FULL PAPER

BJC

British Journal of Cancer (2013) 109, 2453–2461 | doi: 10.1038/bjc.2013.609

Keywords: early breast cancer; HER1/EGFR; HER2; HER3; exemestane; oestrogen receptor; tamoxifen

Do type 1 receptor tyrosine kinases inform treatment choice? A prospectively planned analysis of the TEAM trial

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Background: Epidermal growth factor receptors contribute to breast cancer relapse during endocrine therapy. Substitution of aromatase inhibitors (AIs) may improve outcomes in HER-positive cancers.

Methods: Tissue microarrays were constructed. Quantitative analysis of HER1, HER2, and HER3 was performed. Data were analysed relative to disease-free survival and treatment using outcomes at 2.75 and 6.5 years.

Results: Among 4541 eligible samples, 4225 (93%) had complete HER1–3 data. Overall, 5% were HER1-positive, 13% HER2-positive, and 21% HER3-positive; 32% ($n=1351$) overexpressed at least one HER receptor. In the HER1–3-negative subgroup, the hazard ratio (HR) for upfront exemestane *vs* tamoxifen at 2.75 years was 0.67 (95% confidence interval (CI), 0.52–0.87), in the HER1–3-positive subgroup, the HR was 1.15 (95% CI, 0.85–1.56). A prospectively planned treatment-by-marker analysis demonstrated a significant interaction between HER1–3 and treatment at 2.75 years (HR = 0.58; 95% CI, 0.39–0.87; $P=0.008$), as confirmed by multivariate regression analysis adjusting for prognostic factors (HR = 0.55; 95% CI, 0.36–0.85; $P=0.005$). This effect was time dependent.

Conclusion: In the 2.75 years prior to switching patients initially treated with tamoxifen to exemestane, a significant treatment-by-marker effect exists between AI/tamoxifen treatment and HER1-3 expression, suggesting HER expression could be used to select appropriate endocrine treatment at diagnosis to prevent or delay early relapses.

Aromatase inhibitors (AIs) confer a disease-free survival (DFS) benefit over and above that achieved with adjuvant tamoxifen in postmenopausal women with early oestrogen-or progestrone-receptor (ER/PgR)-positive breast cancer (Thurlimann *et al*, 2005; Forbes *et al*, 2008; van de Velde *et al*, 2011). Recent data from the Tamoxifen and Exemestane Adjuvant Multinational (TEAM) and Breast International Group (BIG) 1–98 trials suggest that DFS is similar in patients treated with either an AI for 5 years

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Previous publication: Portions of these data were presented by Dr Bartlett at the 31st, 32nd, and 33rd Annual SABCS Meetings in 2008 (abstract 81), 2009 (abstract 75), and 2010 (abstract S2-4), and the abstracts were published in *Cancer Research* supplements.

Received 25 June 2013; revised 3 September 2013; accepted 12 September 2013; published online 3 October 2013

or 'switched' to an AI following 2–3 years of tamoxifen (Thurlimann *et al*, 2005; van de Velde *et al*, 2011).

This observation has generated debate regarding the optimal treatment strategy (upfront AIs *vs* switch) for postmenopausal ER/PgR-positive breast cancer. As, for all strategies, the benefit of AIs *vs* tamoxifen is modest when compared with tamoxifen *vs* no endocrine treatment (Abe *et al*, 2005; Viale *et al*, 2009), there is considerable impetus for translational studies aimed at identification of those patients most likely to benefit from upfront AIs.

The differences in benefit between hormonal regimens may be explained, in part, by the diverse biology of breast cancer, particularly the differences between luminal A and luminal B cancers (Perou *et al*, 2000). Clearly, multiple factors may influence differential response to hormonal treatments. Including HER2, Ki-67, and RAS/RAF or PI3K/Akt signalling (Beeram *et al*, 2007; Viale *et al*, 2008). Future selection of optimal endocrine therapy for early breast cancer will need to be personalised based on studies identifying an increasing number of patient subsets with unique molecular profiles, for example (Curtis *et al*, 2012).

Selecting optimal adjuvant endocrine therapy and/or chemotherapy is currently influenced by measures of residual risk (Van Belle *et al*, 2010). However, selection between endocrine agents (AIs *vs* tamoxifen) requires specific markers indicating differential benefit from these agents. We have previously shown, within TEAM, that quantitative analysis of ER and PgR expression combined with clinicopathologic factors (age, tumour size and grade, and nodal status) can identify patients at higher risk for early recurrence (Bartlett *et al*, 2011). We confirmed previous data indicating that PgR, although prognostic, is not a predictive marker of benefit from AI *vs* tamoxifen (Dowsett *et al*, 2008; Simon *et al*, 2009). Our study was, unlike previous studies, based on an adequately powered and prospectively planned treatment-by-marker analysis, satisfying criteria for high-level evidence (Simon *et al*, 2009).

Type 1 receptor kinase expression (HER1, HER2, and HER3 (HER1–3)) is associated with a higher probability of early relapse in tamoxifen-treated patients (Tovey *et al*, 2004, 2005), consistent with both preclinical and clinical data suggesting overexpression of HER2, and HER1/EGFR confer resistance to tamoxifen (Benz *et al*, 1992; Carlomagno *et al*, 1996; Houston *et al*, 1999). Conversely, neoadjuvant studies suggest that AIs are effective regardless of HER1 or HER2 overexpression (Ellis *et al*, 2001; Dixon *et al*, 2004). On the basis of these observations, we hypothesised that overexpression of HER1, HER2, and/or HER3 is associated with a differential benefit of an AI compared with tamoxifen in the adjuvant setting, and that outcome in patients with HER1–3-positive tumours would be improved by initiating treatment with an AI rather than tamoxifen. The analysis presented here was prospectively planned and powered to test the hypothesis, within the TEAM study, that HER1–3 status acts as a predictive biomarker for benefit of exemestane *vs* tamoxifen during the 2.75 years prior to the switch point.

