Impact of HER2 copy number in IHC2 þ /FISH-amplified breast cancer on outcome of adjuvant trastuzumab treatment in a large UK cancer network

A Borley *,1, T Mercer 2, M Morgan 3, P Dutton 4, P Barrett-Lee 1, M Brunelli 5 and B Jasani *,6

1Velindre Cancer Centre, Whitchurch, Cardiff CF14 2TL, UK; 2Cardiff University School of Medicine, Heath Park, Cardiff CF14 4YU, UK; 3Department of Cellular Pathology, University Hospital of Wales, Heath Park, Cardiff CF14 4YU, UK; 4Wales Cancer Trials Unit, Neuadd Meirionnydd, Heath Park, Cardiff CF14 4YS, UK; 5Department of Pathology and Diagnostic, University of Verona, Verona 37134, Italy and 6Institute of Cancer & Genetics, Cardiff University School of Medicine, Heath Park, Cardiff CF14 4XN, UK

Background: Adjuvant trastuzumab with chemotherapy is standard treatment for HER2-positive breast cancer, defined as either HER2 IHC3 þ or IHC2 þ and FISH amplified. The aim of this study was to investigate the degree to which HER2 amplification in terms of HER2 gene copy numbers in HER2 þ IHC2 þ cancers affected the outcome in a community setting.

Methods: Case records of 311 consecutive patients with early breast cancer presenting between 1st January 2005 and 31st December 2008 were reviewed. Progression-free survival and overall survival were calculated with the Kaplan–Meier method using STATA 13.

Results: Among 3 þ cases (n = 230) 163 received T vs 67 no-T. Among 2 þ cases (n = 81) 59 received T vs 22 no-T. Among 59 IHC2 þ treated cases n = 28 had an average of >12, n = 13 had >6 to <12, and n = 18 had 6 to <6 HER2 gene copies, respectively. The time of progression and overall survival of high and low copy number patients was similar and better than the intermediate copy number and the untreated cohorts.

Conclusions: High HER2 copy number (>12) appears to be associated with consistently better response compared with patients with intermediate HER2 copy numbers (6–12). In light of emerging data of patients showing insensitivity to trastuzumab therapy, we propose that the HER2 gene copy number value should be included as an additional indicator for stratifying both the management and the follow-up of breast cancer patients.

Trastuzumab is a humanised monoclonal antibody directed at the human epidermal growth factor receptor 2 (HER2), which is overexpressed in ~15% of newly diagnosed invasive breast cancers (HER2 þ) (Slamon et al, 1987). Adjuvant trastuzumab, in conjunction with chemotherapy, is now the standard of care for these patients, following the significantly improved outcomes demonstrated in several large multinational clinical trials. (Piccart-Gebhart et al, 2005, Romond et al, 2005, Slamon et al, 2006, Joensuu et al, 2006). A similar magnitude of benefit has been confirmed in a ‘community’ setting in a UK Cancer network (Webster et al, 2012).

Accurate diagnosis of HER2 þ cancers is important to maximise the benefit of targeted therapy. All breast cancers should now be tested for HER2 expression and two methods are used. Firstly, immunohistochemistry detects HER2 protein expression on the cell membrane, and is described on a scale of 0–3 based on
the Hercept Test Score (Dowsett et al, 2003). Thus, scores 0 and 1+ are considered negative, and score 3+, meaning that >30% of invasive tumour cells demonstrate uniform intense membrane staining, considered to be positive. An equivocal result represented by score 2+, requires further tests for confirming the presence or absence of HER2 gene amplification and this is achieved using the second method, in situ hybridisation or most commonly fluorescent in situ hybridisation (FISH). The most recent guidelines produced by the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) (Wolff et al, 2007) define a FISH result of more than six copies of the HER2 gene per nucleus, or a FISH ratio (HER2 gene signals to chromosome 17 signals) of more than 2.2 as a positive result. A ratio of HER2/CEP17 >2.0 was required to enter the large adjuvant trastuzumab trials, and was approved by the US FDA.

