Pathological complete response and survival according to the level of HER-2 amplification after trastuzumab-based neoadjuvant therapy for breast cancer

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BACKGROUND: We analysed whether the level of human epidermal growth factor receptor-2 (HER-2) amplification significantly influenced either pathological complete response (pCR) or recurrence-free survival (RFS) and overall survival (OS) after trastuzumab-based neoadjuvant therapy.

METHODS: In all, 99 patients with an HER-2-amplified breast tumour treated with trastuzumab-based neoadjuvant therapy were included. Tumours were classified as low amplified (LA; 6–10 signals per nuclei) or highly amplified (HA; > 10 signals). Pathological response was assessed according to Chevallier’s classification (pCR was defined as grade 1 or 2). Median follow-up lasted 46 months (6–83). Cox uni- and multivariate analyses were performed.

RESULTS: In all, 33 tumour samples were LA and 66 were HA. The pCR in HA tumours was significantly higher than in LA tumours (55% vs 24%, P = 0.005), whereas no association was found between the pCR rate and tumour stage, grade or hormone receptor status. In multivariate analysis, the pathological nodal status (P = 0.005) and adjuvant trastuzumab (P = 0.037) were independently associated with RFS, whereas the level of HER-2 amplification nearly reached statistical significance (P = 0.057). There was no significant difference between LA and HA tumours for OS (P = 0.22, log-rank).

CONCLUSION: The level of HER-2 gene amplification significantly influenced pCR but not RFS or OS in non-metastatic breast cancer treated with trastuzumab-based neoadjuvant therapy. However, RFS in patients with HA tumours tended to be shorter.

British Journal of Cancer (2010) 103, 1335 – 1342. doi:10.1038/sj.bjc.6605939 www.bjcancer.com © 2010 Cancer Research UK

Keywords: pathological response; survival; fluorescence in situ hybridisation; HER-2 level amplification; trastuzumab

The human epidermal growth factor receptor-2 (HER-2) gene is amplified in 10–26% of human breast cancers (Gown et al., 2008). HER-2 gene amplification is associated with the over-expression of the HER-2 protein in >95% of cases (Wolff et al., 2007). Both HER-2 over-expression and HER-2 gene amplification have been correlated with poor clinical outcome (Slamon et al., 1987; Kallioniemi et al., 1991; Press et al., 1997). The HER-2 status is also a strong predictor of a clinical benefit from HER-2-targeted therapy, such trastuzumab (Herceptin; Roche, Neuilly-sur-Seine, France), a humanised monoclonal antibody directed against the external domain of HER-2 protein (Yamauchi et al., 2001). Several randomised trials have proved the efficacy of trastuzumab in metastatic (Slamon et al., 2001; Marty et al., 2005) and adjuvant (Piccart-Gebhart et al., 2005; Romond et al., 2005; Joensuu et al., 2006; Smith et al., 2007) settings for HER-2-positive breast cancer in terms of response rate, recurrence rate and a decrease in mortality. In the neoadjuvant setting, trastuzumab in association with chemotherapy had also shown a clinical benefit in terms of pathological complete response (pCR; Van Pelt et al., 2003; Buzdar et al., 2005; Coudert et al., 2006, 2007; Gianni et al., 2007).

American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines recommend using either immunohistochemistry (IHC) assays for initial evaluation of HER-2 status followed by reflex testing by fluorescence in situ hybridisation (FISH) of some IHC categories or FISH in initial testing (Wolff et al., 2007). The level of over-expression of HER-2 protein with IHC assays is a known predictive factor of response to trastuzumab (Slamon et al., 2001) and we have previously shown a positive correlation between the level of HER-2 amplification assessed by FISH and the rate of pCR to trastuzumab-based neoadjuvant treatment (Arnould et al., 2007). However, the relationship between the level of HER-2 amplification and the outcome of patients given neoadjuvant trastuzumab remains unclear.

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Received 9 June 2010; revised 31 August 2010; accepted 9 September 2010
The aim of this study was to determine whether the level of HER-2 gene amplification using FISH assays significantly influenced recurrence-free survival (RFS) and overall survival (OS) in non-metastatic breast cancer treated with trastuzumab-based neoadjuvant therapy.