MATERIALS AND METHODS

Study design. The TEAM trial, an international, open-label, phase III trial in postmenopausal women with ER/PgR-positive early breast cancer (van de Velde *et al*, 2011), included two prospectively planned and powered pathology studies. This, the second TEAM pathology study, tests the hypothesis that upfront exemestane improves DFS compared with tamoxifen in patients with HER1–3-positive tumours, defined as tumours expressing high levels of at least one of HER1, HER2, or HER3 proteins. Outcomes in patients with high levels of HER1–3 expression were compared with those in patients without high HER1–3 expression. This intent-to-treat analysis was planned at 2.75 years follow-up and was not event-driven (van de Velde *et al*, 2011). Using a two-sided $\alpha = 0.05$ assuming a hazard ratio (HR) of 1.93 and HER1–3-positive prevalence of 25%, a sample size of 4000 patients would give $>90\%$ power to detect a treatment–biomarker interaction. Secondary exploratory analyses including DFS at 2.75 years, censoring patients at the actual time of switching, and DFS at a median follow-up of 6.5 years were performed.

Patients. Overall samples from 4781 patients were received from the United Kingdom (1097), The Netherlands (2722), Belgium (122), Germany (745) and Greece (95). Patient demographics and tumour characteristics were similar between the analysed subset and all patients in the pathology substudy; patients in the pathology subset were at slightly higher risk than the entire TEAM population (Bartlett *et al*, 2010) (Supplementary Table 1: CONSORT table).

In general, patients had histologically or cytologically confirmed T1-3 N0-2 M0 breast adenocarcinoma and were treated with surgical resection followed by radiotherapy and/or adjuvant chemotherapy. Among countries participating in the TEAM trial, five (United Kingdom/Ireland, The Netherlands, Belgium, Germany, and Greece) provided tumour samples for this substudy after appropriate ethical review.

Staining methodology. Tissue microarrays (TMAs) were constructed as reported previously (Bartlett *et al*, 2011), in line with current guidelines (Leyland-Jones *et al*, 2008). Standard immunohistochemical techniques were used to stain TMAs for HER1–3 (HER1 clone 31G7: Invitrogen, Paisley, UK; HER2, HercepTest, and HER3 clone DAK-H3-IC: Dako, Cambridgeshire, UK) and Ki67 (Clone MIB1, 1 : 50 dilution, Dako). Assays were performed to good laboratory practice (GLP) in a GLP-monitored laboratory using single batches of each antibody and reagent; incubations were rigorously controlled for temperature. In each assay, quality controls with varying HER expression were included as described previously for ER/PgR (Bartlett *et al*, 2011). Cores were scored manually for HER1–3 using highly trained observers (Kirkegaard *et al*, 2006). Histoscores for HER1–3 (membrane staining only) were recorded. For Ki67, scoring was performed using the Ariol SL-50 Image analysis system (Genetix, New Milton, UK) as previously described for ER/PgR (Bartlett *et al*, 2011) using an algorithm developed specifically for Ki67. Results for Ki67 were recorded as the percentage of Ki67 positive cells. HER2 status was confirmed by fluorescence *in situ* hybridisation (FISH) with 95% concordance between immunohistochemical and FISH results (Wolff *et al*, 2007).

Statistical analysis. Disease-free survival, was defined as time from randomisation to earliest documentation of disease relapse (primary tumour recurrence (locoregional or distant) and ipsilateral/contralateral breast cancer) or death from any cause. The primary aim of this study was evaluation of the interaction between HER1–3 expression and treatment at 2.75 years on an intent-to-treat basis. Exploratory intent-to-treat analyses of interactions between HER1–3 expression and treatment were performed with 6.5 years median follow-up (van de Velde *et al*, 2011). A sensitivity analysis of the impact of censoring patients at the recorded time of treatment switch (Tam-AI) was performed. Exploratory analysis of DFS from 2.75 years postrandomization (excluding patients without follow-up after 2.75 years) until last recorded follow-up was performed. Data included in these analyses were locked on 1 February 2013.

The predictive value of HER expression was assessed using Cox proportional hazards regression models. Interactions between treatment arms and HER expression levels were evaluated using the Wald chi-square (χ^2) statistic. The predictive value of HER expression was further investigated in multivariate analyses,

consistent with REMARK guidelines (McShane *et al*, 2006), adjusting for known prognostic factors: patient age (continuous variable); tumour size (continuous variable); number of positive nodes (continuous variable); treatment with chemotherapy (yes/no); treatment arm (tamoxifen/exemestane); and expression of HER1–3 (negative/positive), ER, PgR, and Ki67 (each a continuous variable). Continuous variables were evaluated for nonlinearity by applying simple log transformations followed by more complex fractional polynomials, and the best-fitting transformation was applied as assessed by the change in Akaike's information criterion between univariate Cox proportional hazard models of transformed and untransformed data (Collett, 1994). Treatment allocation was included as a time-dependent covariate to investigate the impact of switching on the tamoxifen randomised arm. The proportional hazards assumption was investigated and time and covariate interactions were analysed to evaluate changing effects with time. All data were analysed using SAS/STAT statistical software (SAS Institute, Cary, NC, USA).

