in gastric cancer seem to have a role the adjuvant setting and in patients with locally advanced disease.

Key-words (MESH): stomach neoplasms, esophageal neoplasms, trastuzumab, ERBB2, amplification, immunohistochemistry, in situ hybridisation

RESUMO

O cancro gástrico é a 4ª neoplasia mais frequentemente diagnosticada e a 2ª causa de morte por cancro no mundo. O oncogene *HER2* (human epidermal growth factor receptor 2), pertencente à família HER dos factores de crescimento, encontra-se sobre-expresso no cancro gástrico. O trastuzumab é um anticorpo monoclonal dirigido ao HER2, que demonstrou prolongar a sobrevivência em associação com quimioterapia nos doentes que sobre-expressam o *HER2,* no ensaio clínico ToGA (Trastuzumab for GAstric Cancer). Estes dados contribuíram para a aprovação do trastuzumab em terapêutica combinada no tratamento do adenocarcinoma metastático do estômago e da junção esófago-gástrica (JEG). No intuito de perceber a realidade existente no Instituto Português de Oncologia do Porto (IPOP), foi desenhado um estudo de coorte retrospectivo, com o objectivo primário de estimar a proporção de doentes potencialmente elegíveis para terapêutica com trastuzumab.

Noventa e oito doentes admitidos durante 2005 e 2006 com o diagnostico de adenocarcinoma do tipo intestinal do estômago ou JEG foram incluídos e analizados quanto a variáveis clinicopatológicas. O *status* HER2 foi determinado por imunohistoquímica, tendo os casos ambíguos sido analisados por hibridização *in situ* com prata.

A sobrevivência global foi de 36 meses [24-48], com uma sobrevivência livre de doença de 12 meses [8-16]. A positividade global para o HER2 foi de 16,3%. O *status* HER2 correlaciona-se significativamente com o estádio patológico, positividade ganglionar e razão de gânglios metastizados / gânglios excisados *($p < 0.05$).* A sobrevivência global em doentes HER2- foi de 43 vs. 19 meses em doentes HER2+ *($p=0.032$)*.

Os dados sugerem um papel importante para o HER2 como factor de prognóstico. Os métodos utilizados para a determinação do *status* HER2 demonstraram ser eficazes. A terapêutica HER2 no cancro gástrico parece ter um papel no contexto adjuvante e em doentes com doença localmente avançada.

"É preciso fazer um esforço contínuo para amar o presente. Viver pelo passado, pelo que se fez, pelo que se conseguiu, é o mesmo que alimentar uma fome premente com banquetes de outrora."

Miguel Torga

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I**NTRODUCTION**

Epidemiology of Gastric Cancer

Gastric cancer is the fourth most commonly diagnosed cancer and the second leading cause of cancer death worldwide, with an estimated 990.000 new cases and 738.000 cancer deaths in 2008 (1). In the northern region of Portugal, gastric cancer has an incidence rate of about 47.4/100.000 person-years in men and 28/100.000 person-years in women (2). Incidence rates show considerable geographic variation, varying from 3.3 per 100.000 in men and 2.0 in women in Egypt to 65.9 in men and 25.9 in women in Korea. Highest rates are found in Asian and South American countries. These large regional variations possibly reflect different prevalences of *Helicobacter pylori* infection, which is responsible for more than 60% of gastric cancer globally (3).

About 85% of gastric cancers are adenocarcinomas, with 15% comprised of lymphomas, gastrointestinal stromal tumors (GIST) and leiomyosarcomas. Gastric adenocarcinomas can be subdivided into diffuse and intestinal types based on the Lauren classification, which constitute pathological entities with distinct epidemiological and prognostic features (4). In diffuse type gastric adenocarcinoma cell cohesion is absent; they occur throughout the entire stomach and more often in younger patients than in intestinal type adenocarcinoma and carry a worse prognosis. Intestinal type gastric adenocarcinoma presents cohesive cells arranged in glandlike structures; they occur more commonly in the antrum and lesser curvature of the stomach (4-6). Different etiologic factors seem to be involved, as incidence of diffuse type is similar in most populations while intestinal type predominates in high risk regions. A decrease in gastric cancer incidence in the United States in the last 70 years has been mainly observed for the intestinal type, associated with *Helicobacter pylori* infection and therefore reflecting improved sanitary conditions and food conservation methods, including refrigeration; on the other hand, the incidence of diffuse type gastric cancer has remained constant over time and seems to be increasing recently, both in Western and Eastern parts of the world (7-9).

Tumors at the junction of the esophagus with the stomach are oftentimes of either gastric or esophageal origin and are therefore referred as gastroesophageal junction (GEJ) cancers. Although the incidence and mortality of gastric cancer are globally decreasing, the incidence of GEJ cancer is increasing in Europe and the United States (10, 11). This increase is thought to reflect an increase in gastroesophageal reflux disease associated with the increasing prevalence of obesity in these countries. In the last three decades, a tendency towards more proximal located gastric cancer (cardia

vs. corpus and pylorus) is emerging, probably explained by a parallel increase of Barrett's esophagus and the decline in *Helicobacter pylori* infection (10, 12).

In western countries, 50 - 80% of patients present with inoperable advanced or metastatic disease, which in combination with patient co-morbidities, age (the majority of patients is > 65 years old) and the relative chemoresistance of the disease contributes to poor overall survival (OS). A prognostic index for locally advanced and metastatic esophago-gastric cancer (the Royal Marsden Hospital Prognostic Index) was initially published in 2004, with data supporting its validity presented in 2009 (13, 14). Four independent poor prognostic factors are taken in consideration: performance status ≥ 2, liver metastasis, peritoneal metastasis, and serum alkaline phosphatase ≥ 100 U/L. Patients are subdivided into good (no risk factor), moderate (1 or 2 risk factors), and poor (3 or 4 risk factors) risk groups. Applying this index to the data of the REAL-2 study (described below) resulted in statistically significant differences between the 3 risk groups in terms of median survival and 1-year survival rates (median survival times for good, moderate, and poor risk groups were 12.7, 8.6, 4.3 months respectively; 1-year survival rates were 52.4%, 33.1% and 13.7% respectively (14).

Two-thirds of patients amenable to curative surgical resection will have recurrence within 2 years (15-17). The late presentation contributes to the grim prognosis associated with gastric cancer, with 5-year survival rate for metastatic gastric cancer being estimated at 5-20% and median survival times ranging from 7 to 10 months (18, 19). The survival rates of patients with resectable disease ranges from 10-30% (20). Combination chemotherapy has been shown to improve overall survival in comparison with single-agent chemotherapy or best supportive care (21). Current management typically consists of fluoropyrimidine and platinum-based combinations with or without a third drug (usually docetaxel or epirubicin) (22). However, data available have been conflicting when it comes to evaluate the clinical outcome with different treatment strategies, which can explain the lack of global consensus for adjuvant chemotherapy (23, 24). Considering the poor prognosis of advanced gastric or GEJ cancer, new therapeutic regimens with acceptable toxicity have been actively pursued.

Pathology of gastric cancer

Sporadic intestinal type gastric adenocarcinoma is thought to arise in a multi-step process of intestinal metaplasia – dysplasia – carcinoma, involving mutations or deregulation of genes regulating key events in the cancerization process such as apoptosis, cell cycle regulation, proliferation and metabolism, in a similar fashion of the progression of colon adenomas to carcinoma (25). Therefore, accumulation of molecular genetic alterations involving activation of oncogenes and inactivation of

tumor suppressor genes are likely involved in the process of carcinogenesis. Other implicated genes include *p53*, *CHD1*, *SMAD4*, *FHIT* and *CDKN2A*. On the other hand, no precursor cells have been clearly identified in diffuse type gastric adenocarcinoma, but aberrations in the *FGFR2/ErbB3/PI3* kinase pathway are frequent in this kind of neoplasia (26-29).

Information on the specific pathways involved is scarce; however, HER2 has been shown to be overexpressed/amplified in gastric cancer and correlation between HER2 expression and intestinal type gastric cancer has been reported (30). HER2 is a member of the epidermal growth factor receptor (EGFR) family of growth factors, with intrinsic protein tyrosine kinase activity and its increased activity is an assumed mechanism underlying cell transformation. The EGFR family includes four structurally related members, ErbB1 (HER1), also known as EGFR, ErbB2 (HER2), ErbB3 (HER3) and ErbB4 (HER4) (31). These receptors are known to be activated by homo- or heterodimerization induced by ligands. However, no ligand has as far been identified for HER2, which is therefore an orphan receptor. It is believed that the receptor homodimerizes independently of a ligand or heterodimerizes with another ligand-bound member of the EGFR family for activation (32). Ligand-independent homodimerization will occur in the setting of HER2 overexpression. The 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 (see Fig. 1).

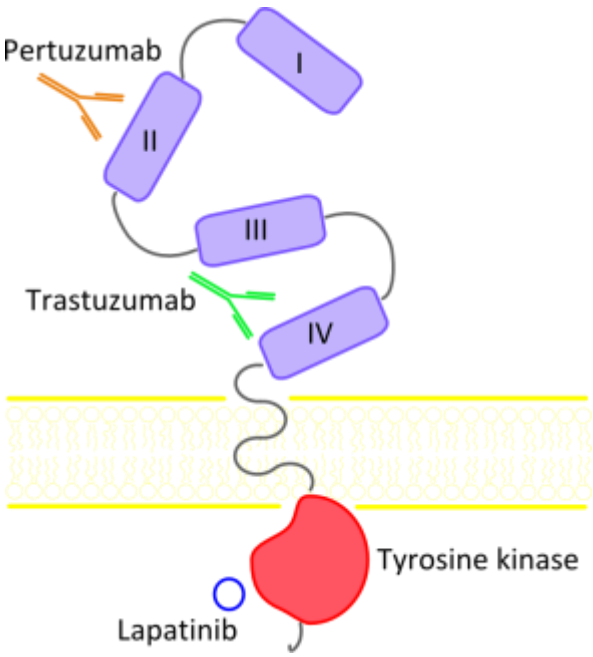


Figure 1
The HER2 receptor

As aforementioned, the receptor is activated through homodimerization or heterodimerization, leading to a cascade of events that involves autophosphorilation and activation of the tyrosine kinase domain, Ras/Raf/mitogen-activated protein kinase (MAPK) pathway, phospholipase C-γ (PLC γ) and phosphatidylinositol-3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) (see Fig.2).

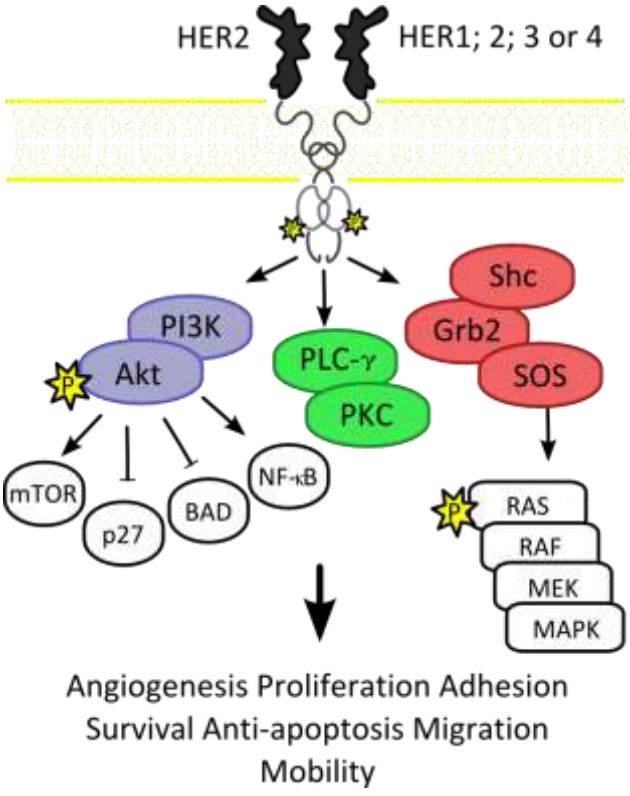


Figure 2
HER2-activated pathways

Furthermore, HER2 receptors have been found in nuclear localization, where they can act as transcription factors for cycline D1 and p53 (33, 34). Therefore, *HER2* (also known as *c-erbB-2/neu*) is an oncogene that is involved in the regulation of cell proliferation, differentiation, motility and apoptosis (35-39). Despite being an orphan receptor it is known that, if overexpressed, the receptor becomes a preferential binding partner for other family members, leading to ligand-independent hetero- and homodimerization. This ligand-independent dimerization is better understood in the light of crystal structure studies, which show fixed conformations resembling a ligand activated state and interaction with other family members in the absence of any ligand and therefore activating the mentioned oncogenic pathways (27, 40-43). 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 own ligands (32, 44-46).

Understanding the molecular structure of HER2 has allowed integrating the mechanisms through which the receptor can be approached as a therapeutic target (see Figs. 1 and 3).

