Expert Forum
Gastric Cancer Workshop – HER2 testing
Making Education Easy
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Michael Bilous holds the appointment of Clinical Associate Professor at the University of Sydney. Michael has had a long-standing interest in breast pathology and is a member of a number of federal and state committees and advisory boards concerned with best practice in breast pathology. Michael is a member of the International HER2 Testing Advisory Board and is Chairman of the Australian HER2 Testing Advisory Board. The latter was responsible for developing the National HER2 Testing Programme that operates in Australia to ensure reliable, reproducible and uniform testing methods in each state and territory. In addition to having authored more than 80 papers, Michael is on the Editorial Board of The Breast and is a regular reviewer for a number of journals including, among others, the Medical Journal of Australia, European Journal of Cancer, Pathology, and The Breast Journal.

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About Expert Forums

Expert Forum publications are designed to encapsulate the essence of a local meeting of health professionals who have a keen interest in a condition or disease state. These meetings are typically a day in duration, and will include presentations of local research and discussion of guidelines and management strategies.

Even for local events it is not always possible for everyone with a similar therapeutic interest to attend. Expert Forum publications capture what was said and allows it to be made available to a wider audience through the Research Review membership or through physical distribution.

Welcome

to this review of the recent Gastric Cancer Workshop held in Auckland, which focused on human epidermal growth factor receptor 2 (HER2) testing.

This review is a summary of presentations by Dr Christopher Jackson and Associate Professor Michael Bilous. Dr Jackson spoke on the management of advanced gastric cancer and the implications of the ToGA study, while A/Prof Bilous spoke on HER2 testing to drive patient selection, and on ensuring quality of HER2 testing in gastric cancer.

Management of Advanced Gastric Cancer

Implications of the ToGA study

Presented by Dr Christopher Jackson

Incidence of gastric cancer

Gastric cancer is an important disease in NZ. In men, the incidence of gastric cancer ranks in the top 10 of all cancers, accounting for 2.1% of all male cancer registrations in 2005 (Ministry of Health data)[1]; percentages reported for women were slightly lower. The mortality rate is high, with over two-thirds of patients diagnosed with localised gastric cancer dying of the disease (in NZ in 2005, there were 341 new cases and 256 deaths).[1] The disease is more common in Maori and Pacific Island individuals, with the death rate from gastric cancer being twice that in Maori males compared with European males, and the death rate for Maori and Pacific Island women being 4-fold that of European women.[1] Furthermore, Guilford and colleagues discovered that mutations in the *E-cadherin* gene were associated with the development of gastric cancer in a large familial cohort in NZ.[2]

While the incidence of gastric tumours in NZ is declining, the incidence of tumours in the gastro-oesophageal junction (GOJ) and the lower third of the oesophagus appears to be increasing. Dr Jackson says that this increase appears to be related to dietary changes, obesity, alcohol consumption and reflux disease, and that the trend is observed world-wide.

The anatomical distribution of gastro-oesophageal junction tumours

Siewert classified GOJ tumours as type I, II or III depending on their relative involvement of the oesophagus, true junction and proximal cardia.[3] Dr Jackson says that many large studies of gastric cancer exclude patients with type I GOJ tumours. He points out that the pathophysiology and clinical outcomes of the three types appears to be similar, and that in future studies we should consider systemic therapies for these junctional tumours as a group rather than with this anatomical distinction. Given that oesophageal tumours cause dysphagia and can be locally erosive into pericardium, lungs, bronchi and pleura, local control is also a challenging issue in management.

Chemotherapy

Since the early 1990s, it has been very clear that chemotherapy plays a key role in the management of advanced gastric cancer, with chemotherapy extending and improving quality of life in appropriately selected individuals.[4-7] In four key studies, the median overall survival (OS) in patients with advanced gastric cancer treated with chemotherapy was ≈8-11 months, compared with ≈3-5 months for those treated with best supportive care.[4-7]

From this follows the question of selecting the optimal systemic regimen. Multiple differening chemotherapy combinations including monotherapy, doublets and triplets have been tested, with cisplatin + 5-fluorouracil or capecitabine (CF/X) and epirubicin + cisplatin + 5-fluorouracil (ECF) obtaining the most widespread acceptance and use in the US and Europe. In human epidermal growth factor receptor 2 (HER2)-positive advanced breast cancer, the addition of trastuzumab [Herceptin] to chemotherapy improved survival.[8] A proportion of gastric cancers also over-express HER2 and this provided the rationale for the phase III ToGA trial (discussed in more detail on page 2).[9]

Assessment of HER2 status in gastric cancer

In breast cancer, immunohistochemistry techniques are used as initial screening for HER2 overexpression. However, the presence of an apical lumen in gastric epithelial cells results in a different staining pattern than in breast cancer, and so required validation. The validation study,[10] designed to establish a HER2 scoring system for gastric cancer in order to identify patients for enrollment in the ToGA study,[9] revealed tumour heterogeneity and the occurrence of basolateral membrane staining. Results from this study also showed that a small number of immunohistochemistry (IHC) 0 tumours are fluorescence in situ hybridisation (FISH) positive, a small number of IHC 1+ tumours are FISH positive, a slightly larger number of IHC 2+ tumours are FISH positive, and almost all IHC 3+ tumours are FISH positive (see **Figure 1**). This appears to be different to the situation in breast cancer, where there is greater concordance between IHC 0/1+ and FISH-. For enrollment in the ToGA registration study, patients could be either IHC 3+ or any IHC grade and FISH positive.