RESULTS

Study population. Of 4541 eligible samples (Bartlett *et al*, 2011), 4225 (93%) had complete HER1–3 data: 199 (5%) were HER1-positive; 547 (13%) HER2-positive, and 875 (21%) HER3-positive. Altogether, 1351 (32%) tumours were positive for at least one HER1–3 biomarker. Patient demographics and tumour characteristics were similar between the analysed subset and all patients in the pathology substudy; patients in the pathology subset were at slightly higher risk *vs* the entire TEAM population (Bartlett *et al*, 2010) (Supplementary Table 1: CONSORT table).

Efficacy

At 2.75 years, prospectively powered intent-to-treat analysis of treatment by HER1–3 marker expression. Among patients analysed in this substudy ($n=4225$), 408 DFS events were recorded over 2.75 years of follow-up. A trend towards DFS benefit of exemestane *vs* tamoxifen (HR = 0.84; 95% confidence interval (CI), 0.69–1.02) similar to the entire TEAM trial population was observed at 2.75 years (van de Velde *et al*, 2011). Among 2874 patients with HER1–3-negative tumours, 237 (8%), and among the 1351 patients with HER1–3-positive tumours, 171 (13%) DFS events occurred. HER1–3-positive patients had a 57% increased risk of a DFS event *vs* HER1–3-negative patients (HR = 1.57; 95% CI: 1.29–1.91; $P<0.0001$). In the pre-planned treatment-by-marker analysis, there was a significant interaction between HER1–3 expression and treatment (HR = 0.58; 95% CI, 0.39–0.87; $P=0.008$) in favour of increased benefit from exemestane in HER1–3-negative patients (Figure 1A). Among HER1–3-negative patients, there was a DFS benefit associated with exemestane *vs* tamoxifen (HR = 0.67; 95% CI, 0.52–0.87; Figure 1B). Conversely, there was no marked treatment effect between exemestane and tamoxifen in HER1–3-positive patients (HR = 1.15; 95% CI, 0.85–1.56; Figure 1C). In multivariate regression analysis, this treatment-by-marker interaction remained significant ($P=0.005$; HR = 0.55; 95% CI, 0.36–0.85), along with nodal status, tumour grade/size, patient age, ER, PgR, Ki67 (all as continuous variables); HER1–3 expression; and treatment (Table 1). Prior chemotherapy did not contribute significantly to risk at 2.75 years. Supplementary analysis at 2.75 years stratified by country shows similar interaction HRs across all countries (Table 2).

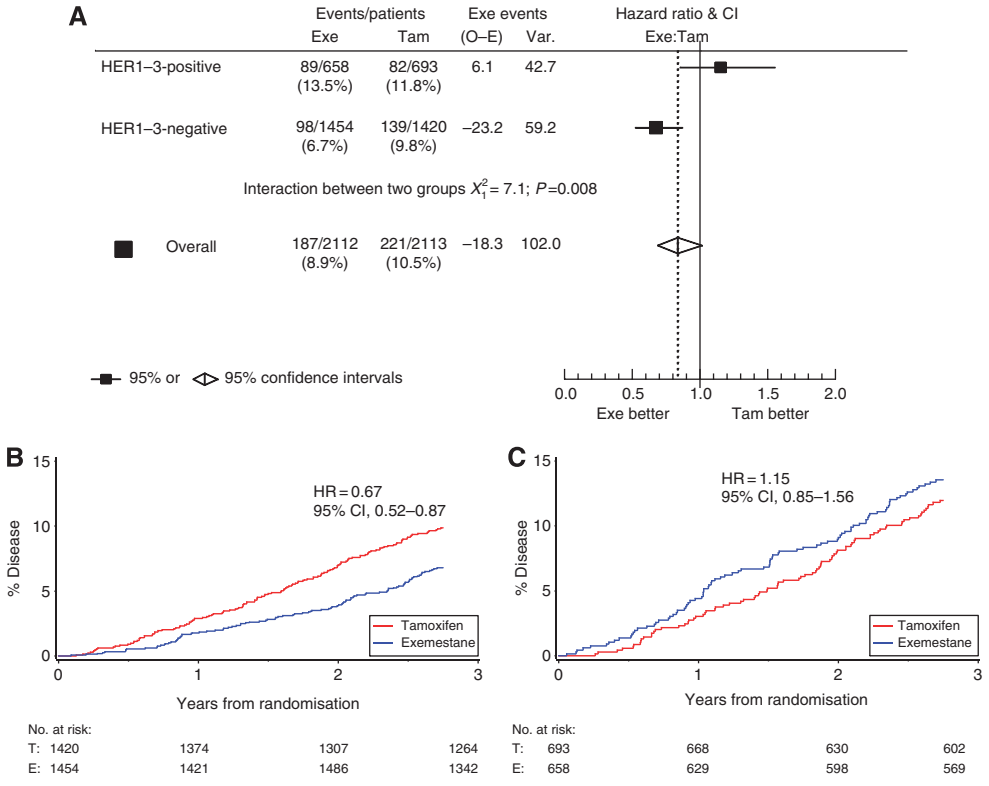

Figure 1. Disease-free survival in the intent-to-treat population at 2.75 years: (**A**) hazard ratio plot of treatment/biomarker interaction; (**B**) tumours negative for HER1, HER2, or HER3 ($n=2874$; 68%); and (**C**) tumours positive for HER1, HER2, or HER3 ($n=1351$; 32%). Abbreviations: CI = confidence interval; Exe = exemestane; HER = human epidermal growth factor receptor; HR = hazard ratio; O–E = observed minus expected; Tam = tamoxifen; Var = variance.