Despite the undoubted benefits of trastuzumab relapses do still occur and clearly not all patients derive the same benefit from treatment. HER2 gene amplification in those cancers considered HER2+ is variable and raises the question—does the degree of HER2 amplification influence outcome?

To answer this question, researchers have looked to the data sets of the original landmark adjuvant studies. For example, a retrospective analysis of the HERA trial dataset (Dowsett et al, 2009) investigated whether IHC status (2+ vs 3+), the degree of FISH amplification or polysomy of chromosome 17 affected clinical outcome and the conclusion was that there was no evidence for reduced benefit for adjuvant trastuzumab in IHC2+ FISH+ cases.

This study is a follow on to the previously published South East Wales Network experience (Webster et al, 2012), and our aim was to examine, again in a ‘community’ setting, whether the degree of HER2 positivity affected the outcome of adjuvant treatment.

MATERIALS AND METHODS

Patients diagnosed with HER2+ early breast cancer (defined as IHC3+, or IHC2+ FISH amplified with a HER2/CEP17 ratio >2.0) in the South East Wales Cancer Network between 1 January 2005 and 31 December 2008 were identified via the histopathology department records in the University Hospital of Wales, where all testing was carried out. All patients considered eligible for adjuvant treatment were included in the study. The electronic case record, CANISC, was reviewed to record the HER2 IHC result. The cancers with IHC grade 2+ results underwent FISH analysis and were reported as HER2 amplified. The HER2 gene copy number and HER2/CEP17 ratio did not feature in the issued reports but this information was recorded in the pathology department records and was retrieved manually for each IHC2+ case.

The date of disease recurrence or death from any cause was updated and follow up censored at the date of the last entry in the electronic case record.

STATA 13 (StataCorp LP, www.stata.com) was used to generate Kaplan–Meier survival curves.

RESULTS

A total of 311 patients were included in the study. The patient characteristics are described in Figure 1A and included a preponderance of high grade ductal cancers mostly of early stage I and II, and a high proportion of ER-negative cancers.

Of these, 230 were diagnosed as HER2+ on the basis of grade 3+ IHC results, whilst 81 scored IHC2+ and were HER2 amplified on FISH (HER2/CEP17 ratio >2.0). Of the 311 patients, 222 received trastuzumab, of whom 163 were IHC3+, and 59 were IHC2+ FISH+, as illustrated in Figure 1B.

Figure 2A shows the disease free survival and overall survival for all 311 patients according to whether trastuzumab treatment was given or not. Trastuzumab resulted in a statistically significant improvement is DFS and OS which was maintained over a long duration of follow up.

To determine whether the IHC status had an effect on outcome, an analysis was performed comparing treated IHC2+ FISH+ patients with treated IHC3+, illustrated in Figure 2B. This showed no significant difference between the two groups.

To further investigate if HER2 gene amplification had any effect on outcome in IHC2+ FISH+ cases, the 59 cases who received trastuzumab were split into 3 groups according to average copy number: low copy number cases, between 2.0 and 6.0; intermediate copy number, average between >6.0 and 12.0; and high copy number >12.0. The low copy number group were all defined as HER2+ on the basis of a HER2/CEP17 ratio >2.0, according to the standard practice of HER2 testing prevalent at the time.

The results are shown in Figure 2C. These clustered into three groups according to HER2 gene copy number. The low copy number group, with between 2.0 and <6.0 HER2 gene copies per nucleus appeared to have a good prognosis. The high copy number group also did well. This was in striking contrast to the intermediate copy number group which appeared to have a worse outcome with a lower DFS and OS when compared with the other two groups.
DISCUSSION

We have previously published our local community level experience of adjuvant trastuzumab treatment of breast cancer patients, showing that outcomes similar to those derived from large scale multinational trials can be reproduced in a community setting and this updated study confirms those benefits with extended follow up.

