The significance of the HER-2 status in esophageal adenocarcinoma for survival: an immunohistochemical and an in situ hybridization study†

M. J. D. Prins¹, J. P. Ruurda¹, P. J. van Diest², R. van Hillegersberg¹ & F. J. W. ten Kate²

Departments of ¹Surgery; ²Pathology, University Medical Center Utrecht, Utrecht, The Netherlands

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Background: In esophageal adenocarcinoma (EAC), concordance and prognostic significance of human epidermal growth factor 2 (HER-2) protein overexpression and gene amplification are equivocal, which led us to reevaluate this by immunohistochemistry (IHC) and in situ hybridization.

Methods: One hundred and fifty-four patients were included in a tissue micro array (TMA). HER-2 gene amplification was assessed by fluorescence and silver-enhanced in situ hybridization (FISH and SISH) and expression with the HercepTest™.

Results: HER-2 was amplified in 16% by SISH and 18% by FISH. HER-2 positivity (IHC 3+ or 2+ with ISH+) was seen in 12% and overexpression (IHC 2+/3+) in 14%. Concordance was 92% between SISH/IHC, 90% between FISH/IHC and 95% between SISH/FISH. All IHC 3+ cases were amplified by SISH and in 93% by FISH. Of the IHC 2+ cases, this was 33% (SISH) and 50% (FISH). Of the IHC 1+ cases, still 6% (SISH) and 8% (FISH) showed amplification. HER-2 positivity, overexpression and amplification were all associated with poor cancer-specific survival, in univariate analysis.

†All authors contributed to the study.

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Furthermore, HER-2 positivity and amplification (SISH) were independently associated with poor survival (hazard ratio, HR 6.343; 95% CI 1.218–36.234; \( P = 0.029 \) and HR 3.231; 95% CI 1.092–9.663; \( P = 0.034 \)).

**Conclusion:** HER-2 positivity and gene amplification are fairly frequent and independently associated with poor survival.

**Key words:** esophageal adenocarcinoma, gene amplification, HER-2, protein

**introduction**

In the Western world, esophageal cancer constitutes the seventh leading cause of tumor death [1]. Advances in surgical techniques and introduction of neoadjuvant chemo (radio) therapy have already improved patient’s prognosis [2, 3]. Further improvements might come from molecular therapy that targets biological markers that play a key role in oncological processes.

The proto-oncogene, human epidermal growth factor 2 (HER-2) receptor, can be specifically targeted by monoclonal antibody trastuzumab. Activation of HER-2 plays a pivotal role in cell proliferation, inhibition of apoptosis and tumor progression, which is mainly mediated through the Ras/Raf/mitogen-activated protein kinase and the phosphatidylinositol-3-kinase/Akt mammalian target of rapamycin pathway [4, 5].

In this, HER-2 protein overexpression and gene amplification represent features of an adverse clinical outcome.

In the ToGa trial (trastuzumab for gastric cancer) on patients with advanced gastric or gastro-esophageal junction (GEJ) carcinomas, who had a positive HER-2 status, it was shown that addition of trastuzumab to chemotherapy significantly improved survival [6]. Because of the similarities between gastric and esophageal carcinoma with respect to risk factors and molecular characteristics [7], evaluation of HER-2 in esophageal carcinoma might have clinical consequences as well.

There are several methods to determine the HER-2 status: immunohistochemistry (IHC) to evaluate protein expression and in situ hybridization (ISH) techniques to investigate the amplification status. Post-hoc analysis of the ToGa trial showed that patients with HER-2 protein overexpression derived the greatest benefit from trastuzumab [6]. Therefore, IHC was recommended as an initial HER-2 testing method [8].

According to the guidelines for gastric and breast cancers, tumors with an IHC 3+ score are considered as positive and IHC 1+ as negative for HER-2 expression. Tumors with IHC 2+ are considered equivocal and are subjected to validated ISH techniques like fluorescence and silver-enhanced in situ hybridization (FISH and SISH, respectively). Patients with IHC 3+ or 2+ with HER-2 gene amplification are then considered eligible for trastuzumab [8].