Table 1. Multivariate analysis of disease-free survival at 2.75 years ($N = 3779$; 360 DFS events)

Variable	Hazard ratio (95% CI)	Wald χ^2	*P*-value
Age (per 10 years)	1.35 (1.20–1.52)	26.0	<0.001
ER (per 50 histoscore units)	0.88 (0.81–0.96)	9.2	0.002
PgR (per 50 histoscore units)	0.87 (0.81–0.92)	18.7	<0.001
Ki67 (per 10%)	1.07 (1.01–1.13)	4.6	0.03
Tumour size[a]	NA	6.2	0.01
Number of positive nodes[a]	NA	50.6	<0.001
Second degree transformation (^2)	NA	18.4	<0.001
Tumour grade			
1 2 3	1.00 1.0 (0.68–1.47) 1.33 (0.90–1.99)	6.7	0.04
HER1–3	0.94 (0.70–1.27)	0.1	0.7
Treatment	1.24 (0.90–1.71)	1.7	0.2
HER1–3 treatment interaction	0.55 (0.36–0.83)	7.8	0.005

Abbreviations: CI = confidence interval; ER = oestrogen receptor; HER = human epidermal growth factor receptor; PgR = progesterone receptor; NA = not available.
[a]Nonlinear transformations for number of positive nodes (^2), tumour size (^–0.5).
Prior chemotherapy (Y/N) was a nonsignificant variable excluded from the model. Units (see text) Age = years, ER/PgR = histoscore (0–300), and Ki67 = per cent positive cells. Hazard ratios for continuous variables (Age, ER/PgR, and Ki67) are expressed for an interval of 10 years (Age), 50 histoscore units (ER/PgR) or 10% change in positivity (Ki67).

Table 2. Hazard ratios for the individual countries for HER1–3 expression and interaction with treatment arm

	HER1–3 negative		HER1–3 positive		
Country	**HR**	**95% CI**	**HR**	**95% CI**	**HR (95% CI) *P*-interaction**
The Netherlands/ Belgium	0.66	0.49–0.90	1.01	0.70–1.46	0.66 (0.41–1.06) $P = 0.082$
Germany	1.36	0.53–3.51	1.85	0.61–5.66	0.73 (0.17–3.16) $P = 0.67$
UK/IRE	0.63	0.34–1.15	1.22	0.65–2.30	0.51 (0.21–1.23) $P = 0.13$
Greece[a]	—	—	—	—	—

Abbreviations: HR = hazard ratio; CI = confidence interval; *P* interaction = *P*-value for treatment-by-marker interaction in individual countries. 2.75 years median follow-up.
[a]Only five events in the Greek subset of patients, analysis not possible.

Exploratory analyses: HER1, HER2, and HER3 expression. Further exploratory analyses were performed for the individual HER receptors; benefit from upfront exemestane *vs* tamoxifen treatment was apparent in HER1-negative (HR = 0.80; 95% CI, 0.65–0.98) *vs* HER1-positive tumours (HR = 1.60; 95% CI, 0.79–3.25; interaction test HR = 0.50; 95% CI, 0.24–1.03; $P = 0.06$). Similarly, HER2-negative tumours indicated benefit from upfront exemestane *vs* tamoxifen (HR = 0.71; 95% CI, 0.57–0.89), *vs* HER2-positive tumours (HR = 1.67; 95% CI, 1.09–2.55; interaction test HR = 0.43; 95% CI, 0.26–0.70; $P < 0.001$). However, there was no apparent differential benefit among HER3-negative or HER3-positive patients (HR = 0.80; 95% CI, 0.64–0.99 *vs* HR = 1.00; 95% CI, 0.65–1.53; interaction test HR = 0.80; 95% CI, 0.50–1.29; $P = 0.36$; Figure 2A). In a second exploratory analysis, tumours expressing either HER1 or HER2 were assumed (by the formation of active homo- or heterodimers) to exhibit 'active HER signalling', whereas tumours lacking HER1, HER2, and HER3 expression or expressing HER3 only were assumed to exhibit limited 'HER signalling' (Bartlett *et al*, 2010). A significant (exemestane *vs* tamoxifen) treatment-by-marker ('active HER signalling' *vs* cases without 'active HER signalling') interaction (HR = 0.42; 95% CI, 0.27–0.65; $P < 0.001$; Figure 2B) suggests that patients with active HER signalling do not derive benefit from early exemestane treatment.

