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SPECIAL ARTICLE

Consensus of the Spanish Society of Medical Oncology (SEOM) and Spanish Society of Pathology (SEAP) for *HER2* testing in gastric carcinoma

Carlos Gómez-Martín · Ángel Concha · José María Corominas · Tomás García-Caballero · Elena García-García · Mar Iglesias · José Antonio López · Santiago Ramón y Cajal · Federico Rojo · José Palacios · Francisco Vera-Sempere · Enrique Aranda · Ramon Colomer · Pilar García-Alfonso · Pilar Garrido · Fernando Rivera · Fernando López-Ríos

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Abstract The identification of HER2 alterations in advanced gastric carcinomas is of critical importance in daily clinical practice as such neoplasms require specific treatment with trastuzumab. For these reasons, pathologists and oncologists with expertise in gastric carcinomas and HER2 testing from both organisations (SEAP and SEOM) have endeavoured to discuss and agree on national guidelines for HER2 testing in gastric carcinomas. These guidelines are based on the experience of those who participated in the discussions and also on experience published internationally. These agreed guidelines give the minimum requirements that a pathological anatomy laboratory must fulfil in order to guarantee adequate HER2 testing in daily practice. Any laboratories which do not meet the minimum standards set out in the guidelines must make every effort to achieve compliance.

Keywords Gastric carcinoma · HER2 · Standardisation · Immunohistochemistry · *In situ* hybridisation · Quality control

The affiliations are listed at the end of the article

C. Gómez-Martín (✉)
GI Cancer Research Unit
Clinical Research Programme
National Cancer Research Centre (CNIO)
C/ Melchor Fernández Almagro, 3
ES-28029 Madrid, Spain
e-mail: cgomezm@cnio.es

Introduction

The need for consensus guidelines for HER2 testing in gastric carcinoma

Following publication of the ToGA trial, it has become clear that reliable testing of the HER2 status of patients with gastric carcinoma is an indispensable requirement for the correct use of anti-HER2 treatment [1]. However, as we learned in the case of breast carcinoma, in daily reality, HER2 testing presents numerous difficulties on account of its complexity. It is necessary to follow strict criteria in methodology and interpretation. Recent studies suggest that one in five HER2 tests is inaccurate [2]. Furthermore, important questions remain to be answered such as the predictive significance of the level of amplification and of chromosome 17 polysomy.

For these reasons, pathologists and oncologists with expertise in HER2 testing and in gastric carcinomas, representing both organisations (SEAP and SEOM), have endeavoured to discuss and agree on national guidelines for HER2 testing in patients with gastric carcinomas. These proposals are based not only on the experience of those participating in the discussions but also on the scientific evidence available in the literature. It must be stressed that, to the best of our knowledge, this is the first set of guidelines of this nature to be published. Figure 1 shows a timeline marking the crucial events in the development of trastuzumab in gastric carcinoma [3–8].

The result is this set of guidelines, created to serve as a foundation for daily use by pathologists and clinicians in our country. The objective is two-fold. Firstly, it gives pathologists detailed recommendations on the different phases of HER2 testing in gastric carcinoma. This will lead

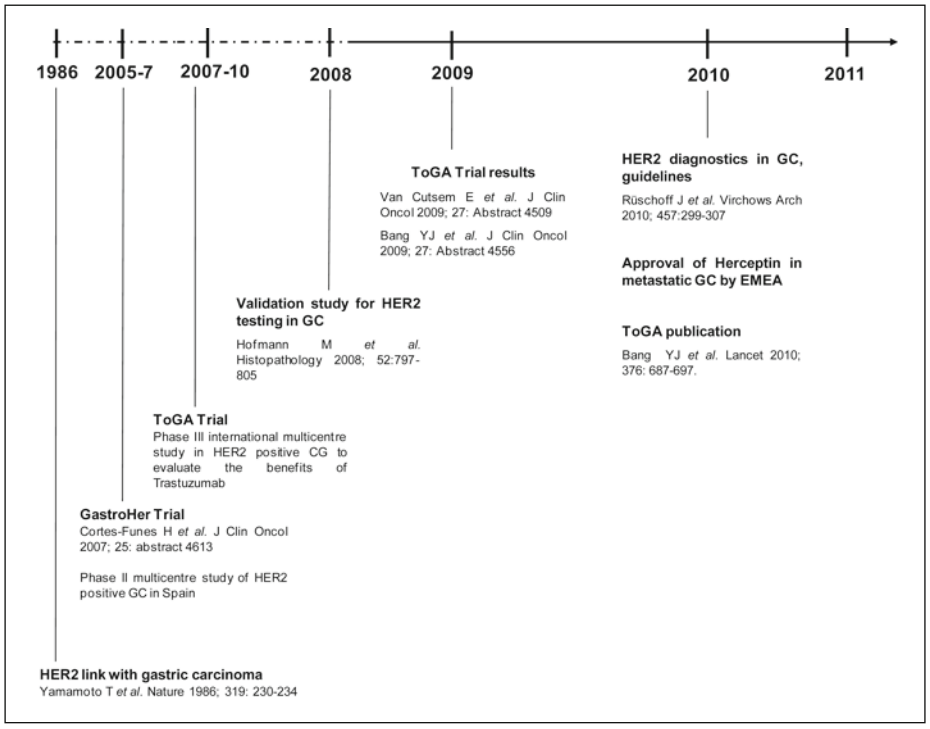

Fig. 1 Timeline of the principal events in the development of trastuzumab in gastric carcinoma

to a standardised application of this practice in healthcare centres throughout the country. Furthermore, problems of methodology and interpretation, which have been common in the case of HER2 testing in breast carcinoma, will be avoided. Secondly, it provides information to clinicians about current indications and limitations of testing, thus enabling the opening of two-way communications between pathologists and oncologists. This in turn will contribute to improving treatment for patients with gastric carcinoma.

What differences exist with breast carcinoma?

When assessing HER2 overexpression and/or amplification in gastric carcinoma, it is necessary to take into account a series of facts which are fundamentally different from those learned in the experience of assessing HER2 in breast cancer. These are:

1. The prevalence of heterogeneous distributions of HER2 amplification in gastric carcinoma is greater than that detected in breast carcinoma [5, 9].

2. The correlation between levels of *HER2* expression detected by immunohistochemistry and the state of the gene is lower than that observed in breast carcinoma [5, 10]. A significant number of gastric tumours with levels of expression classified as 0/1+ present *HER2* amplification. In the ToGA Trial, as many as 18.6% of 1+ gastric carcinomas presented *HER2* amplification [1].

3. The proportion of tests rejected for pre-analytical reasons among gastric carcinoma samples could be reduced by frequent use of small-sized diagnostic biopsies [10].

4. According to EMEA approval for trastuzumab, immunohistochemistry is more predictive than hybridisation and, as such, the latter should be reserved for re-classifying 2+ cases [1, 8].

