Effect of HER2 on prognosis and benefit from peri-operative chemotherapy in early oesophago-gastric adenocarcinoma in the MAGIC trial


1Department of Medicine, The Royal Marsden NHS Foundation Trust, London and Surrey; 2Department of Histopathology, The Royal Marsden NHS Foundation Trust, London; 3The Medical Research Council Clinical Trials Unit, London; 4The Breakthrough Breast Cancer Research Centre, The Institute of Cancer Research, London, UK

Background: Perioperative epirubicin, cisplatin and fluorouracil (ECF) chemotherapy improves survival in operable oesophago-gastric cancer [Adjuvant Gastric Cancer Infusional Chemotherapy (MAGIC) trial HR 0.75 (0.6–0.93)]. HER2 amplification is reported to predict enhanced benefit from anthracyclines in breast cancer. We sought to define whether HER2 predicts benefit from ECF in oesophago-gastric cancer.

Patients and methods: Diagnostic biopsies and/or resection specimens were collected from 415 of 503 MAGIC trial patients (82.5%). HER2 was evaluated by immunohistochemistry (IHC) and brightfield dual in situ hybridisation (BDISH) in tissue microarrays. The prognostic and predictive impact of HER2 status was investigated.

Results: Concordance between HER2 over-expression (IHC3+) and amplification was 96%. Results of HER2 assessment in biopsy and resection specimens were concordant in 92.9% (145/156). HER2 positive rate (IHC3+, or IHC2+/BDISH positive) was 10.9% in the whole cohort and 10.4% in resection specimens. A further 4.0% of resections were IHC negative/BDISH positive. HER2 status was neither prognostic, nor (in pre-treatment biopsies) predicted enhanced benefit from chemotherapy [HER2 positive HR 0.74 (0.14–3.77); HER2 negative HR 0.58 (0.41–0.82), interaction P = 0.7]. However, the power of the predictive analysis was limited by the small number of HER2 positive pre-treatment biopsies.

Conclusions: HER2 status is not an independent prognostic biomarker in early oesophago-gastric adenocarcinoma.

Key words: adenocarcinoma, gastric, hER2, immunohistochemistry, in situ hybridisation, oesophageal

Introduction

Survival after surgery alone (S) for oesophago-gastric cancer is poor except for very early (stage I) disease; therefore, multimodality therapy has been investigated for stage II–III disease. The UK Medical Research Council’s MAGIC (ST02) trial evaluated the addition of peri-operative epirubicin, cisplatin and infused 5-fluorouracil (ECF) to surgery for localised oesophago-gastric adenocarcinoma. Peri-operative chemotherapy (CSC) improves 5-year overall survival from 23% with S to 36% with CSC; HR 0.75 (0.6–0.93) [1]. A second study of peri-operative cisplatin/5-FU confirmed the benefit of this strategy [2]. Despite multimodality therapy, almost two-thirds of patients will relapse and die of their disease. Additionally, some patients exhibit primary resistance, progressing during chemotherapy, demonstrating that alternative strategies are needed. ECF is generally well tolerated, but can result in significant morbidity and even death. Reliable biomarkers are therefore needed to improve outcomes by allowing selection of those who will benefit from CSC, S or investigation of new therapies.

The anti-HER2 monoclonal antibody trastuzumab combined with cisplatin-fluoropyrimidine chemotherapy is a standard treatment of HER2-positive advanced oesophago-gastric adenocarcinoma, mandating routine testing of HER2 in this setting [3]. Targeting HER2 in early-stage disease is now being investigated, including evaluation of the small molecule inhibitor of EGFR and HER2, lapatinib, within the MRC’s ongoing ST03 study.

In breast cancer, HER2 amplification is a potential predictive biomarker of epirubicin sensitivity [4–7], probably due to co-amplification with the Topoisomerase IIa gene (TOP2A), located close to HER2 on chromosome 17q21 [8, 9]. TOP2A cleaves and re-ligates double-stranded DNA, is a key enzyme in DNA replication and repair, and the molecular target of anthracyclines, which stabilise the cleavable complexes formed by topoisomerases; causing DNA breaks and cell death [10]. The role of HER2 and TOP2A gene status as predictive...
markers for response to anthracycline-based chemotherapy has proven controversial. In gastric cancer, the significance of HER2 as a prognostic biomarker remains unclear, with conflicting reports [11–14], possibly reflecting variation in HER2 testing and scoring before the introduction of a standardised scoring system [15, 16]. We therefore sought to determine whether HER2 status would constitute a predictive marker for response to ECF, and/or a prognostic marker, in localised oesophago-gastric cancer.