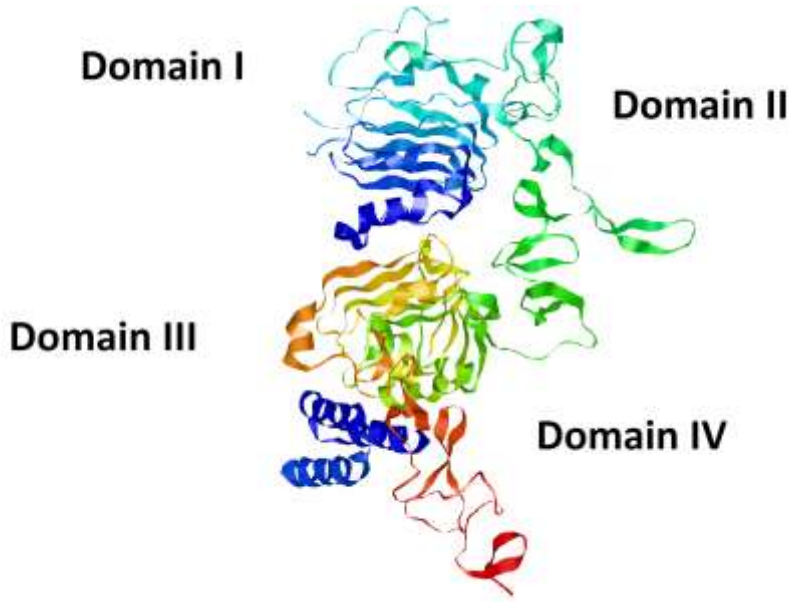


Figure 3
HER2 ultrastructure

The extracellular domain can be subdivided into four subdomains. Subdomains II and IV are involved in the process of dimerization and are the binding sites for pertuzumab and trastuzumab respectively, two of the most well studied HER2 inhibitors, whereas subdomains I and III are binding sites of potential ligands. Crystallographic data confirm that most receptors exist in an open configuration, where subdomains I and III associate to leave the dimerization arm protruded outside of subdomains II and IV. This might explain the role of HER2 as a preferred dimerization partner among the HER family. However, interesting data suggests that there is no lack of autoinhibitory interdomain interactions in HER2, as these are maintained and even extended when compared to EGFR. Some authors therefore suggest that HER2 signalling may be regulated by ligands that we do not know yet, just as in EGFR (26, 47). The transmembrane domain of HER2 plays an important role in the process of dimerization and oncogenic mutations in this region are known. At last, the intracellular domain contains the active enzyme site and activates different downstream pathways by phosphorylation (42, 48-50).

Treatment of gastric cancer

Surgery constitutes a fundamental modality in treatment of resectable gastric cancer, with endoscopic submucosal dissection constituting a valid alternative to surgery in early gastric cancer (51). However, the optimal extent of lymph node dissection is a matter of debate, with different strategies applied in the western and eastern world, particularly Japan (27). Japanese practice advocates a D2 resection (*en-bloc* resection of perigastric and celiac axis nodes). Even with radical node dissection, survival remains poor, with 5-year survival rates typically < 50% for stage II disease with surgery alone (52). In a Dutch trial comparing D1 vs. D2 node dissection, 15-year survival was reported to be 33% for stage II and 19% for stage IIIA disease (53). While D2 dissection was associated with lower recurrence of the disease, higher surgical mortality and complication rate was observed and the authors found no difference in OS. Likewise, D2 resection + para-aortic nodal dissection seems not to improve OS or relapse-free survival; however a recent systematic review found no increase in post-operative mortality with this technique, favouring D2 resection (53-56). Although surgery constitutes the only potentially curative procedure, most patients eventually recur in regional or distant sites, even after radical resection as mentioned above.

Outcomes of operable cancer have improved since the introduction of multimodality therapies in the neoadjuvant/adjuvant setting (57).

In 1999, Nakajima *et al.* presented the results of a phase III clinical trial of adjuvant chemotherapy after curative gastrectomy in macroscopically serosa-negative gastric cancer. A total of 579 patients were enrolled in the study, randomly allocated for adjuvant chemotherapy with mitomycin +5-fluorouracil (5-FU) for 3 weeks past surgery and uracil + tegafur for 18 months. No significant difference in survival between the groups was found (OS 82.9% in control group vs. 85.8% in treated patients) (58).

Adjuvant chemoradiation has been shown to improve OS compared to surgery alone (59), but in the ARTIST (Adjuvant Chemoradiation Therapy in Stomach Cancer) trial, which compared adjuvant chemotherapy with adjuvant chemoradiation after D2 resection, the addition of radiotherapy to chemotherapy with capecitabine + cisplatin did not significantly reduce recurrence after curative resection and D2 lymph node dissection (60).

Perioperative chemotherapy was investigated by the MAGIC (Medical Research Council Adjuvant Gastric Infusional Chemotherapy) trial, where patients were treated with 3 cycles of 5-FU + cisplatin + epirubicin before and after radical surgery experienced a significant improvement in 5-year OS (23% vs. 36.3%), higher likelihood of progression-free survival (PFS), higher rate of curative surgery, reduced tumor size and less advanced nodal disease in comparison with the surgery-only group (27, 60,

61). In the French FNLCC Accord07-FFCD 9703 study, R0 resection rate after neoadjuvant therapy (cisplatin + 5-FU) was improved compared to surgical resection alone (84% vs. 73%, p = 0.04). Gain in the 5 year-DFS rate was 13% (34% vs. 21%, p = 0.0033) and in the 5- year OS rate was 38% vs. 24% (p = 0.021), favouring neoadjuvant chemotherapy (62).

In another trial, adjuvant oral fluoropyrimidine monotherapy (with S-1) showed promising results, with 3-year OS rates of 80.1% in the chemotherapy group and 70.1% in the surgery-only group (63). The potential of adjuvant chemotherapy was addressed by a metanalysis with data from 3838 patients, demonstrating a median OS of 7.8 years following adjuvant chemotherapy vs. 4.9 years following surgery alone, with the authors concluding that adjuvant 5-FU-based chemotherapy is associated with improvement in OS and is therefore recommended for patients who have not received perioperative treatments after gastric cancer resection (24).

In metastatic gastric cancer, combination chemotherapy improves OS in comparison with single-agent chemotherapy or best supportive care (21). Objective response rates are estimated as 10 – 30% for single-agent therapy and 30 – 60% for combination therapy (64, 65). Current management typically consists of fluoropyrimidine-based and platinum-based combinations with or without a third drug (usually docetaxel or epirubicin) (22). In a meta-analysis comprising 13 randomized trials, drug regimens containing 5-FU, anthracyclines and cisplatin achieved superior survival results compared to cisplatin + 5-FU or antracycline + 5-FU combinations; furthermore, regimens including irinotecan demonstrated a non-significant trend towards better survival (66).

ECF (epirubicin + cisplatin + 5-FU) demonstrated response rates of 42.4%, with median survival of 9.4 months. MCF (mitomycin + cisplatin + 5-FU) had equivalent efficacy, but quality of life was superior with ECF (67). In the REAL-2 (Randomized ECF for Advanced and Locally Advanced Esophagogastric Cancer 2) trial conducted by Cunningham *et al.*, 1002 patients were randomly assigned to receive triplet therapy with epirubicin and cisplatin + either 5-FU (ECF) or capecitabine (ECX) or triplet therapy with epirubicin and oxaliplatin + either 5-FU (EOF) or capecitabine (EOX). It demonstrated OSs between 9.3 and 11.2 months (with epirubicin + oxaliplatin + capecitabine). Toxic side-effects of capecitabine and 5-FU were comparable. Compared with cisplatin, oxaliplatin was associated with lower incidences of grade 3 or 4 neutropenia, alopecia, renal toxicity, and thromboembolism but with higher incidences of grade 3 or 4 diarrhea and neuropathy (22, 68). Similar values were found in a recent single-center trial with an overall response rate of 51.1% and median OS of 10.4 months (69). A median OS of 9.2 months was reported by Van Cutsem *et al.* in a

phase III clinical trial with patients receiving a combination therapy of docetaxel + cisplatin + 5-FU (vs. 8.6 months with cisplatin + 5-FU, p = 0.02) (70). OSs of 10.5 and 10.7 months were reported in phase III trials with capecitabine + cisplatine and 5-FU + leucovirin + oxiplatin respectively (70-72). Irinotecan showed promising results as 2nd line therapy in metastatic gastric or GEJ cancer. In a phase III trial involving 40 patients, irinotecan 250mg/m^2 every 3 weeks was compared to best supportive care. Stable disease was found in 58% of patients; median survival in the irinotecan arm was of 123 days and 72.5 days in the best supportive care arm (73). The SPIRITS trial, a phase III trial which enrolled 305 patients, compared S-1 + cisplatin vs. S-1 alone in chemotherapy-naïve patients with advanced gastric cancer. Median OS was longer in patients assigned to S-1 + cisplatin (13 months) than in those assigned to S-1 alone (11 months). PFS was also significantly longer in patients assigned to S-1 + cisplatin than in those assigned to S-1 alone (median PFS of 6 months vs 4 months) (74).

However, taken together, data available have been conflicting when it comes to evaluate the clinical outcome with different treatment strategies, which can explain the lack of global consensus for the choice of chemotherapy regimens (21, 23, 24). Even if adenocarcinomas of the GEJ have different clinicopathologic features as compared with distal gastric cancers, the chemotherapy approaches remain similar for both entities (10, 11, 75-77). Despite the benefits of chemotherapy in the palliative setting, the prognosis of advanced gastric cancer remains poor (78, 79).

New therapeutic regimens with acceptable toxicity have been actively pursued, namely molecular targeting agents. Several drugs are objects of current interest, such as bevacizumab, cetuximab, erlotinib and sorafenib. In this context, bevacizumab (a monoclonal antibody against VEGF-A) added to docetaxel + cisplatin + 5-FU showed a median OS of 16.2 months and 6-month PFS of 79% in metastatic gastroesophageal adenocarcinoma (80). With bevacizumab added to a regimen of docetaxel + cisplatin + irinotecan, a partial response in 63% and stable disease in 30% of patients was achieved (81). Cetuximab, a recombinant monoclonal antibody, binds to the extracellular domain of the human EGFR, competitively inhibiting the binding of epidermal growth factor and other ligands. In pre-treated patients, cetuximab shows poor response rates, in the order of 6% (82). Better results have been observed in the first line metastatic setting combining cetuximab with various chemotherapy regimens, yielding response rates between 41% and 69% and median OS between 9.0 and 16.6 months (79). Erlotinib is a small molecule TKI that inhibits EGFR autophosphorilation and therefore downstream signal transduction (79). In a phase II trial of erlotinib in GEJ junction and gastric adenocarcinomas, an overall response probability of 9% was estimated, all of them occurring in GEJ cancer patients. Median survival was 6.7

months in GEJ and 3.5 months in gastric cancer. Interestingly, no somatic mutations of the EGFR exons 18, 19, or 21 or amplification of EGFR were detected. The authors concluded that erlotinib showed activity in GEJ adenocarcinoma, but appeared inactive in gastric cancer (83). Sorafenib is a multi-target TKI, against RAFK, PDGFR-β, VEGF-2 and VEGFR-3. In a phase II trial by the SWOG, 53 patients with metastatic or locally advanced unresectable disease were treated with sorafenib + docetaxel + cisplatin. Median OS was 14.9 months with a median PFS of 5.8 months (84).

HER2 as a therapeutic target

The importance of addressing HER2 as a therapeutic target is underscored by a number of molecular and pathological findings. Upregulated HER2 level causes tumorigenesis and the level of HER2 gene expression is much higher in cancer cells than that in non-malignant adult cells. HER2 overexpression is found in both primary tumours and metastasized organs. (85) Furthermore, HER2 is the preferred dimerization partner for other HER receptors in the activation of HER signaling pathways, and the HER2 containing heterodimers have the highest mitogenic potential among all HER complexes. (35) HER2 overexpression has been reported in breast, lung, salivary gland, ovary, colon, prostate and pancreatic cancers. (86)

About 10–34% of invasive breast cancers present HER2 overexpression, which 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, which has shown survival advantage in early and metastatic disease and is a part of standard care. (87). Trastuzumab proved to be effective as adjuvant treatment in breast cancer with HER2 overexpression, with various chemotherapy regimens (88-94). In the adjuvant setting, the HERA trial showed that trastuzumab treatment for one year after chemotherapy correlates with improved status at four-year follow up and improved outcome (95). Neoadjuvant chemotherapy is increasingly becoming standard for patients with inflammatory or locally advanced breast cancer, being currently increasingly used in patients with operable disease (96). In the 2010 NOAH phase III trial, the addition of trastuzumab to anthracycline and taxane-based chemotherapy was assessed in two arms of HER2-positive patients with locally advanced and inflammatory breast cancer. The complete pathological response was higher in the trastuzumab-treated arm than in the standard arm (87% vs. 74%) (97). The GeparQuattro phase III trial showed a better complete pathological response in the trastuzumab arm when compared with the standard chemotherapy arm 31.7% vs. 15.7% respectively (98).