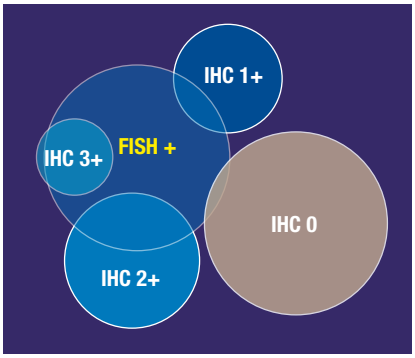

Figure 1: Assessment of HER2 status in Gastric Cancer. (Adapted from Hofmann et al, 2008).[10]

The ToGA study[9]

Given extensive prior use in breast cancer, regulators did not require phase I/II testing of CF/X chemotherapy with trastuzumab prior to the commencement of a phase III trial for gastric cancer (despite that this combination had not formally been tested). In the phase III, randomised, open-label, international, multicentre ToGA study, 3803 patients with inoperable locally advanced, recurrent, or metastatic adenocarcinoma of the GOJ or stomach, were screened for HER2 status. A total of 810 patients (22.1%) were found to be HER2-positive (IHC 3+ and/or FISH+) and 594 patients were randomised to one of two arms: cisplatin with 5-fluorouracil/capecitabine (n = 290) or 5-fluorouracil/capecitabine + cisplatin + trastuzumab (n = 294). The study did not allow crossover and Dr Jackson says that this feature is important for a phase III trial.

The study was undertaken in Asia, South America, North America and some parts of Europe. Geographical heterogeneity in gastric cancer is noted, for example in South America gastric cancer is predominantly located in the body and distal stomach, while in North America and Europe tumours are predominantly centred on the GOJ and proximal stomach (as is the case in NZ). In Asia, gastric cancer is often detected early via a screening programme.

The primary endpoint of the study was OS, which Dr Jackson says is a challenging yet important endpoint for any study to achieve in the modern era, particularly because of the confounding influence of second and subsequent lines of therapy. However, the study achieved its primary endpoint demonstrating an improvement in OS with the addition of trastuzumab from a median of 11.1 to 13.8 months (HR 0.74; 95% CI 0.6-0.91). Dr Jackson points out that this correlates to a 26% relative improvement with trastuzumab + chemotherapy compared with chemotherapy alone. For progression-free survival, the improvement was surprisingly shorter than the benefit in OS.

An analysis by HER2 status revealed that OS was longer in patients with higher expression of HER2 protein, and a post-hoc exploratory analysis that divided patients into two groups, either high (IHC 2+/FISH positive or IHC 3+) or low (IHC 0 or 1+/FISH+) levels of HER2, showed a median OS of 16 months in patients with a high HER2 level who received trastuzumab + chemotherapy, compared with 11.8 months in patients with high HER2 levels who received chemotherapy alone (HR 0.65; 95% CI 0.51-0.83). See **Figure 2**.

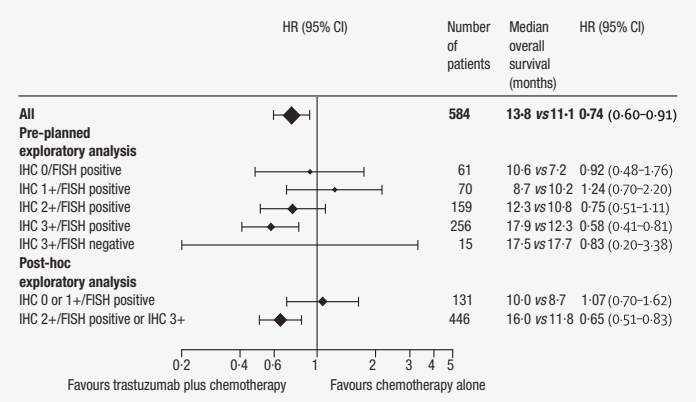

Figure 2: Overall survival according to HER2 status in patients receiving either chemotherapy + trastuzumab or chemotherapy alone. Pre-planned and post-hoc analyses. (Adapted from Bang et al, 2010).[9]

Is this a new standard of care?

Dr Jackson says that a median OS of 16 months has never been reported before in any phase III trial in gastric cancer, and adds that the ToGA study provides a new `high water mark' in this disease. The question arises as to whether HER2 overexpression may be prognostic, as well as predictive, and whether the long OS was achieved because this group of patients had a more favourable outlook. In breast cancer, HER2 overexpression is an adverse prognostic factor. The literature on gastric cancer is mixed. The chemotherapy-only arm achieved an OS of 11 months, which is comparable to other recently reported phase III studies, arguing that HER2 overexpression may not be an adverse feature, and suggests therefore that the 16 month OS was not achieved simply by selecting a more favourable group.[11-13]