In the present study we have examined whether the degree of HER2 overexpression had influenced the outcome in our cohort of patients which reflected the typical spread of HER2+ patients

Figure 2. Progression free and overall survival according to (A) trastuzumab vs no trastuzumab (P = 0.27) (B) IHC3+ vs IHC2+ FISH+ (P = 0.505) and (C) HER2 gene copy number 2.0–6.0, 6.1–12.0, >12.1 (P = not possible to analyse).
seen in other studies, with a preponderance of high grade ductal cancers mostly of early stage I and II, and as expected, given the inverse relationship between ER and HER2 expression (Konecny et al., 2003), a high proportion of ER negative cancers.

When considered together, there was no apparent difference in DFS or OS between IHC2 + FISH + and IHC3 +, as reported in the HERA trial analysis (Dowsett et al., 2009). However, when the IHC2 + FISH + groups were further analysed according to HER2 gene copy number intriguing differences were observed, suggesting there may be a group with a low degree of HER2 amplification who derive less benefit from trastuzumab.

Although the numbers of patients in each of the three groups described above were small, there was a difference demonstrated between the three groups according to HER2 gene copy number. The low copy number group, with between 2.0 and 6.0 HER2 gene copies per nucleus appeared to have a good prognosis and this could reflect the fact that if defined by copy number alone (rather than HER2/CEP17 ratio > 2.0) these cancers would be considered HER2 non amplified and therefore essentially HER2 negative with generally a better prognosis compared with HER2-positive cases. The high copy number group (>12 copies on average) also did well presumably owing to a consistently good response to trastuzumab. The intermediate copy number group appeared to have a worse outcome with a lower DFS and OS when compared with the other two groups.

The precise degree of HER2 overexpression that translates into benefit from trastuzumab has remained controversial since the emergence of this treatment in the early 2000s. In 2002 a single agent trastuzumab study was reported in women with metastatic breast cancer overexpressing HER2 and no found improvement for patients whose cancers were graded IHC2 + compared with those graded IHC3 + (Vogel et al., 2002). Conversely, an analysis of the NSABP B31 trial (Paik et al., 2007) showed that 174 patients who were considered centrally HER2 negative by IHC and FISH appeared to derive a benefit from trastuzumab. A similar retrospective analysis of N9831 (Perez et al., 2010) showed no difference in outcome between IHC3 + and FISH + cases, but also suggested an apparent benefit for centrally HER2-negative cases by IHC and FISH, although this was not significant and the numbers were small.

In the neoadjuvant setting, Guiu et al (2010) demonstrated that the level of HER2 gene amplification influenced the pathological complete response rate (pCR), with high amplified cancers (>10.0 HER2 gene copies per cell) showing a significantly improved pCR rate, although this did not translate into a RFS or OS benefit.

Since a definition of HER2 positivity is a HER2/CEP17 ratio of >2.0 (or 2.2 as more recently stipulated), therefore the number of copies of chromosome 17 per nucleus is also important, as a cancer with polysomy of chromosome 17 as well as HER2 amplification will have a lower HER2/CEP17 ratio than a similarly HER2-amplified cancer in the presence of monosomy 17. The influence of chromosome 17 polysomy was studied in both the retrospective analyses of HERA and N9831 (Perez et al., 2010) and no correlation between polysomy 17 and benefit from trastuzumab was found.

Our small-scale study contrasts with the large scale retrospective analyses cited above, in that it has the advantage of a longer period (up to 5 years) of follow up compared with, for example, the HERA study with the median follow up to only 2 years. We also took a different approach to the other studies by examining the IHC2 + FISH + cases in isolation, rather than analysing by FISH ratio including IHC2 + and 3 + cases, and this may account for the discrepancy between the two studies.

We hypothesise that low amplified HER2 cancers, as defined by an average HER2 gene copy number >6 and <12 in our study, may represent a different subset of HER2-positive breast cancers, characterised by polysomy of chromosome 17 and intra-tumoural heterogeneity (Figure 3). The well recognised presence of G2 cells or/and polyploidization owing to chromosomal instability often justify, at least in part, the DNA ploidy count variation and the consequent skewing of the expected value from the two wild molecular signals.