In metastatic breast cancer, a therapeutic role for trastuzumab is also established, particularly in combination regimens. In a phase I study by Nakayama *et al.*, the

association of S-1 and trastuzumab attained overall response and disease control rates of 33.3 and 83.3% respectively (99). Morrow *et al.* evaluated the combination of trastuzu-mab and everolimus (a mTOR inhibitor), in HER2-positive metastatic breast cancer. This association showed good performance in patients who had previously been treated with a trastuzumab-based regimen, with a clinical benefit rate of 34%, a partial response in 15% of patients and a stable disease rate of 19% (100).

In the particular case of breast cancer, recognition of the molecular signature of HER2 overexpression / *HER2* amplification using immunohistochemistry (IHC) or in situ hybridization (ISH) is widely used to tailor therapeutic regimens involving trastuzumab.

Clinical application of HER2 targeted therapy

Prognostic significance

The most important prognostic factor for gastric cancer is the TNM stage which evaluates depth of invasion, involvement of lymph nodes and distant metastasis (64, 88). HER2 overexpression/amplification is currently demonstrated to occur in about 7-34% of gastric and GEJ cancers, with considerable variation regarding the assay used. However, the correlation between the expression of HER2 protein and the prognosis of gastric cancer is still controversial. (101-103)

Initial works addressing the prognostic significance of *HER2* amplification reported a negative effect on OS and a role as marker of poor short term prognosis began to emerge (57, 104). However, conflicting results regarding the prognostic value of HER2 have been published in the last twenty years. While some studies found a negative effect of HER2 on prognosis with reduction in OS (30, 88, 104-111), others found no relationship (112-115) and a trend towards improved survival was found in one cohort (116). A recent systematic analysis by Jørgensen *et al.* found that the majority of publications (71%) 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 (117). Chua and Merrett recently reviewed 49 studies with data regarding the relation of HER2 with clinicopathological variables and survival and concluded that HER2 overexpression is associated with poorer survival; results pertaining other variables were not conclusive (118). Furthermore, HER2 overexpression has been suggested as a molecular abnormality in the development of intestinal type gastric cancer and HER2 expression increases with disease progression in some studies, leading to the suggestion that the initial timing of this event probably occurs in early stages. Barros-Silva *et al.* found overexpression and amplification in both components of mixed tumours (with intestinal

and diffuse types) and *HER2* amplification in early stages, supporting the idea of amplification in an early stage of carcinogenesis (88). This idea is further supported by the high levels of concordance between primary tumours 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 (119). Kataoka *et al.* on the other hand 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 (105, 120, 121). Although these data tend to establish HER2 as a potential negative prognostic factor in gastric cancer, the relation seems not to be as consistent as in breast cancer (117). In fact, recent studies demonstrate no significant prognostic effect. In a study involving 381 metastatic gastric/GEJ cancer patients, Yanjigian *et al.* 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 (122). Okines *et al.* analysed the prognostic role of HER2 in the MAGIC trial (see above), concluding that HER2 status is not an independent prognostic factor in early esophagogastric adenocarcinoma (123). Terashima *et al.* found no correlation with OS in 829 stage II/III resected gastric cancer patients (124). Hsu *et al.* 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 failed to do so on multivariate adjustment (125).

Despite these conflicting results, it seems likely that HER2 is not associated with an adverse prognosis in gastric and GEJ cancer in an extent similar to breast cancer; nevertheless, inhibition of the HER2 pathway in patients with HER2 amplification demonstrated clinical benefits, as will be discussed in the following section.

Preclinical data

Overexpression of HER2 in gastric cancer cells was first related in 1986 by Sakai *et al.* and Fukushige *et al.* (126, 127). Preclinical models of gastric cancer were successful in proving the inhibitory effect of trastuzumab on human gastric cancer cell lines in vitro and in mice xenografts in vivo, with additive and synergistic antineoplasic effects in combination with chemotherapy (128-132). A study by Tanner *et al.* 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* (30), while Matsui *et al.* reported suppression of tumor growth in a xenograft model (130). Enhanced antineoplasic effects were observed with capecitabine, cisplatin, docetaxel, paclitaxel and irinotecan (128), and a further synergistic effect with cisplatin has been found by Kim *et al.* (131).

Clinical data

As noted earlier, 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 carcinogenesis in gastric cancer, with studies pointing out amplification or overexpression in 7-34% of tumours, mainly in the intestinal type and in GEJ and proximal tumours (30, 64, 133). The correlation of intestinal type histology with HER2 overexpression (75% vs. 9% in diffuse type) may be in part explained by E-cadherin mutations (134). In gastric cancer, HER2 gene amplification associates inversely with E-cadherin mutations, which are far more common in diffuse type gastric cancer than in the intestinal type (134).

The broad variation in numbers concerning amplification/overexpression is due in part to a lack of a standardized definition of HER2 positivity in gastric cancer, histological variation within gastric cancer tissues, different scoring systems and laboratorial methods. Taking into account the investigational data and clinical experience from breast cancer and considering the possible benefits of introducing HER2 targeted therapy in gastric cancer, a standardized scoring system was developed and validated for the ToGA (Trastuzumab for Gastric Cancer) trial.

The ToGA trial constitutes a milestone, establishing trastuzumab as the first biological therapy that demonstrated survival benefits in gastric cancer exhibiting HER2 expression (128, 130). ToGA was a multicenter, international trial, undertaken in 24 countries (101). 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 and GEJ 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/m² was given on day 1 by intravenous infusion. Capecitabine 1000 mg/m² was given orally twice a day for 2 weeks followed by a 1-week rest or 5-FU 800 mg/m² 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 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 Fig. 4.

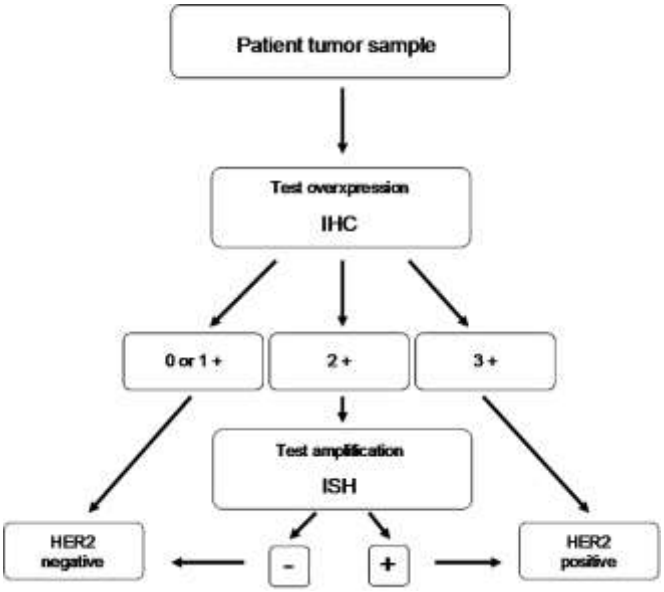


Figure 4
HER2 assesment in the TOGA trial

The highest rate was observed in 34% of GEJ cancer and 20% of gastric cancer samples, (135) 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%. (12, 30, 64, 136, 137)

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 months against 11.1 months 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. (26, 101) Median PFS (6.7 vs. 5.5 months) 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 medial survival of 4.2 months, comparable to the figures in breast cancer (57). In fact, patients with strongest HER2 expression (IHC 3 + FISH +) gained the greatest benefit, with a median survival of 17.9 months in patients treated with trastuzumab vs. 12.3 months with chemotherapy alone. A summary of selected clinical trials of trastuzumab in gastro-esophagic cancer can be found in table 1.

Reference	Phase	Treatment	*n*	OS (mo)	PFS (mo)	%RR	%CR	%PR
Bang *et al.* (101)	III	5FU + cisplatin or capecitabine + cisplatin	290	11.1	5.5	34.5	N/A	N/A
		Trastuzumab + 5FU + cisplatin or trastuzumab + capecitabine + cisplatin	294	13.8	6.7	47.3	N/A	N/A
Cortés-Funes *et al.* (138)	II	Trastuzumab + cisplatin	21	N/A	N/A	41.1	5.8	35
Egamberdiev *et al.* (139)	II	Trastuzumab + leucovirin + cisplatin + 5FU	16	N/A	8.3	54.5	N/A	N/A
		Leucovirin + + cisplatin + 5FU	18	N/A	5.2	33.3	N/A	N/A
Gravalos *et al.* (140)	II	Trastuzumab + cisplatin	22	N/A	5.1	32	N/A	N/A

Table 1
Trials of trastuzumab in gastro-esophagic cancer
Abbreviations: RR, response rate; CR, complete response; PR, partial response; N/A, not available.

In 2007, Cortés-Funes *et al.* 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 days 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 (138). Data from another preliminary phase II study involving 16 gastric cancer patients were presented by Egamberdiev *et al.* in 2011. Trastuzumab 6mg/kg was administered once in addition to cisplatin 100 mg/m^2 during 3 days + 5-FU 1000 mg/m^2 3 days + leucovirin 100 mg/m^2 3 days, every 3 weeks. 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 vs. 5.2 months (139). In a recent phase II study

carried out by Gravalos *et al.*, 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 weeks. 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 months. 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 (140).

In more recent studies, HER2 overexpression was found to be lower than previously reported, especially in distant gastric cancers (141). 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 (142, 143). 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 (27). Trials have been initiated which intend to investigate anti-HER2 therapeutics in this setting (144, 145). 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 (146-148).

Anti-HER2 agents

Trastuzumab

Trastuzumab is a fully humanized monoclonal antibody that binds to the extracellular domain of the receptor and acts by blockage of HER2 receptor cleavage and inhibiting dimerization, as well as by the induction of antibody-dependent cellular cytotoxicity (ADCC), increasing endocytosis of the receptor and possibly through anti-angiogenic effects (149-151). 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 (129, 152, 153).

In January 2010, based on ToGA trial results as previously discussed, the European Medicines Agency (EMA) granted approval to trastuzumab plus chemotherapy in the treatment of with IHC 3+ or 2+/metastatic adenocarcinoma of the stomach or GEJ (154). The U.S. Food and Drug Administration (FDA) approved trastuzumab for HER2 overexpressing patients, without further specification (155).

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/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/week, trastuzumab achieves and maintains serum minimum concentrations greater than 20 µg/mL. Recent results demonstrate that trastuzumab 6 mg/kg every 3 weeks 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 days (156, 157). No relevant drug interactions have been reported to date and elimination pathways remain largely unknown (158). Targeted delivery systems involving anti-HER2 antibody mediated nano-scaled systems, drug conjugates, and fusion proteins are under active investigation (35, 159, 160).

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 (78). 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% or a drop > 10% from baseline LVEF are generally regarded as counter-indications for trastuzumab use (57). 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 (ESMO) (161), 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 (162).

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-week intervals (163).

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 (101). In a phase II study with trastuzumab and cisplatin as first-line therapy in GEJ and gastric cancer, trastuzumab showed a favourable toxicity profile (140).

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 months) (134, 164-166).

Proposed resistance mechanisms include aberrations in the PI3K/AKT/mTOR pathway with or without loss of the PTEN (phosphatase and tensin homologue protein) 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 (MUC4) and cyclin E overexpression (96, 134, 166).

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 (167). 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 (100, 168-170).

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 tumours. 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 antiboby. p95HER2 is ex-pressed in approximately 30% of HER2-positive breast tumours and is correlated with poor disease-free survival (DFS) and increased nodal metastasis when compared with patients that express full-length HER2 (96, 171). 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 (172).

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 weeks was observed in 24.7% of patients in the combination arm vs. 12.4% in the monotherapy arm (173, 174). According to some authors this combination could provide a chemotherapy-free option after first line chemotherapy + trastuzumab (166).

Increased signalling through other receptor TKIs including EGFR, HER3, MET and IGF-1R has been found in cells resistant to HER2-targeting treatments (166). PI3K/AKT/mTOR pathway activation through upregulation of HER3 signalling was demonstrated after exposure of breast cancer cells to HER TKIs (175). On the other hand, pertuzumab, a HER2-HER3 dimerization inhibitor has demonstrated activity against trastuzumab resistant breast cancer cells (176). 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 (177, 178).

Overcoming resistance to trastuzumab

Strategies to overcome trastuzumab resistance imply the important fact that many HER2-positive gastric tumours 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 (179, 180). 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 (181-183).

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 (27). Trials have been initiated which intend to investigate anti-HER2 therapeutics in this setting (144, 145). 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 (146-148).

Maintenance therapy

From a clinical perspective, data known from breast cancer suggest that trastuzumab administration after disease progression might have benefits in OS (91, 134). In an observational of study of 623 patients, median time to progression was longer in patients who continued trastuzumab beyond progression than in those who stopped (10.2 vs. 7.1 months) (184). Data from an interventional study involving 156 patients revealed OS rates of 20.4 vs. 25.5 months and response rates of 27 vs. 48.1% in patients who stopped and continued trastuzumab beyond progression, respectively. Continuation of trastuzumab beyond progression was not associated with increased toxicity (185). 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 (186-188).

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 (61, 123). In addition, some data indicate that response to neoadjuvant treatment is a major predictive factor of survival after curative surgical resection (189).

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 (190-192).

Other anti-HER2 agents

Lapatinib

Lapatinib is a dual tyrosine kinase inhibitor (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. (57, 193, 194) Wainberg *et al.* 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 (195).