However, in esophageal adenocarcinoma (EAC), the selection criteria for trastuzumab therapy are unclear. It is unknown which HER-2 testing method can be best used to identify a HER-2 positive tumor and published data on the prognostic significance of HER-2 in EAC are conflicting [9–17]. A recent meta-analysis, however, showed a significant association between the HER-2 positive status and poor survival [18]. Moreover, HER-2 gene amplification is usually seen in patients with IHC 3+, but the percentage of HER-2 gene amplification in patients with IHC 0, 1+ and 2+ is unclear.

We hypothesized that HER-2 overexpression and gene amplification are seen in ~22% of patients [6, 18] and that a HER-2 positive status results in a poor prognosis. The aim of the study was to evaluate the percentage of HER-2 gene amplification in all HER-2 IHC groups, and to confirm their prognostic value in EAC using different HER-2 testing methods.

**materials and methods**

**study population**

Data of all patients who underwent esophagectomy for cancer between August 1988 and November 2009 at the University Medical Center Utrecht were collected from a database. Patients with histologically proven EAC were included, whereas patients under neoadjuvant treatment were excluded. Positive resection margins (R1) according to the College of American Pathologists (CAPs) criterion [19] and patients with pathological T4 disease were also excluded because these pathological features represent advanced stage of the disease and are associated with a very poor survival [20] irrespective of the HER-2 status. Furthermore, patients who died in hospital or within 30 days from operation were excluded from analyses.

A HER-2-positive status was defined as IHC 2+ or 3+ staining (IHC), or ≥6 HER-2 gene copy numbers (SISH), or a HER-2/CEP17 ratio of ≥1.8 (FISH) or IHC 3+ and 2+ staining in combination with gene amplifications (according to the gastric scoring method). This modified HER-2 scoring system was also applied in the current study, because gastric cancer and EAC share similarities with regard to etiology and risk factors [7].

All tumor resection specimens were reviewed by an experienced pathologist (F.J.W.t.K.). Tumors were staged according to the tumor node–metastasis (TNM) staging system (7th edition) [21]. Patients were followed up until death or up to June 2012. The primary and secondary outcome was the prognostic value of a positive HER-2 status and the agreement between the different HER-2 testing methods. The study was carried out in accordance with the local ethical guidelines concerning informed consent using patient’s material after surgical resection.

**TMA construction**

Formalin-fixed, paraffin-embedded tumor blocks were used for the construction of a tissue micro array (TMA) (Figure 1) as described previously [22].

**IHC, SISH and FISH**

HER-2 IHC staining was carried out using an automated staining machine (Bond™ System, Leica Microsystems GmbH, Wetzlar, Germany) and a biotin-free Bond™ Polymer Define Detection system (Leica Microsystems GmbH, Catalog no. DS98000). Automated staining steps included antigen retrieval (Bond™ epitope retrieval solution 2) and incubation with the...
primary antibody (Neomarkers SP3, RM9103-s, 91035906D, 1:100, Fremont) for 15 min.

SISH analysis was carried out using the automated SISH staining machine (BenchMark XT, Ventana). The staining procedure steps included deparaffinization, pre-treatment (based on ‘heat unmasking’ using a reaction buffer during 36 min), incubation with ISH protease 3 (8 min), incubation with HER-2 probe (SISH V-Probe, 2 drops for 4 min), hybridization (52°C during 6 h), denaturation (72°C, during 24 min), washing procedures [stringency saline-sodium citrate buffer (pH 7.0) 1, 2 and 3], incubation with Silver C (one drop, 8 min) and counterstaining with hematoxylin.

FISH was carried out with the automated FISH staining procedure (Menarini Diagnostics, Leica) which included several steps. Between all steps, slides were washed in Bond Wash Solution (150 µl) (1–12 min). In the first step, Bond Dewax Solution (150 µl) was added to paraffin-embedded slides for 2 min. Subsequently, the slides were washed with alcohol (150 µl) for 1 min. Antigen retrieval was achieved using Bond ER Solution (150 µl, 32 min) followed by incubation with enzyme 5 (ID 2006, reagent UPI 03045025) for 2 min. Subsequently, the slides were incubated using an LSI HER-2/CEP17 probe 3D test (ID 2006) for 19 min. The slides were rinsed with post hybridization wash 2 (ID 2006) (150 µl) for 2 min. The slides were then washed in deionized water (150 µl) for 103 min and were dried.