**materials and methods**

**study population and design**

All patients gave written informed consent to participate in the MAGIC trial (results previously published) [1]. In brief, 503 patients with potentially operable oesophago-gastric adenocarcinoma were randomised to surgery (n = 253) or peri-operative ECF chemotherapy (n = 250). The TransMAGIC study was subsequently designed to determine whether tumour biomarkers could predict benefit from peri-operative ECF. Additional consent was not sought from surviving patients; therefore samples were studied anonymised; identifiable by histology number, study number and initials only. This amendment to the MAGIC study was approved by the South East Research Ethics Committee (Ref 11/LO/0566).

Paraffin-embedded samples from the diagnostic biopsy and resection (where applicable) were requested for all 503 patients randomised from 55 centres predominantly in the UK. Approval was obtained from institutional review boards according to local and national requirements. All tumours were reviewed, and classified according to World Health Organisation classification [17] by one specialist gastrointestinal cancers pathologist (AW) who was blinded to treatment allocation and outcome.

**histological review and tissue microarray construction**

Forty-nine of 55 centres agreed to participate in the study and paraffin-embedded blocks from the diagnostic biopsy, resection or both were received for 415 of 503 patients. Of these, 404 had sufficient material for tissue microarray (TMA) construction. Two cases found to be neuroendocrine tumours on central review (both allocated to S) were excluded. Of 402 remaining patients (79.9%), pre-treatment biopsies were included for 244 of 503 patients (48.5%), and resections or post-treatment biopsies for 337 patients (67.0%). Paired samples were available for 179 of 503 patients (35.6%); shown by treatment arm in Figure 1.

TMAs were constructed from representative blocks from the trial specimens, and were composed of replicate 1-mm cores from each case and controls (i.e. samples of non-neoplastic kidney, liver, placenta, small bowel or normal stomach) as previously described [18]. For biopsy specimens, 0.5-mm-diameter cores were used due to limited available tissue.

**immunohistochemistry and brightfield dual in situ hybridisation**

Immunohistochemical (IHC) testing was carried out on all available resection specimens and biopsies with confirmed adenocarcinoma. HER2 expression was detected using the PATHWAY anti-HER2/neu (4B5) antibody on an automated immuno-stainer (BenchMark® XT Ventana, Medical Systems Inc., Tucson, Arizona) was used for the BDISH assay for HER2 and centromere probe on chromosome 17 (CEP 17) DNA targets, which was previously validated against FISH [19].

Full-face tissue sections (where available) were employed for IHC and/or BDISH using adjusted protocols in cases where results were unavailable due to technical failures (e.g. differences in fixation of the paraffin-embedded samples).

**microscopic analysis and scoring**

One pathologist (AW) examined representative TMA sections subjected to IHC with antibodies against HER2 according to published guidelines [15, 16]. In brief, for IHC, all samples evaluated on the TMAs were scored as initially. A ratio of <1.8 was considered negative and >2.2 was positive. For ratios 1.8–2.2, a further 20 cells were counted, and the ratio re-calculated for 40 cells. Samples with a HER2:CEP17 ratio ≥2.0 in 40 cells were then considered positive.

**definitions of HER2 positive**

For biopsies, HER2 positive was defined by IHC3+ or IHC2+ and BDISH positive (EMEA definition for approval of trastuzumab [20]). For
resections, two definitions of HER2 positive were analysed; (i) IHC3+ or IHC2+ and BDISH positive (EMEA) or (ii), IHC3+ or BDISH positive with any IHC result, (US FDA definition for trastuzumab approval [21]), as all samples had been subjected to both IHC and BDISH.

**statistical considerations**

Sample size was dictated by availability of samples; a priori power calculations indicated that with ~160 deaths and a marker prevalence of ~10%, power to detect an interaction effect (ratio of HRs) of 0.25 (0.5) was ~80% (~70%).