Clinical importance of HER2 testing in gastric carcinoma

The oncogene *HER2/neu* (c-erb-B2) is situated on chromosome 17 (17q11.2-q12) and codifies a protein (HER2) of 185 kDa with three domains, one extracellular, rich in cysteine residues, one transmembrane and one intracellular domain with tyrosine-kinase activity. HER2 acts as a receptor on the surface of the cell and belongs to the erbB family, which comprises four members: HER1 or EGFR, HER2 itself, HER3 and HER4. Activation of these receptors requires the formation of homodimers or heterodimers after the binding of a ligand with its specific receptor. Ligands specific to HER2 have not been identified. Therefore, the cause of HER2 homodimer formation has been ascribed to alternative mechanisms such as overexpression of the receptor due to genetic amplification of the *HER2* oncogene itself; defects in the internalisation and degradation of HER2 on the membrane; and the absence or mutation of the suppressor gene FOXP3 [11]. *HER2* can also be activated in aberrant form by spontaneous mutations in its sequence [12, 13]. Regardless of the causal mechanism, overexpression of HER2 molecules facilitates the spontaneous formation of dimers on the surface of the tumour cell leading to the activation of different intracellular signalling pathways. This, in turn, leads to an increase in cellular

Table 1 Summary of reported clinical trials of gastric cancer patients treated with herceptin combinations

Trial	Rx	N (screening)	ORR (%)	CBR (%)	Med PFS (months)	Med OS (months)
Gastroher	C+herceptin	22 (228)	31	63.6	5.1	12.9
ToGA	CF±herceptin	584 (3807)	34.5 CF 47.3 CF+H	ND	5.5 CF 6.7 CF+H	11.1 CF 13.8 CF+H
Nicholas	TC+herceptin	9 (55)	80	100	ND	ND
Rech	2nd-line herceptin	3 (33)	33 (1 pt)	ND	ND	ND

Rx, treatment; ORR, overall response rate; CBR, clinical benefit rate; med PFS, median progression-free survival; med OS, median overall survival; ND, not determined; C, cisplatin; F, 5Fu/capecitabine; T, docetaxel

proliferation; greater cell survival through apoptosis avoidance; loss of control of the cell cycle; greater dedifferentiation; and an increase in cellular migration.

In the case of breast cancer, the percentage of cases of HER2 overexpression or amplification is relatively well established and testing is now routine practice. It is different in the case of identifying HER2 alteration in other epithelial tumours where there is significant disparity of results [14, 15]. The first studies on the amplification of the *HER2* gene and its protein overexpression in gastric carcinomas were published in 1986 [3]. Since then, a multitude of studies have confirmed the presence of amplification and overexpression of this oncogene and of the receptor for which it codes in gastric cancer. The range of results in the studies based on different series analysed by immunohistochemistry is very broad, varying between 8.25% [16] and 53.4% [17]. Additionally, numerous studies have investigated *HER2* amplification in gastric cancer using *in situ* hybridisation techniques, basically FISH or CISH. Although somewhat lower than in the case of receptor overexpression, there is still a certain disparity in the range of amplification cases, with values that vary between 16% [18] and 27.1% [19]. Tables 1 and 2 summarise the published studies with the greatest number of cases [revised in 10]. Similarly to initial occurrences in studies on malignant mammary neoplasms, the correlation between HER2 receptor overexpression and the presence of amplification of its gene is a question of some controversy. Currently, in the case of breast cancer, protein overexpression is considered to be due principally to the amplification of the gene, leading to an increase in transcription and, consequently, an increase in the number of receptors in the cell membrane [20]. In the case of gastric adenocarcinomas, correlation between protein expression and *HER2* amplification has

Table 2 Gastric cancer HER2 overexpression studies including more than 100 cases each

Reference	*N* '	Location	% Cases with HER2 overexpression	% cases of intestinal type	Antibody anti HER2
Yonemura Y 1991	260	Japan	11.9	Not determined	Polyclonal pAB-1 (Triton Bioscience)
Uchino S 1993	214	Japan	9.8	Not determined	Polyclonal (Nichirei)
Lee HR 1996	225	Korea	27.4	Not determined	Monoclonal TAB-250 (Triton Bioscience)
Shun CT 1996	112	Taiwan	30.3	56	Monoclonal 3B5 (Oncogene Science)
Ooi A 1998	396	Japan	10.1	Not determined	Monoclonal CB11 (Novocastra)+polyclonal (Nichirei)
Ishikawa T 1997	375	Japan	10.4	57	Monoclonal CB11 (Novocastra)
Wu MS 1997	163	Taiwan	26.4	54	Monoclonal 3B5 (Oncogene Science)
Allgayer H 2000	189	Germany	53.4	53	Monoclonal 3B5 (Oncogene Science)
Ougolkov A 2000	116	Japan	16.0	Not determined	Polyclonal (Nichirei)
Sanz-Ortega J 2000	143	Spain	31.0	75.5	Monoclonal CB11 (Novocastra)
Wang YL 2002	100	Taiwan	32.0	85	Polyclonal A0485 (Dako)
Ghaderi A 2002	146	Iran	16.4	65	Monoclonal ICR12 (Santa Cruz Biotech)
Takehana T 2002	352	Japan	8.2	Not determined	Polyclonal (Nichirei)
Pinto-de-Sousa J 2002	147	Portugal	15.3	51	Polyclonal A0485 (Dako)
Lee KE 2003	6141	Korea	17.0	38	Monoclonal CB11 (Novocastra)
Yano T 2006	200	Japan	23.0	100	Herceptest (Dako)
Park DI 2006	182	Corea	15.9	48	Polyclonal (Zymed Labs)
Hofmann M 2008	168	China, Mexico, Germany	10.7	71	Herceptest (Dako), modified
Barros-Silva JD 2009	463	Portugal	9.3	40	Monoclonal CB11 (Novocastra)
Marx AH 2009	166	Germany	16.9	63	Heceptest (Dako)
Yu GZ 2009	1143	China	28	Not determined	Monoclonal SP3 (Lab Vysion)
Bang YJ 2009	3807	Global	10.97	52	Herceptest (Dako)
Grabsch 2010	924	Germany, England	6–10	62	Monoclonal CB11 (Novocastra)

Table 3 Gastric cancer *HER2* amplification studies with more than 100 cases each

Reference	N '	Location	% Cases with *HER2* overexpression	% cases of intestinal type	*In situ* hybridisation method
Park D 2006	182	Korea	3	48	FISH PathVysion (Vysis), CISH SPOT-Light (Zymed)
Ishikawa T 1997	120	Japan	18.1	Not determined	FISH 17q11.2-12 specific Cosmid probe (Oncor)
Tanner M 2005	131	Finland	17.3	49	CISH SPOT-Light (Zymed)
Yano T 2006	199	Japan	27.1	100	FISH PathVysion (Vysis)
Hofmann M 2008	168	China, Mexico, Germany	17.4	71	FISH pharmaDx (Dako)
Marx AH 2009	166	Germany	16.0	63	FISH PathVysion (Vysis)
Barros-Silva JD 2009	463	Portugal	8.23	40	HER2 FISH probe (MP Biomedicals)
Bang YJ 2009	3807	Global	23.05	52	FISH PharmDx (Dako)
Garcia-Garcia E 2010	166	Spain	17.5 (FISH), 21 (SISH)	52	FISH PathVysion (Vysis) modified+ SISH INFORM™ (Ventana)

been examined in several studies with varying results [5, 10, 18, 21]. The majority of these studies appear to indicate that *HER2* oncogene amplification determines receptor protein overexpression, in the same way as occurs in the case of breast cancer.

The potential prognostic value of the *HER2* oncogene in gastric carcinomas is a controversial topic [1, 22–24]. Historically HER2 overexpression and/or amplification has been considered a feature of poor prognosis, associated with diagnosis in more advanced stages, lymphatic involvement or lower survival rate after surgery. However, the most recent studies appear to cast doubt on this negative nature [10]. It remains to be explained whether the greater survival rate achieved with standard chemotherapy in patients with HER2 overexpression and/or amplification is related to the molecular alteration itself, the greater percentage of cases among these patients being intestinal-type, or if it is due to factors as yet unknown.

With regard to the predictive value of HER2 in response to biological treatments targeting the receptor, the ToGA Trial alone has demonstrated a clear association between the alteration and the response.