**scoring of the tissue micro array (TMA)**

Immunohistochemical scoring was carried out by two readers (F.J.W.t.K. and M.J.D.P.). Interobserver variation was <5%. For the SISH score, the TMA slides were simultaneously evaluated by the same readers. Thereafter, the FISH score was evaluated by one reader (M.J.D.P.). Because FISH has a low interobserver variation [23], it was not thought to be beneficial to check again for this well-established low interobserver variability. In all cases, the readers were blinded to the other HER-2 testing methods. Immunohistochemical scoring of HER-2 expression was based on the modified and validated Hercep Test™ [24].
HER-2 heterogeneity

HER-2 heterogeneity was defined as the presence of both HER-2 protein overexpression/gene amplification in one to two tumor core(s) (not expressed as a percentage) and low HER-2/absence of gene amplification in the other core(s), observed in one tumor.

statistical analysis

Retrospective power analyses (power 80%, α = 0.05) based on previously published data (10) showed that a minimum of 108 patients needed to be included to detect a difference in survival between HER-2-positive and negative patients.

Associations between clinical features such as age, gender, recurrence of disease and pathological features such as T-stage and positive lymph nodes on the one hand, and a positive HER-2 status on the other hand were evaluated using the Pearson’s chi-square test. Kaplan–Meier function was used to compare the cancer-specific survival (CSS, defined as the period between surgery and death due to cancer) among patients with a positive and negative HER-2 status.

The clinical and pathological features summarized in Table 2 (left column) were included in univariate analysis, in which the HER-2 ratio by FISH indicates HER-2 copy numbers to chromosome 17 centromere (HER-2/CEP17 ratio; ≥1.8 versus <1.8) and the lymph node ratio indicates the number of positive nodes divided by the total number of resected nodes; ≤25% versus >25%. Variables with a P value of <0.10 were included in multivariate analysis (Cox proportional hazards regression analysis). All analyses were carried out using standard statistical software (SPSS version 20.0; SPSS inc, Chicago, IL).

results

In total, 290 patients were operated for EAC between 1988 and 2009. Patients with R1 resection (N = 26), T4 disease (N = 7), patients who received neoadjuvant chemotherapy (N = 49) and patients with incomplete follow-up data (N = 54) were excluded from analysis, leaving 154 patients (Table 1). Follow-up was complete in 144 (94%) patients and the median follow-up was 27.1 months (range 2.7–271.1). The 5-year CSS was 38%.

HER-2 protein expression

Tumor cores of 149 (97%) patients were available for immunohistochemical evaluation. In five cases, the cores were missing because of the absence of tumor cells (N = 3) or missing cores (N = 2). In total, 89 patients (60%) were negative for HER-2; 39 (26%) patients showed IHC 1+; 6 (4%) had IHC 2+ and 15 (10%) patients had an IHC 3+ score. HER-2 overexpression was seen in 14% of patients and was significantly associated with poor CSS (hazard ratio, HR 1.897; 95% CI 1.098–3.271; P = 0.022). The median survival was 20.7 months in patients with HER-2 overexpression and was 35.6 months in patients with low protein expression (P = 0.020, log rank) (Figure 2A). Furthermore, HER-2 protein overexpression was associated with a lymph node ratio of >25% (P = 0.042) and with distant metastasis (P = 0.044, chi-square, supplementary Table S1A, available at Annals of Oncology online). HER-2 protein overexpression was more frequently observed in GEJ tumors than in distal esophageal tumors (17.1% versus 7.3%); however, this was statistically not significant (P = 0.128).