Analyses of the biopsy and resection samples were carried out separately as the resection rate and, therefore, availability of resection specimens was potentially affected by the allocated treatment arm. Baseline characteristics and survival of relevant patients with and without specimens were compared to assess the representativeness of the sample populations. For subjects with pre-treatment biopsy samples, the comparative population comprises those without biopsy samples, and survival time is calculated from randomisation to date of death from any cause, or last follow-up. For patients with resection specimens the relevant comparative population is all resected patients with no available surgical sample, and survival time is dated from surgery. Oesophageal or OGJ primary tumour [22, 27] have been previously associated with HER2 over-expression; these associations were assessed using Fisher’s exact test.

**Introduction**

Marker analysed is HER2

Objectives; to determine whether HER2 is predictive for benefit from ECF or prognostic

Hypotheses; HER2 positivity is associated with a negative prognosis but enhanced benefit from ECF

**Materials and Methods**

| Patients | Patients with potentially operable gastric, OGJ or lower oesophageal adenocarcinoma randomised to surgery or surgery and perioperative ECF chemotherapy |
| Specimen characteristics | Paraffin-embedded tissue from biopsies and/or resection specimens |
| Assay method | Standard IHC and BDISH scored according to gastric cancer guidelines [15, 16]. Standard controls used with each BDISH and IHC run, using BDISH-positive and -negative controls and IHC tissue known to be 1+, 2+ and 3+, respectively. |
| Study design | Retrospective analysis of paraffin-embedded material from patients treated within a prospective randomised study. Clinical end-point of overall survival used Sample size determined by the availability of tissue from the 503 randomised patients; with the observed number of deaths power calculations indicated that with 80% (~70%) for 0.5 and ~70% for 0.5 |
| Statistical methods | Biopsies and resection samples assessed separately. Associations with site of primary tumour, histological subtype and differentation assessed using Fisher’s exact test Predictive value of HER2 status at biopsy assessed using tests of heterogeneity of the subgroup treatment HRs, displayed in forest plots Prognostic value of HER2 status assessed in each treatment arm separately using Kaplan–Meier survival curves compared with the log-rank test Multivariate analysis using Cox’s proportional hazards regression model to assess the independent prognostic value of HER2 status Cohen’s x coefficient used to measure concordance between HER2 expression for biopsy and resection specimens, and IHC and BDISH |

**Results**

Data analysis and presentation

Flow of patients detailed in Figure 1; baseline characteristics in Table 2 Relationship of HER2 with standard prognostic variables displayed in supplementary Table S1, available at Annals of Oncology online

Univariate and multivariate analyses results reported. Kaplan–Meier survival curves for effect of HER2 on survival in Figures 2 (predictive effect demonstrated by treatment arm within HER2 status subgroups) and 3 (prognostic effect in all patients)

Discussion

No prognostic effect in keeping with the majority of recent studies. No clear predictive effect. Study limited by sample size, low HER2 positivity rate and the use of combination chemotherapy

The prognostic value of HER2 status was assessed in each treatment arm separately using Kaplan–Meier survival curves compared with the log-rank test; the treatment groups were combined if a test of heterogeneity of the HER2 hazard ratios (HRs) showed no evidence of differential prognostic effect (heterogeneity P > 0.1). Adjusted multivariable Cox proportional hazards regression models were used to assess the independent prognostic value of HER2 status. All variables that we allow for an effect of, on the prognostic value of HER2 status, were forced into the model along with HER2 status and the prognostic value of HER2 status assessed again. Formal evaluation of HER2 status as a predictor of benefit from ECF chemotherapy could only be undertaken using pre-treatment biopsy specimen results. Here, the treatment HRs in HER2-negative and -positive patients were calculated and tested for heterogeneity, where a P < 0.1 was taken as evidence of potential predictive value.

Cohen’s x coefficient was used to measure concordance between HER2 expression for biopsy and resection specimens, and IHC and BDISH results [28].

This study is compliant with REMARK guidelines (Table 1) [29].

**patients’ characteristics**

Baseline characteristics and survival for the tumour analysis patients and comparable populations without samples are
shown in Table 2 and in supplementary Figure S1, available at Annals of Oncology online, respectively, and are broadly similar.

HER2 and correlation with clinicopathological and prognostic factors

HER2 results were available on 217 of 244 biopsies and 15 of 217 (7%, 95% CI 4% to 11%) were positive. Of the resection specimens, 34 of 332 were IHC3+ or IHC2+/BDISH+ positive. A further 13 of 332 exhibited HER2 amplification (BDISH ≥ 2) but not protein over-expression (IHC0-1+) (Table 3).