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 (196). In a phase II trial conducted by Galsky *et al.*, patients with HER2 amplified gastro-esophageal, bladder, ovarian, or uterine tumours 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 tumours were enrolled in the study. At 3 months, 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 (197). 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 months, less than that seen with conventional cytotoxic chemotherapy (198). 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 (199). 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% (200).

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 (201). Two phase III studies evaluating the role of lapatinib in combination with chemotherapy in advanced esophagogastric cancer are currently being conducted, the LOGIC trial (202, 203) (combination of lapatinib with oxaliplatin and capecitabine as first-line treatment) and the TYTAN trial (204, 205) (lapatinib in combination with weekly paclitaxel in second-line setting).

Data from the TYTAN trial were presented at ASCO GI 2013. 430 patients were randomized, with an OS of 11 months for the experimental arm vs. 8.9 months for the paclitaxel-alone arm; the subgroup of patients with HER2 3+ expression score attained an OS of 14 months.

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 (174); a clinical case reported durable stable disease in a patient treated with this strategy despite progression during prior chemotherapy with trastuzumab (206).

Pertuzumab

Pertuzumab is a monoclonal antibody targeting HER2 in domain II (see Fig. 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 (207). In the phase III CLEOPATRA 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 months in the control group vs. 18.5 months 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

(208). Pre-clinical results show potentiation of trastuzumab antitumour activity when combined with pertuzumab (209). 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 (210).

T-DM1

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 (166). 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 (159). In a phase II study by Burris *et al.*, T-DM1 had robust single-agent activity in patients with heavily pretreated, HER2-positive metastatic breast cancer, with a favourable toxicity profile (211). 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 months with T-DM1 versus 6.4 months with lapatinib plus capecitabine; with an objective response rate of 43.6% for T-DM1 (212). Taken together, results from preclinical studies and in breast cancer clinical trials point out T-DM1 as a promising agent to be evaluated in gastric cancer. Currently, a phase II-III study is ongoing to evaluate T-DM1 versus taxane in patients with previously treated locally advanced or metastatic HER2+ gastric and GEJ cancer. (213)

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 (214). Currently investigated pan-HER TKIs include dacomitinib and afatinib. Dacomitinib (PF00299804) is a pan-HER small molecule inhibitor, with antitumor activity reported in HER2-positive gastric cancer cell lines and xenografts, and synergy observed with several commonly used cytotoxics (5-FU, cisplatin, docetaxel and paclitaxel), targeted agents such as trastuzumab (214).

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.* 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 (215). Successful repression of the HER2 gene by the means of adenovirus constructs rises expectations for possible applications in cancer treatment (216). Radioimmunotherapy is another possible application of HER2 directed homing, namely 212Pb conjugated with trastuzumab in intraperitoneal cancer showed interesting results (217, 218).

HER2 testing in gastric cancer

As mentioned before, HER2 overexpression is currently estimated to occur in about 7-34% of gastric and GEJ cancers as a whole, with considerable variation regarding the assay used (101-103). There is some controversy about the concordance between HER2 overexpression and *HER2* amplification in gastric cancer. In breast cancer, standardized methods of FISH and IHC assessments have been developed, with concordance rates between the two methods around 73-98%, and overexpression is regarded as achieved primarily through gene amplification (219, 220). In gastric cancer however, earlier studies did not observe high concordance between the two methods and overexpression without amplification was described in some studies, with some authors postulating possible alternative mechanisms of overexpression by transcriptional activation by other genes or post-transcriptional events (64, 221).

Recent studies report high concordance between overexpression assessed by IHC and amplification by FISH or chromogenic ISH (CISH), with both surgical resected material and biopsy specimens suitable for evaluating gastric cancer for HER2 status (222). Tsapralis *et al.* recently concluded that in gastric cancer *HER2* amplification is the main mechanism of HER2 overexpression, as happens in breast cancer (223).

It is important to stress the recent development of validated methods in identifying suitable patients for trastuzumab therapy, which differ from the methods used in breast cancer (16, 36, 224, 225). Testing of HER2 status by IHC differs from breast cancer in fundamental aspects: the IHC 2+/3+ score is attributed even though membranous staining is incomplete if membrane staining is clearly detectable even at low

magnification or medium magnification; membrane staining at the appropriate intensity found in at least 10% of tumor cells is restricted to resection specimens (see table 2).

Score	Surgical specimen – staining pattern	Biopsy specimen – staining pattern	HER2 overexpression assessment
0	No reactivity or membranous reactivity in <10% of tumour cells	No reactivity or no membranous reactivity in any tumour cell	Negative
1+	Faint or barely perceptible membranous reactivity in ≥10% of tumour cells; cells are reactive only in part of their membrane	Tumour cell cluster with a faint or barely perceptible membranous reactivity irrespective of percentage of tumour cells stained	Negative
2+	Weak to moderate complete, basolateral or lateral membranous reactivity in ≥10% of tumour cells	Tumour cell cluster with a weak to moderate complete, basolateral or lateral membranous reactivity irrespective of percentage of tumour cells stained	Equivocal
3+	Strong complete, basolateral or lateral membranous reactivity in ≥10% of tumour cells	Tumour cell cluster with a strong complete, basolateral or lateral membranous reactivity irrespective of percentage of tumour cells stained	Positive

Table 2
IHC scoring for HER2 in gastric and GEJ cancer, adapted from ref. (101)

Evaluation of *HER2* amplification by *in situ* hybridization is similar to breast cancer with *HER2* / CEP17 ratio (*HER2* gene copy number per tumor cell to the chromosome 17 copy number) values ≥2.0 indicating *HER2* gene amplification. Using methods developed for breast cancer in gastric cancer can yield markedly different results according to some authors. Therefore, new scoring criteria have been validated and refined during the ToGA (101) trial and are internationally regarded as standard in the determination of HER2 positivity (226).

Some particularities in the laboratorial assessments are still under debate. For example, heterogeneity in gastric cancer tissue is higher than in breast cancer (5% vs. 1.5%), and therefore CISH, by allowing easier morphological examination, may have an advantage over FISH (224, 227). This also raises questions about the adequacy of biopsy specimens and the number that need to be analysed (228). Concordance between IHC HER2 status between biopsies and gastrectomy material was found to be 74.1% in one study (229). Using CISH, amplification can only be assessed semiquantitatively and quantitative polymerase chain reaction (PCR) techniques have been proposed as alternative. Multiplex ligation-dependent probe amplification (MLPA) has been tested in breast cancer and could be a possible technique in gastric cancer also (12). Another important question refers to the differences between HER2 results on primary tumours and corresponding metastases. In a study by Bozzeti *et al.* a high concordance between HER2 status on primary tumours and metastases is described, suggesting that in gastric cancer HER2 status is maintained in most cases unchanged during the metastatic process (119). On the other hand, Kim *et al.* found discordance in *HER2* amplification between primary tumours and metastatic lesions and attributed this to heterogeneity within primary tumors (230). The debate on hetero- or homogeneity of *HER2* amplification by its turn raises the question on the feasibility of tissue microarrays vs. whole-tissue sections in practice. In a study of 2009 cases, *HER2* positivity was detected in 12.3% of whole-tissue sections and in 17% of tissue microarrays (231). Marx *et al.* described *HER2* amplification as being highly homogenous (232), while Yang *et al.* describe intratumoral heterogeneity (233).

Discussion about the ideal methodology for HER2 testing in gastric cancer is ongoing, (234, 235) with many studies evaluating new methods, including dual colour silver ISH (SISH) (236). Kim *et al.* found high concordance between methods in a study where *HER2* amplification by FISH and real-time PCR and HER2 expression by IHC were performed (237). Other studies revealed similar results and CISH and SISH are emerging as a reasonable alternative to FISH, with less expensive costs and necessary equipment (222, 238). A high concordance was comproved using the latter method compared to FISH in breast cancer (96%) (239). Advantages of SISH include

the possibility of being realized in automatic processing equipment, the use of a conventional clear-field light microscope (as opposed to fluorescence microscopy in FISH) and the easy archiving of glass slides with less signal loss compared to other ISH methods, contributing to reduction of costs (240).

The primary objective was to estimate the proportion of patients with gastric cancer or GEJ cancer admitted at IPOP eligible for trastuzumab therapy. Secondary objectives were the characterization of the study population according to clinicopathological variables and to obtain values of OS and PFS.

MATERIAL AND METHODS

Type of study

Quantitative, non-interventional, retrospective cohort study

Inclusion criteria

The study population consists of patients with diagnosed gastric or GEJ cancer admitted at IPOP from 01.01.2005 to 31.12.2006, as retrieved from the centre database. The following selection criteria are applied:

- Patients with stage IV (according to AJCC 7th Edition) and locally advanced disease are selectable, with locally advanced disease being defined as T4NxM0, T3NxM0, T2N+M0 or T1N+M0
- Patients without clearly defined date of first observation, without available tumour samples in the institutional archive or lacking variables essential for the clinicopathological characterization described below are excluded

In a first step, this population is characterized according to clinicopathological variables such as age, gender, treatment, TNM stage, survival and N-ratio. A second step involves the selection of cases potentially eligible for trastuzumab treatment according to EMA approval (metastatic, i.e. stage IV adenocarcinoma of the stomach or GEJ) and locally advanced disease. In a third step, cases of this latter group are analyzed for HER2 overexpression/amplification according to the methods used in the ToGA trial.

Tumour sample inclusion criteria

Tumour samples fulfil the following criteria:

- Histologically confirmed gastric or GEJ adenocarcinoma of the intestinal type;
- Surgical specimens or biopsy specimens with sufficient invasive tumour tissue for HER2 overexpression assessment and
- Specimens must be available at the Department of Pathology of IPOP.

HER2 status testing

HER2 testing is applied according to the methods described in the ToGA trial (Fig 5). (16, 36, 101, 226)

Immunohistochemistry

IHC targeting the HER2 protein was carried out in 3µm thick tissue sections, on adhesive glass slides (Super Frost Plus Menzel®), using a Ventana® Benchmark

ULTRA (Ventana Medical Systems®, S.A. Illkirch, France) automatized equipment. The Her2/neu TesT (4B5) antibody and the DAB Ultraview Universal detection kit, both by Ventana Medical Systems® were used.

Silver in-situ hybridization

SISH was processed using the mentioned Ventana® Benchmark ULTRA system, with the following kits: Ultraview SISH DNP detection kit, Ultraview RED ISH DIG detection kit and Inform Her2 Dual ISH DNA probe cocktail.

Pathological evaluation

The glass slides used for HER2-status analysis were prepared by Ana Sousa Tavares, Pathology Technician and afterwards selected by Pathologist Dr. Luís Pedro Afonso, both working at IPOP. The slides were processed by Dr. Dina Leitão at Instituto de Patologia e Imunologia Molecular da Universidade do Porto (IPATIMUP). Pathological evaluation was performed by Professor Fernando Schmitt.

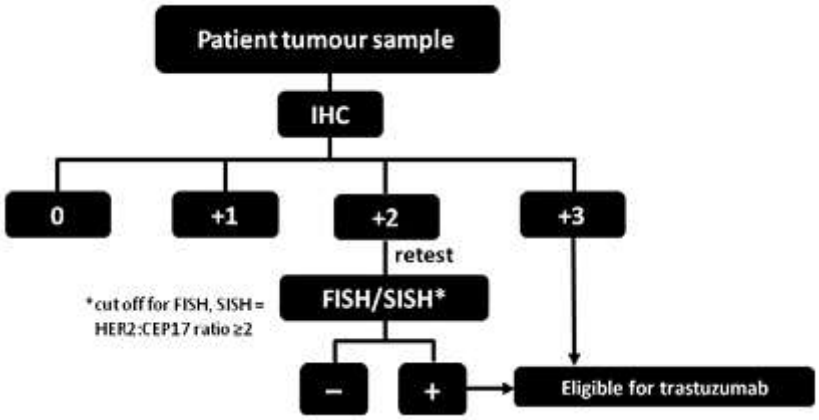


Figure 5

HER2 testing algorithm, adapted from ref. (135)

IHC scoring refers to the criteria outlined in table 1. FISH is considered positive if the ratio of the HER2 gene copy number per tumour cell to the chromosome 17 copy number (HER2 / CEP17 ratio) is ≥ 2.

Statistical analysis

Categorical data was analyzed with the χ^2 - test. Survival curves are obtained according to the Kaplan-Meier method and the significance of differences between survival curves is determined using the log-rank test. Patients without known date of death will are censored at the date last known to be alive. Values o $p < 0.05$ are

considered statistically significant. Analysis was performed using the computer program IBM® SPSS Statistics for Windows®, Version 20.0 (Armonk, USA).