MATERIALS AND METHODS

Patients

Breast biopsies from 116 patients, who had received neoadjuvant trastuzumab in combination with chemotherapy for locally HER-2-positive breast cancer were retrospectively collected from 19 centres in France. All of the patients provided written, informed consent for their tissue material and clinical data to be centrally collected and used for research purposes. This study was approved by our institutional review board.

The patients were aged from 26 to 76 years (mean, 46.6 years) and had histologically confirmed, unilateral, unicentric, non-metastatic, HER-2-positive (in IHC) invasive ductal breast carcinoma. Most of the patients were treated in the framework of two open-label phase II clinical trials: GETN(A)-1 (n = 63; Coudert et al, 2007) and TAXHER-S01 (n = 21; Coudert et al, 2006). The remaining patients (n = 32) had an equivalent preoperative regimen to that used in the TAXHER-S01 trial.

The 63 patients included in the GETN(A)-1 trial had received weekly neoadjuvant trastuzumab (4 mg kg\(^{-1}\) loading dose followed by 2 mg kg\(^{-1}\)) in combination with docetaxel (75 mg m\(^{-2}\)) and carboplatin (area under the curve of six) every 3 weeks for six cycles. Adjuvant trastuzumab was also administered in responding patients. The 21 patients included in the TAXHER-S01 trial had received the same preoperative schedule of trastuzumab in association with docetaxel (100 mg m\(^{-2}\)) every 3 weeks for six cycles, but no adjuvant trastuzumab was scheduled in this study. Additional patients (n = 32) received trastuzumab every 3 weeks (8 mg kg\(^{-1}\) loading dose followed by 6 mg kg\(^{-1}\)) instead of weekly trastuzumab (Leyland-Jones et al, 2003) and adjuvant trastuzumab was also administered.

In all patients, 3 weeks after the last administration of neoadjuvant trastuzumab, tumours were surgically removed and pCR was assessed according to Chevallier's classification: pCR was defined as no evidence of carcinoma either in the breast or in the lymph nodes, without (grade 1) or with (grade 2) in situ carcinoma. In accordance with institutional practices, adjuvant hormone therapy in patients with hormone receptor-positive tumours and adjuvant radiotherapy were mandatory.

HER-2 status

The 84 patients included in the GETN(A)-1 or TAXHER-S01 trials were initially tested IHC 3+ or 2+ for HER-2 status. For all of the patients in this study, HER-2 status was re-analyzed centrally using both IHC and FISH assays by an experienced pathologist who was blinded to patient information, including the original IHC test results.

HER-2 status in IHC was evaluated with A485 polyclonal antibody (Dako, Glostrup, Denmark) or 4B5 monoclonal antibody (Ventana Medical Systems Inc., Tucson, AZ, USA) on the BenchMark XT system (Ventana Medical Systems Inc.). biopsies were graded according to the HercepTest (Dako) scoring system (0+, 1+, 2+, or 3+).

FISH analyses were carried out using the HER-2 Probe (Oncor, Gaithersburg, MD, USA) and BenchMark XT system. For each biopsy, HER-2 signals were counted in ≥60 tumour cell nuclei and the mean HER-2 signals per nuclei was calculated. The level of HER-2 amplification in tumours was classified as follows: no amplified (NA; mean, <6 signals per nuclei), low amplified (LA; mean, 6–10 signals per nuclei), or highly amplified (HA; mean, >10 signals per nuclei or uncountable because of clusters of signals). The cutoff of 10 gene copies per nuclei to distinguish between LA and HA was chosen because it is the same as that proposed with chromogenic in situ hybridisation and also because above this cutoff, it is almost impossible to count signals precisely because of clusters and small aggregates. Borderline tumours (mean between four and eight signals per nuclei) were analysed by double-color FISH using a HER-2-gene-specific probe and a centromeric probe for chromosome 17 (PathVyon HER-2 DNA Probe kit, Vysis-Abbott, Abbott Park, IL, USA) to determine HER-2 amplification. In these cases, HER-2 amplification was defined by a ratio of HER-2 to chromosome 17 centromeric signals (HER-2/CEP17) of ≥2.2 (Wolff et al, 2007). All the tumours with ≥6 HER-2 signals per nuclei had a HER-2/CEP17 ratio ≥2.2 and therefore, were amplified tumours. All the tumours with <6 HER-2 signals per nuclei had a HER-2/CEP17 ratio ≤1.8 and thus, were NA tumours.