Significantly more moderately differentiated ($P = 0.002$) and intestinal cancers ($P = 0.034$) were HER2 positive (supplementary Tables S1a and S1b, available at Annals of Oncology online).

concordance of expression and amplification of HER2 (resection specimens only)

Concordance between HER2 positive by IHC and BDISH was high; 26 of 27 IHC3+ resection specimens were also BDISH positive (96%). By contrast, 11 of 264 (4.2%) IHC0 and 2 of 22 (9%) IHC 1+ resections were BDISH positive, of which 5 of 13 cases (3 of 11 IHC0 and 2 of 2 IHC1+) harboured ≥6 copies of HER2. Overall, concordance between non-equivocal IHC and BDISH results was substantial ($\kappa = 0.789, P < 0.001$). For the 16 IHC equivocal (IHC2+) samples, using BDISH, 7 were positive, 7 negative and 2 not assessable due to technical failure, despite repeating the test with an adjusted digestion (supplementary Table S2, available at Annals of Oncology online).

HER2 positive (EMEA definition; IHC 3+ or IHC 2+ and BDISH+) paired sample concordance

HER2 status in pre-treatment biopsies and resection specimens was concordant in 145 of 156 (93%) of assessable cases ($\kappa = 0.608, P < 0.001$, substantial agreement). In discordant cases, an apparent gain of HER2 over-expression was more common than apparent loss of expression. Nine patients were HER2 negative on biopsy but positive on resection, whereas only two were HER2 positive on biopsy, but negative on resection specimen. Eight of the 11 discordant cases were in patients randomised to S. The discordance is most likely due to the (EMEA) definition of HER2 positive employed (supplementary Table S3, available at Annals of Oncology online): Both cases that apparently lost HER2 protein expression on the resection were HER2 amplified but IHC negative (0–1+) on resection, and six of nine patients that apparently gained HER2 expression were HER2 amplified but IHC0 on biopsy (one was not assessable by BDISH, two BDISH negative).

Of 46 BDISH-positive resection specimens, HER2 amplification was confirmed in 10 paired biopsies, with 5 discordant (BDISH negative) biopsies (33.3%).

Table 2. Baseline characteristics by tissue available compared with all randomised patients

| Age | Median (years) | 62 | 62 | 62 | 62 |
| Range (years) | 23–81 | 29–85 | 29–81 | 23–81 |
| Sex, n (%) | Male | 202 (78) | 194 (80) | 77 (79) | 258 (78) |
| | Female | 57 (22) | 50 (20) | 20 (21) | 74 (22) |
| Site of tumour, n (%) | Lower oesophagus | 39 (12) | 34 (11) | 14 (14) | 36 (11) |
| | OGJ | 31 (15) | 27 (14) | 14 (14) | 41 (12) |
| | Stomach | 189 (73) | 183 (75) | 69 (71) | 255 (77) |
| WHO PS, n (%) | 0 | 180 (69) | 162 (66) | 77 (79) | 229 (69) |
| | 1 | 79 (31) | 82 (34) | 20 (21) | 103 (31) |
| Histological subtype, n (%) | Intestinal | Not assessable | 179 (73) | Not assessable | 260 (78) |
| | Diffuse | 50 (20) | 15 (6) | 12 (4) | 3 (1) |
| | Mixed | 15 (6) | 12 (4) | 3 (1) | 3 (1) |
| Differentiation, n (%) | Well | Not assessable | 0 (0) | Not assessable | 0 (0) |
| | Moderately | 134 (45) | 143 (45) | 143 (45) | 108 (57) |
| | Poorly | 110 (55) | 186 (57) | 186 (57) | 186 (57) |
| | Not assessable | 0 (0) | 3 (1) | 3 (1) | 3 (1) |

*Only patients that underwent resection are included; five post-treatment biopsies in patients that did not undergo resection are excluded.*
prognostic impact of HER2 positivity