The UK National Cancer Research Institute Randomized ECF for Advanced and Locally Advanced Esophagogastric Cancer 2 (REAL-2) trial was a 2 x 2 factorial study to test non-inferiority of capecitabine for 5-fluorouracil, and oxaliplatin for cisplatin, and individual arm comparisons were performed to determine the most effective regimen to take forward for future studies.[11] The study found that patients receiving epirubicin, oxaliplatin and capecitabine had the longest median OS at 11.2 months; whilst the best results from leading US and Asian research groups have only achieved 9 and 10-11 month OS.[12,13]

Dr Jackson points out that the populations included in the studies were slightly different, with the UK group including a higher proportion of patients with locally advanced disease. Patients with locally advanced disease would tend to survive longer than those with metastatic disease, potentially extending the median OS found in the REAL-2 study. The ToGA study included more patients with metastatic disease than the other studies, and yet their patients still achieved an OS of 16 months. Progression-free survival rates were similar between the ToGA study (median 6.7 months) and the other studies (5.6-7 months).

Dr Jackson says that the findings seen in the ToGA study are an improvement over what has been seen in comparable phase III studies by respected international groups.

Contamination from second-line therapy?

In the ToGA study, 42% of trastuzumab recipients received second-line therapy after disease progression, while in the UK study, only 14% received second-line therapy.[9,11] It is therefore possible that in the UK, the OS results are worse than others by the fact that the vast majority of patients did not receive second-line therapy.

Progress in the treatment of gastric cancer

Looking back at phase III randomised trials for the treatment of gastric cancer over the previous 15-20 years, we see that the situation has vastly improved from `best supportive care' to the use of three-drug chemotherapy regimens. However, even with such regimens, the best median OS time was 11 months. Dr Jackson says that with the addition of trastuzumab, the 11-month barrier has been broken and that we can now expect a median OS of 16 months in this patient group.

A paradigm for advanced oesophagogastric cancer

Dr Jackson recommends a new paradigm, outlined in **Figure 3**, for the treatment of advanced oesophagogastric cancer. He strongly recommends that patients have their tumours tested for HER2 positivity and that he would recommend that his HER2-positive patients receive cisplatin, fluoropyrimidine and trastuzumab, with the expectation that this regimen could push their survival beyond a year.

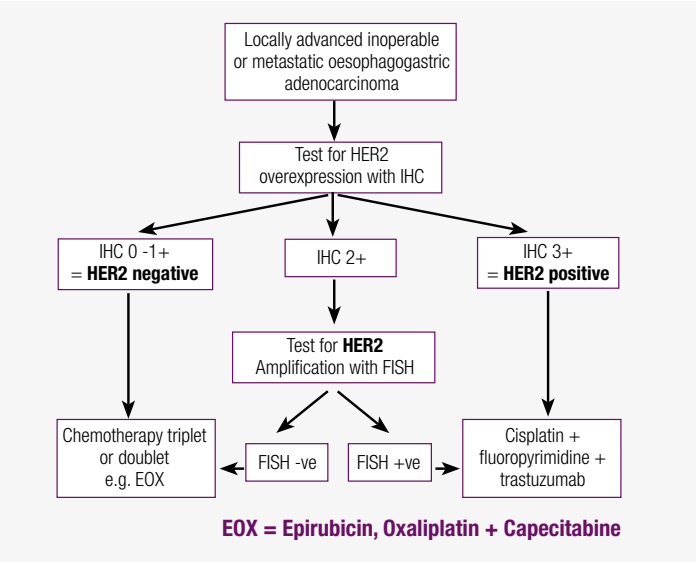

Figure 3: Paradigm for the treatment of advanced oesophagogastric cancer in 2010. (Adapted from Okines and Cunningham, 2010).[14]

Future directions

- LOGiC study: Lapatinib (an oral HER2 inhibitor) and Capecitabine/Oxaliplatin in gastric cancer (n = 410)
- Lapatinib + capecitabine/paclitaxel
- Identification of mechanisms of resistance (primary and acquired)
- Pre-operative and adjuvant therapy
- Treatment beyond progression.

In Summary

Dr Jackson says that the ToGA study provides evidence for a new standard of care in gastric cancer. He points out that while there are many agents available for the treatment of gastric cancer, it is an aggressive cancer and the prognosis is poor. He says that trastuzumab adds to the available armamentarium against this disease in a clinically meaningful way.

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HER2 screening to drive patient selection in gastric cancer – lessons from the ToGA study

Presented by Associate Professor Michael Bilous

TNM staging of gastric cancer

The American Joint Committee on Cancer (AJCC) separates stages of gastric cancer into groups defined in terms of combinations of three component classifications, tumour (T), lymph nodes (N) and distant metastasis (M).[1] Using these classifications, and their sub-classifications, seven distinct stages of gastric cancer have been defined (0, IA, IB, II, IIIA, IIIB, IV). An analysis by Hundahl et al, of patients in the US who were diagnosed with gastric cancer between 1985 and 1996, employed the AJCC classification system and calculated stage-stratified 5-year survival rates (see **Figure 4**).[2] Their findings showed poor survival rates in gastric cancer, for anything other than very early disease.

A/Prof Bilous points out that, in comparison to breast cancer, gastric cancer is an infrequent cancer in NZ and Australia, but that it is an extremely aggressive cancer, associated with very high morbidity and mortality rates.