Only patients with centrally confirmed HER-2 amplification were finally included in this study to evaluate pathological response rate (Figure 1). For RFS and OSs, NA tumours were also included.

Statistical analysis

Qualitative variables were described using frequency and percentages. \(\chi^2\) and Fisher’s exact tests were used to compare patient or tumour characteristics according to the level of HER-2 amplification with FISH assays (NA, LA, and HA). For these analyses, Bonferroni adjustments were carried out to prevent inflation of type one error (the significant level was 0.016 for 3 comparisons).

Associations between tumour size, tumour grade, hormone–receptor status, level of HER-2 amplification, and the presence or absence of pCR were evaluated using univariate and multivariate logistic regression. To take into account the trial effect (GETN(A)-1, TAXHER-S01, and GFLCC database), analyses were adjusted for this factor.

The median follow-up was calculated using the reverse Kaplan–Meier method. Recurrence-free survival was defined as the time from the date of histology to the date of the first recurrence of breast cancer at any site or death from any cause. Surviving patients without recurrence were censored at the last follow-up. The OS was defined as the time from the date of histology to death from any cause. Survival distributions were estimated with the
Kaplan–Meier method and compared using the log-rank statistic. Univariate (RFs and OS) and multivariate (RFs) Cox proportional hazards models stratified on the trial were fitted to test for an association between classical prognostic variables, the level of HER-2 amplification, pCR, adjuvant trastuzumab, and RFS or OS. Given the small number of events, multivariate analysis for OS was not performed. Aikaie information criterion was computed for the goodness of fit for multivariate models and Harrell’s C-statistic for discrimination (a Harrell’s C-index = 0.5 indicates no predictive discrimination and a Harrell’s C-index = 1.0 indicates perfect separation of patients) for each variable and for final multivariate Cox models. The multivariate models were internally validated using bootstrapping (100 replications). P-values were two-tailed and considered significant when less than 0.05. All analyses were performed using Stata V11 software (StataCorp LP, College Station, TX, USA).

RESULTS

Patients and tumours

Baseline patient and tumour characteristics are summarised in Table 1. In all, 99 (85%) tumours were considered amplified after central FISH analyses, among which 33 were classified as LA

tumours and 66 HA tumours. There were no significant differences between these two groups in terms of tumour stage, nodal status, hormone receptor status, or treatment given. The HA tumours had a higher histological grade than the LA tumours (P = 0.01).

Analysis of pCR

According to Chevallier's classification (Table 2), 44 (44.5%) patients had a pCR, whereas 55 (55.5%) had no or only a partial response. In univariate logistic analysis, only the level of HER-2 amplification (FISH) was related to pCR (P = 0.005). In multivariate analysis, this variable was independently associated with pCR (P = 0.024), whatever the trial (P = 0.632).

Recurrence-free survival according to HER-2 amplification

Median follow-up was 46-months (range, 6–83 months). Local or regional recurrences occurred in six patients (two HA tumours, two LA tumours, two NA tumours); one of these had a pCR. Metastatic recurrence (alone or with locoregional recurrence) occurred in 18 patients (14 HA tumours, 2 LA tumours, 2 NA tumours) among whom 5 had an initial pCR. Three patients died without a diagnosis of recurrent disease.

Table 1 Patient and tumour characteristics according to the level of HER-2 amplification