In patients with pre-treatment biopsies, the HER2 status (EMEA definition) HRs were similar in S and CSC patients (HR = 0.54 and 0.63, respectively) with no evidence of heterogeneity (P = 0.7). Combining treatment arms, there was a non-statistically significant association (HR = 0.58, 95% CI 0.32–1.06, log-rank P = 0.08) between HER2 positive status on pre-treatment biopsy (n = 15/217) and longer survival. However, this was not confirmed by HER2 status (EMEA definition) on resection specimens, where there was no clear prognostic effect in either treatment group alone (HER2 HRs 1.0 and 0.99 in S and CSC patients respectively, test for heterogeneity P = 0.99) or combined (HR = 0.99, 95% CI 0.62–1.59, log-rank P = 0.97). When patients who were HER2 BDISH positive but IHC negative (0–1+, n = 13) were included (FDA definition), similar results were found (Figure 3) combined arms HR = 1.17 (0.77–1.77). In both datasets, multivariate analysis including HER2 status with performance status, histology, differentiation, tumour site and, for resection samples only, T-stage and N-stage, yielded HER2 HRs similar to the unadjusted data, and remained non-significant. On pre-planned trend analysis, there was no clear survival trend with increasing amplification (log-rank test for trend <2 (n = 272) versus 2–5 (n = 23) versus >5 (n = 23), P = 0.73). An exploratory analysis of the prognostic impact of HER2 in intestinal-type cancers (n = 255; supplementary Figure S2, available at Annals of Oncology online) also demonstrated no significant effect (HR = 1.26 (0.80–1.97), P = 0.32).

discussion

The MAGIC study reported a clinically and statistically significant benefit from peri-operative chemotherapy compared with S. Although tissue was not collected from all enrolled subjects, the Kaplan–Meier survival curves indicate that the patients with available specimens were representative of the comparable intention to treat biopsy and resection populations.

The rate of HER2 positive cancers (14.4% IHC3+ or BDISH+) reported here is lower than reported in advanced disease; both in the screening study for the ToGA trial (22.1%) [3], and an international collaborative biomarker study (20.6%) [27]. Our results are more comparable to a biomarker analysis of the ACTS-GC study in early gastric cancer, where 13.6% of 829 resected gastric cancers were HER2 positive, defined by IHC3+ or IHC2+ and BDISH+ [24]. Using this definition, only 10.4% of our resections were HER2 positive, possibly reflecting ethnic variation; a large European study reported HER2 expression in <10% of gastric cancers evaluated, albeit using breast cancer HER2 scoring [12]. The differences observed suggest that HER2 over-expression/HER2 gene amplification may be more frequent in advanced than early disease. The age of the paraffin-embedded samples, however, may also have affected our results, as the reliability of immunohistochemical results for HER2 have been reported to decline with age, leading to false negatives [30].

Our concordance between HER2 over-expression (IHC3+) and amplification was high (26 of 27 patients, 96%), consistent with previous reports [25]. However, 13 of 47 HER2-positive

Table 3. HER2 immunohistochemistry and BDISH results

<table>
<thead>
<tr>
<th></th>
<th>Pre-treatment biopsy (n = 244)</th>
<th>Resection (n = 332)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>IHC not assessable</td>
<td>27/244 (11.1)</td>
<td>3/332 (0.9)</td>
</tr>
<tr>
<td>BDISH–</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>BDISH+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Not assessable</td>
<td>27</td>
<td>3</td>
</tr>
<tr>
<td>IHC assessable</td>
<td>217</td>
<td>329</td>
</tr>
<tr>
<td>IHC 0 Total</td>
<td>194/217 (89.4)</td>
<td>264/329 (80.2)</td>
</tr>
<tr>
<td>BDISH–</td>
<td>241</td>
<td>91.3</td>
</tr>
<tr>
<td>BDISH+</td>
<td>11</td>
<td>4.2</td>
</tr>
<tr>
<td>Not assessable</td>
<td>N/Aa</td>
<td>12 (4.3)</td>
</tr>
<tr>
<td>IHC 1+ Total</td>
<td>7 (3.2)</td>
<td>22 (6.7)</td>
</tr>
<tr>
<td>BDISH–</td>
<td>N/Aa</td>
<td>20 (90.9)</td>
</tr>
<tr>
<td>BDISH+</td>
<td>2</td>
<td>9.1</td>
</tr>
<tr>
<td>IHC 2+ Total</td>
<td>4 (1.8)</td>
<td>16 (4.9)</td>
</tr>
<tr>
<td>BDISH–</td>
<td>1 (25.0)</td>
<td>7 (43.8)</td>
</tr>
<tr>
<td>BDISH+</td>
<td>3 (75.0)</td>
<td>7 (43.8)</td>
</tr>
<tr>
<td>Not assessable</td>
<td>0</td>
<td>2 (12.5)</td>
</tr>
<tr>
<td>IHC 3+ Total</td>
<td>12 (5.5)</td>
<td>27 (8.2)</td>
</tr>
<tr>
<td>BDISH–</td>
<td>N/Aa</td>
<td>1 (25.0)</td>
</tr>
<tr>
<td>BDISH+</td>
<td>26</td>
<td>96.3</td>
</tr>
<tr>
<td>IHC 3+ or IHC 2+ and BDISH+</td>
<td>15 (6.9)</td>
<td>34/327 (10.4)b</td>
</tr>
<tr>
<td>IHC 3+ or BDISH+, any IHC result</td>
<td>N/Aa</td>
<td>47/327 (14.4)</td>
</tr>
</tbody>
</table>