Disease	Stage	TNM[1]	5-yr survival Rates[2]
Early	0	Tis, N0, M0	89%
	IA	T1, N0, M0	78%
	IB	T1, N1, M0 or T2, N0, M0	58%
	II	T1, N2, M0 or T2, N1, M0 or T3, N0, M0	34%
Locally Advanced	IIIA	T2, N2, M0 or T3, N1, M0 or T4, N0, M0	20%
	IIIB	T3, N2, M0	8%
	IV	T1-3, N3, M0 or T4, N1-3, M0 or any T, any N, M1	7%
Metastatic			

Tis = *in situ* carcinoma; T1 = tumour invades lamina propria; T2 = tumour invades muscularis propria or subserosa; T3 = tumour penetrates serosa; T4 = tumour invades adjacent structures; N0 = no metastases to regional lymph nodes; N1 = metastases in 1-6 regional lymph nodes; N2 = metastasis in 7-15 regional lymph nodes; N3 = metastasis in >15 regional lymph nodes; M0 = no distant metastasis; M1 = distant metastasis.

Figure 4: Gastric cancer stage and US survival rates. (Adapted from Greene et al, 2002[1] and Hundahl et al, 2000[2])

Differences in HER2 testing in breast and gastric cancers

The pre-ToGA international validation study by Hofmann et al investigated HER2 testing in 168 gastric cancer samples.[3] As outlined by Dr Jackson above, the findings of the Hofmann study revealed histological differences between gastric and breast cancers, namely, tumour heterogeneity where there can be a positive area of IHC-stained gastric cells in an area of negative staining, and incomplete membrane staining where only the basolateral aspect of the gastric cells are stained, in a cup-like pattern (see **Figure 5**). These findings necessitated modifications to the HER2 breast cancer scoring criteria for use in gastric cancer. A/Prof Bilous points out that in breast cancer, the incomplete staining shown in **Figure 5** would be classified as IHC 1+, but in gastric cancer, such staining would be classified as IHC 3+.

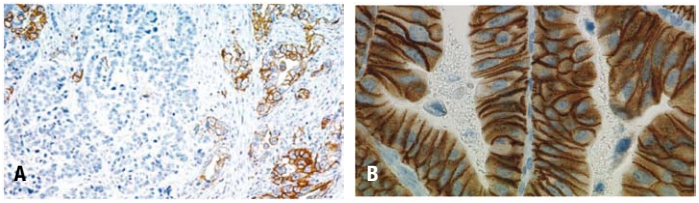

Figure 5: Histological appearance of IHC-stained gastric cancer tumour samples, showing heterogeneity of staining (a) and incomplete membrane staining (b). (Adapted from Hofmann et al, 2008).[3]

Findings of the ToGA study

The IHC scoring for HER2 in gastric and GOJ cancer was defined separately for the type of diagnostic specimen (surgical or biopsy). In surgical specimens, faint or barely perceptible membranous reactivity in ≥10% of tumour cells was considered a positive finding, while in biopsy specimens, the presence of any IHC stained cells was considered a positive result. In the study, those patients who were IHC 3+ and/or FISH+ were considered to be HER2 positive, and a total of 810/3665 patients (22.1%) had this result.[4]

An analysis of data from the ToGA study by country, revealed that Australia had the highest rate of HER2 positivity at ≈34%. When the distribution of tumour location by country was analysed, it became clear that the rates of gastric and GOJ cancer varied greatly among countries; Belgium and Denmark having equal numbers of both types of cancer, but the majority of countries having vastly greater numbers of gastric cancer than GOJ cancer (in Asia, almost all cancer was of the gastric type). GOJ tumours were found to exhibit a significantly ($p < 0.001$) higher rate of HER2 positivity than gastric tumours (33.2% vs 20.9%). Furthermore, intestinal-type gastric cancers showed a significantly higher HER2-positivity rate than diffuse tumours (32.2% vs 6.1%). A/Prof Bilous says that while the rates of HER2 positivity were high in the ToGA study, which included only patients with advanced disease, we don't know what the rates will be when we look at all gastric cancers.

Assessment of HER2 status by sample type revealed a significantly ($p = 0.03$) higher rate of HER2 positivity in biopsy specimens than surgical specimens (23.1% vs 19.9%). A/Prof Bilous says that this finding is probably due to the fact that biopsy specimens generally undergo better fixation and processing than do surgical specimens.

Another interesting finding of data from the ToGA study was the correlation between IHC and FISH scores. Out of 2519 IHC 0/1+ cases, 7.5% were FISH+, while out of 761 IHC 2+/3+ cases, 74.4% were FISH+.

The primary end-point in the ToGA study, OS, revealed that all of the patients showed some benefit from chemotherapy + trastuzumab when compared with chemotherapy alone, but that the group of patients who were IHC 2+/FISH+ or IHC 3+ experienced the most benefit (median OS 16 months vs 11.8 months: HR 0.65; 95% CI 0.51-0.83). See **Figure 2**.

