Prognostic and Predictive Value of HER2 Expression in Ductal Carcinoma *In Situ*: Results from the UK/ANZ DCIS Randomized Trial

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ABSTRACT

Purpose: HER2 is overexpressed more frequently in ductal carcinoma *in situ* (DCIS) than in invasive breast cancer but its prognostic significance and predictive role for radiotherapy has not been clearly established. We investigated the prognostic and predictive value of HER2 overexpression in DCIS.

Experimental Design: HER2 expression was evaluated by IHC using the HercepTest™ in samples from UK/ANZ DCIS trial participants ($n = 755$) with IHC 3+ expression categorized as HER2 positive for primary analyses. Sensitivity analyses included HER2 categorization as negative (IHC 0,1+), equivocal (IHC 2+), and positive (IHC 3+) and analyses restricted to a nested case–control component where 181 cases (with recurrence) were matched to 362 controls by treatment arm and age.

Results: Two-hundred and forty-five (34.4%) of evaluable 713 samples [181 ipsilateral breast events (IBE)] were HER2 positive. HER2 overexpression was associated with significantly increased risk of IBE [HR = 2.29; 95% confidence interval (95% CI), 1.64–3.14; $P < 0.0001$] and *in situ* IBE (DCIS-IBE; HR = 2.90; 95% CI, 1.91–4.40; $P < 0.0001$), but not of invasive IBE (I-IBE; HR = 1.40; 95% CI, 0.81–2.42; $P = 0.23$; $P_{\text{heterogeneity}} = 0.04$). Inclusion of HER2 significantly improved [$\Delta\chi^2$ (1d.f.) 12.25; $P = 0.0005$] a prognostic model of clinicopathological and treatment variables, HER2 being an independent predictor of IBE (multivariate HR = 1.91; 95% CI, 1.33–2.76; $P = 0.0004$). Radiotherapy benefit in preventing DCIS-IBE was significantly greater ($P_{\text{heterogeneity}} = 0.04$) in HER2-positive DCIS (HR = 0.16; 95% CI, 0.07–0.41) compared with HER2-negative DCIS (HR = 0.58; 95% CI, 0.28–1.19).

Conclusions: HER2 overexpression is associated with significantly increased risk of *in situ* recurrence and is also predictive of radiotherapy benefit, with greater reductions in *in situ* but not invasive recurrences in HER2-positive DCIS.

Introduction

HER2 is a well-established predictive and prognostic biomarker in invasive breast cancer (1). A higher proportion (around 40%) of ductal carcinoma *in situ* (DCIS) overexpresses HER2, compared with around 15%–20% of invasive breast cancers (2). The biological and clinical significance of this higher proportion of DCIS tumors overexpressing HER2 than invasive cases is unclear (3). Consequently, HER2 expression is not routinely evaluated in DCIS (4).

Twenty-seven studies (Supplementary References S1–S27) have investigated the relationship between HER2 expression and recurrence in DCIS. Ten of these (5–14) reported a positive relationship between HER2 expression and ipsilateral recurrence risk, that is, ipsilateral breast event (IBE) risk; in four studies (7, 9, 11, 14), HER2 overexpression was associated with an increased risk of *in situ* IBE (DCIS-IBE) but not invasive IBE (I-IBE) whereas Visser and colleagues (12) investigated I-IBE risk alone and reported a statistically significant association. Wang and colleagues (15) pooled data from the studies by Provenzano (5) and de Roos (6), and reported that HER2 overexpression was associated with a 3-fold increase in IBE risk [relative risk (RR), 3.07; 95% confidence interval (CI), 1.32–7.12]. Other pooled analyses of four studies (7, 9, 10, 16) performed by Zhang and colleagues (17) found that HER2 overexpression was associated with only a nonsignificant increase in the I-IBE risk (RR, 1.25; 95% CI, 0.70–1.81).