*a* As only IHC 2+ biopsies or those without paired resections were tested by BDISH.

*b* Denominator excludes two IHC2+ patients without BDISH results.

Figures in bold indicate the total number of patients (and percentage of all assessable samples these comprise) for each IHC score (0, 1+, 2+ or 3+).

predictive biomarker analysis: chemotherapy and HER2

For the 217 patients with established baseline HER2 status (overall treatment HR in patients with biopsy samples 0.69 (95% CI 0.42–0.81), the treatment HR in the HER2-positive population (EMEA definition; n = 15) was 0.74 (95% CI 0.14–3.77) in favour of CSC and 0.58 (95% CI 0.41–0.82) in the HER2-negative population (n = 202) (Figure 2). Power for testing heterogeneity was limited given the low HER2-positive rate, but there was no evidence that pre-treatment HER2 status predicted survival outcome (heterogeneity P = 0.7). As concordance between biopsy and resection was high (93%), an indirect assessment of potential predictive effect in resection specimens was also undertaken, looking for evidence of heterogeneity in the prognostic impact of HER2 status following resection across treatment groups, with again no significant effect (heterogeneity P = 0.988 using EMEA definition; P = 0.821 using US FDA definition).
resections (27.7%) were HER2 amplified, without detectable HER2 protein over-expression; similar to the 22% (131 of 594) FISH positive, IHC negative (0–1+) patients reported in ToGA [3]. Further studies are warranted to investigate the biological significance of 17q21 amplification encompassing the HER2 gene locus in the absence of HER2 protein over-expression, particularly if routine FISH testing is limited to IHC2+ patients thus excluding a quarter of patients from receiving.

Figure 2. Predictive marker assessment in pre-treatment biopsies (A, B and C) and exploratory analysis in resections (D and E). (A) Kaplan–Meier curve of OS by treatment arm in HER2 positive (IHC3+ or IHC 2+ and BDISH+) patients (B) Kaplan–Meier curve of OS by treatment arm in HER2 negative patients (C) Forest plot of treatment effect by HER2 subgroup, (interaction P = 0.77) (D) Forest plot of HER2 effect by treatment subgroup in resection specimens (IHC3+ or IHC 2+ and BDISH+) (E) Forest plot of HER2 effect by treatment subgroup in resection specimens (IHC3+ or BDISH+).
trastuzumab. Although the assessment of HER2 was centralised and carried out using validated immunohistochemical and BDISH methodologies, scoring was carried out by a single pathologist experienced in gastrointestinal pathology. Given the inter-observer variability reported for HER2 assessment [16], one potential limitation of this study is single-observer bias.

Our concordance between biopsies and resections was 92% for negative results and 83% for positive results. A previously published comparison of HER2 expression between biopsies and resections predated the standardisation of HER2 scoring for gastric cancer, but reported 88.5% concordance for IHC in 200 paired samples. FISH was carried out only in biopsy specimens from patients with HER2-amplified resection specimens; with 62.2% concordance (66.6% in our study). In contrast to our study, no patients received neoadjuvant therapy between the diagnostic biopsy and resection [31]. In breast cancer, changes in HER2 status between pre-treatment biopsies and resections following neoadjuvant chemotherapy have been reported in 7.6%–30% of cases, most commonly gain of expression (reviewed in [32]). We detected a change in HER2 status in 11 patients; however, the majority (8 of 11) had concordant BDISH amplification and only discordant HER2 protein expression, likely representing heterogeneous HER2 expression rather than a true change in the biology of the disease. Noteworthy, only three patients whose HER2 status differed from biopsy to resection specimen had received chemotherapy.