A/Prof Bilous says that most algorithms or programmes developed from the findings of the ToGA study have honed in on the group who showed the most benefit from treatment. He presented a treatment algorithm for HER2 testing in gastric and GOJ cancer, based on findings of the ToGA study (see **Figure 6**). He points out that in the algorithm, patients who are IHC 3+ are automatically eligible for trastuzumab, while those who are IHC 2+ should be retested for FISH, and if they are positive, then they too are eligible for trastuzumab.

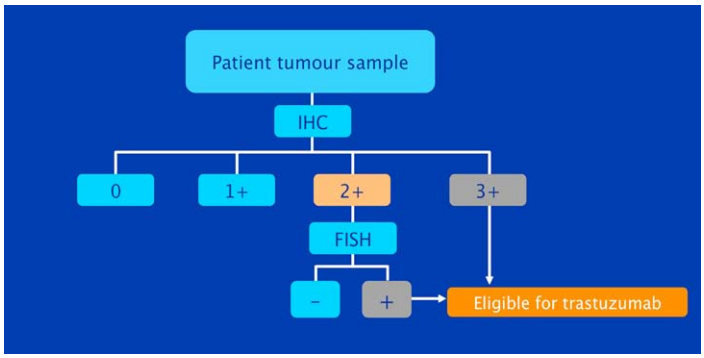

Figure 6: Recommended HER2 testing algorithm in gastric and GOJ cancer.

Summary of HER2 testing in gastric cancer

- All patients with gastric or GOJ carcinomas should be tested for HER2 in the advanced setting
- IHC should be the primary testing modality
  - Allows localisation of HER2-positive areas in heterogeneous tumour tissue
  - Higher predictive value of IHC
  - The gastric cancer scoring criteria should be applied
  - IHC 3+ considered HER2 positive
- FISH retesting required for IHC 2+ tumours
  - IHC 2+/FISH+ tumours considered HER2 positive
- Approximately 16% of gastric tumours are HER2 positive.

The need for multiple biopsies

In gastric cancer tumours, HER2 status heterogeneity has been seen to occur in 5-30% of all cases.[3,5] For this reason, it is essential that multiple biopsies are taken from individual patients for the assessment of HER2 status. A/Prof Bilous says that endoscopists need to be made aware of the necessity for this. An example of the importance of multiple biopsies is shown in **Figure 7**, where specimens from a single patient are shown to exhibit different IHC status. A/Prof Bilous emphasises that this is a complicated example and that not every patients samples are going to look like this.

From a processing perspective, he says that every laboratory varies with regard to the maximum number of samples that they will process per paraffin block. He says that a good embedder will be able to successfully put 4 samples in a block. He adds that the recommended number of biopsy samples per case is 6-8.

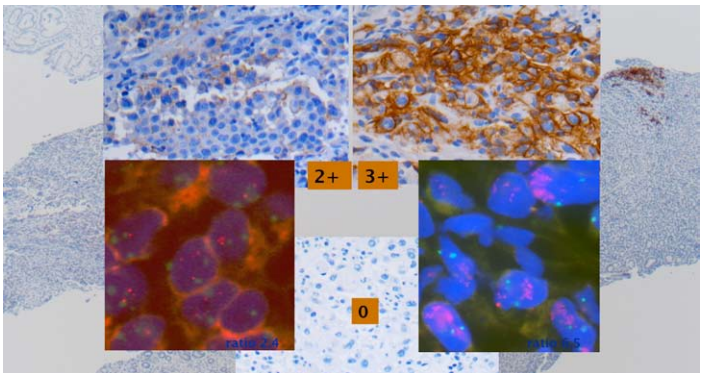

Figure 7: IHC and FISH testing showing heterogeneity and low-level amplification/ protein expression of HER2 in gastric cancer biopsy samples from a single patient.

Recommended HER2 testing algorithm in gastric and GOJ cancer (from the European Medicines Authority)

- All patients with gastric or GOJ cancer should be accurately tested for HER2 status
- IHC should be the primary testing modality
- Samples with an equivocal score (IHC 2+) should be retested with FISH
- IHC2+/FISH+ tumours are considered HER positive
- HER2 testing should be performed by laboratories demonstrating proficiency in the above-mentioned technologies
- Validated assays for detection of HER2 are mandated.

Key points

- There are significant differences in HER2 distribution and testing between gastric and breast cancers
- Overall rates of HER2 overexpressesion in advanced gastric and breast cancer are similar
- Heterogeneity of the distribution of HER2 is common in gastric cancer
- GOJ cancers have a higher rate of HER2 overexpression than gastric cancers
- The diffuse type of gastric cancer has a lower rate of HER2 overexpression than the intestinal type
- Testing algorithms need to reflect breast/gastric cancer differences
- Testing experience is limited and the ToGA trial is the best source of data – so far.[4]

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HER2 testing in gastric and GOJ cancer: a guide to best practice

Presented by Associate Professor Michael Bilous

EMA/NICE/TGA approvals for trastuzumab in gastric cancer

The European Union approved Herceptin for use in gastric cancer in January 2010. The European Medicines Agency (EMA) full indication for Herceptin use is as follows:

- Herceptin in combination with capecitabine or 5-fluorouracil and cisplatin is indicated for the treatment of patients with HER2-positive metastatic adenocarcinoma of the stomach or GOJ who have not received prior anti-cancer treatment for their metastatic disease.
- Herceptin should only be used in patients with metastatic gastric cancer whose tumours have HER2 overexpression as defined by IHC 2+ and a confirmatory FISH+ result, or IHC 3+, as determined by an accurate and validated assay.