| Characteristic                    | Total (n = 116) | NA (FISH; n = 17) | LA (FISH; n = 33) | HA (FISH; n = 66) | P (3 groups; Fisher exact test) | P (NA vs LA/HA; Fisher exact test) | P (LA vs HA) (Fisher exact test) |
|----------------------------------|----------------|------------------|------------------|------------------|---------------------------------|-----------------------------------|---------------------------------|
| Mean age (Range), year           | 46.6 (26.5–76.4) | 46.6 (32–62)     | 48.5 (29–76.4)   | 45.6 (26.5–66)   | 0.699                           | 0.474                             | 0.710                           |
| Tumour stage                     |                |                  |                  |                  |                                 |                                   |                                 |
| T1                               | 16 (14%)       | 3 (18%)          | 4 (12%)          | 9 (14%)          |                                 |                                   |                                 |
| T2                               | 73 (63%)       | 12 (70%)         | 19 (58%)         | 42 (64%)         |                                 |                                   |                                 |
| T3–T4                            | 27 (23%)       | 2 (12%)          | 10 (30%)         | 15 (22%)         |                                 |                                   |                                 |
| Nodal status                     |                |                  |                  |                  |                                 |                                   |                                 |
| N0                               | 55 (47%)       | 11 (65%)         | 12 (36%)         | 32 (49%)         | 0.330                           | 0.358                             | 0.636                           |
| N1                               | 59 (51%)       | 6 (35%)          | 20 (61%)         | 33 (50%)         |                                 |                                   |                                 |
| N2                               | 2 (2%)         | 0                | 1 (3%)           | 1 (1%)           |                                 |                                   |                                 |
| Tumour grade                     |                |                  |                  |                  |                                 |                                   |                                 |
| SBR1                             | 5 (4%)         | 1 (6%)           | 0                | 4 (6%)           | 0.057                           | 0.890                             | 0.010                           |
| SBR2                             | 57 (49%)       | 9 (53%)          | 23 (70%)         | 25 (38%)         |                                 |                                   |                                 |
| SBR3                             | 47 (41%)       | 6 (35%)          | 10 (30%)         | 31 (47%)         |                                 |                                   |                                 |
| Unknown                          | 7 (6%)         | 1 (6%)           | 0                | 6 (9%)           |                                 |                                   |                                 |
| Hormone receptor status          |                |                  |                  |                  |                                 |                                   |                                 |
| Positive                         | 68 (59%)       | 13 (76%)         | 20 (61%)         | 35 (53%)         | 0.072                           | 0.017                             | 0.782                           |
| Negative                         | 43 (37%)       | 2 (12%)          | 12 (36%)         | 29 (44%)         |                                 |                                   |                                 |
| Unknown                          | 5 (4%)         | 2 (12%)          | 1 (3%)           | 2 (3%)           | 0.315*                          | 0.145*                            | 0.670*                          |
| Neoadjuvant treatment            |                |                  |                  |                  |                                 |                                   |                                 |
| TDC                              | 63 (54%)       | 12 (70%)         | 18 (55%)         | 33 (50%)         | 0.154*                          | 0.033*                            | 0.044*                          |
| TD                               | 53 (46%)       | 5 (30%)          | 15 (45%)         | 33 (50%)         |                                 |                                   |                                 |
| Central IHC score                |                |                  |                  |                  |                                 |                                   |                                 |
| 1+                               | 10 (8%)        | 10 (59%)         | 0                | 0                | <0.001                          | <0.001                            | 0.003                           |
| 2+                               | 11 (10%)       | 6 (35%)          | 5 (15%)          | 0                |                                 |                                   |                                 |
| 3+                               | 95 (82%)       | 1 (6%)           | 28 (85%)         | 66 (100%)        |                                 |                                   |                                 |
| Pathological response            |                |                  |                  |                  |                                 |                                   |                                 |
| pCR                              | 45 (39%)       | 1 (6%)           | 8 (24%)          | 36 (55%)         | <0.001*                         | 0.003*                            | 0.004*                          |
| Non-pCR                          | 71 (61%)       | 16 (94%)         | 25 (76%)         | 30 (45%)         |                                 |                                   |                                 |
| Adjuvant trastuzumab             |                |                  |                  |                  | 0.769                           | 0.557                             | 0.872*                           |
| No                               | 32 (28%)       | 6 (35%)          | 9 (27%)          | 17 (26%)         |                                 |                                   |                                 |
| Yes                              | 84 (72%)       | 11 (65%)         | 24 (72%)         | 49 (74%)         |                                 |                                   |                                 |