**HER2** gene amplification [4, 5, 33] has been reported to predict for benefit from anthracycline therapy in breast cancer studies; in a meta-analysis, treatment HRs (epirubicin versus no epirubicin) were 0.73 in patients with HER2-amplified
tumours and 0.91 in the rest [34]. In our analysis, the estimated benefit from ECF chemotherapy in HER2-positive and -negative patients was similar (HR 0.74 and 0.58, respectively). This may be a function of the combination chemotherapy, thereby not only testing anthracycline sensitivity. However, while our power to detect an interaction was limited by our sample size of pre-treatment biopsies and the low frequency of HER2 positivity, the observed HRs did not suggest the existence of a subgroup of patients with oesophago-gastric cancer who should not be offered ECF chemotherapy. Further studies are planned to investigate whether a combination of potential anthracyline/platinum/fluoropyrimidine biomarkers is predictive in this setting.

In contrast to breast cancer, the prognostic effect of HER2 in gastric cancer is controversial (reviewed in [35]). This can be partially explained by the heterogeneous scoring methods before the standardised system proposed in 2008 [15]. While a recent series of resected patients with non-metastatic stage I–IV gastric cancer (n = 221) reported that HER2 amplification, but not over-expression, correlated significantly with worst survival (P = 0.023) [26], large series of both early [24, 25] and advanced gastric cancer [27] have reported no significant prognostic impact. The latter study is consistent with observations from the ToGA study, where the original statistical calculations, based upon an assumption that HER2 conferred an adverse prognosis, were revised when a lower-than-expected event rate was observed by the IDMC [3]. The power of our study is limited given the low frequency of HER2 positivity. Based on the pre-treatment biopsy specimens, an absolute increase in survival at 2 years of >30%, and a decrease of >2%, could be ruled out and using resection specimens, absolute differences in 2-year survival after surgery greater than a 15% increase or 21% decrease can be reliably excluded. Our results therefore, support the conclusions from recent studies that HER2 is not an independent predictor of poor prognosis in oesophago-gastric adenocarcinoma.

Taken together, our results demonstrate that HER2 status is neither a clinically useful biomarker to select patients for perioperative ECF chemotherapy nor a prognostic biomarker in early oesophago-gastric cancer.

acknowledgements

We thank the patients who participated in the MAGIC trial and all the investigators and pathologists who kindly participated in TransMAGIC.

funding

TransMAGIC is funded by CRUK (grant reference number C20023/A7217), with support from the Medical Research Council through the MRC Clinical Trials Unit, who sponsored and coordinated the MAGIC trial, and analysed the TransMAGIC data. JSR-F is funded by Breakthrough Breast Cancer. Alicia Okines, David Cunningham and Tom Waddell acknowledge NHS Funding to the NIHR Biomedical Research Centre.

disclosure

AFCO has previously received an honorarium and travel support from Roche and travel support from Amgen and Bayer. DC has served on an advisory board for Roche and Amgen, acted as expert witness for Amgen (uncompensated) and received research funding from Amgen, Merck Serono, Roche and Sanofi Aventis. TSW has received travel support from Pfizer, Roche, Amgen Ltd and GSK. RW has previously received an honorarium from Roche. JSR-F, SS, REL, AW, ZE, LCT and DN have no relevant disclosures.

references


22. Bang Y, Chung H, Xu J et al. Seoul National University Hospital, Seoul, Republic of Korea; Yonsei University College of Medicine, Seoul, Republic of Korea; Affiliated Hospital (307 Hospital) Cancer Centre, Beijing, China; National Centre for Tumour Diseases, Heidelberg, Germany; Aichi Cancer Center, Nagoya, Japan; Bashkirian Republican Clinical Oncology Dispensary, Ufa, Russia; F. Hoffmann-La Roche, Basel, Russia; Roche Products Ltd, Welwyn Garden City, United Kingdom; TARGOS Molecular Pathology GmbH, Kassel, Germany; University Hospital Gasthuisberg, Leuven, Belgium. Pathological features of advanced gastric cancer (GC): relationship to human epidermal growth factor receptor 2 (HER2) positivity in the global screening programme of the ToGA trial. J Clin Oncol 2009; 27: 15s (Suppl; abstr 4556).