The National Institute for Health and Clinical Excellence (NICE) UK approved Herceptin for use in the UK in September 2010:

- Herceptin approved for use in combination with chemotherapy for patients with metastatic gastric or GOJ cancer who overexpress HER2 at the IHC 3+ level and are retested positive by ISH.

The Australian Therapeutic Goods Administration (TGA) approved indication and testing listing for Herceptin use in gastric cancer, that has been put forward for approval for funding, states the following:

- Herceptin is indicated in combination with cisplatin and either capecitabine or 5-FU for the treatment of patients with HER2-positive advanced adenocarcinoma of the stomach or GOJ who have not received prior anti-cancer treatment for their metastatic disease.
- Herceptin treatment is only appropriate if there is HER2 overexpression, as described by a IHC 3+ score. For cases with an IHC score of <3+, confirmation of HER2-positive status by ISH is mandatory.

Gastric HER2 testing in Australia

In Australia, there are to be five reference laboratories for ISH testing, and these will be funded by Roche. All IHC 0, 1+ and 2+ results must be retested at one of the five reference laboratories, and IHC 3+ will be regarded as a positive result with no retesting required.

Unique features of HER2 testing in gastric cancer

A/Prof Bilous presented the EMA model for HER2 testing in gastric cancer (see **Figure 8**). He says that this model is most likely to be the one used in NZ.

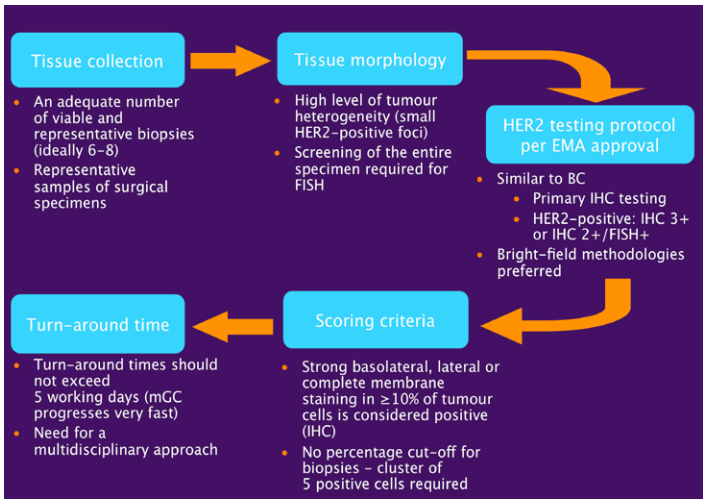


Figure 8: Unique features of HER2 testing in gastric cancer: the EMA model

BC= breast cancer; IHC = immunohistochemistry; mGC = metastatic gastric cancer

A/Prof Bilous says that successful and timely HER2 testing will require a multidisciplinary approach, involving the patient, oncologist/gastroenterologist, surgeon/endoscopist, medical technician and the pathologist. Furthermore, laboratory personnel should be specifically trained before embarking on HER2 testing and interpretation in gastric and GOJ cancer.

Tissue preparation

Standardised tissue processing is essential for HER2 testing and key tissue preparation steps include: collection → fixation → paraffin embedding → sectioning → deparaffinisation of sections → IHC/FISH testing.

Collection: Both surgical and biopsy samples are acceptable for HER2 testing. If biopsies are taken, it is recommended that 6-8 viable and representative specimens are processed (preferably in two blocks). There are currently no specific guidelines available, therefore, the SIGN (Scottish Intercollegiate Guidelines Network) 2006 is the most practicable reference to work with.[1]

Fixation: Tissue should be fixed within 20 minutes of surgery and transported promptly to the laboratory. It is essential that the tissues are fixed properly, as poor tissue fixation is the most common source of error in HER2 testing.[2] For fixation, 10% neutral-buffered formalin is preferred and this should be replaced regularly. Surgically excised and biopsy samples should be fixed for 8-48 hours (a minimum of at least 1 h/mm tissue).[3]

Paraffin embedding: Tissue samples are dehydrated in an ethanol/xylene substitute series and embedded in paraffin. Fresh paraffin should be used and prolonged incubation in molten paraffin should be avoided as high temperatures may degrade epitopes. Paraffin-embedded samples can be stored indefinitely prior to sectioning. The minimum area of the paraffin block should be 1cm^2, in order to enable proper sectioning.

Sectioning and deparaffinisation: Ideally sections should be cut from the tissue block immediately prior to HER2 testing. Sections must be cut from a representative area of the tumour and confirmed with a haematoxylin and eosin section. The thickness of the section can affect the interpretation of results and sections should be cut to 4µM. Sections are mounted on slides and dried for 12-24 hours at room temperature or for 1 hour at 60˚C. Sections are then deparaffinised and rehydrated in an ethanol/xylene substitute series. It is important to ensure the complete removal of paraffin, as residual paraffin will increase non-specific staining.