RESULTS

Histology

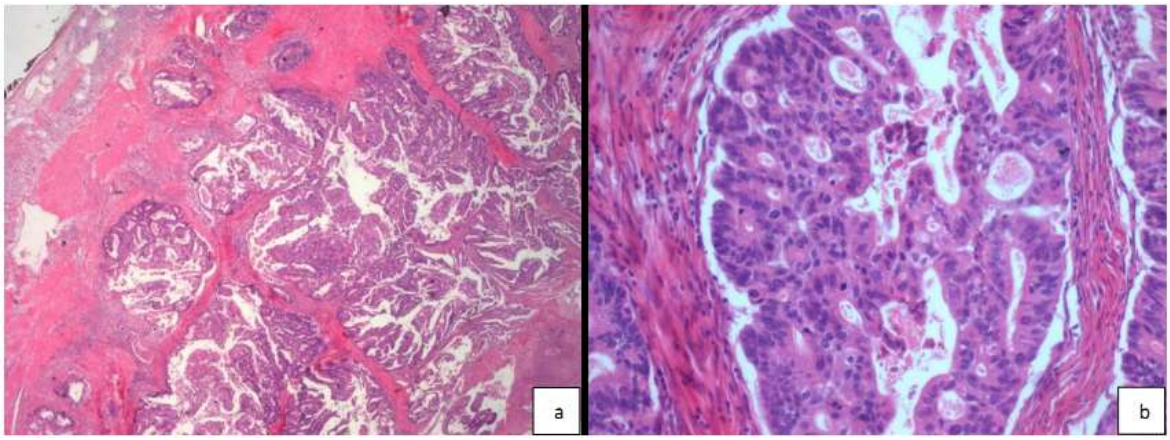


Image 1
Hematoxilin-eosin staining of gastrectomy sample, with intestinal-type adenocarcinoma (a – 10x, b – 40x amplification)

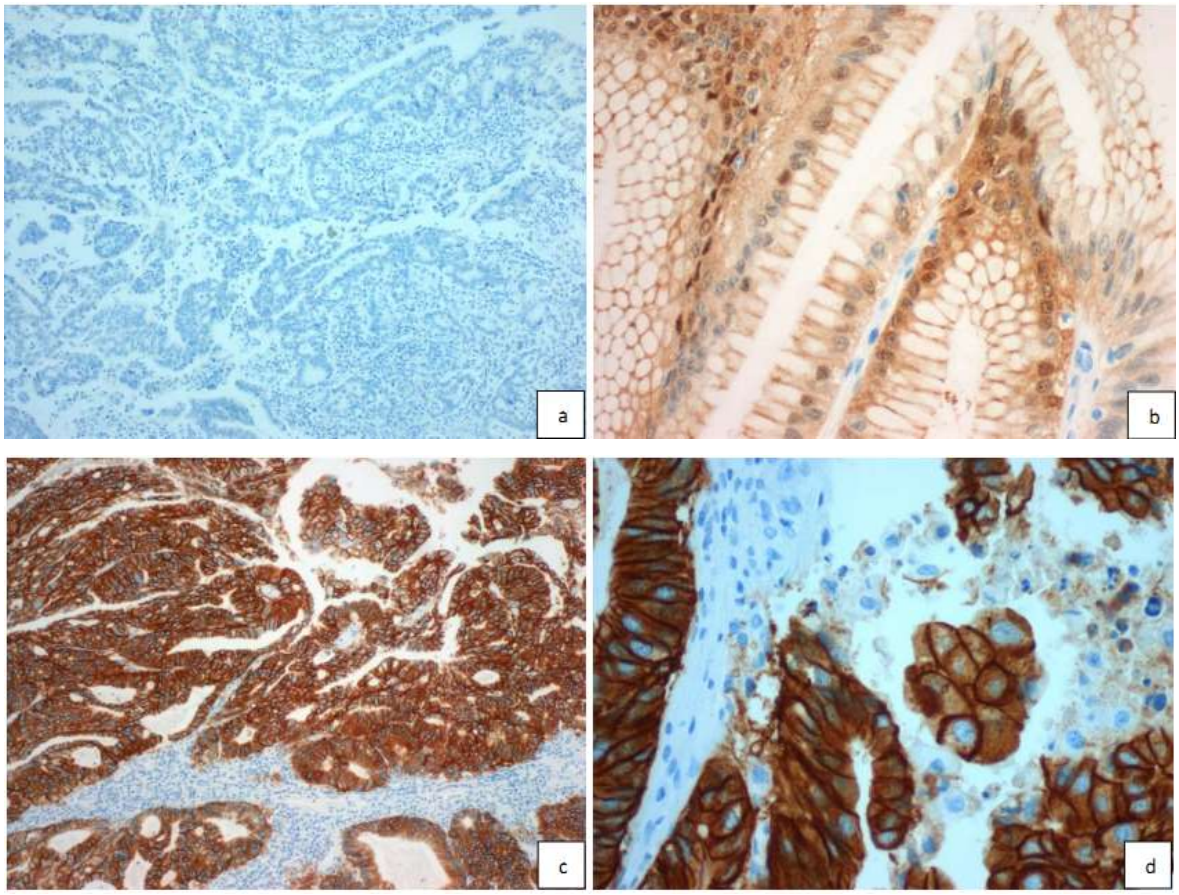


Image 2
IHC for HER2 a – negative (10x), b – 1+ (40x), c – 3+ (10x), d – 3+ (40x)

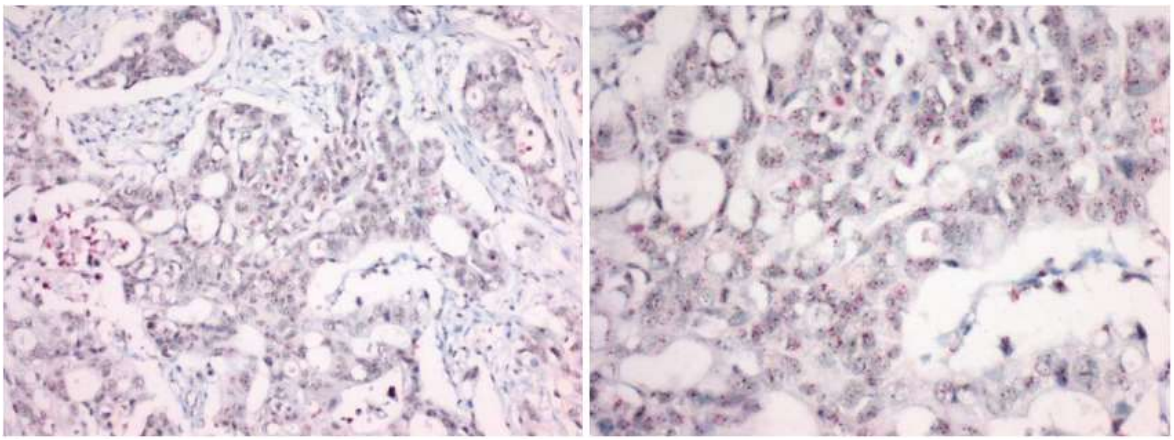


Image 3
SISH for HER2. Chromosome 17 marked red and the HER2 gene in black, 20 and 40x

Description of the sample

General sample

In the years of 2005 and 2006, 720 patients were admitted with a diagnosis of gastric cancer. Male patients comprise 60.6% of the sample. Table 3 depicts the proportion of the different histophatological types (missing cases in the following tables due to lack of clinical information).

Histophatological classification	Proportion
Adenocarcinoma	88.8%
Intestinal	42.2%
Diffuse	22.2%
Mixed	8.3%
Mucinous	5.3%
NOS	10.6%
Lymphoma	5.6%
GIST	1%
Other	4.6%

Table 3

Study sample

According to the selection criteria previously enumerated (MATERIALS AND METHODS), 98 patients were included.

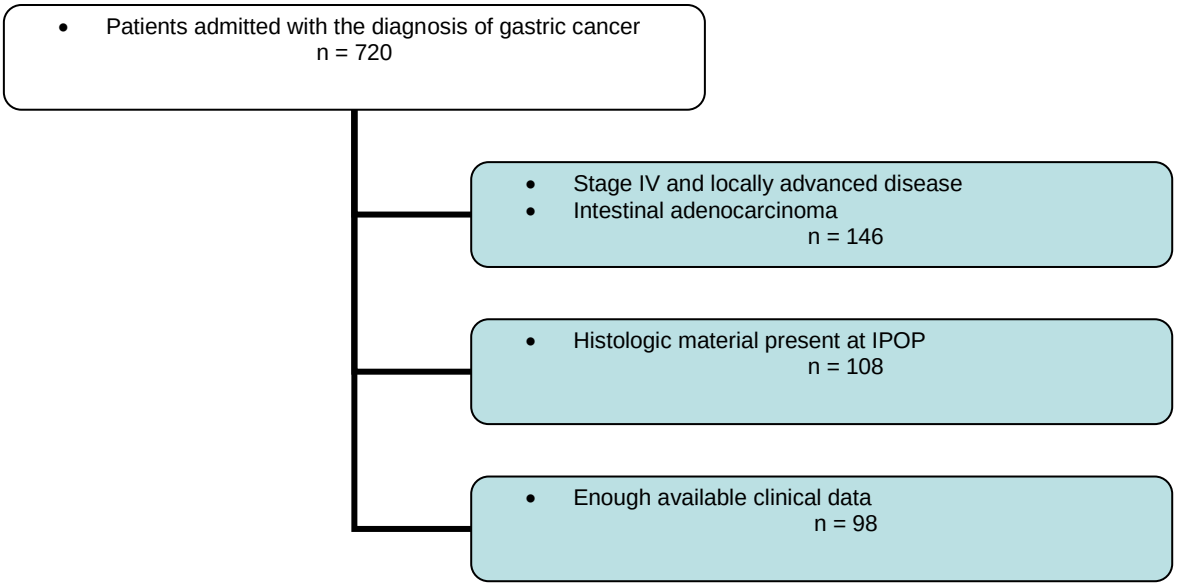


Figure 6

Selection criteria flow-chart

Male patients constitute 57.1% of the sample. Median age at diagnosis was 66 years [35 – 89]. No statistically significant difference was found between genders regarding age at diagnosis (p = 0.9). Properties of the study sample can be found in table 4.

Gender	n / %
Male	56 (55.1%)
Female	42 (44.9%)
Age	Median 66 years
Tumour	
Grade	
1	6 (6.1%)
2	51 (52%)
3	34 (34.7%)
Stage	
I	3 (3.1%)
II	44 (44.9%)
III	37 (37.8%)
IV	14 (14.3%)
Topography	
Body (including curvatures)	38 (38.8%)
Antrum and pylorus	38 (38.8%)
Overlapping	12 (12.2%)
GEJ	6 (6.1%)
Fundus	2 (2%)
Undefined	2 (2%)

Table 4

Treatment

- Surgery

Primary surgery was realized in 93.4% of patients; intent was curative in 68 (80%) cases and palliative in 17 (20%). Lymphadenectomy was reported as D2 in 2/3 of surgeries. Extent of resection was classified as R0 in 71.8%, R1 in 10.3% and R2 in 12.8% of cases, with undetermined extent in 5.1%. Mean N-ratio was 20%. A correlation between resection and surgical intent is depicted in table 5.

Surgical intent	Resection			*p*
	R0	R1	R2	
Palliative	0 (0%)	5 (35.7%)	9 (64.3%)	*p < 0.001*
Curative	55 (91.7%)	4 (6.7%)	1 (1.7%)	

Table 5

- Chemotherapy

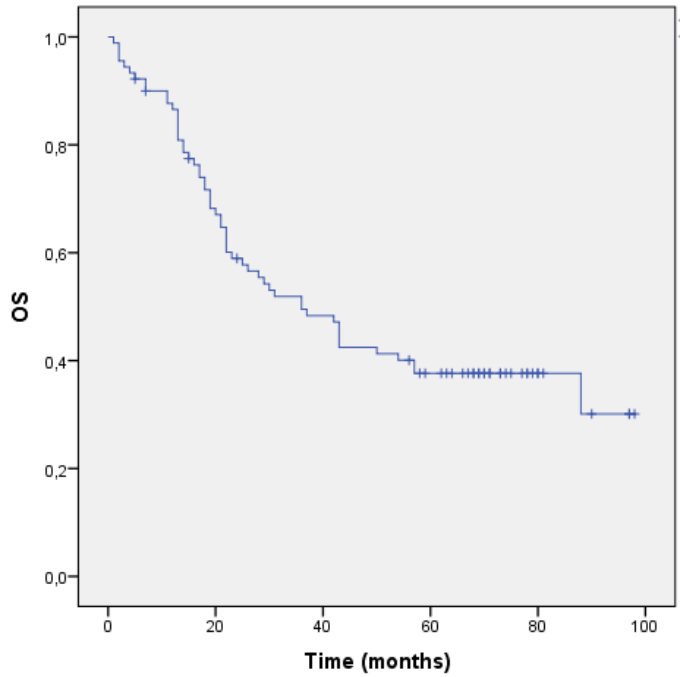
Adjuvant chemotherapy was administered in 6 patients submitted to surgery, while palliative chemotherapy following after surgery was administered in 10 patients, with a cisplatin/5-FU regimen (cisplatin 100 mg/m^2 on day 1 + 5-FU 1000 mg/m^2/day during 5 days, cycles interval 28 days). Primary palliative chemotherapy (without prior surgery) was used in 2 patients; with the same regimen described above. Neoadjuvant chemotherapy was used in 2 patients, also with a cisplatin/5-FU regimen.
Table 6 describes the use of adjuvant chemotherapy according to stage, N+, resection, node sampling and N+ status.