In the development of trastuzumab (Herceptin®, Roche, Basel), the first monoclonal antibody targeting the HER2 receptor used in humans, various preclinical trials showed the antitumour activity of this drug both in monotherapy and in combination with cytotoxic agents in gastric adenocarcinoma cell lines [25]. Apart from the GastroHER and ToGA clinical trials [1, 4], information on the use of trastuzumab in patients with gastric carcinoma is based on a few individual published cases [26, 27] and on non-randomised phase II clinical trials from which only preliminary information is available [28, 29] (Table 3). The GastroHER study, the first phase II study conducted in HER2-positive gastric carcinoma [4], was conducted in Spain as a multicentre study. In the study, HER2 receptor status was tested by immunohistochemistry and possible amplification of the gene by FISH in 228 patients with untreated metastatic gastric carcinoma. Of these, 22 patients (10%) showed moderate or strong amplification and/or overexpression by immunohistochemistry and received treatment with trastuzumab and cisplatin. There was an objective response rate of 33% and disease control was achieved in 64% of cases. The average time to tumour progression obtained was 5.1 months and the overall survival was 12.9 months, clearly better than that obtained with standard chemotherapy regimens to date.

The first phase III randomised clinical trial was the so-called ToGA Trial [1], which compared treatment with a standard chemotherapy regimen such as cisplatin/5-FU or cisplatin/capecitabine vs. a combination of chemotherapy and an agent targeted at HER2 (trastuzumab) in patients with gastric metastatic carcinoma. This multicentre study (24 countries) included patients with 3+ HER2 overexpression measured by immunohistochemistry or with *HER2* amplification determined by FISH. Tests were conducted in a centralised reference laboratory. A consensus was established among the participating pathologists prior to the study being implemented [5]. Screening took place of 3803 potential candidates using immunohistochemistry and FISH. Of these candidates, 810 ultimately presented receptor overexpression or amplification of the *HER2* gene. Five hundred and ninety-four patients were then included in the study. These were randomised to receive cisplatin and 5-FU or capecitabine or the same chemotherapy regimen plus trastuzumab. The principal objective of the study was to find a significant difference in the patients' overall survival rate in favour of the experimental arm with trastuzumab. Among the secondary objectives was finding an increase in the rate of objective responses to the treatment and in the tumour progression-free interval. After an average course of 17.1 months for the total study population, the results were presented at the American Clinical Oncology Congress in June 2009 [6, 7] and finally published in August 2010 [1].

This study found a statistically significant link between HER2 overexpression and/or amplification with intestinal-type vs. diffuse adenocarcinomas (32.3% and 61% respectively) and with tumours situated in the gastroesophageal junction in comparison with other tumour

locations (33.2% vs. 20.9%). The correlation between the results of the analysis with immunohistochemistry and the FISH analysis of HER2 status was 87.3%. The principal result of the study was attained, with the addition of trastuzumab to standard chemotherapy treatment achieving an increase in overall survival rate of 2.7 months (13.8 vs. 11.1, HR 0.74, p=0.0046). Similarly, the group which received trastuzumab and chemotherapy attained a longer tumour progression-free period (6.7 vs. 5.5 months, HR 0.71, p=0.002) and an increase in the objective tumour response rate (47.3% vs. 35.5%, p=0.0017). A subanalysis of this study, which included only those cases with an immunohistochemistry result of 3+, 2+ and amplification detected by FISH, concluded that the efficacy of the treatment might be greater in the subgroup with more intense receptor expression, on observing an increase in the overall survival rate of 4.2 months in the group which received trastuzumab (11.8 months in the control arm vs. 16.0 months in the experimental arm with HR 0.65, CI 95% 0.51–0.85). Tolerance of the combined trastuzumab and chemotherapy treatment in the ToGA Trial was adequate. Significant differences were not shown in haematological or extra-haematological toxicity between the two arms of the study with the exception of a greater incidence of asymptomatic left ventricular disfunction in the group which received trastuzumab. Subsequent analysis of the quality of life of the patients included in the trial did not find any differences between the two arms of the study [30].

Recently, results have been published which show that lapatinib (a dual HER1/HER2 tyrosine kinase inhibitor) is synergistic with trastuzumab when used in combination to treat gastric carcinoma cell models with *HER2* oncogene amplification, achieving tumour regression in some of the tumour xenografts studied [31].

Given the increase in the overall survival rate, a survival which requires the administration of trastuzumab in combination with chemotherapy in patients with HER2 overexpression or amplification, correct determination of HER2 status is essential. This enables accurate selection of those patients with greater potential to respond to treatment directed at this molecular target.

The ToGA Trial methodology

Optimisation of HER2 testing prior to the ToGA Trial

As a criterion for inclusion in the ToGA trial, it was necessary to confirm HER2 overexpression and/or amplification in patients with gastric carcinoma. For the HER2 study, the criteria adopted were those established by Hofmann et al. in an earlier exploratory study in which a total of 178 tumours were analysed, producing 168 valid samples for HER2 testing [5]. The location of the tumours analysed was the stomach in 149 cases, the gastroesophageal junction in 16, the oesophagus in three cases, along with two samples of recurrent tumours and one metastatic. The tumours were classified according to the Lauren Classification. Distribution was as follows: 120 intestinal-type, 39 diffuse and 9 mixed.

The samples came from tumours that were formalin-fixed and paraffin-embedded. The study to identify the HER2 protein by immunohistochemistry was conducted with HecepTest™ using a manual procedure, following the manufacturer's instructions (Dako, Glostrup, Denmark). Analysis of HER2 gene status was conducted by fluorescence *in situ* hybridisation (FISH) with the *HER2* pharmDx™ (Dako). Significant changes were made to the protocol as follows: (a) conditions for the proteolytic pretreatment with pepsin which were set at two minutes at 37°C and (b) use of a thermostatic oil bath at 107°C in place of the water bath at 95–98°C for ten minutes, which is stipulated in the denaturation protocol [5].

In the first attempt, the immunohistochemistry study was scored according to the HercepTest™ assessment criteria approved for breast cancer, with a cut-off point of 10% of tumour cells with expression. The modifications established in the ASCO/CAP guidelines published in 2007 [32] were not included. The immunohistochemistry study revealed very significant differences between the patterns of protein expression in gastric carcinoma and breast carcinoma. Firstly, incomplete staining (basolateral) could be observed frequently, which resulted in a large number of cases being classified as HER2 negative. This can be attributed to the particular features of gastric secretory cells and to the formation of glandular lumens being greater in gastric carcinoma than in breast carcinoma. Secondly, a greater level of heterogeneity was identified in the HER2 expression (4.8% of cases) than that observed in the case of breast carcinoma. This resulted in negative classification of tumours with intense expression but with a percentage of cellular positivity lower than 10%. Taking these two circumstances into account, the cases in this series were classified as follows: 121 (72%) tumours were considered HER2 negative; 29 (17.3%) tumours HER2 equivocal and 18 (10.7%) HER2 positive.

The FISH study gave a result of a total of 29 cases with *HER2* amplification, which represented 17.26% of the total gastric carcinomas analysed. It was considered a gastric carcinoma with amplification of the *HER2* gene when the *HER2*/chromosome 17 centromere ratio was >2.

In this preliminary study of Hofmann et al., and following the adapted criteria for gastric carcinoma, correlation between the techniques of immunohistochemistry and FISH was 93.5%. This recommends both as appropriate techniques for HER2 testing in patients with gastric carcinoma for the purpose of selection for treatment with trastuzumab [5].