Antigen retrieval

Antigen retrieval is a crucial step prior to IHC, and is a frequent source of variation in HER2 testing. The extent of antigen retrieval is dependent on tissue fixation and primary antibody. The two tests used in the IHC testing regimen in the ToGA study were Ventana CONFIRM HER2/neu (antibody 4B5; Roche Tissue Diagnostics) and HerceptTest™ (antibody A0485; Dako). It is highly recommended that manufacturer's instructions are followed for validated IHC kits.

Excessive antigen retrieval is detected by assessing normal tissue surrounding the tumour on the same section. If normal tissue stains positive, the assay should be rejected and repeated.

IHC sample exclusion criteria

Poorly preserved tumour tissue samples should be excluded from analysis, as too should those with non-specific staining within tumour tissue or within non-tumour tissue.

HER2 scoring criteria in gastric cancer

There are separate criteria for scoring surgical and biopsy specimens, and these are outlined in **Figure 9**.

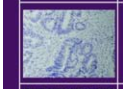
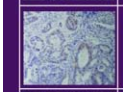
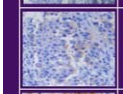
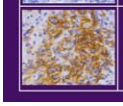
	Score	Surgical specimen – staining pattern	Biopsy specimen – staining pattern	HER2 overexpression assessment
	0	No reactivity or membranous reactivity in <10% of tumour cells	No reactivity or no membranous reactivity in any tumour cell	Negative
	1+	Faint/barely perceptible membranous reactivity in ≥10% of tumour cells; cells are reactive only in part of their membrane	Tumour cell cluster with a faint/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

Figure 9: HER2 scoring criteria in gastric cancer. (Images Courtesy of Targos Molecular Pathology GmbH)

As a supplementary assessment method, Targos suggested a third technique, magnification. With this system, an IHC 3+ result is one that is visible at 5x magnification, an IHC 2+ result is visible at 10x or 20x magnification, and an IHC 1+ result requires 40x magnification before it is visible. A/Prof Bilous says that this is not a substitute for the scoring system, but rather a helpful guide. (See **Figures 10** and **11**)

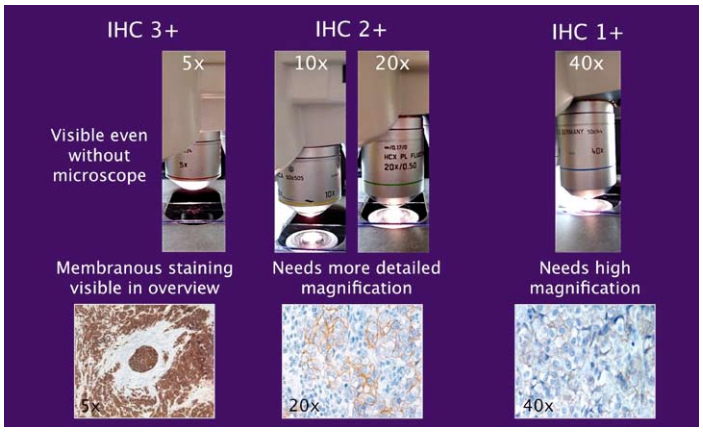


Figure 10: HER2 scoring and magnification

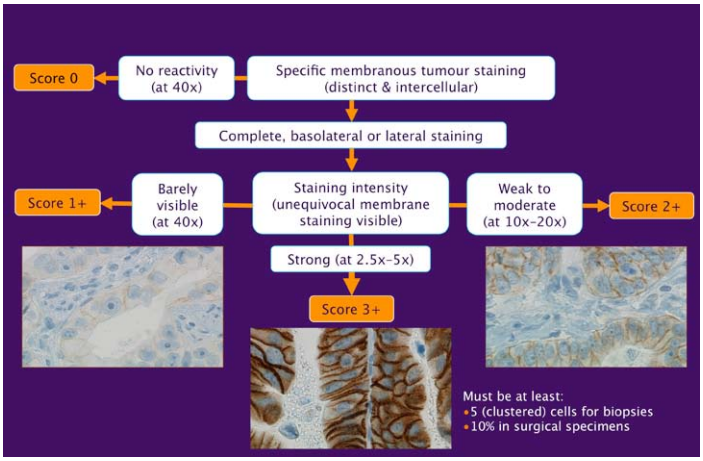


Figure 11: HER2 IHC scoring system with magnification

Interpretation of IHC scoring in gastric cancer

- Tumour cells showing complete, basolateral or lateral membrane staining should be scored
- Cytoplasmic staining should not be included when interpreting results
- Normal epithelial cells should not be scored
- Artifacts may lead to false positive interpretation.

Examples of HER2 staining by IHC in gastric cancer

Figure 12 shows low and higher magnification examples of IHC 1-3+ staining in surgical and biopsy gastric cancer specimens.