Abbreviations: FISH = fluorescence in situ hybridization; HA = highly amplified tumours; IHC = immunohistochemistry; LA = low-amplified tumours; NA = no amplified tumours; non-pCR = absence of complete pathological response; pCR = pathological complete response; SBR = Scarff–Bloom–Richardson; TD = trastuzumab–docetaxel; TDC = trastuzumab–docetaxel–carboplatin. *p*-test. Values in bold: P < 0.05.
RFS was not statistically different according to the pCR rate ($P = 0.145$, log-rank test; Figure 2A), or according to the HER-2 copy number ($P = 0.313$, log-rank test; Figure 2B) or the level of HER-2 amplification ($P = 0.006$) and adjuvant trastuzumab treatment ($HR = 3.247$ (CI 95%, 1.396–7.552), $P = 0.006$) and adjuvant trastuzumab treatment was not significantly associated with RFS ($Table 3$), whereas the pathological nodal status ($HR = 7.118$ (CI 95%, 1.864–27.177), $P = 0.004$) were. In multivariate analysis, pathological nodal status and adjuvant trastuzumab were independently associated with RFS, whereas the level of HER-2 amplification nearly reached statistical significance ($HR = 2.819$ (CI 95%, 0.970–8.197), $P = 0.057$). Internal validation using bootstrapping confirmed the results only for the pathological nodal status.

There was no significant difference in RFS according to both pCR and level of HER-2 amplification ($P = 0.09$, log-rank test). However, the subgroup of HA tumours without pCR had a significantly shorter RFS than did the other subgroups ($P = 0.01$, log-rank test; Figure 2C).

Overall survival according to amplification of HER-2

During follow-up, 11 patients, including 8 with a metastatic recurrence (6 HA tumours, 2 LA tumours), died. There was no significant difference between NA, LA, and HA tumours subgroups for OS ($P = 0.111$, log-rank test; Figure 3) or between LA and HA tumours subgroups ($P = 0.22$, log-rank test). With Cox univariate analysis, only tumour stage ($HR = 0.158$ (CI 95%, 0.039–0.636), $P = 0.034$) and pathological nodal status ($HR = 7.118$ (CI 95%, 1.864–27.177), $P = 0.004$) were significantly associated with OS, whereas the level of HER-2 amplification was not ($HR = 1.974$ (CI 95%, 0.413–9.425), $P = 0.394$).

DISCUSSION

Systemic neoadjuvant therapy is the treatment of choice for locally advanced or inflammatory breast cancer. It also facilitates breast conservation in selected patients with operable disease (Kaufmann et al, 2006). For patients with HER-2-positive breast
cancer (IHC 3+ and/or FISH positive), the addition of trastuzumab to chemotherapy increases pCR rates (Buzdar et al., 2005; Gianni et al., 2007). We confirm in this larger study our previous results (Arnould et al., 2007) regarding the positive correlation between the level of HER-2 amplification determined by FISH and the rate of pCR after trastuzumab-based neoadjuvant therapy. Indeed, we report 55% vs 24% (P = 0.005) of pCR in the subgroup of HA vs LA tumours, respectively. In light of these results, the level of HER-2 amplification could be a useful tool to decide whether to administer neoadjuvant therapy and could therefore also increase the rate of conservative surgery. However, this interesting predictive factor needs to be validated in further studies. Indeed, our results contrast with those of a smaller subgroup of HA tumours, respectively. In light of these results, the level of HER-2 amplification could be a useful tool to decide whether to administer neoadjuvant therapy and could therefore also increase the rate of conservative surgery. However, this interesting predictive factor needs to be validated in further studies.

To date there are no data suggesting another mechanism than HER-2 gene amplification to explain the over-expression of the HER-2 protein. Consequently, both IHC and FISH can be used to determine HER-2 status and the benefit of trastuzumab in breast cancer (Wolff et al., 2007). In this study, after centralised analyses, 10 (8%) tumours were subsequently scored IHC 1+ (no pCR was observed in this subgroup, data not shown). This highlights the modest inter-laboratory reproducibility of IHC results. This observation is in line with a recent critical review of the ASCO/CAP guidelines (Sauter et al., 2009), which concluded that inherent technical properties strongly argue for primary HER-2 FISH testing. Furthermore, although IHC and FISH have shown high concordance in some studies, reproducibility remains insufficient in others (Paik et al., 2002; Roche et al., 2002; Mass et al., 2005). In our study, 6 out of 11 (55%) tumours with an IHC score of 2+ were finally considered NA with the FISH assay (with only one pCR) and the patient with a 3+ tumour in IHC, which was NA in FISH did not benefit from trastuzumab (data not shown).