Table 1. Baseline characteristics (N = 154)

<table>
<thead>
<tr>
<th>Variable</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>126 (81.8)</td>
</tr>
<tr>
<td>Female</td>
<td>28 (18.2)</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>64.0 (33.8–81.3)</td>
</tr>
<tr>
<td>Tumor extent (T stage)</td>
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</tr>
<tr>
<td>T1,T2</td>
<td>52 (33.8)</td>
</tr>
<tr>
<td>T3</td>
<td>102 (66.2)</td>
</tr>
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<td>LNN involvement</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>104 (67.5)</td>
</tr>
<tr>
<td>No</td>
<td>46 (29.9)</td>
</tr>
<tr>
<td>Unknowna</td>
<td>4 (2.6)</td>
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<tr>
<td>LNN ratio &gt;25%</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>48 (31.2)</td>
</tr>
<tr>
<td>No</td>
<td>102 (66.2)</td>
</tr>
<tr>
<td>Unknowna</td>
<td>4 (2.6)</td>
</tr>
<tr>
<td>Perinodal extensionc</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>62 (59.6)</td>
</tr>
<tr>
<td>No</td>
<td>42 (40.4)</td>
</tr>
</tbody>
</table>

aData are n (%), unless noted otherwise.

HER-2 gene amplification by SISH

Cores of 153 patients (99%) were available for SISH analysis. In one patient, the cores were not assessable because of the absence of tumor cells. Low-level amplification (6–10 copies) was seen in 8 (5%) patients, and 17 (11%) had high-level amplification (>10 copies). In total, 25 (16%) patients had HER-2 gene amplification (>26).

Gene amplification was significantly associated with T-stage (P = 0.011), lymph node ratio >25% (P = 0.001), and with recurrence of disease (P = 0.044, supplementary Table S1B, available at Annals of Oncology online).

Patients with HER-2 gene amplification (>26) had a significantly worse CSS (HR 1.691; 95% CI 1.014–2.820, P = 0.044) (Table 2). The median survival was 21.0 months in patients with, and 39.4 months in individuals without HER-2 gene amplification (P = 0.042, log rank, Figure 2B).

HER-2 gene amplification by FISH

The cores of 149 patients (97%) were available for FISH evaluation. Tumor cores of two patients were absent, and in three cases the cores did not contain tumor cells. Amplified tumors (HER-2/CEP17 ratio ≥1.8) were seen in 27 patients (18%). A high HER-2 ratio (≥1.8) was significantly associated with worse CSS (HR 1.828, 95% CI 1.102–2.711, P = 0.020) (supplementary Figure S1, available at Annals of Oncology online), with median age (P = 0.029), T-stage (P = 0.048), vasoinvasive tumor growth (P = 0.046), lymph node metastases (P = 0.036), lymph node ratio >25% (P = 0.004) and with recurrence of disease (P = 0.047, supplementary Table S1C, available at Annals of Oncology online).
Table 2. Univariate and multivariate analysis of associations between clinical and pathological features and cancer-specific survival (CSS)

| Table 2. Univariate and multivariate analysis of associations between clinical and pathological features and cancer-specific survival (CSS) |
|---|---|---|---|---|
| **Gender (male/female)** | Median CSS in months | Unadjusted HR (95% CI) (Univariate) | P value | Adjusted HR (95% CI) a  
**Multivariate** | P value |
| Gender (male/female) | 33.9 | 26.7 | 1.051 (0.593–1.864) | 0.865 | 3.465 (1.310–9.169) | 0.012 |
| Gender (male/female) | 15.6 | 18.7 | 2.280 (1.310–3.953) | 0.004 | 6.643 (1.218–36.234) | 0.029 |

| **T stage (T3/T1,T2)** | Median CSS in months | Unadjusted HR (95% CI) (Univariate) | P value | Adjusted HR (95% CI) a  
**Multivariate** | P value |
| T stage (T3/T1,T2) | 22.9 | 26.4 | 9.865 (4.283–22.723) | 0.000 | 3.465 (1.310–9.169) | 0.012 |

| **G grade (G3/G2,G2)** | Gender (male/female) | 21.2 | 26.4 | 3.084 (1.877–5.070) | 0.000 | 2.450 (1.381–4.344) | 0.002 |