Exploratory 2.75-year censored analysis. The primary intent-to-treat analysis (DFS at 2.75 years) evaluated benefit of exemestane and tamoxifen with relation to HER1–3 expression at the expected 'switch point' (tamoxifen patients switching to exemestane) of 2.75 years. However, 45% (949/2113) of tamoxifen patients switched treatment before the 2.75-year follow-up, whereas 21% (439/2113) discontinued tamoxifen early and did not switch. Among exemestane-treated patients, 12% (257/2112) stopped treatment early. A sensitivity analysis censored all patients at the actual time of switch, at treatment cessation, or at 2.75 years, whichever occurred first. The time until treatment cessation (excluding those who switched) for patients who stopped treatment early was different in the two treatment arms (median treatment duration, 0.94 *vs* 0.67 years for tamoxifen-treated and exemestane-treated patients, respectively), leading to potential bias in this analysis. Among patients included in the sensitivity analysis ($n = 4225$), 278 events were recorded. Comparing DFS at 2.75 years between HER1–3-negative and HER1–3-positive patients, as with the 2.75-year analysis, HER1–3-positive patients had significantly increased risk of a DFS event (HR = 1.61; 95% CI, 1.27–2.04; $P < 0.001$). Analysis revealed that significant treatment-by-marker interaction between HER1–3 expression and treatment with exemestane/tamoxifen remained significant in both univariate (HR = 0.50; 95% CI, 0.31–0.81; $P = 0.0049$; Figure 3), and multivariate analyses (HR = 0.44; 95% CI, 0.26–0.73; $P = 0.002$).

Extended follow-up. In an unplanned analysis, data were also evaluated at a median follow-up of 6.5 years, including the period when all patients were treated with exemestane (unless they discontinued treatment). Among patients analysed ($n = 4225$), 1021 DFS events were recorded. No significant interaction between HER1–3 and treatment with tamoxifen *vs* exemestane was observed (HR = 1.05; 95% CI, 0.82–1.36; $P = 0.68$). Among HER1–3-negative patients ($n = 2874$), 632 (22%) DFS events occurred, and among HER1–3-positive patients ($n = 1351$), 389 (29%) DFS events occurred (Figure 4A). There was no significant treatment effect (exemestane/tamoxifen) in HER1–3-positive patients (HR = 0.93; 95% CI, 0.76–1.14; Figure 4B). In HER1–3-negative patients, there was no longer a significant treatment effect between exemestane and tamoxifen (HR = 0.98; 95% CI, 0.84–1.15; Figure 4C). Relapses in the exemestane monotherapy group increased after the 2.75-year assessment and did not follow an extrapolation of the 2.75-year curve.

Analysis of treatment-by-marker interaction as a time-dependent covariate. By including each of the covariates as an interaction term with time in a Cox proportional hazard model, it is possible to investigate how the effect of each covariate on outcome varies with time. There is no significant interaction with time for either treatment or HER1–3 positivity. However, the hazard associated with the interaction between treatment and HER1–3 positivity significantly increases with time suggesting that HER1–3-negative patients treated with exemestane have an increased relapse risk as