As for the unexpected better response observed in the low HER2 copy number group, we speculate that these cases apart from representing false negative results on technical grounds, may relate to the observations of Paik et al (2008) demonstrating a subset of patients who are HER2 negative benefiting from trastuzumab therapy. At pathological level, there are emerging data to suggest that at least some of the observed discordance may be owing to an inappropriate selection of patients who could benefit from antiHER2 treatments. Cumulative worldwide experience gained over a decade of HER2 testing of breast cancer patients has suggested that a subset of patients scoring negative (score 0, 1 +) may show gene amplification (Dendukuri et al, 2007; Starczynski et al, 2010; Iorfida et al, 2012; Brunello et al, 2013). This has led to the need for an alternative way of interpreting such discords (Starczynski et al, 2012).

There is the potential to answer these questions by re-examining the existing datasets from the large adjuvant studies, and work is planned to try and validate our results within the HERA trial. As the number of IHC2 + FISH + patients in each trial are small conducting a meta-analysis would be ideal.

All these examples serve to emphasise the importance of recognising the wide variation of HER2 overexpression and amplification associated with ‘HER2-positive’ breast cancer. This could help the clinicians treating the HER2-positive-women to better appreciate the variable responses to anti HER2 therapy observed in routine practice. The data of the present study suggest that a small proportion of the patients with HER2 IHC2+/intermediate HER2 copy number amplification status are not likely to derive the full benefit expected from adjuvant trastuzumab.

Resistance to targeted agents is an emerging feature, and understanding of the molecular mechanisms predicting response or failure has become a crucial issue to optimise treatment and select patients who are the best candidates to respond (Tortora, 2011). Although the actual study is based on small number of patients, we think the inclusion of the HER2 gene copy number value may be an additional useful indicator (side-by-side with other clinicopathological parameters) to stratify both the management and follow up of breast cancer patients treated with trastuzumab.
Impact of HER2 copy number

REFERENCES

Brunello E, Bogina G, Bria E, Vergine M, Zamboni G, Pedron S, Daniele I, Furlanetto J, Carbognin L, Marconi M, Manfrin E, Ibrahim M, Miller K, Tortora G, Molino A, Jasani B, Beccari S, Bonetti F, Chilosi M, Martignoni G, Brunelli M (2013) The identification of a small but significant subset of patients still treatable with anti-HER2 inhibitors when affected by triple negative breast carcinoma. J Cancer Res Clin Oncol 139(9): 1563–1568.

Dendukuri N, Khetani K, McSaac M, Brophy J (2007) Testing for HER2-positive breast cancer: a systematic review and cost-effectiveness analysis. CMAJ 176(10): 1429–1434.

Dowsett M, Bartlett J, Ellis I, Salter J, Hills M, Mallon E, Watters A, Cookie T, Paish C, Wenczyk PM, Pinder S (2003) Correlation between immunohistochemistry (HercepTest) and fluorescence in situ hybridisation (FISH) for HER-2 in 426 carcinomas from 37 centres. J Pathol 199(4): 418–423.

Dowsett M, Procter M, McCaskill-Stevens W, de Azambuja E, Dafni U, Rueschoff J, Jordan B, Doherty D, Piccart-Gebhart M, Leyland-Jones B (2009) Correlation between HER-2 amplification after trastuzumab-based neoadjuvant therapy for HER2-overexpressing breast cancer and from the FinHer Study Investigators (2006) Adjuvant Docetaxel or Cyclophosphamide and Fluorouracil, Doxorubicin, and Cyclophosphamide in Operable HER2-Positive Breast Cancer with Favorable Prognostic Features. J Clin Oncol 23(5): 1371–1372.

Fortina P, Kim C, Jeong J, Geyer C, Romond H, Meija-Mejia O, Mamounas P, Wickerham L, Costantini J, Wolmark N (2007) Benefit from adjuvant trastuzumab may not be confined to patients with high HER2+/low HER2− tumors: clinical results from NSABP B-31. J Clin Oncol 25(suppl; abstr 511): 5s.