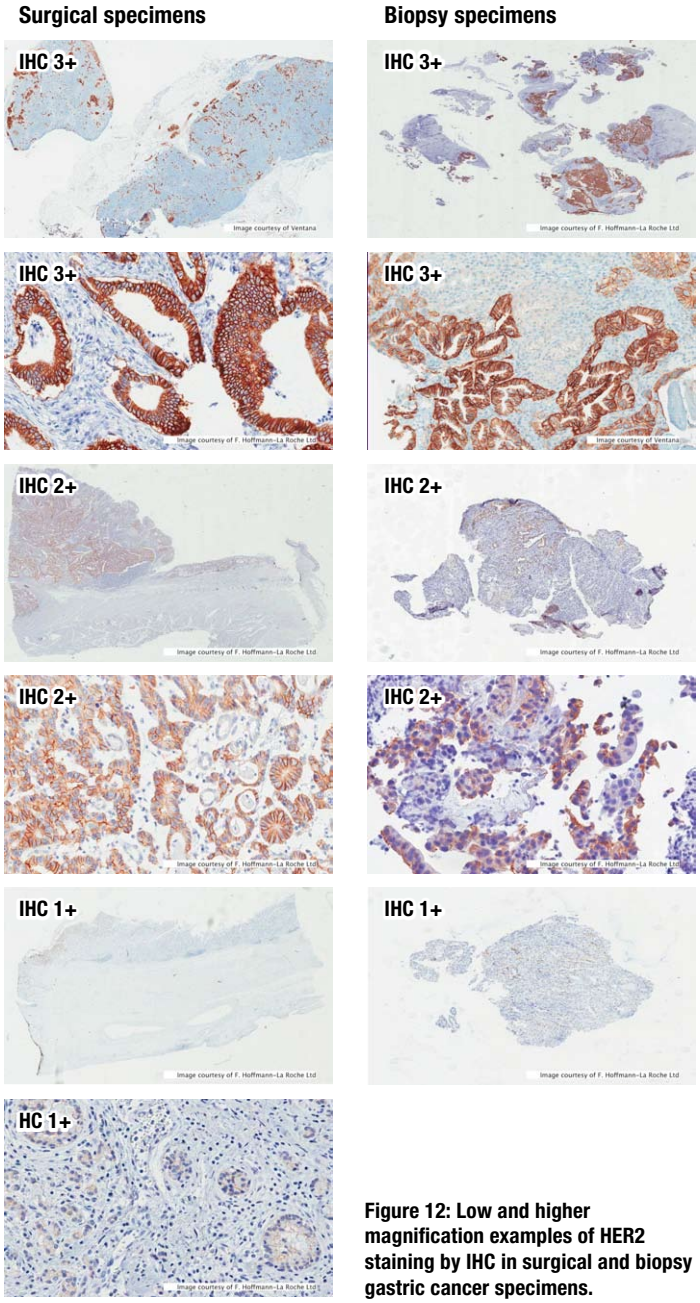


Figure 12: Low and higher magnification examples of HER2 staining by IHC in surgical and biopsy gastric cancer specimens.

If specimens are IHC 3+, then they are considered to be HER2 positive and no further testing is required. Samples that are IHC 2+ need to be FISH tested.

FISH testing

FISH quantifies the level of HER2 gene amplification. Dual-colour FISH uses two labelled DNA probes, a HER2 probe that targets the HER gene locus on chromosome 17, and a CEP_{17} probe that targets the centromeric region of chromosome 17. Results are expressed as a ratio of HER2 gene copies per number of chromosome 17 copies. The definition of FISH positive is HER2:CEP_{17} ratio ≥2.

FISH has some limitations in that it does not allow for simultaneous assessment of tumour morphology, it is difficult and time consuming to assess and evaluate and requires the use of a dark field microscope, and there is a need for photographic record due to fading of the signal. To this end, a bright-field approach for ISH has some advantages and may be used in the future. However, the ToGA study used FISH for their analysis.

Standardisation and validation of HER2 testing techniques in gastric cancer

A/Prof Bilous says that the quality of HER2 testing is crucial to ensure the best outcome for patients, and that ring studies should focus on validating and standardising HER2 testing methods. He adds that further studies to assess the reproducibility of HER2 testing in gastric cancer are encouraged, and should look at both inter-laboratory and inter-observer variation.

The GaTHER study (unpublished) was recently undertaken in Australia by A/Prof Bilous and colleagues to evaluate the accuracy and agreement of HER2 testing for gastric cancer between different laboratories and between different testing methods (IHC vs FISH vs Silver ISH [SISH] vs Chromogenic ISH [CISH]). The study also aimed to establish guidelines for HER2 testing in gastric cancer, to confirm the HER2 testing algorithm and to establish a network of HER2 reference laboratories for gastric cancer. While there was very good concordance between ISH methods, as anticipated, IHC concordance was not as good between labs.

Ring studies, undertaken in Germany and France, revealed good reproducibility of HER2 testing between different pathologists as long as they adhered to guidelines and they had undergone specific training for HER2 status determination in gastric cancer.[4]

HER2 testing in gastric and GOJ cancers: Questions for NZ

- Who to test?
  - Advanced/metastatic patients only
  - All patients with gastric of GOJ cancer
- What test(s) to use?
  - IHC followed by FISH or SISH
- Which testing algorithm to use?
- Where to test?
  - Reference laboratory
  - Peripheral laboratory.

Immunohistochemistry as a quantitative test

- By its nature, IHC is semi-quantitative
- Scoring systems are only as good as the stainer and the interpreter
- Always exercise caution in evaluating cases at the borderlines (1+/2+ and 2+/3+) where this will make a difference to the next step (retest or treatment versus no treatment).

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