HER2 testing in the ToGA Trial

In the ToGa Trial, of a total of 594 patients, 584 were included in the principal study analysis [1]. HER2 status was

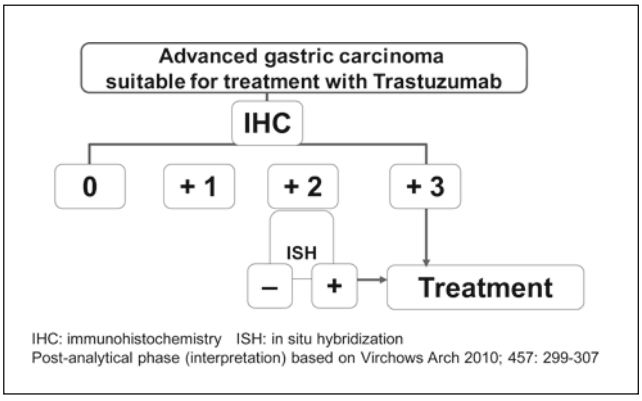

Fig. 2 Algorithm for HER2 testing in gastric carcinoma

distributed as follows: 131 (22.4%) with FISH amplified and negative immunohistochemistry (0 and 1+); 159 patients (27.2%) with FISH amplified and immunohistochemistry 2+; 256 patients (43.8%) with FISH amplified and positive immunohistochemistry (3+); 15 patients (2.7%) with FISH non-amplified and positive immunohistochemistry; and 23 patients (3.9%) showed positive results for FISH or immunohistochemistry. In analysing the type of sample assayed, it was observed that the percentages of positivity for HER2 were greater in the samples obtained from endoscopic biopsies, both for immunohistochemistry (11.26%) and for FISH (24.36%), in comparison with samples obtained from surgical specimens: 10.39% with immunohistochemistry and 20.52% with FISH. This difference was statistically significant in the FISH analysis.

The diagnostic algorithm proposed in this study for determining HER2 status in gastric carcinoma recommended immunohistochemistry as the technique of first choice. Patients suitable for treatment with trastuzumab are those with expression scored as 3+ and those with expression scored as 2+ in which *HER2* amplification was confirmed using an *in situ* hybridisation technique (Fig. 2).

HER2 testing after the ToGA Trial

The objective of the post-ToGA study was to validate the procedure for HER2 analysis in gastric carcinoma, firstly by analysing the reproducibility of the immunohistochemistry method used by numerous pathologists in different laboratories [8]. For this, variability among laboratories was analysed using a tissue microarray of 30 gastric carcinoma samples on which different immunohistochemistry methods were used. With the data obtained, guidelines were created for reading HER2. Secondly, there was analysis of the reproducibility of the interpretation criteria among different observers. A wider sample of cases was analysed on which immunohistochemistry had been performed in the same laboratory. Finally, in order to validate the established guidelines, another series of cases from the daily routine of five laboratories was analysed.

In order to ensure that the HER2 reading is reproducible, the consensus group recommends considering as valid microscope magnification which permits clear visualisation of lateral or basolateral staining of the membrane (linear and intercellular): "microscope magnification rule".

As a positivity criterion, the authors stipulate the presence of at least 10% of tumour cells with HER2 expression in a resection specimen. In cases from endoscopic biopsies, a minimum of five cells with expression of the receptor is stipulated as valid given that, with fewer than five cells, the correlation among observers was very low.

The use of assays accepted by the FDA and/or EMEA was recommended in selecting HER2-positive patients in gastric carcinoma. In comparing different immunohistochemical methods, the antibody 4B5 (Ventana Medical Systems) showed at least the same sensitivity as HercepTest (Dako) with a greater correlation of the interpretation of results among laboratories and a greater correlation among cases with immunohistochemistry 3+ and amplified HER2 [8].

Furthermore, participation in quality control programmes is recommended, for both immunohistochemistry and *in situ* hybridisation techniques, specifically for gastric carcinoma, given that the reading of HER2 differs from that applied in breast cancer.

The application of these post-ToGA rules of interpretation to a series of 153 tumours, in parallel with the study on the same rules of interpretation of HER2 amplification by SISH, showed a complete correlation in cases with immunohistochemistry 3+ and positive *in situ* hybridisation. Despite this, in neither the ToGA Trial nor in this study [1, 8] is it clearly established whether there is a response-predictive correlation between treatment with trastuzumab and the ratio of amplification of the *HER2* gene in cases of gastric carcinoma.

A final observation makes reference to the frequent heterogeneity in HER2 expression in gastric carcinoma. For this reason, it is recommended that screening of the whole tumour area is conducted, especially if the FISH method is employed. The authors suggest that, as it offers better morphological control, the use of bright field hybridisation techniques would facilitate detection of foci of HER2 amplification in heterogeneous cases.

Pre-analytic phase in HER2 testing in gastric carcinoma

When to test

Determination of HER2 status will be conducted in advanced-stage adenocarcinomas of the stomach and of the gastroesophageal junction. This is because therapeutic use of trastuzumab is currently approved for this stage only (Table 4). It is probable that, in the near future, if the clinical trials underway establish it, routine analysis of HER2

Table 4 HER2 testing in gastric carcinoma

Recommendation
HER2 Testing should be performed to all patients diagnosed with advanced stage adenocarcinoma of the stomach or gastroesophageal junction before deciding any combination treatment including
Types of samples
Biopsies or surgical specimens from tumours
In suboptimal samples, use of *in situ* hybridisation techniques is recommended
Cytological samples are only recommended for *in situ* hybridisation studies and only when no alternative tissue sample is available

status will be necessary in all cases which might potentially receive anti-HER2 treatment, regardless of the stage, as has already occurred in breast cancer.

Sample types

Samples obtained by endoscopic biopsy and those provided through surgical resection are equally acceptable for HER2 testing by immunohistochemistry and, similarly, *in situ* hybridisation. In the case of advanced-stage tumours, the available material will generally be an endoscopic biopsy. Although initially it could be considered that sections provided by surgical specimens might be more representative, given the heterogeneity of HER2 expression in gastric adenocarcinomas, endoscopic biopsies are equally useful for conducting these tests, always assuming that there is a minimum number of samples available (6–8 fragments). In fact, indications exist which suggest a greater sensitivity in endoscopic biopsies when positive cases are diagnosed in comparison with surgical specimens [10]. This surprising finding may be explained, at least in part, by the following reasons:

• The cases biopsied by endoscopy, without prior surgical resection, largely correspond to advanced tumours, metastatic or recurrent, which might present HER2 activation with greater frequency than localised tumours if the HER2 activation is not an early event in carcinogenesis of the gastric mucosa.

• The criteria for assessing HER2 in endoscopic biopsies are different from those used on surgical specimens. The threshold for considering a case positive is also different. It is more strict for surgical specimens than for endoscopic biopsies [8].

• Fixation and processing of endoscopic biopsies is better than for surgical specimens. The latter present a high rate of false negatives due to inadequate fixation. However, endoscopic biopsies can present retraction artifacts, instrumental crushing or the "margin effect" which lead, with greater frequency, to false positives.

Cytological samples are not recommended for HER2 studies although cell extensions can be used where no other more suitable types of samples are available. In such cases, *in situ* hybridisation techniques are recommended as these give better results than immunohistochemistry techniques. This is because cell membrane integrity is often damaged in cytological preparations and assessment of immunostaining is difficult or impossible.

If samples of metastatic lesions are available to us, where the primary tumour is not available or has proved negative, study of HER2 in the aforesaid lesions is recommended.

Where samples are suboptimal from a pre-analytic point of view, *in situ* hybridisation is recommended as the first technique of choice. In our experience, there is no less analytical value in the case of *in situ* hybridisation on paraffin blocks of some years of age as some authors have suggested [33].

Fixation

Fixation shall always use 10% neutral-buffered formalin at constant room temperature. Once the sample has been obtained, fixation shall be completed as soon as possible. In the case of surgical specimens, this shall be within 30 minutes of excision [34]. It is advisable to process a gastrectomy specimen (opening and placing it in a suitable container) when the laboratory receives it in order to facilitate fixation. It is recommended that the volume of fixative should be at least four times the volume of the specimen.

Techniques for HER2 testing using immunohistochemistry and *in situ* hybridisation are validated for formalin-fixed, paraffin-embedded tissue samples. Therefore, conducting such techniques on other types of samples should be avoided.