Piccart-Gebhart M, Proctor M, Leyland-Jones B, Goldhirsch A, Untch M, Smith I, Gianni L, Baselga J, Bell R, Jackisch C, Cameron D, Dowsett M, Barrios C, Steger H, Huang C, Andersen M, Lichinitser M, Lang I, Nitz U, Iwata H, Thomssen C, Lohrisch C, Suter T, Rüschoff J, Sítow T (2005) Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. N Engl J Med 353(16): 1659–1672.

Romond EH, Perez EA, Bryant J, Suman VJ, Geyer Jr CE, Davidson NE, Tan-Chiu E, Martino S, Paik S, Kaufman PA, Swain SM, Pisansky TM, Feuerhanger L, Kuttch LA, Vogel VG, Visscher DW, Yothers G, Jenkins RB, Brown AM, Dakhil SR, Mamounas EP, Lingle WL, Klein PM, Ingle JN, Wolmark N (2005) Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. N Engl J Med 353(16): 1673–1684.

Slamon D, Eiermann W, Robert N (2006) BCIRG 006: Second interim analysis phase III randomized trial comparing doxorubicin and cyclophosphamide followed by docetaxel with doxorubicin and cyclophosphamide followed by docetaxel and trastuzumab with docetaxel, carboplatin and trastuzumab in HER2+ breast cancer patients. In: Annual San Antonio Breast Cancer Symposium. pp S1–299 (abstract 52) San Breast Cancer Res Treat: Antonio, TX, USA.

Slamon D, Clark G, Wong S, Leven W, Ullrich A, McGuire W (1987) Human Breast Cancer: correlation or relapse and survival with amplification of the HER2/neu oncogene. Science 235(4785): 177–182.

Starzynski J, Atkey N, Connelly Y, O’Grady T, Campbell FM, di Palma S, Wencyk P, Jasani B, Gandy M, Bartlett JM. (2012) HER2 gene amplification in breast cancer: a rogues’ gallery of challenging diagnostic cases: UKNEQAS interpretation guidelines and research recommendations. Am J Clin Pathol 137(4): 595–605.

Starzynski JL, Campbell FM, Jones P, Gilbert J, Dowds JC, Miller K, Ibrahim M, Jasani B (2010) Audit of the Accuracy of Immunohistochemical (IHC) Testing of HER2 Status of Breast Cancer in the United Kingdom: An Interim Analysis. Cancer Res 70(24 Suppl): Abstract nr P3-10-21.

Tortora G (2011) Mechanisms or resistance to HER2 target therapy. J Natl Cancer Inst Monogr 2011(43): 95–98.

Webster RM, Abraham J, Palaniappan N, Caley A, Jasani B, Barrett-Lee P (2012) Exploring the use and impact of adjuvant Trastuzumab for HER2-positive breast cancer patients in a large UK cancer network: Do the results of international clinical trials translate into a similar benefit for patients in South East Wales? Br J Cancer 106: 32–38.

Wolf AF, Hammond ME, Schwartz JN, Hagerty KL, Allred DC, Cote RJ, Dowsett M, Fitzgibbons PL, Hanna WM, Langer A, McShane LM, Paik S, Pegram MD, Perez EA, Press MF, Rhodes A, Sturgeon C, Taube SE, Tubbs Raymond, Vance Gail H., van de Vijver M, Wheeler TM, Hayes DF (2007) American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. J Clin Oncol 25: 118–145.

Vogel C, Cobleigh M, Tripathy D, Gutheil J, Harris L, Feuerhanger L, Slamon D, Murphy M, Novotny W, Burchmore M, Shak S, Stewart S, Press M (2002) Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer. J Clin Oncol 20: 719–726.

This work is published under the standard license to publish agreement. After 12 months the work will become freely available and the license terms will switch to a Creative Commons Attribution-NonCommercial-Share Alike 3.0 Unported License.