Currently, 52 weeks of adjuvant trastuzumab are recommended for the treatment of HER-2-positive breast cancer with a high risk of relapse. This regimen has improved both RFS and OS (Piccart-Gebhart et al., 2005; Romond et al., 2005; Smith et al., 2007; Slamon et al., 2009). Several trials in progress are comparing this standard with a shorter exposure to trastuzumab: 9 weeks as in the SOLD study (NCT00593697) and the Short HER study (NCT00629278) or 26 weeks (PHARE study). In a recently published study, a brief course of trastuzumab (9 weeks) administered concomitantly with docetaxel followed by three cycles of FEC tended to improve RFS but not OS, compared with the same regimen without trastuzumab (Joensuu et al., 2009). In our study, adjuvant trastuzumab, administered in addition to the 18 pre-operative injections, significantly improved RFS (P = 0.003). All these results are not in favour of lightening adjuvant trastuzumab. The above mentioned studies should resolve this question.

Figure 2 Recurrence-free survival according to the pathological response (A), the HER-2 copy number (B) and both pathological response and level of HER-2 amplification (C). Kaplan–Meier estimate. Abbreviations: HA, highly amplified tumours; LA, low-amplified tumours; NA, no amplified tumours; pCR+, pathological complete response; pCR−, absence of pathological complete response.
Pathological complete response is often considered a surrogate marker of outcome after neoadjuvant chemotherapy. Indeed, in several large trials with anthracycline and/or taxanes-based neoadjuvant therapy, RFS and OS rates were significantly improved when pCR had been achieved (Fisher et al., 1998; Kuerer et al., 1999; Guarneri et al., 2006). These studies were performed before the assessment of HER-2 status and the use of trastuzumab. In more recent studies with trastuzumab-based neoadjuvant therapy, the association between pCR and RFS has been inconclusive, sometimes statistically associated (Buzdar et al., 2007), but sometimes not (Hurley et al., 2006; Shimizu et al., 2009) as was the case in our study, despite a long follow-up. This prognostic factor thus remains controversial.

To our knowledge, this is the first study to report the outcome of patients after trastuzumab-based neoadjuvant therapy according to the level of HER-2 amplification in FISH. Although the increase in the number of HER-2 gene copies had a significant positive impact on the pCR rate, there was no significant difference between HA tumours (4–10 HER-2 gene copies per nuclei) and LA tumours (6–10 HER-2 gene copies per nuclei) for either RFS or OS after a median follow-up of 46 months. However, RFS tended to be

| Table 3 Univariate and multivariate Cox analysis of predictive factors of recurrence-free survival |

| Recurrence or death | Univariate analysis | Multivariate analysis | Bootstrapping* |
|---------------------|---------------------|-----------------------|----------------|
|                     | HR                  | 95% CI                | P              | HR, N = 98 | 95% CI | P | 95% CI | P |
| Tumour stage        |                     |                       |                |            |        |    |        |    |
| T1                  | 0.116               | 0.471                 | 0.978          |            |        |    |        |    |
| T2                  | 0.446 (0.160–1.241) | 0.642 (0.181–2.279)   | (0.007–56.910) |
| T3–T4               | 0.215 (0.048–0.972) | 0.344 (0.062–1.911)   | (0–6 160 872) |
| Tumour grade        | 0.260               |                       |                |            |        |    |        |    |
| SBR1                | 0.279 (0.058–1.351) |                       |                |            |        |    |        |    |
| SBR2                | 0.414 (0.082–2.076) |                       |                |            |        |    |        |    |
| SBR3                | 2/2                 |                       |                |            |        |    |        |    |
| Unknown             | 0.175               |                       |                |            |        |    |        |    |
| Hormone receptor    |                     |                       |                |            |        |    |        |    |
| Negative            | 0.547 (0.229–1.308) |                       |                |            |        |    |        |    |
| Unknown             | 2/1                 |                       |                |            |        |    |        |    |
| Pathological nodal status | 0.0006 | 0.005                | 0.022          |            |        |    |        |    |
| Negative            | 3.247 (1.396–7.552) | 3.928 (1.524–10.127)  | (1.219–12.659) |
| Positive            | 1/0                 |                       |                |            |        |    |        |    |
| Unknown             | 1.000               |                       |                |            |        |    |        |    |
| Amplification (FISH) | 0.199               | 0.057                 | 0.883          |            |        |    |        |    |
| LA                  | 1.918 (0.710–5.180) | 2.819 (0.970–8.197)   | (0–2 667 202) |
| HA                  | 48/18               |                       |                |            |        |    |        |    |
| pCR                 | 0.161               |                       |                |            |        |    |        |    |
| No                  | 0.523 (0.212–1.293) |                       |                |            |        |    |        |    |
| Yes                 | 36/8                |                       |                |            |        |    |        |    |
| pCR and FISH        | 0.146               |                       |                |            |        |    |        |    |
| No+HA               | 0.385 (0.120–1.228) |                       |                |            |        |    |        |    |
| No+LA               | 0.401 (0.148–1.090) |                       |                |            |        |    |        |    |
| Yes+LA              | 0.204 (0.025–1.669) |                       |                |            |        |    |        |    |
| Adjuvant Trastuzumab | 0.003               | 0.037                 | 0.779          |            |        |    |        |    |
| No                  | 0.157 (0.045–0.539) | 0.214 (0.050–0.914)   | (0–10 456 280) |
| Yes                 | 62/11               |                       |                |            |        |    |        |    |
| Harrell's C-statistic | 0.7745             |                       |                |            |        |    |        |    |
| AIC                 | 137                 |                       |                |            |        |    |        |    |