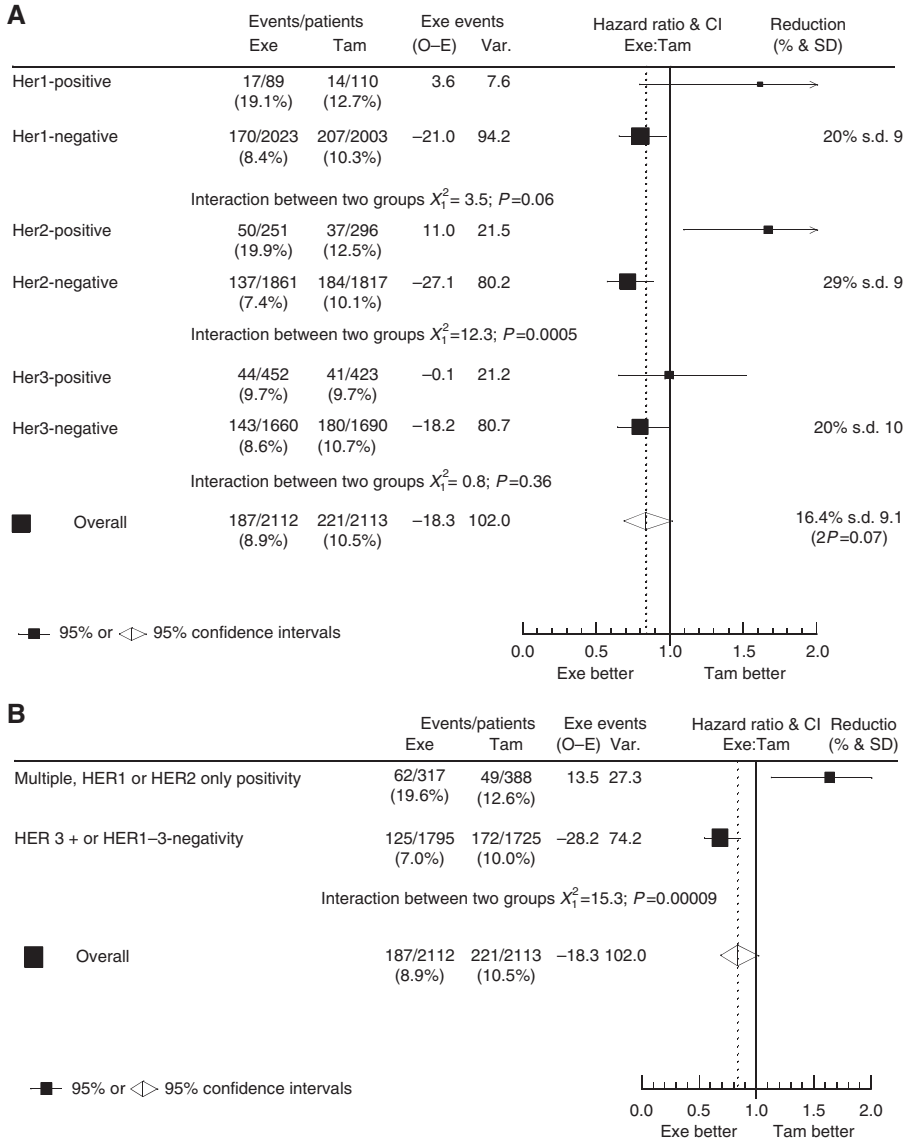

Figure 2. Contribution of each HER to disease-free survival benefit by treatment group: (**A**) interaction for individual HER receptors; and (**B**) activity of HER 'active' *vs* 'inactive' signalling. Abbreviations: CI = confidence interval; Exe = exemestane; HER = human epidermal growth factor receptor; HR = hazard ratio; O–E = observed minus expected; Tam = tamoxifen; Var = variance.

time progresses (HR = 1.07, 95% CI 1.01–1.14, $P = 0.017$). Relapse risk increases by 7% per annum for HER1–3-negative exemestane-treated patients when compared with all other patients.

As expected, given the time dependence of the HER1–3/treatment interaction term, assessment of the proportional hazard assumptions of the Cox model shows that the hazard of disease is not proportional between the two groups (Figure 5). Relapse risks clearly diverge between 0–3 years indicating that the hazard of disease during this time period for the two groups are not proportional. After 3 years, the proportionality assumptions are met. Therefore, the inclusion of the interaction with time of the treatment-by-marker interaction term is justified and explains the lack of evidence for treatment-by-marker interaction with extended follow-up without the use of a time-dependent model.

DISCUSSION

The results of this prospectively planned translational study show that expression of HER1, HER2, or HER3 predicts a differential benefit from initial adjuvant therapy with an AI compared with tamoxifen, which is shown to be both real and time dependent. In a prospectively planned and powered analysis, a significant DFS benefit in favour of initiating treatment with exemestane was observed among patients with HER1–3-negative tumours, in both univariate and multivariate analyses including the treatment-by-marker interaction (Figure 1, Table 1). Strikingly, this study did not show any benefit associated with initial exemestane treatment *vs* tamoxifen in patients with HER1-, HER2-, or HER3-positive tumours suggesting these tumours are partially resistant to endocrine therapy (Shou *et al*, 2004; Folgiero *et al*, 2008; Massarweh *et al*, 2008; Osborne *et al*, 2011). However, lack of overexpression of HER1, HER2, or HER3 is confirmed as an independent predictive biomarker for early AI benefit in patients with ER/PgR-positive early breast cancer. Exploratory analyses suggested that this effect was largely driven by HER1/HER2 expression, consistent with predicted HER signalling activity (HER3 lacks significant signalling potential) (Yarden and Pines, 2012). Therefore, assessing HER1/HER2 could provide valuable information in clinical practice in ER-positive disease (Hudelist *et al*, 2003; Sassen *et al*, 2008). Finally, in an exploratory

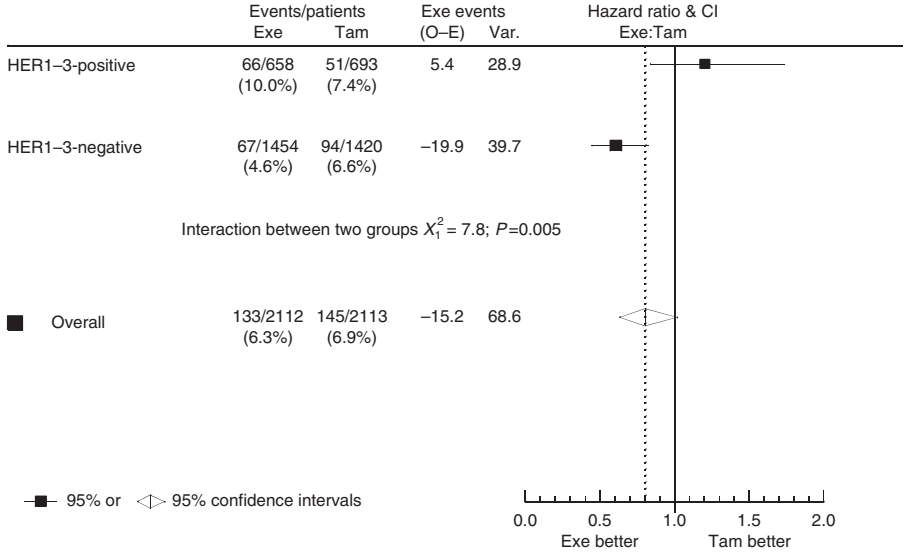

Figure 3. Hazard ratio plot of treatment-by-marker analysis, censoring patients at the time of treatment switch. This analysis presents disease-free survival at 2.75 years; data in this figure are censored = whereas Figure 1A data are not. Abbreviations: CI = confidence interval; Exe = exemestane; HER = human epidermal growth factor receptor; HR = hazard ratio; O–E = observed minus expected; Tam = tamoxifen; Var = variance.