In general, for immunohistochemistry, fixation of samples is not recommended at less than 6 hours [35] or more than 48 hours [34]. The optimum time for fixation for gastric biopsies is between 6 and 24 hours. Surgical specimens must be fixed between 24 and 48 hours. It must be taken into account that formalin penetration (1 mm/h) does not mean fixation and that the reaction for the formation of methylene glycol bonds is not immediate but is quantified in hours [36]. Defective fixation is considered more serious than over-fixation in immunohistochemistry [35]. Excessive fixation tends to cause a decrease in immunostaining and false negatives. Insufficient fixation, on the other hand, produces a proportionally predominant influence of alcohol-based fixatives and, as such, greater immunoreactivity and false positives. Rapid fixation methods using microwaves are not advisable. They tend to result in

Table 5 Reasons for rejecting HER2 tests in gastric carcinoma

When the recommended pre-analytical and analytical requirements are not met (fixation other than formaldehyde buffered at 10%)
Unsatisfactory results in the controls
Lack of tumour component in the histological section
Presence of a marked technical or procedural artifact
Negative results in sections prepared during a period in excess of four to six weeks (which have not been paraffinised and/or stored at 4°C)

heterogeneous fixation, which varies with sample size and tissue type [35].

Processing and sectioning

For an effective procedure, in the case of surgical specimens, it is recommended to embed tissues which do not exceed 2 cm in length and 3 mm in thickness [34]. Samples shall be processed routinely, avoiding the use of rapid embedding methods based on the use of microwaves.

Procedures for HER2 testing shall be performed on high-quality sections of 4 μm, avoiding irregularities of thickness, scratches, bubbles or any other artifact which hinders the conducting or interpreting of the procedures. Sections shall be mounted on treated slides to prevent cleavage during the procedures. Sections shall be dried at 60ºC for one hour or at 45ºC overnight.

Any incident occurring during the process of fixation or embedding in paraffin must be documented in order to be taken into consideration at the point of scoring. Such incidents must also be included in the report if sufficiently important.

Use of recent sections (from the previous day) is recommended for HER2 testing. Sections which have been stored for more than six weeks shall never be used when the study of HER2 expression is conducted by immunohistochemistry. When the study is conducted by *in situ* hybridisation, the maximum storage period is six months. Otherwise, there is a greater probability of false negatives. In all cases, whenever there is a delay in conducting the procedures, it is useful to apply a protective coating of paraffin to the sections [37] in order to prevent loss of antigenicity caused by photo-oxidation and drying of the tissues [38].

The paraffin block selected for conducting the procedures must be chosen after all the preparations have been examined. It must be representative of the neoplasm, avoiding hypocellular areas, areas with necrosis, haemorrhage, poor tissue quality, autolysis and artifacts. In mixed tumours, areas with intestinal-type histology must be selected as the probability of observing HER2 overexpression is much greater than in areas of diffuse-type.

In order to avoid problems of irregular or background staining, it is essential to deparaffinise the samples properly. The first xylol bath must be discarded and an additional xylol bath applied before the alcohols. It should be remembered that, when HER2 testing is to be conducted on sections protected by a paraffin coating, more thorough deparaffinising is necessary. In this case, it is recommended either to double the deparaffinising time or to place the preparations in xylol in a heater at 60ºC for ten minutes prior to performing the usual deparaffinising process.

Guidelines for HER2 testing by immunohistochemistry

Method

For HER2 testing by immunohistochemistry, use of diagnostic kits certified by the FDA and/or EMEA is recommended. Use of standardised kits requires strict adherence to the manufacturers' instructions with no variations. Even when using these approved methods, at the start of their use, it is recommended that each laboratory conduct a technical validation. For this, it is recommended that the results of at least 25 positive and 25 negative cases should be compared with a reference centre, achieving a correlation of 95%.

The number of annual tests considered optimum for guaranteeing the technical sufficiency of a laboratory is 250, including HER2 testing for breast cancer.

Controls

Kits for conducting standardised tests provide a number of positive and negative controls from cell lines. As the number of controls is limited, grouping cases of breast carcinoma and gastric carcinoma is recommended.

In addition to the commercial controls mentioned, use of internal controls is recommended with the fixing and processing conditions of the laboratory. A case with weak–moderate (2+) immunostaining should be used which enables us to detect mild losses of sensitivity easily.

Reasons for rejecting a test

The reasons for rejecting a test are summarised in Table 5.

Assessment of the results

As recommended in the guidelines for HER2 testing in breast cancer, assessment of immunohistochemical positivity for HER2 must be conducted by a pathologist. Similar criteria shall be followed in interpretation as in assessment

Table 6 Interpretation of immunohistochemistry testing of HER2

Negative (0)	Absence of staining or staining in less than 10% of cells (surgical specimens)[a]
Negative (1+)	Membrane staining, laterally at least, almost imperceptible (visible only with a 40× objective) in >10% of cells (surgical specimens)[a]
Indeterminate (2+)	Moderate membrane staining, laterally at least, (visible with objectives of 10–20×) in >10% of cells (surgical specimens)[a]
Positive (3+)	Intense membrane staining, laterally at least, (visible with objectives of 2.5–5×) in >10% of cells (surgical specimens)[a]

Only the infiltrating component and membrane staining shall be evaluated

[a]In endoscopic biopsies, tumour nests of at least five cells are considered positive regardless of the staining percentage (for example, less than 10%)

exclusively in the tumour component and in considering the membrane staining in terms of number of cells and intensity.

However, there are some differences with the assessment of breast carcinoma. These are summarised below [5, 8].

Membrane staining

In carcinoma of the stomach, the staining pattern of the membrane is very frequently basolateral or lateral without staining of the luminal pole. For the purpose of assessment, it was decided to consider as positive circumferential or complete membrane staining and/or basolateral even if incomplete.

Staining intensity is considered: negative, barely perceptible, moderate and intense.

Percentage of positive cells

HER2 overexpression in gastric carcinoma is much more heterogeneous than in breast cancer. As such, the criteria differ according to whether a surgical specimen or an endoscopic biopsy is being assessed.

In assessment of endoscopic samples, a test shall be considered positive when a group of at least five cells is detected with intense membrane staining (3+), regardless of the percentage which it represents. When the study is conducted on surgical resection specimens, HER2 positivity must be in >10% of the tumour component.

Scoring

Table 6 sets out the guidelines on scoring.

Automated post-analytical (interpretation) phase

According to some authors, use of automated image analysis systems minimises interobserver variability in interpretation. As such, these systems are considered accurate and effective tools for assessing HER2 immunostaining [39, 40].

Quality control

For quality control in HER2 testing in different laboratories, it is advisable to bear in mind that the endoscopic biopsies assessed must contain a minimum of six tumour fragments.

Incidentally, it is important to consider the percentages of positive cases published in various series which indicate that:

– Gastric adenocarcinomas show positivity for HER2 in around 15–17% of cases.

– Positivity is greater in intestinal-type adenocarcinomas (30%) than in diffuse-type (5.5%), while in mixed type it is approximately 20%.

– Positivity is more frequent in adenocarcinomas of the gastroesophageal junction (more than 30%).

As such, it is highly advisable, every year, for laboratories to calculate the percentage of cases corresponding to each score (0, 1+, 2+ and 3+) in order to confirm whether the results match those obtained in the large series. The aforesaid percentages are important when assessing whether testing is being conducted properly.

Similarly, it is very important to remember that approximately 25% of positive cases by FISH are negative (0 or 1+) in studies by immunohistochemistry.

Finally, and as discussed in the section on quality control, every laboratory must add internal controls and participate in external evaluations with other laboratories or accredited centres.