Factor	Adjuvant chemotherapy		*p*
	No	Yes	
Stage			
I	3 (100%)	0 (0%)	NS
II	44 (100%)	0 (0%)	
III	31 (83.8%)	6 (16.2%)	
Resection			
0	54 (96.4%)	2 (3.6%)	NS
1	6 (75%)	2 (25%)	
2	8 (80%)	2 (20%)	
N sampling			
≥ *15*	65 (92.9%)	5 (7.1%)	NS
< *15*	13 (92.9%)	1 (7.1%)	
N+ status			
N-	27 (100%)	0 (0%)	NS
N+	51 (89.5%)	6 (10.5%)	

Table 6

Survival analysis - overall survival

Median OS was 36 months [24-48], 87% at 1 year, 59% at 2 years and 37% at 5 years.

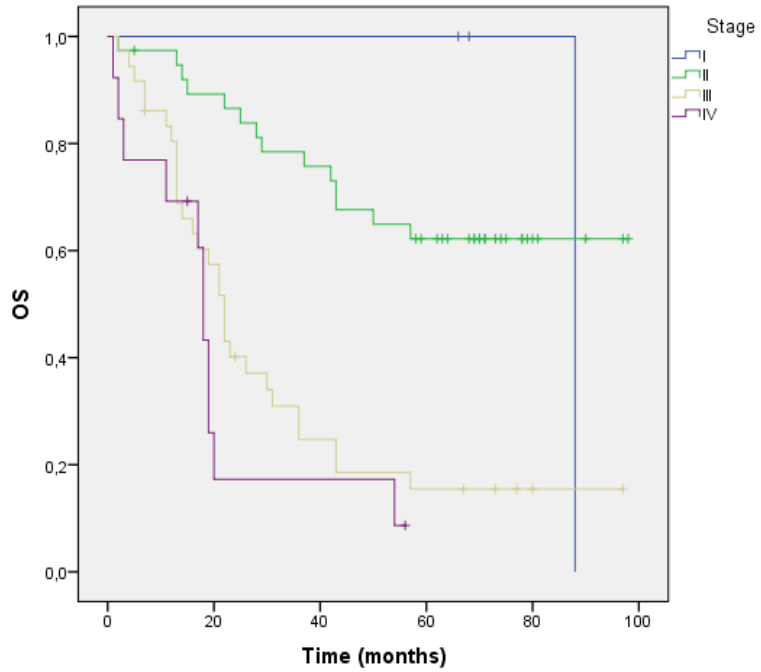


Graph 1

OS by stage

Stage	Median OS (months)	
I	88	
II	72 [61-83] (mean)	*p < 0.001*
III	22 [19-25]	
IV	18 [16-20]	

Table 7

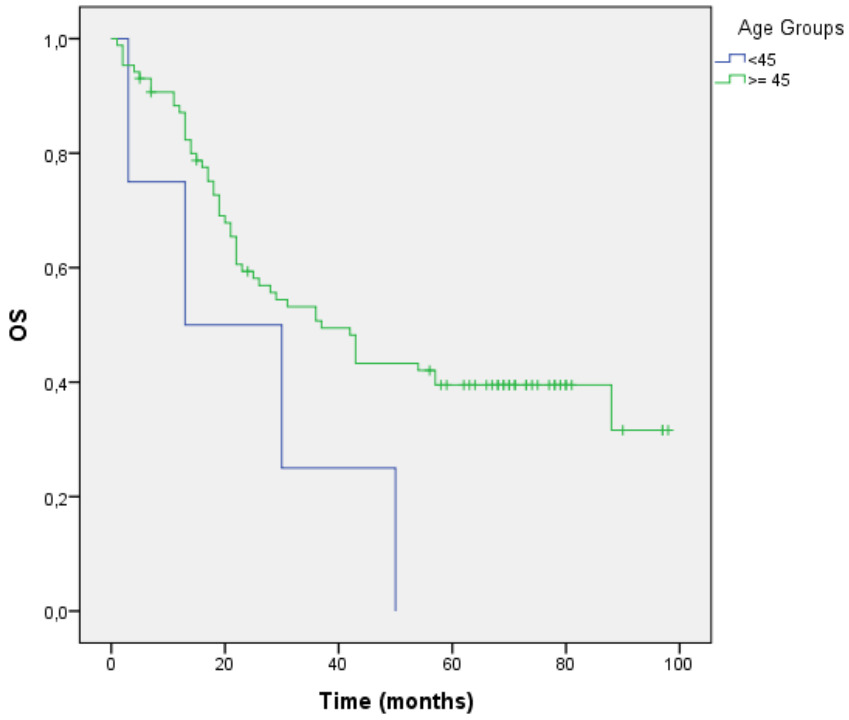


Graph 2

OS by age (early-onset gastric cancer)

Age group	n	Median OS (months)	
≥ 45	86	37 [24-50]	*p = 0.081*
< 45 (early-onset)	4	13 [6-40]	

Table 8

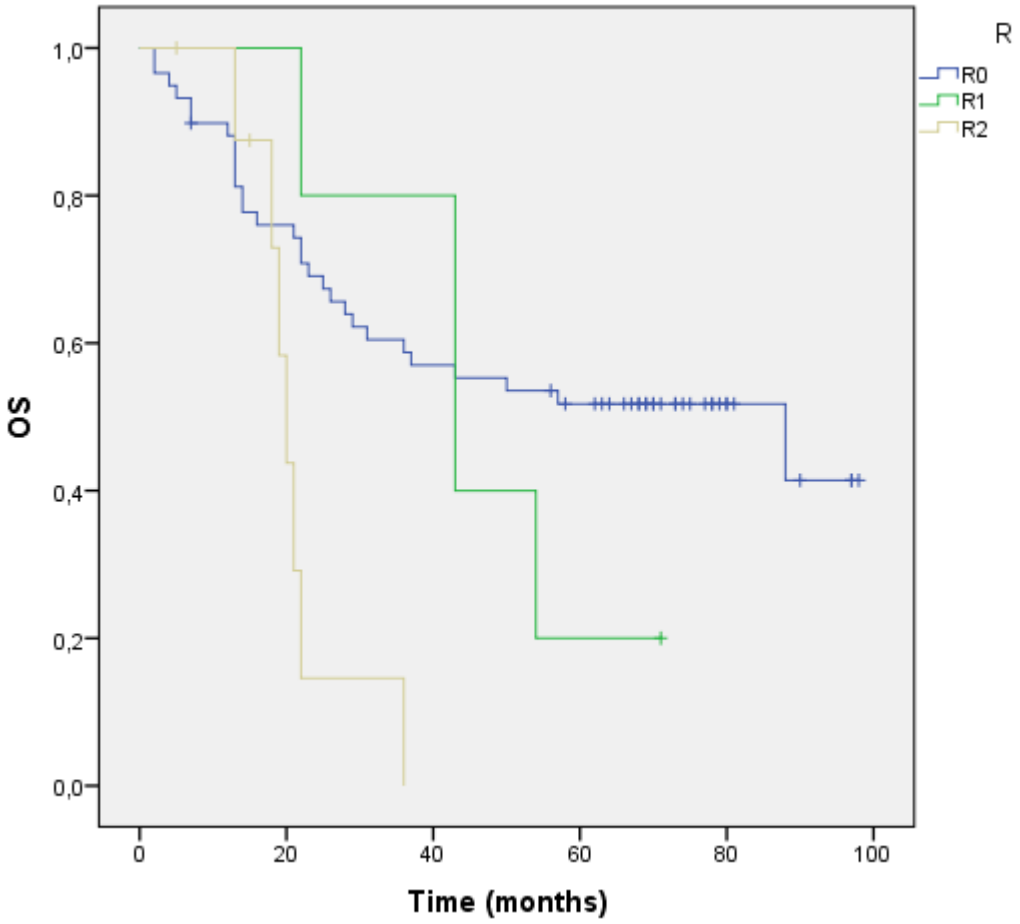


Graph 3

OS by resection

R	n	Median OS (months)	
0	56	88 [20-156]	*p = 0.011*
1	8	43 [21-66]	
2	10	20 [18-23]	

Table 9

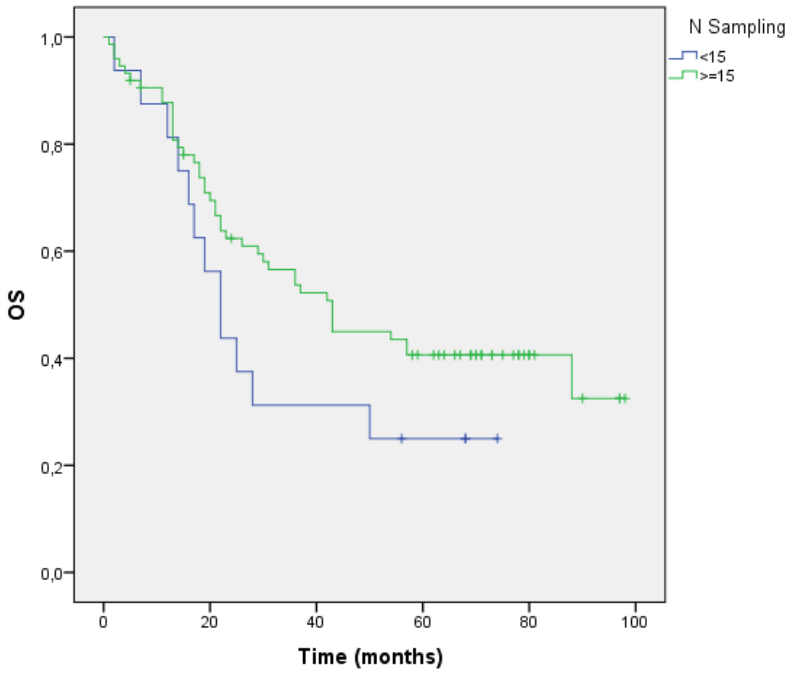


Graph 4

OS by N sampling

N sampling	n	Median OS (months)	
≥ 15	16	43 [31-55]	*p = 0.145*
< 15	74	22 [16-28]	

Table 10

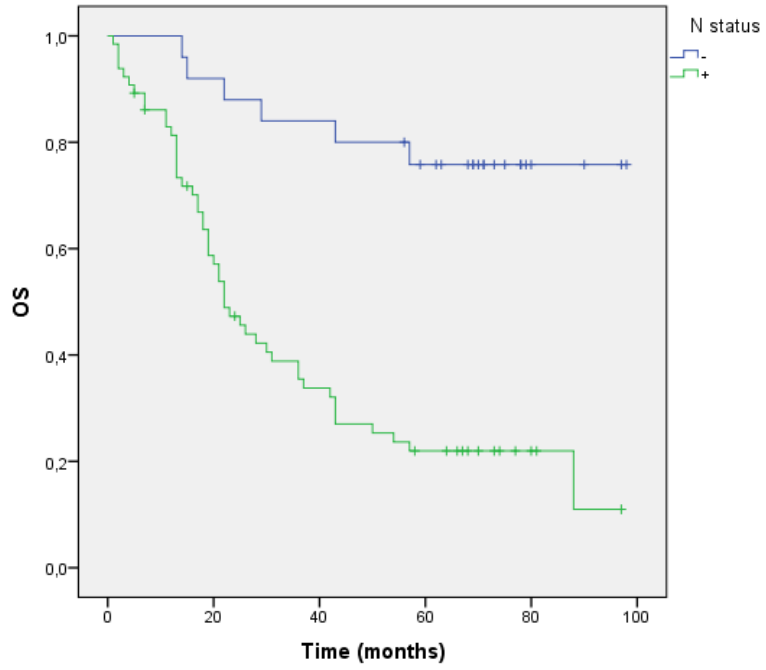


Graph 5

OS by N status

N status	Mean OS (months)	
N -	80 [67-92]	*p < 0.001*
N +	40 [32-48]	

Table 11



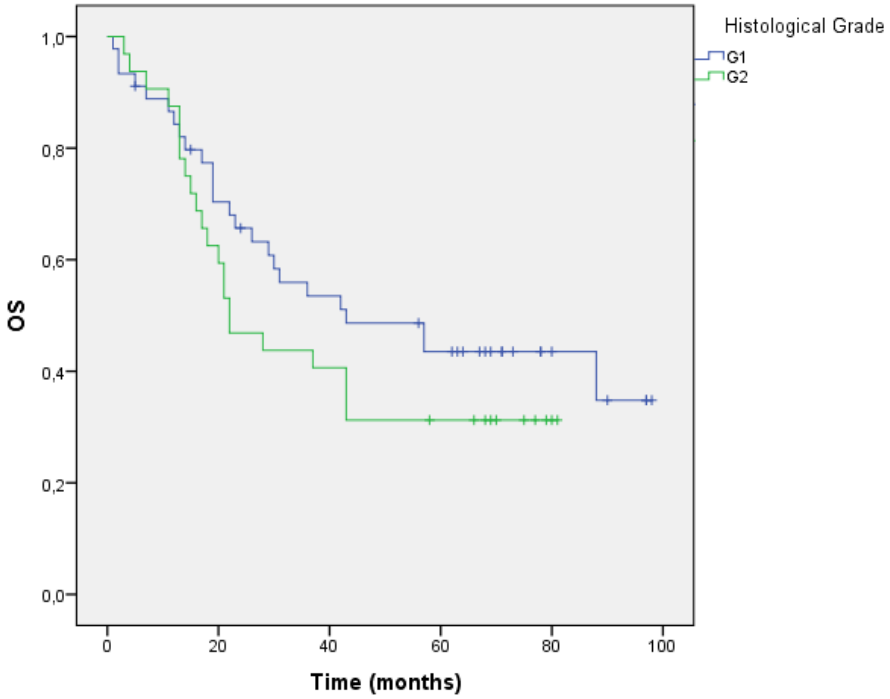
Graph 6

OS by histological grade

Only 7 tumours were classified as G1 and 6 tumours had undetermined histological grade. A comparison between grades 2 and 3 shows no statistically significant difference in OS.