Abbreviations: AIC = Akaike information criterion; CI = confidence interval; FISH = fluorescence in situ hybridisation; HA = highly amplified tumours; HR = hazard ratio; LA = low-amplified tumours; N = number; pCR = pathological complete response; SBR = Scarff–Bloom–Richardson. *100 replications. Values in bold: P < 0.05.

Pathological complete response is often considered a surrogate marker of outcome after neoadjuvant chemotherapy. Indeed, in several large trials with anthracycline and/or taxanes-based neoadjuvant therapy, RFS and OS rates were significantly improved when pCR had been achieved (Fisher et al., 1998; Kuerer et al., 1999; Guarneri et al., 2006). These studies were performed before the assessment of HER-2 status and the use of trastuzumab. In more recent studies with trastuzumab-based neoadjuvant therapy, the association between pCR and RFS has been inconclusive, sometimes statistically associated (Buzdar et al., 2007), but sometimes not (Hurley et al., 2006; Shimizu et al., 2009) as was the case in our study, despite a long follow-up. This prognostic factor thus remains controversial.

Figure 3 Overall survival according to the HER-2 copy number (Kaplan–Meier estimate). Abbreviations: HA, highly amplified tumours; LA, low-amplified tumours; NA, no amplified tumours.
better in the HER-2 LA group (P = 0.057). A large population-based cohort, treated before the use of trastuzumab, had already shown that OS in breast cancer was not significantly different according to the level of HER-2 amplification, for patients with a HER-2/CEP17 ratio > 2.2 (Jensen et al, 2008). Since the era of trastuzumab therapy, the link between the level of HER-2 amplification and the outcome of patients has been investigated only in the metastatic and adjuvant settings. A retrospective analysis was performed in 33 patients with HER-2-positive metastatic breast cancer receiving trastuzumab (Gullo et al, 2009): patients with a high HER-2/CEP17 ratio had shorter time-to-progression and OS than did those with lower ratios (Gullo et al, 2009), although the difference did not reach statistical significance, probably because of the sample size and the relatively short follow-up. Dowsett et al (2009) analyzed whether the degree of HER-2 amplification (HER-2 gene copy number and HER-2/CEP17 ratio) influenced the clinical outcome in patients with HER-2-positive breast cancer randomized in the two HERA trial (Piccart-Gebhart et al, 2005) arms with or without 1 year of trastuzumab after adjuvant chemotherapy. Although there was an apparent trend towards shorter disease-free survival with increasing HER-2 gene copy numbers or increasing HER-2 FISH ratios, the differences were not statistically significant. However, in this study, the median follow-up was only 2 years.

In conclusion, the level of HER-2 gene amplification using FISH assays significantly influenced pCR but neither RFS nor OS in non-metastatic breast cancer treated with trastuzumab-based neoadjuvant therapy. However, the subgroup of patients with HA tumours (> 10 signals per nucle) tended to have a shorter RFS. This result suggests that a high level of HER-2 amplification could be a poor prognostic factor even though it was associated with a good initial sensitivity to trastuzumab. Further larger and longer studies are needed to confirm this hypothesis.

ACKNOWLEDGEMENTS

We thank Philip Bastable for revising the English in this paper.

Conflict of interest

The authors declare no conflict of interest.

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