| **LNN ratio (≥25/≤0.25)** | Gender (male/female) | 18.5 | 88.9 | 3.524 (2.279–5.451) | 0.000 | 2.497 (1.405–4.438) | 0.002 |

| **Vasooinvasion (yes/ no)** | Gender (male/female) | 21.6 | 26.4 | 3.310 (2.212–5.165) | 0.000 | 1.580 (0.914–2.732) | 0.102 |

| **Perineural growth (yes/ no)** | Gender (male/female) | 21.2 | 49.4 | 2.543 (1.641–3.940) | 0.000 | 1.238 (0.727–2.109) | 0.432 |

| **HER-2 protein (IHC) (2,3+/0,1+)** | Gender (male/female) | 20.7 | 35.6 | 1.897 (1.098–3.277) | 0.022 | 1.434 (0.401–5.121) | 0.579 |

| **HER-2 gene (ISH) (≥2+/<6)** | Gender (male/female) | 20.7 | 35.6 | 1.828 (1.126–2.913) | 0.014 | 3.465 (1.310–9.169) | 0.012 |

| **HER-2 ratio (FISH) (≥1.8/<1.8)** | Gender (male/female) | 21.0 | 41.3 | 1.828 (1.102–3.033) | 0.020 | 1.103 (0.432–2.815) | 0.838 |

**CEP17 copy number increase (i.e. ≥3 copy numbers of centromere 17) was seen in 18 of 149 (12%) cases. Furthermore, 16 of 18 (89%) patients with CEP17 copy number increase had low HER-2 expression, whereas only 2 of 18 (11%) patients had HER-2 overexpression, this result was statistically not significant (P = 0.655). CEP17 copy numbers (≥3) were neither associated with clinical or pathological features nor with CSS (HR 1.318, 95% CI 0.679–2.556, P = 0.415).**

**HER-2 positivity**

According to the current standard used in gastric cancer, 12% of patients had HER-2 positivity. This HER-2 positivity was not associated with any of the included clinical and pathological variables (data not shown), but was significantly associated with a poor CSS (HR 1.828, 95% CI 1.010–3.309; P = 0.046). Patients with HER-2 positivity had a median survival of 20.7 versus 35.6 months in patients who were negative (P = 0.043, log rank) (Figure 2C).

**Concordance between IHC, FISH and SISH**

Concordance between IHC and SISH was 92% (137 of 149), was 90% between IHC and FISH (131 of 145) and was 95% between SISH and FISH (141 of 149) (data not shown). More specifically, all patients (15 of 15, SISH) and 93% (14 of 15, FISH) with IHC 3+, had gene amplification. In patients with IHC 2+, gene amplification was seen in 33% (2 of 6, SISH) and in 50% (3 of 6, FISH). However, also in the IHC 1+ group, gene amplification was seen in 6% (8 of 128, SISH) and in 8% (10 of 124, FISH) (data not shown). Patients with IHC 0 did not have HER-2 gene amplification.

Patients with HER-2 IHC 3+ expression can be considered as ‘true’ HER-2-positive tumors, because in gastric, GEJ and breast cancer, these patients are eligible for trastuzumab therapy and no further analysis is required. The sensitivity of FISH to detect an IHC 3+ HER-2 tumor was 93% (14 of 15) and was 100% (15 of 15) when SISH was applied (data not shown). Furthermore, SISH interpretation was less time consuming compared with FISH.

**HER-2 heterogeneity**

HER-2 protein intratumoral heterogeneity was observed in 10 of 148 (7%) cases. HER-2 gene copy number heterogeneity was found in 3% (5 of 153) using SISH, and was found in 6% (9 of 149) using FISH (e.g. Figure 1K).

**Multivariate analysis**

In multivariate analysis, HER-2 positivity (HR 6.343; 95% CI 1.218–36.234; P = 0.029), HER-2 gene amplification (SISH) (HR 3.231; 95% CI 1.092–9.563; P = 0.034), T-stage (HR 3.465; 95% CI 1.310–9.169; P = 0.012), grade of tumor differentiation (HR 2.450; 95% CI 1.381–4.344; P = 0.002) and lymph node ratio (HR 2.497; 95% CI 1.405–4.438; P = 0.002) were independent prognostic markers for poor CSS, but not HER-2/CEP17 ratio by FISH and HER-2 protein overexpression (HR 1.103; 95% CI 0.432–2.815; P = 0.838 and HR 1.434; 95% CI 0.401–5.121; P = 0.579, respectively) (Table 2).