Histological grade	n	Median OS (months)	
2	45	43 [11-75]	*p = 0.231*
3	32	22 [13-31]	

Table 12

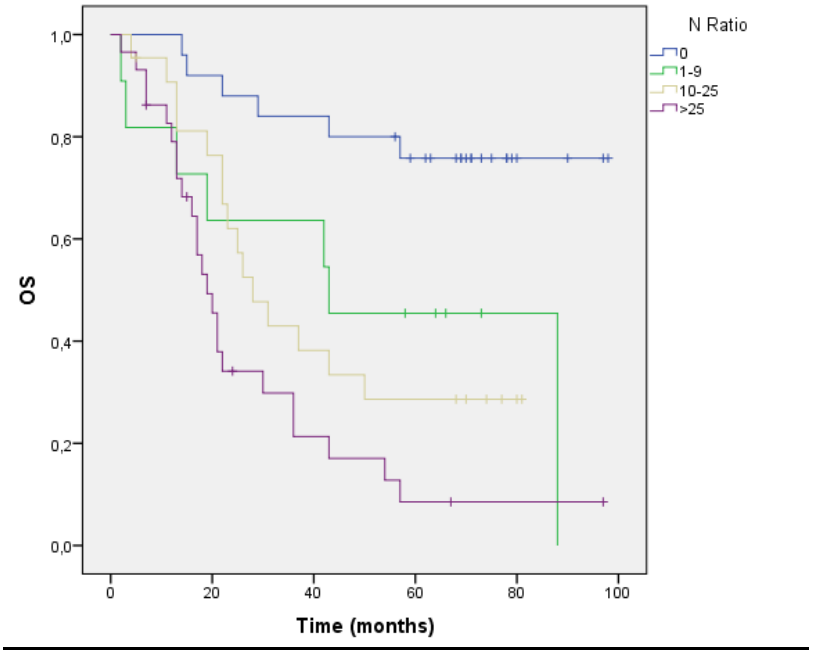


Graph 7

OS by N-ratio (ratio between metastatic and examined lymph nodes)

N-ratio	n	Median OS (months)	
0	25	81 [70-93] (mean)	
1-9%	11	43 [11-75]	*p < 0.001*
10-25%	22	28 [19-37]	
>25%	29	19 [15-23]	

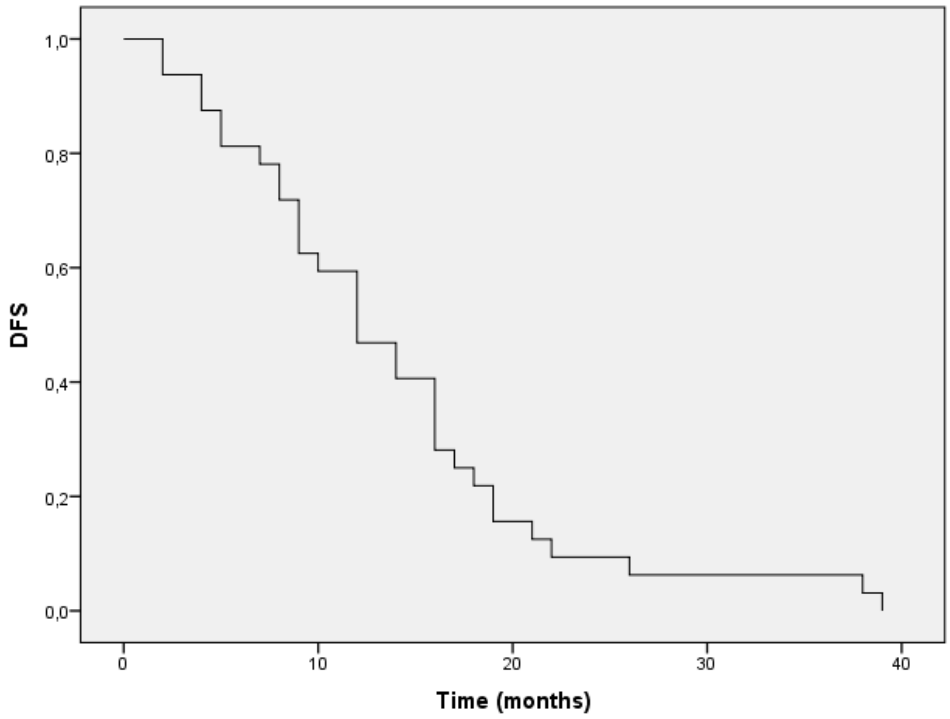
Table 13



Graph 8

Survival analysis - Disease-free Survival (stages I-III)

Median DFS was 12 months [8-16], 53% at 1 year, 12% at 2 years.



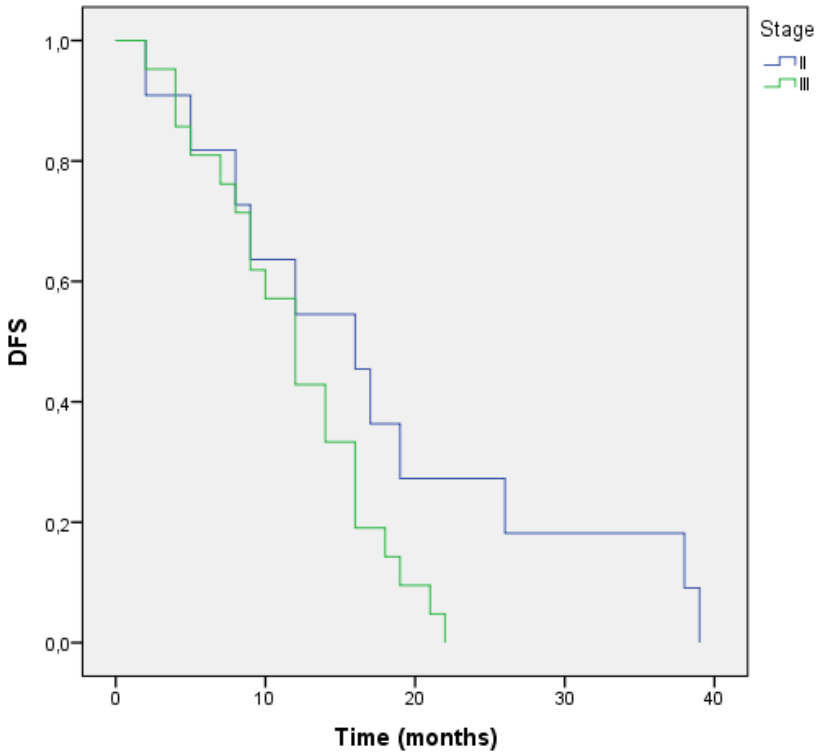
Graph 9

DFS by stage

Only 3 patients with stage I disease were included into the sample.

Stage	n	Median DFS (months)	
II	11	16 [7-25]	*p = 0.090*
III	21	12 [9-15]	

Table 14

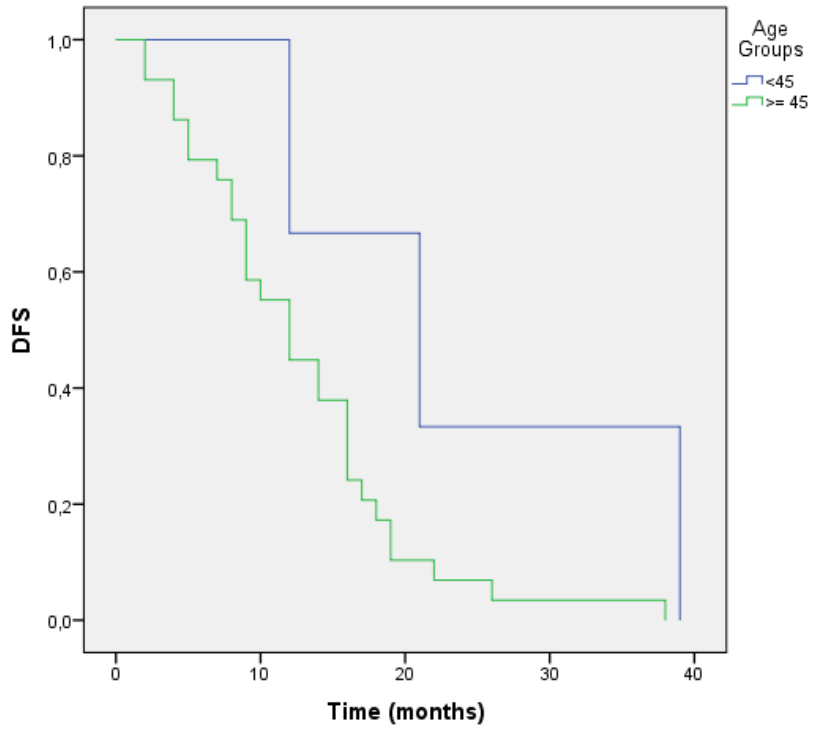


Graph 10

DFS by age (early-onset gastric cancer)

Age group	n	Median DFS (months)	
≥ 45	29	12 [9-16]	*p = 0.074*
< 45 (early-onset)	3	21 [7-35]	

Table 15

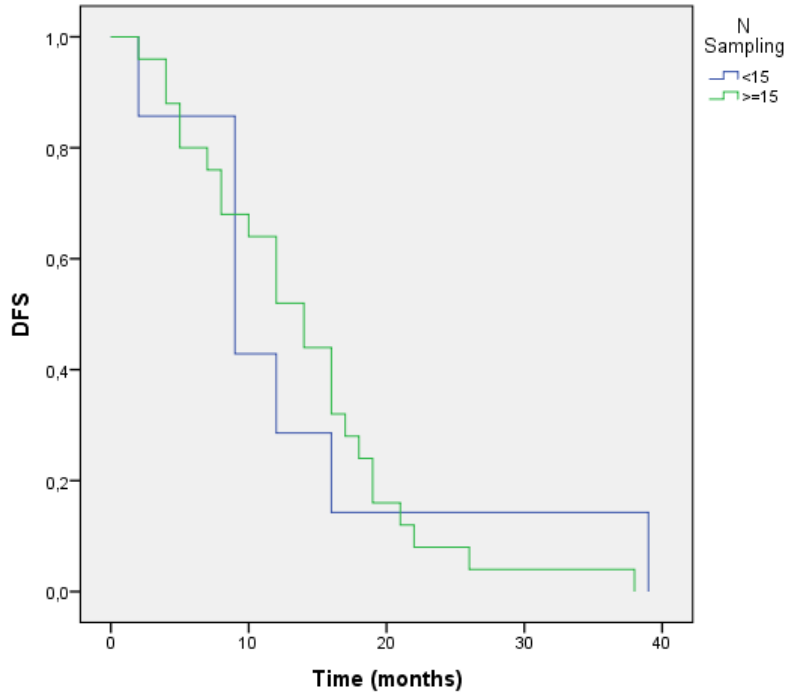


Graph 11

DFS by N sampling

N sampling	n	Median DFS (months)	*p = 0.914*
≥ 15	25	14 [10-18]	
< 15	7	9 [3-15]	

Table 16

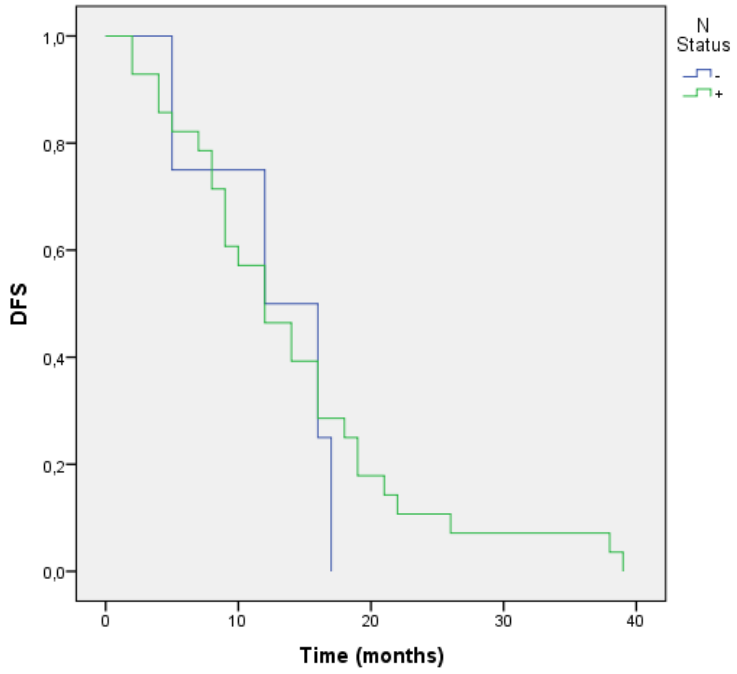


Graph 12

DFS by N status

N status	n	Median DFS (months)	
N -	4	12 [1-23]	*p = 0.673*
N +	28	12 [8-16]	