Personnel

The number of laboratory technicians involved in conducting tests and the number of pathologists who interpret them must be as low as possible in order to guarantee the effectiveness of the undertaking. Both the technicians and the pathologists must have undergone training. For assessing the post-analytical (interpretation) phase, correlation with the reference result of at least 95% in 50 cases is recommended. Periodically, the aforesaid training should be refreshed in dedicated working sessions.

Report

The data required in the report are summarised in Table 7. The recommended turnaround time is ≤7 days.

Guidelines for HER2 testing by *in situ* hybridisation

Method

Use of diagnostic kits certified by the FDA and/or EMEA is recommended, with prior validation in the laboratory

Table 7 Required data in the report on HER2 testing by immunohistochemistry

Patient identification
Identification of doctor making request
Date of request and test
Identification of sample (case and block number)
Type of sample (endoscopic biopsy, surgical specimen or other) and anatomical origin
In the case of endoscopic biopsies, specification of number of tumour fragments
Type of fixative (obligatory), time until fixation (recommended) and type of fixation (recommended)
Antibody and method used (clone, supplier, specification of whether it is approved by the FDA or other regulatory agency)
Method of assessment (semi-quantitative, image analysis)
Suitability of the sample (suitable/unsuitable for diagnosis)
Interpretation of the results
Percentage of positive tumour cells
Scoring: 0, 1+, 2+, 3+ or non-interpretable (according to Ref. 8)
Heterogeneous vs. homogeneous staining
Specification of whether there was participation in any external quality control and its name
Name of the person who conducted the procedure
Name and signature of the pathologist responsible for the study
Inclusion of a sentence expressing the idea that the report has been compiled according to the recommendations of the SEAP-SEOM National Guidelines for HER2 testing in gastric carcinoma

when the technique is implemented. This validation can be conducted with 25 positive cases and 25 negative. The results are compared with a reference centre and a correlation of at least 95% should be obtained. When a laboratory authorised to conduct FISH adopts another bright field *in situ* hybridisation for diagnostic purposes, an internal validation can be conducted in the laboratory comparing the new technique with FISH. Again, a correlation of 95% must be obtained. The use of standardised kits requires strict adherence to the manufacturers' instructions with no variations. Use of kits which include a centromeric probe is highly recommended for adequate diagnosis of polysomic cases.

The number of annual tests considered optimal for guaranteeing the technical sufficiency of a laboratory is 100 ISH tests on any type of tumour. Use of complete histological sections of 4–5 μm thickness is recommended in order to minimise signal loss on the Z axis. In FISH procedures, it is essential to use fluorescence filters with excitation and emission wavelengths appropriate to the fluorochromes of the probes contained in the kit. For probes labelled with FITC, excitation at 495 nm and emission at 520 nm; for Texas Red, excitation at 596 nm and emission at 615 nm; for Rodamina, excitation at 540 nm and emission at 570 nm.

Controls

With *in situ* hybridisation techniques, the case for study serves as a control when consistently presenting signals, both in tumour cells and in the accompanying normal ones (lymphocytes, fibroblasts, non-tumorous gastric epithelium, etc.). Nevertheless, the use of an internal control with the laboratory fixation and processing conditions will assist us in interpreting whether cases with an absence of hybridisation are due to problems with the technique or with the sample under study itself.

Reasons for rejecting a test

A test will not be considered sufficient for assessing the number of copies of the *HER2* gene where the reported pre-analytical requirements (fixation) and analytical requirements (microtomy, intensity of hybridisation signals, presence of both signals in the dual probe test) have not been met.

Cases without representation of cells corresponding to a gastric carcinoma in sufficient number for assessment will also be rejected.

Assessment of the results

Interpretation of the results must be conducted by a pathologist. If some other person undertakes the reading, then it is essential that a pathologist coordinates, validates and signs off the interpretation.

In gastric carcinoma, *HER2* signals in *in situ* hybridisation techniques can present different patterns of distribution. It is therefore important, if possible, to conduct an assessment of the whole histological section and an appropriate selection of the fields for quantifying the signals. In an intermediate increase (×200), it is recommended to cover the surface of the tumour and assess signal distribution. The distribution of HER2 signals is, in general, homogeneous within the entire tumour surface although a heterogeneous distribution may be detected. In this situation, the cells which show amplification can group, in the case of focal amplification, or can intermingle with non-amplified tumour cells, defined as mosaic amplification.

Table 8 Interpretation of *HER2* testing by *in situ* hybridisation

Only the tumour component shall be evaluated
At least 20 cells (surgical specimen) shall be evaluated
Interpretation (dual probe techniques):
Non-amplified: Ratio of *HER2* gene signals to chromosome 17 signals less than two
Amplified: Ratio of *HER2* gene signals to chromosome 17 signals greater than two
Polysomy: Number of centromere 17 signals per nucleus greater than three
Monosomy: Number of centromere 17 signals per nucleus >1.5[a]
Non-interpretable: If at least one of the following occurs:
No presence of signals of one or other of the probes in at least 20 cells
If these signals are weak or non-existent in more than 25% of cells
The controls do not show the expected result

[a]In some cases with monosomy 17, the *HER2*/CEN17=2 relationship is caused by the existence of only one copy of centromere 17 and two copies of the *HER2* gene. Therefore, these cases should not be interpreted as amplified

In either of these situations, it is necessary to quantify at least 20 consecutive cells in the area of greatest amplification (i.e., signal intensity). In endoscopic biopsies, a minimum of five assessable cells is required. In order to select the areas, it can be very useful to observe the pattern of immunohistochemistry staining beforehand. Assessment must be conducted exclusively on nuclei with sufficient hybridisation quality, in a consecutive manner, and with the microscope focus adjusted on each nucleus in order to correctly identify all the signals present in the nucleus of the cells.

When conducting FISH as an *in situ* hybridisation technique, given the difficulty of dark field assessment, the following are recommended:

a) Before conducting the procedure, a pathologist shall assess a section consecutive to the one used for the hybridisation procedure. This section will be stained with H&E in order to avoid areas of intense inflammation, necrosis, defective fixation or artifacts. A tumour area of 1 cm^2 can be selected for hybridisation, marked with a diamond pencil or indelible marker on the reverse of the hybridisation section. In order to select the areas, it can be very useful to observe the pattern of immunohistochemistry staining beforehand.

b) Verification of the representative area of the tumour on the H&E and/or immunohistochemistry section is recommended before and during visualisation of the technique. In cases with intestinal metaplasia, special care must be taken in correctly identifying the tumour component.

When a dual probe is used, the ISH procedure assessment shall be conducted according to the following criteria (Table 8) on the overall calculation of the cells assessed:

a. A result shall be considered non-amplified when the ratio of the *HER2* gene signals to chromosome 17 signals is less than two.

b. A result shall be considered amplified when the ratio of the *HER2* gene signals to chromosome 17 signals is greater than or equal to two.

c. A result shall be considered polysomy when the number of centromere 17 signals per nucleus is >3 and monosomy at <1.5. Monosomy 17 can cause false positives in interpretation when a dual probe is used. As such, in some cases with monosomy 17, it is evident that the *HER2*/CEN17 >2 ratio is caused by the existence of only one copy of centromere 17 and two copies of the *HER2* gene. Therefore, these cases should not be interpreted as amplified [41].

d. Two signals of the same size, separated by a distance smaller or equal to the signal size, must be considered as one single signal.

e. It is recommended that evaluation of the following be discounted: (1) nuclei with low intensity signal; (2) areas with intense background; (3) nuclei with signs of excessive digestion; and (4) areas of intense signal in the bacterial DNA in macrophages and mast cells.

f. A result shall be considered uninterpretable if at least one of the following circumstances occurs: (1) There is no presence of CEP17 or *HER2* signals in at least 20 cells. (2) If these signals are weak or non-existent in more than 25% of cells. (3) The controls do not show the expected result.

g. It must be remembered that tumour nuclei which present very high *HER2* amplification can present gene signal clusters.