**Discussion**

To our knowledge, this is the first study in EAC that included several techniques to evaluate HER-2 in one tissue microarray, showing that HER-2 positivity (12%) and HER-2 gene amplification as assessed with SISH (16%) were independently associated with poor CSS. Concordance between IHC and SISH was 92%, between IHC and FISH 90% and between SISH and FISH 95%, which is similar to previously published IHC/ISH concordance rates [25, 26].

Most discordant cases were found in patients with low IHC HER-2 expression, who still had HER-2 gene amplification (6–8%). In esophageal cancer, this phenomenon has been reported before with percentages ranging from 2 to 33% [10, 15, 16, 25, 26]. The IHC/ISH disagreement observed might be explained...
by erroneous post-translational processes, such as inappropriate protein glycosylation, incorrect three-dimensional folding and dysfunctional mobilization of the protein to the cell membrane, resulting in decreased HER-2 protein expression, while the HER-2 gene is amplified [27]. In the literature, a number of studies reported the independent prognostic significance of a positive HER-2 status in EAC [10–12]. Also a recent meta-analysis confirmed the results of the present study [18]; however, most studies failed to find any association between HER-2 and survival [13–17]. The absence of standardized HER-2 testing methods might underlie these contrasting results. In EAC, there is no clear definition of a true positive HER-2 tumor. Trastuzumab has not been registered for EAC yet. However, if that was the case, according to the HER-2 scoring guidelines for gastric cancer [24], 12% of patients would be eligible for therapy. When using SISH or FISH as first testing method, 16–18% of patients would have been selected, including patients with IHC 1+ with gene amplification. Therefore, it must be considered to re-evaluate EAC patients with IHC 1+ with in situ hybridization techniques in order to reduce the number of false-negative cases, which results in under treatment of patients. It should be noted here that SISH proved to be equally sensitive as FISH, but was less time consuming and may, therefore, be the preferred technique.

Traditionally, correction for chromosome 17 polysomy was believed to be essential to identify a true HER-2 amplified tumor, because only the latter group is thought to respond to trastuzumab therapy. Polysomy is defined as ≥3 centromere 17 copies, which is assumed to be representative for chromosome 17. A study in breast cancer reported that genes localized on chromosome 17 showed a complex pattern of gains and losses, which was unrelated to centromere 17 copy numbers. It was, therefore, concluded that chromosome 17 polysomy is a rare event [28]. Although the entire chromosome 17 was not evaluated in the present study, true polysomy, defined as a complete gain of chromosome 17, might be a rarity in EAC as well. Increased copy numbers of centromere 17 were seen in 18 of 149 (12%) cases and did not have prognostic significance, which is similar to previous studies [17, 25]. Therefore, evaluation of the absolute HER-2 gene copy numbers might be sufficient to identify HER-2-amplified tumors. Moreover, from the literature it is suggested that if 6 or more gene copies are to compare the differences between survival curves. Patients with a HER-2 protein overexpression had significantly worse CSS compared with patients with low HER-2 expression (P = 0.020). The median survival was 35.6 and 20.7 months, respectively, in patients with low and protein overexpression.

(B). CSS according to low (<6) and high (≥6) HER-2 gene copy numbers. Patients with gene amplification (≥6) had significantly worse CSS compared with patients without HER-2 amplification (P = 0.042). The median survival was 21.0 months in patients with, and 39.4 months in individuals without HER-2 gene amplification. (C). CSS according to HER-2 positivity and negativity. Patients with HER-2 positivity (IHC 3+ or 2+ with gene amplification) had a significantly worse CSS compared with patients with HER-2-negative expression (P = 0.043). The median survival was 41.3 months in the negative and 21.0 months in patients with HER-2 positivity.
seen with a single probe, then the sample can be considered as positive, irrespective of the presence of increased chromosome 17 centromere copies [29].