Table 17



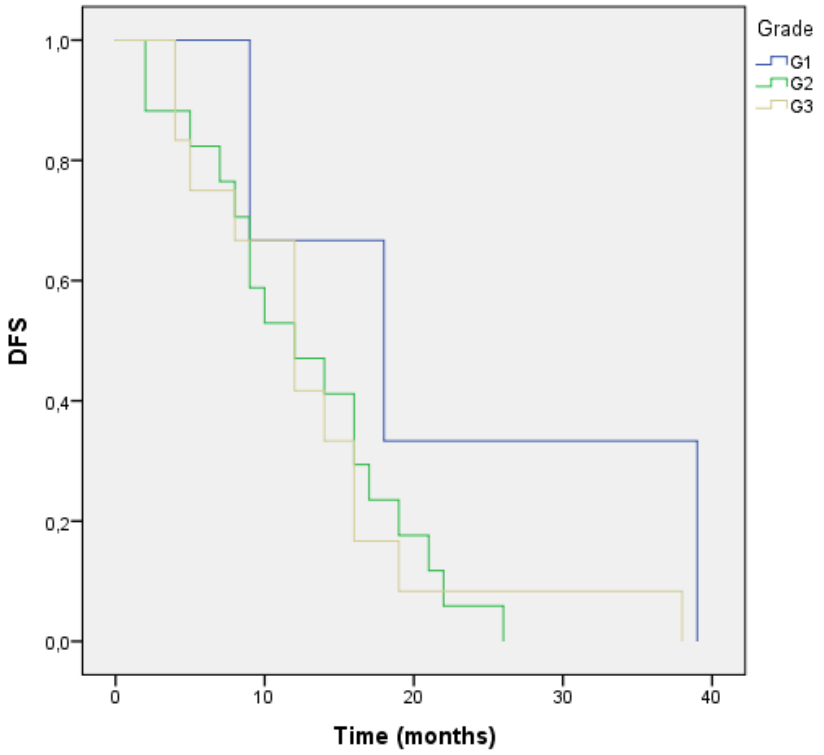
Graph 13

DFS by histological grade

In the subgroup of stage I-III, only 5 tumours were classified as G1, and 4 tumours had undetermined histological grade. A comparison between grades 2 and 3 shows no statistically significant difference in OS.

Histological grade	n	Median DFS (months)	
1	3	18 [6-34]	*p = 0.331*
2	17	12 [5-19]	
3	12	12 [8-16]	

Table 18



Graph 14

HER2

The following tables describe the sample in terms of HER2-status.

HER2 by IHQ			
0	1+	2+	3+
79 (80.6%)	1 (1.0%)	4 (4.1%)	14 (14.3%)
HER2 by SISH			
-		+	
2		2	
HER2 final			
-		+	
82 (83.7%)		16 (16.3%)	

Table 19

HER2	Stage				
	I	II	III	IV	*p = 0.05*
-	3 (3.7%)	42 (51.2%)	29 (35.4%)	8 (9.8%)	
+	0 (0%)	2 (12.5%)	8 (50%)	6 (37.5%)	

Table 20

HER2	N status		
	-	+	*p = 0.006*
-	28 (34.1%)	54 (65.9%)	
+	0 (0%)	16 (100%)	

Table 21

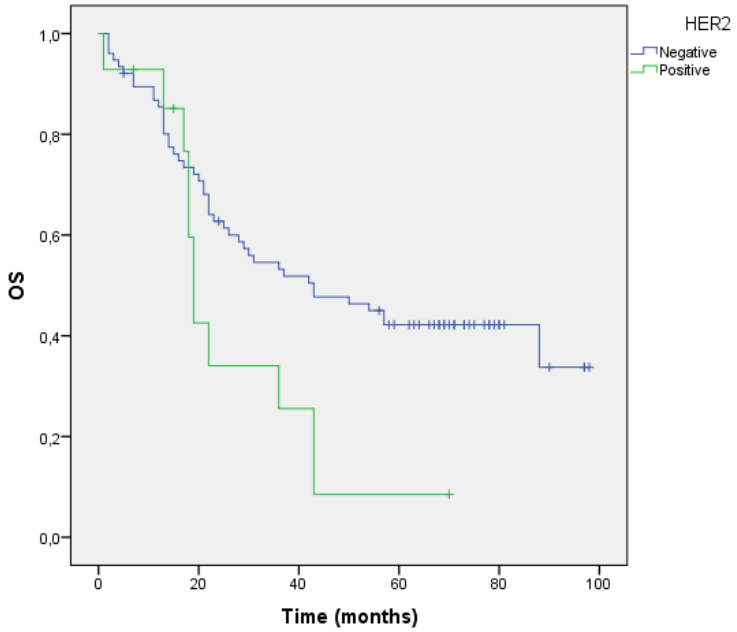
HER2	M status		
	-	+	*p = 0.016*
-	71 (86.6%)	99 (11%)	
+	9 (56.2%)	6 (37.5%)	

Table 22

HER2	n	Mean OS (months)	
-	76	43 [21-65]	*p = 0.032*
+	14	19 [17-21]	

Table 23

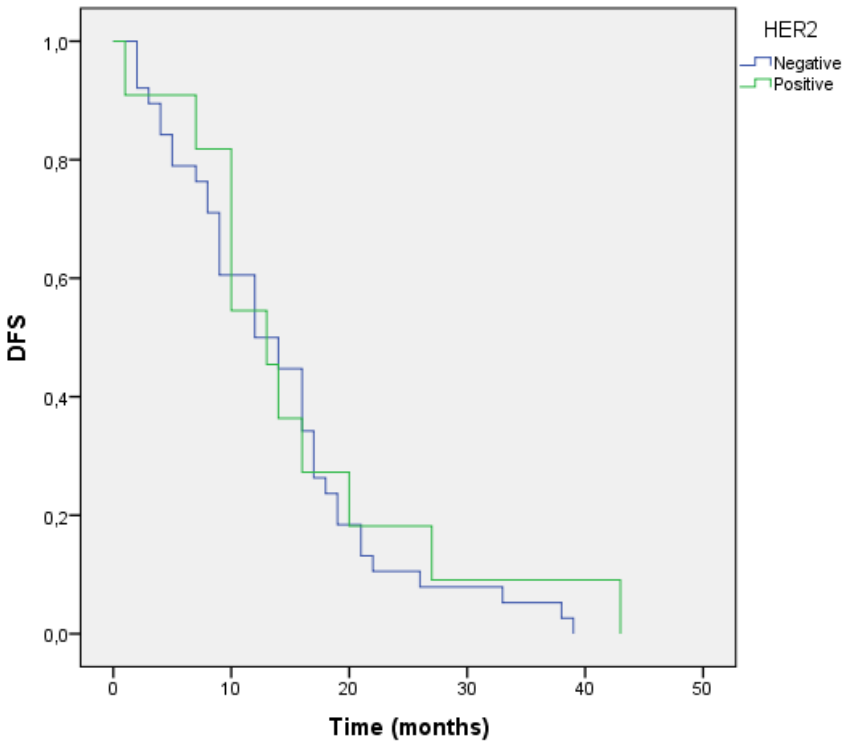
HER2 survival analysis



Graph 15

HER2	Mean DFS (months)	
-	12 [7-17]	*p = 0.572*
+	13 [9-18]	

Table 24



Graph 16

DISCUSSION

Assessing HER2-status has become standard practice in managing breast cancer, as a reliable predictor of response to biological therapeutics with agents targeting the HER2 receptor. Apart from breast cancer, other malignancies were studied for possible therapeutic approaches and gastric cancer followed as the second neoplasia with approved anti-HER2 treatment. Our main objective was to assess the proportion of patients with gastric cancer or GEJ cancer admitted at IPOP eligible for trastuzumab therapy, taking the new histopathological classification standards developed in the context of the ToGA trial into account. On the other hand, this work incorporates a wider project, where the costs of this treatment are determined. Therefore, SISH was selected as the technique employed to assess amplification, due to its inherent lower cost.

The composition of the general sample generally follows a distribution similar to that discribed in the literature. In our study sample, we wanted to include not only metastatic cases but also patients with locally advanced disease, in order to better understand in which way these patients differ from the metastatic cases and if there is a potential role for the use of adjuvant anti-HER2 therapy in this subgroup. In fact, adjuvant treatment with anti-HER2 agents in gastric cancer is currently under discussion and investigation (188).

Primary treatment for gastric cancer remains surgery. In our series, patients that underwent surgery with a curative intent attained a R0 ressection in 91.7%.

Adjuvant and neoadjuvant chemotherapy were not frequently used in our sample, which can be explained taking into account the years involved (2005 and 2006), where the role of this therapeutic modalities was yet to be clarified further. Nevertheless, data reveal a tendency for the use of adjuvant chemotherapy in stage III patients, patients with incomplete ressections and positive N status. Survival analysis regarding adjuvant chemotherapy is limited by the low number of patients that underwent this treatment.

Median OS was 36 months. As expected, TNM staging was highly correlated with different OS between stages, which confirms TNM staging as the most important prognostic factor for gastric cancer. Median survival of 18 months for stage IV is superior than expected, possibly reflecting the low number of cases (n=14). There was a low number of early-onset gastric cancer (n=4); generally in literature gastric cancer diagnosed under 45 is described as making up < 10% of all cases. Data reveal a tendency for a worse prognosis in this subgroup of patients, in line with previously published findings (148). N status significantly influenced OS, therefore further

reinforcing the theoretical advantage of adjuvant therapy in these patients. N-ratio, in accordance with previous publications, significantly influenced OS (241).

Median DFS was 12 months [8-16], 53% at 1 year, 12% at 2 years, in other words, in our sample about half the patients reccur at 1 year and most of the remaining reccured during the following year. Although not statistically significant, there seems to be a tendency confirming the higher odds for early recurrence in stage III vs. stage II patients. As opposed to the role in OS, N-status did not influence recurrence in a statiscally significant way.

Global HER2-positivity was 16.3%, which is in accordance with expected values using the new scoring methods. In a large series published before the new ToGA criteria and with patients from the same centre, positivity rate in intestinal type gastric cancer was about half of the reported in this series (88). Our positivity rate fits into the 9.5-21% positivity rate found in the literature and described in the introduction. The even higher ToGA trial positvity rates are probably due to the higher number of GEJ cancers analysed, which were only in number of 6 in this series.

Analysing HER2-status by stage, it becomes apparent that HER2-positive cases are diagnosed at later stages, with none of the stage I patients in our sample showing HER2 amplification. Further decomposing these analysis, N-status and M-status are independently affected by HER2 status, while T is not ($p= 0.51$). In our sample, OS was negatively affected by HER2-positivity, whereas it had no influence in DFS. The high difference in median OS (43 vs. 19 months) certainly suffers from a small sample size, however, it seem undeniable that patients with locally advanced disease can benefit from anti-HER2 therapy.

As a retrospective work, this study suffers from its inherent limitations. Time and cost restraints imposed selection criteria that deserve a further explanation. While the total number of patients admitted at IPO during the 2 years considered in the study totalizes 720 patients, many had diagnostic biopsy and surgery in other hospitals and were referred for a second clinical opinion or in order to realize chemotherapy after primary surgical treatment. The number therefore decreased to 108 patients, of which 98 had sufficient clinical information accessible in a feasible schedule to be included in the study sample.

Various efforts in detecting potential molecular targets in different cancer cell receptors are being undertaken, which may lead to the development of novel agents directed against kinases (242, 243). As exciting as the evolution of anti-HER2 therapy in gastric cancer may be, strategies to overcome resistance need to be pursued while developing new treatment strategies. A better understanding of the HER signalling pathways and a deeper knowledge about downstream molecules and other signalling pathways

including Wnt/β-catenin and TGF- β/SMAD may contribute to one day achieve multitargeted and network-based therapy possible (179, 244). In such a perspective, a combination of agents will target different crosstalk pathways and contribute to more effective therapies (12).

CONCLUSION

Anti-HER2 therapies have established themselves as valuable partners in the therapeutic strategies against breast cancer and are now part of the standard of care in gastric and GEJ cancer. Our data suggest an important role for theses agents in the adjuvant setting and for locally advanced stages.

However, some open questions remain, regarding the role of these agents in adjuvant therapy, the safety in combination with other chemotherapeutic regimens, the optimal duration of treatment and its usage after disease progression. Addressing resistance and combination therapy with other targeted agents will certainly pose challenges in the future. Further studies in the perioperative and adjuvant settings and in earlier disease stages are warranted, hopefully further extending the survival benefits already found.

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