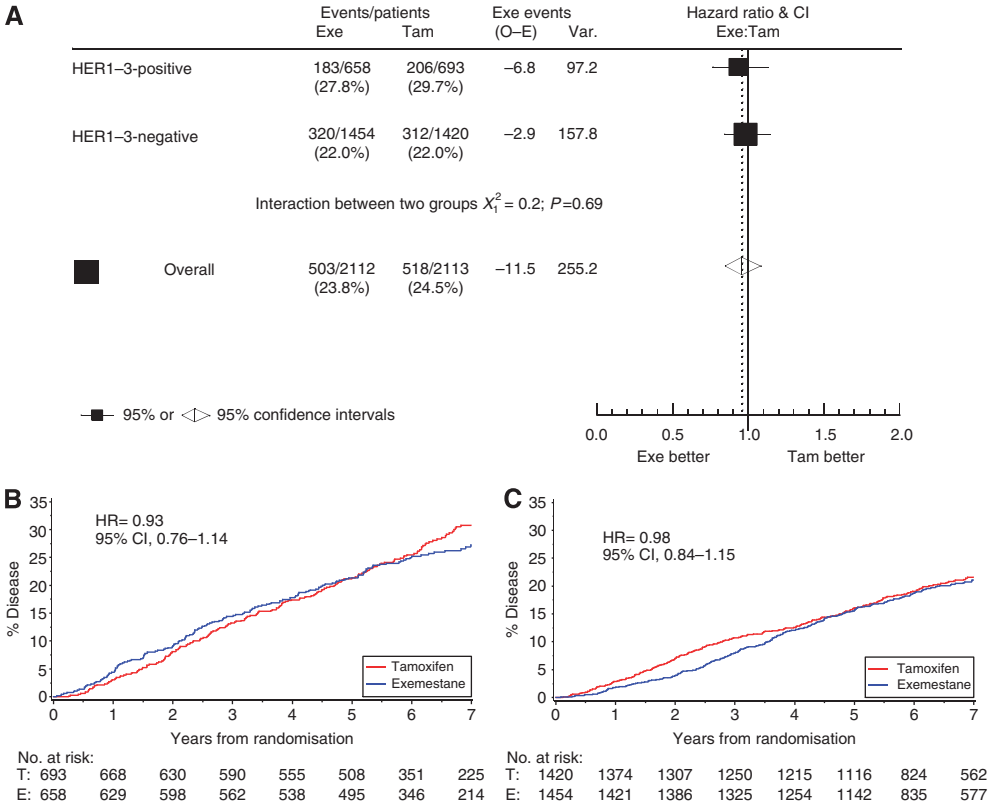

Figure 4. Disease-free survival in the intent-to-treat population at 5 years: (**A**) hazard ratio plot of treatment/biomarker interaction; (**B**) tumours positive for HER1, HER2, or HER3; and (**C**) tumours negative for HER1, HER2, or HER3. Abbreviations: CI = confidence interval; Exe = exemestane; HER = human epidermal growth factor receptor; HR = hazard ratio; O–E = observed to expected; Tam = tamoxifen; Var = variance.

time-dependent analysis (Figure 5), we identified a difference in the risk of relapse, relative to other tumours, associated with HER1–3-negative tumours treated with exemestane as time progressed. This time-dependent effect ultimately negates the treatment benefit observed in the pre-planned analysis (performed at 2.75 years) such that by 6.5 years median follow-up, no significant interaction between initial endocrine treatment and outcome is observed. Strikingly, in an exploratory analysis of DFS from treatment switch time point (2.75 years), the interaction term was inverted owing to the time-dependent effect in the HER1–3-negative exemestane group (data not shown). Nonetheless the time-dependent analysis confirms the statistical robustness of the interaction during the initial treatment period prior to switching from tamoxifen to exemestane.

The lack of DFS benefit associated with exemestane in the HER1–3-positive subset observed in the current study is entirely

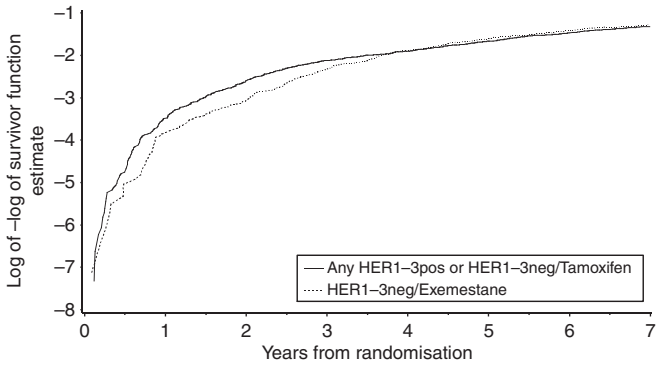

Figure 5. Log–log survivor plot for HER1–3-negative cases treated with exemestane (dashed line) *vs* all other cases (solid line). Divergence of lines prior to 3–4 years postrandomisation is evidence of non-proportional hazard rates between groups at this time. After this time, the hazard of relapse appears to be proportional between groups.

consistent with data from transATAC (an ATAC substudy) indicating no difference between benefit from tamoxifen and anastrozole in HER2-positive cancers (Dowsett *et al*, 2008). In HER2-positive ATAC patients, recurrence rates were 18.8% among tamoxifen-treated patients *vs* 19.8% among anastrozole-treated patients (Dowsett *et al*, 2008). Conversely, in HER2-negative patients, 5-year recurrence rates were 9.0% for tamoxifen-treated *vs* 5.9% for anastrozole-treated patients (Dowsett *et al*, 2008). These data are consistent with a HER2 treatment-by-marker interaction HR of $\sim$0.6, similar to that observed in the present study when patients received either tamoxifen or exemestane (prior to 2.75 years). In BIG-1–98, the possibility of a similar interaction between treatment and HER2 status is suggested by the fact that 48 fewer events were observed in the AI treatment arm for HER2-negative patients *vs* 13 more events in the AI-treated *vs* tamoxifen-treated HER2-positive group (Viale *et al*, 2009). A future meta-analysis of this effect across multiple trials (in collaboration with the AI overview group) will be important in evaluating whether effects of HER2 signalling are consistent across multiple trials.