Personnel

The number of laboratory technicians who conduct tests and the number of pathologists who interpret them must be as low as possible in order to guarantee the effectiveness of the undertaking. Both the technicians and the pathologists must have undergone training. For assessing the post-analytical (interpretation) phase, correlation with the reference result of at least 95% in 50 cases is recommended. Periodically, the aforementioned training should be refreshed in dedicated working sessions.

Report

While the report will be adapted to the different information systems used in different hospitals, it must include, at minimum, the data presented in Table 9. The recommended turnaround time is ≤7 days.

Table 9 Required data in the report on *HER2* testing by *in situ* hybridisation

Patient identification
Identification of doctor making request
Date of request and test
Identification of sample (case and block number)
Type of sample (endoscopic biopsy, surgical specimen or other) and anatomical origin
In the case of endoscopic biopsies, specification of number of fragments
Type of fixative (obligatory), time until fixation (recommended) and type of fixation (recommended)
Probe used (supplier, specification of whether it is approved by the FDA or other regulatory agency)
Method of assessment (semi-quantitative, image analysis)
Number of nuclei assessed
Suitability of the sample (suitable/unsuitable for diagnosis)
Results:
Ratio (to two decimal places, without rounding up or down): *HER2* gene signals against chromosome 17 signals
Scoring: non-amplified, amplified or non-interpretable (according to Refs. 5, 8)
Presence or absence of polysomy or monosomy
Presence or absence of heterogeneity
Specification of whether there was participation in any external quality control and its name
Name of the person who conducted the procedure
Name and signature of the pathologist responsible for the study
Inclusion of a sentence expressing the idea that the report has been compiled according to the recommendations of the SEAP-SEOM National Guidelines for HER2 testing in gastric carcinoma

Algorithm for HER2 testing

The algorithm presented in Fig. 2 is the one proposed following the publication of the ToGA Trial.

Current state of HER2 testing in Spain

The experience of the SEAP Quality Assurance Programme and future perspectives for gastric carcinoma

The Association for Quality Assurance in Pathology of SEAP was established in January 2004 with the aim of fostering and promoting quality control among staff and researchers in anatomical pathology laboratories. Participating laboratories receive unmarked slides from the programme in order to conduct the procedure requested. They return the aforesaid slides together with control slides. These are all assessed by a committee formed of several evaluators. The programme guarantees anonymous participation and confidential communication of results.

Analysis of the data obtained from the module on HER2 in breast carcinoma from the SEAP Quality Assurance Programme, begun in October 2004 and ending October 2009, with a total of 11 cycles, shows that 136 centres participated although only four centres participated in all the cycles (2.9%). Participation data shows that 67 centres (49.3%) participated in five to ten cycles and 65 centres (47.8%) in one to four cycles. The different assessment cycles were conducted on tissue samples with different levels of expression of the HER2 protein, fixed in 10% pH 7 buffered formalin for 24 hours and subsequently embedded in paraffin. The assessment criteria were the same in all the cycles: the highest marks were awarded to those cases in which all the study samples presented correct results, without artifacts in the tissue, which permitted correct interpretation. Acceptable results were those which permitted interpretation but in which the intensity in certain sections was diminished or increased and/or there were artifacts of the procedure which, in some cases, could lead to an inaccurate interpretation. Cases with inadmissible results were those in which, overall, the results obtained were incorrect. This means, for example, negative expression in 3+ and 2+ cases, possibly due to insufficient antigen retrieval or low sensitivity technique. Alternatively it might be due to marked overexpression in negative cases (0 and 1+) often associated with intense and extensive expression of the normal ducts and, on occasion, non-specific staining of stromal cells leading to incorrect interpretations.

Analysis of the results of the four centres which participated in the 11 rounds shows that two of them (50%) obtained optimum and acceptable results with no inadmissible results although only one of these obtained full marks in all the cycles. Of those centres which participated in between five and ten cycles (67 centres), none of these obtained full marks in all the cycles in which they participated. Sixteen centres (23.9%) presented optimum and acceptable results with no inadmissible results. Of those centres that participated in between one and four cycles (65 centres), four of these (6.2%) obtained full marks in all the cycles in which they participated. Thirty centres (46.1%) obtained optimum and acceptable results with no inadmissible results. In summary, depending on the cycle, the number of hospitals with the highest scoring varied between 25.8% and 69.8%; acceptable results ranged between 13.7% and 56.7%; and inadmissible results between 11.1% and 31.1% (Fig. 3). Use of commercial kits approved by the FDA and/or EMEA (HercepTest and Pathway) increased during the various cycles. The percentage of use of such kits in the 11th round was 67.4%.

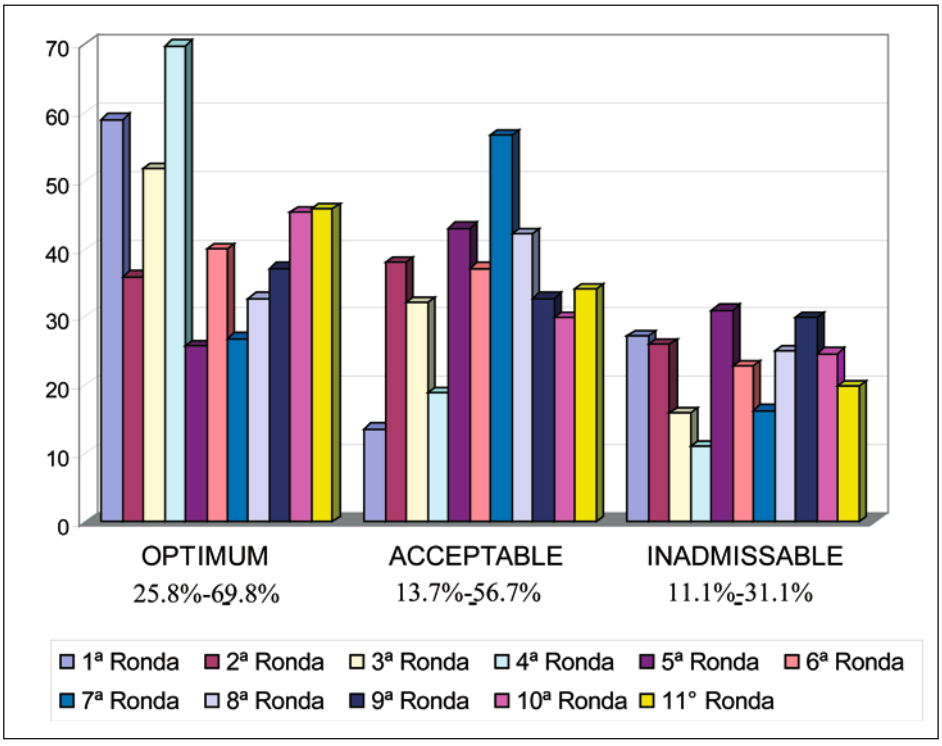

Fig. 3 Comparative results of the 11 cycles of the HER2 module for immunohistochemistry in the SEAP Quality Assurance Programme

In the FISH/CISH/SISH module, the total number of centres which participated was 60. However only one of these participated in the 11 cycles (1.6%). Eighteen centres participated in between five and ten cycles (30%), and 41 centres between one and four cycles (68.4%). Assessment was conducted on a problem slide, which contained various tissue sections with different levels of HER2 amplification. The tissues were fixed in 10% pH 7 buffered formalin for 24 hours and subsequently embedded in paraffin. The assessment criteria were the same in all the cycles. Optimum