In esophageal cancer, there are no definite agreements with regard to ‘borderline’-amplified tumors. In breast cancer, ‘borderline’ amplification is defined as a HER-2 ratio of 1.8–2.2. In the present study, five patients had a HER-2 ratio between 1.8–2.2. SISH showed 6–10 HER-2 copies in 60% (3 of 5) of these cases. This suggests that a HER-2 ratio of 1.8–2.2 represents a minor subgroup that can be incorporated in the amplified category. A future trial should determine whether patients with ‘borderline’ amplification benefit from trastuzumab therapy.

In breast, gastric and GEJ carcinoma, HER-2 is a well-established therapeutic target [6, 30]. To date, in esophageal cancer, only two studies with trastuzumab in combination with chemo(radio)therapy have been carried out. The results of these studies showed a pathological complete response in 3 of 6 (50%) patients who underwent surgical resection [31], and an overall response rate (i.e. complete and partial response) of 32% (7 of 22) of patients with unresectable disease [32]. These results emphasize the need for initiation of a clinical trial with trastuzumab therapy for patients with EAC who have a HER-2 positive status.

In conclusion, HER-2 gene amplification was seen in 16% (SISH) and 18% (FISH), HER-2 protein overexpression seen in 14% and HER-2 positivity seen in 12% of patients. In multivariate analysis, HER-2 positivity and gene amplification (SISH) were independently associated with a poor CSS, but not HER-2/CEP17 ratio (FISH) and HER-2 overexpression. When using the current guidelines for gastric cancer concerning HER-2, patients could be selected who significantly had a worse prognosis, indicating that these guidelines can also be applied for patients with esophageal cancer. However, it must be considered to re-evaluate IHC 1+ patients with ISH, in which SISH is the preferred method. Future clinical trials should determine whether patients with IHC 1+ who have gene amplification benefit from trastuzumab therapy.

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disclosure

The authors have declared no conflicts of interest.

references

Critical review of ‘Public domain application’: a flexible drug approval system in Japan

Y. Ito1, H. Narimatsu2,3*, T. Fukui1, A. Fukao3 & T. Yoshioka1

1Department of Clinical Oncology; 2Advanced Molecular Epidemiology Research Institute, Faculty of Medicine; 3Department of Public Health, Yamagata University
Graduate School of Medicine, Yamagata, Japan

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Background: ‘Public domain application’ is a flexible drug approval system in Japan, similar to the fast track designation in the United States.

Methods: From 1999 to 2009, four drugs and three regimens received approval from ‘Public domain application’. The data from the review reports were extracted, and the reviewing process was critically re-evaluated.

Results: The study drugs were categorized into three groups according to the sizes of the studies and evidence levels in the original articles that were submitted. Carboplatin was categorized into the first group with a large number of study patients and a high evidence level; the review report had studies with more than 15 000 total patients and 8 phase III studies. The ifosfamide and vinblastine regimen was categorized into the second group, with a low number of study patients and a high evidence level; the review report had studies with more than 15 000 total patients and 8 phase III studies. Dacarbazine; cytarabine; methotrexate, doxorubicin, and cisplatin; bleomycin, etoposide, and cisplatin; and fludarabine were categorized into the remaining third group, with a moderate number of study patients and evidence level.

Conclusions: Drugs with various backgrounds, including evidence levels and physicians’ experiences, were approved via ‘Public domain application’. The approvals of most drugs were evaluated to be appropriate.

Key words: anticancer drug, health care service, regulatory science, review authority

introduction

Rigidly designed clinical trials, such as randomized, controlled trials (RCTs), are requested in applications for novel anticancer drugs, but such trials are very expensive and require great effort to complete. Thus, pharmaceutical companies do not have sufficient economic incentives to develop novel anticancer drugs if the expected benefits of the drugs are low. This is one of the main reasons why many novel drugs or indications that are necessary for patients are not approved [1].

A flexible process can be a useful method to provide anticancer drugs to patients immediately [2]. The Food and