Another striking observation in the TEAM study is the time dependency of the interaction between HER1–3 and treatment (Figures 1 and 4). The subgroup of patients with HER1–3-negative tumours treated with exemestane experience a time-dependent increase in risk of disease relapse when compared with all other patients (Figure 5). This increase progressively erodes the benefit of early treatment with exemestane relative to tamoxifen in the HER1–3-negative group. This apparently paradoxical effect does not appear to occur in either the ATAC or BIG-1–98 studies. How then, could it be explained? The key difference between ATAC/BIG-1–98 and TEAM is that the TEAM addresses specifically a switch from tamoxifen to AIs, whereas ATAC/BIG-1–98 predominantly address AIs *vs* 5 years of tamoxifen. Further analysis of the effect observed in the TEAM study could be performed in the relatively small switching arms within BIG-1–98. Exploration of a time-dependent effect of these different strategies is warranted; however, if such a time-dependent effect is not observed, the challenge of explaining our observations remains. We speculate that a proportion of HER1–3-negative early breast cancers are *primed* to develop endocrine resistance, as distinct from those with primary endocrine resistance, and that for a proportion of these cases AIs prevent or delay early recurrence. If our admittedly speculative hypothesis is correct, those cases where AIs delay recurrence may explain the increase in risk for HER1–3-negative patients observed in TEAM, while cases where switching from tamoxifen to AIs provides benefit may explain the convergence of the event rates for HER1–3-negative patients treated with tamoxifen followed by exemestane to those treated with AIs alone. Although we cannot speculate as to the molecular mechanisms relating to these trends, they reflect clinical experience with delayed recurrence following endocrine therapy.

Biomarker analyses raise questions relating to which biomarkers should be included in a risk assessment panel to achieve an optimal result, and how should data be interpreted? Overexpression of HER2 is associated with poor prognosis (Slamon *et al*, 1989), and the current analysis suggests that patients with HER1–3-positive tumours are at increased risk of early relapse regardless of treatment with exemestane *vs* tamoxifen consistent with previous data that signalling through multiple members of the HER family is associated with endocrine resistance (Tovey *et al*, 2004, 2005; Naresh *et al*, 2006). In patients with HER1–3-negative tumours, the question becomes whether these patients benefit from upfront AI treatment rather than tamoxifen. Assessing signalling potential suggests that exemestane provides a significant differential DFS benefit *vs* tamoxifen in tumours with 'inactive' HER1–3 signalling (Figure 3). These results suggest that the receptor activation may better indicate tumour response to adjuvant therapy than simple expression. The TEAM trial was conducted before introduction of adjuvant HER2-directed therapies, and for women eligible for HER2-directed therapy, an additional dimension exists in understanding the impact of such therapy.

In conclusion, upfront exemestane provided a superior DFS benefit compared with tamoxifen in tumours that were HER1–3-negative or had inactive HER signalling. The time dependency of this effect was explained by a progressive increase in relapse risk, over time, in HER1–3-negative patients treated with exemestane. These results warrant further confirmation in meta-analyses. Tumours that are HER1–3-positive appear to be relatively resistant to endocrine therapy. Pragmatically in clinical practice, HER2 results should provide adequate information for selection of early endocrine therapy although the addition of HER1/EGFR results will be of benefit to a small proportion of patients.

ACKNOWLEDGEMENTS

We gratefully acknowledge the support of all pathologists, treating physicians, and the participation of all patients who consented to provide paraffin blocks for the study. Cassandra L Brookes was the lead statistician conducting analyses for the TEAM pathology substudy. Tammy Robson, Nicola Lyttle, and Mary Anne Quintayo were the highly trained observers who manually scored tissue cores. This study was conducted with the support of the Ontario Institute for Cancer Research through funding provided by the Government of Ontario. The TEAM trial is a multinational study supported by an unrestricted research grant by Pfizer Inc., and funding from Cancer Research UK (grant number C7602/A7215). Editorial support was provided by Tamara Fink, PhD, of Accuverus, a division of ProEd Communications, Inc., Beachwood, Ohio, and was funded by Pfizer Inc. Clinical trials registration numbers: NCT00036270, NCT00032136, NCT00279448, NTR 267, Ethics Commission Trial 27/2001, and UMIN C000000057.

CONFLICT OF INTEREST

Annette Hasenburg has received support for travel expenses to meetings for the study, payment for lectures (honoraria), and expert testimony from Pfizer. Dirk G Kieback has received speaker's honoraria and research grant funding from Pfizer. Christos Markopoulos has received speaker's honoraria and educational grants from AstraZeneca (UK), Novartis (Basel, Switzerland), Pfizer Inc. (New York, NY, USA) and Genomic

Health Inc. (USA). Elizabeth A Mallon has received speaker's honoraria from Roche Pharmaceuticals. Daniel W Rea has received consultancy fees from Pfizer, Novartis, and Astra Zeneca. All remaining authors have declared no conflict of interest.

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Supplementary Information accompanies this paper on British Journal of Cancer website (http://www.nature.com/bjc)