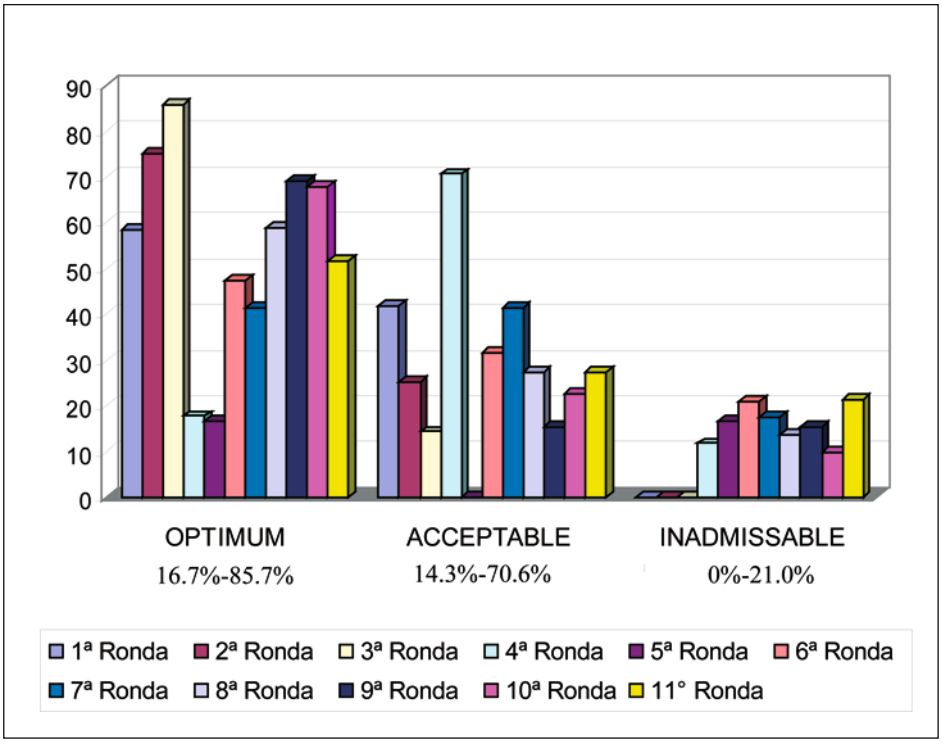

Fig. 4 Comparative results of the 11 cycles of the HER2 module for *in situ* hybridisation in the SEAP Quality Assurance Programme

Table 10 Quality criteria for HER2 testing in gastric carcinoma

Sufficient provision of human resources and infrastructure
Standardisation of procedures (normalised working procedures for handling samples and conducting specific methods)
Use of diagnostic kits approved by regulatory agencies without alteration to the recommended protocol
Initial validation of the testing method: 25 positive cases and 25 negative cases, comparing the results with a reference centre and obtaining a 95% correlation
Validation after any alteration to the testing method
Minimum number of tests recommended annually for quality assurance (including other tumour types):
Immunohistochemistry: 250 tests
In situ hybridisation: 100 tests
Use of appropriate controls in each testing cycle
Initial training and periodic ongoing training of technical and medical staff
Participation in external quality assurance programmes (SEAP, UK NEQAS, NordiQC) with optimum results in 90% of cases assessed
It is recommended that laboratories undertake certification and accreditation processes for their activities

results were those in which all the study samples presented correct results, without artifacts in the tissue, which permitted appropriate interpretation. Results considered acceptable were those which permitted interpretation but in which the intensity of the probes was reduced in some sections which, in certain cases, could lead to defective interpretation. Cases considered inadmissible were those in which, overall, the results obtained were incorrect, principally due to excessive digestion, due to marked non-specific background hybridisation, which led to incorrect interpretations and material unsuitable for reading.

The centre that participated in the 11 cycles obtained optimum and acceptable results in all of them. Of the 18 centres that participated in between five and ten cycles, ten of them obtained optimum or acceptable results without inadmissible assessments (55.5%). Forty-one centres participated in between one and four cycles and 30 of these obtained optimum or acceptable assessments without inadmissible results (73.1%). Of a total of 60 centres, eight (13.3%) obtained full marks in all the cycles in which they participated (between two and eleven). In summary, depending on the cycle, results with the optimum score varied between 16.7% and 85.7%; acceptable results between 14.3% and 70.6%; and inadmissible between 0% and 21% (Fig. 4).

In relation to HER2 testing in gastric carcinoma, as in the case of breast carcinoma, participation is recommended in external HER2 quality assurance programmes. Quality assurance agencies should therefore offer assessment of HER2 in gastric carcinoma. In all the published guidelines on HER2 testing in breast cancer, emphasis is placed on the necessity, and in some cases (UK and Canada) obligation, of participating in external quality assurance programmes in order for laboratories to maintain their accreditation to conduct the said procedures. This set of guidelines for gastric cancer advises participating in quality assurance programmes on a half-yearly basis. It further advises considering it a satisfactory indicator that the procedure is operating correctly when 90% of results are optimum. The best known international quality assurance programmes are UK NEQAS (www.ukneqas.org.uk) and NordiQC (www.nordqc.org). In our country, there is the SEAP quality assurance programme (www.seap.es). The quality criteria for HER2 testing in gastric carcinoma are summarised in Table 10.

Final remarks

This set of SEAP-SEOM guidelines has been produced and published only a few months after the publication of the ToGA Trial and at the same time as the final approval in Spain of the prescription of trastuzumab in the treatment of advanced gastric carcinoma. This speed of publication places limits on the following scientific objectives in this field:

1. Establishing specific quality control in HER2 testing in gastric carcinoma, with special emphasis on quality assurance in the post-analytic (interpretation) phase, taking into account significant differences with breast carcinoma.

2. Promoting studies designed to answer the following questions in relation to the sample of choice for HER2 study:

a) Endoscopic biopsy vs. surgical specimen.

b) Primary tumour vs. locoregional metastasis vs. distant metastasis.

3. Recommending an exhaustive analysis of some subgroups of the ToGA Trial, which would facilitate clarification of the reason for, and predictive significance of, the lack of correlation between levels of HER2 expression detected by immunohistochemistry and the state of the gene determined by *in situ* hybridisation. For this reason, it would be very important to conduct studies that analyse the correlation between the different methodologies of HER2 study.

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The affiliations

Á. Concha
Pathology Service
Hospital Universitario Virgen de la Nieves
Granada, Spain

J.M. Corominas · M. Iglesias
Pathology Service
Hospital del Mar
Barcelona, Spain

T. García-Caballero
Pathology Service
Hospital Universitario de Santiago
Santiago de Compostela, A Coruña, Spain

E. García-García · F. López-Ríos
Pathology Service and Targeted
Therapies Laboratory
Hospital Universitario Madrid Sanchinarro
Universidad San Pablo-CEU
Madrid, Spain

J.A. López
Pathology Service
Hospital Universitario Príncipe de Asturias
Alcalá de Henarés, Madrid, Spain

S. Ramón y Cajal
Pathology Service
Hospital Universitario Vall d'Hebrón
Barcelona, Spain

F. Rojo
Cancer Research Program
IMIM-Hospital del Mar, Barcelona
and Pathology Service
IIS-Fundación Jiménez Díaz
Madrid, Spain

J. Palacios
Pathology Service
Hospitales Universitarios Virgen del Rocío
Sevilla, Spain

F. Vera-Sempere
Pathology Service
University of Valencia
Valencia, Spain

E. Aranda
Medical Oncology Service
Hospital Universitario Reina Sofía
Córdoba, Spain

R. Colomer
Medical Oncology Service
Centro Oncológico MD Anderson España
Madrid, Spain

P. García-Alfonso
Medical Oncology Service
Hospital General Universitario Gregorio Marañón
Madrid, Spain

P. Garrido
Medical Oncology Service
Hospital Universitario Ramón y Cajal
Madrid, Spain

F. Rivera
Medical Oncology Service
Hospital Universitario Marqués de Valdecilla
Santander, Spain

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