The lack of significant association between HER2 and IBE risk in the majority of studies is not unexpected given their limitations, which include both small sample sizes and numbers of events, together with selection bias and adjuvant treatment-related confounding. Treatment-related confounding is of particular importance since HER2 expression is associated with adverse histological features (18–20) and HER2-positive patients are therefore more likely to receive radiotherapy which is effective in reducing IBE (21–24) and thus masking the true association between HER2 expression and IBE risk. Curigliano and colleagues (11) reported that HER2 overexpression was associated with DCIS-IBE (HR = 2.18; 95% CI, 1.28–3.69) and IBE (HR = 1.53; 95% CI, 1.07–2.18) in patients who did not receive radiotherapy. However, in those who received radiotherapy, the risk of

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Translational Relevance

HER2 overexpression is more frequent in ductal carcinoma *in situ* (DCIS) than in invasive breast cancer. In this largest biomarker study in a randomized controlled trial (RCT), HER2 overexpression was associated with almost 2-fold higher ipsilateral recurrence risk but also with a greater radiotherapy benefit. Ipsilateral invasive recurrence risk was not significantly higher in HER2-positive DCIS, and the radiotherapy benefit in reducing this risk did not differ by HER2 status.

Among recurrences, the odds of invasive recurrence were lower for HER2-positive DCIS and it could be hypothesized that HER2 is an early event in DCIS development but without much role in progression and the majority of invasive breast cancers develop from a HER2-negative precursor.

As an early event, HER2 overexpression is likely to be widespread within the sick lobe/breast, thus leading to the development of "new" DCIS lesion(s) and radiotherapy benefit is accrued through eradication of these potential foci.

DCIS-IBE (HR = 1.07; 95% CI, 0.60–1.90) and IBE (HR = 1.01; 95% CI, 0.69–1.49) was not related to HER2 status. Han and colleagues (10) similarly reported a difference in association between IBE risk and HER2 expression according to receipt of radiotherapy. These studies indirectly suggest a greater radiotherapy benefit in HER2-positive DCIS compared with HER2-negative DCIS, that is, HER2 is a predictive factor for radiotherapy benefit in DCIS.

To evaluate robustly the prognostic and predictive role of HER2 expression in DCIS, limitations of earlier observational studies need to be avoided, particularly treatment-related confounding. This can be best achieved through investigation in a randomized controlled trial (RCT) with long-term follow-up. We evaluated prognostic and predictive role of HER2 expression in DCIS using the pathology material from a subset of UK participants of the UK/ANZ DCIS trial (23).

Materials and Methods

Study design

The study was conducted in all UK/ANZ DCIS participants where pathology material was available. Formal power calculations were not performed for this retrospective study; HER2 expression was evaluated in all available samples. A nested case–control design employing matching by treatment allocation was used in sensitivity analyses of the prognostic role of HER2 expression to rule out residual treatment-related confounding (Supplementary Materials and Methods). This study is reported in accordance with the REMARK criteria (25).

Study population

The UK/ANZ DCIS trial (23) was a randomized 2 × 2 factorial design trial investigating the roles of tamoxifen and radiotherapy as adjuvant treatments in DCIS; it enrolled a total of 1,694 patients (Supplementary Fig. S1). After a median follow-up of 12.7 years (23), there have been 162 invasive and 197 DCIS events (17 unknown, total 376). Collection of pathology material and its use in biomarker studies was approved by the National Research Ethics Service—Joint UCL/UCLH Committees on Ethics of Human Research (Committee Alpha).

Formalin-fixed paraffin-embedded (FFPE) tissue blocks were collected from 36 hospitals in the United Kingdom. FFPE blocks containing DCIS were available in 45% (755/1,694) of patients. This subset, labeled biomarker study subset 1 (BSS1) hereafter, was similar to the remaining trial population with regard to treatment allocation and other clinicopathological factors including age and completeness of excision but contained a significantly higher proportion of high-grade DCIS, DCIS with necrosis, and larger lesions (Supplementary Table S1).

IHC assays and evaluation of HER2 expression

HER2 IHC assays were performed on whole sections using the HercepTest™ K5207 (Dako UK Ltd.) on a Dako Autostainer (Dako) as per manufacturer's instructions. HER2 assays were scored following the ASCO-CAP 2013 recommendations (26): 0 (any staining in <10% of tumor cells), 1+ (faint incomplete membrane staining in >10% of tumor cells), 2+ (weak to moderate complete membrane staining in >10% of tumor cells), and 3+ (strong complete membrane staining in >10% of tumor cells). In addition, H-scores (27) were also recorded. *ERBB2* gene-amplification status was not available to reclassify DCIS with equivocal HER2 expression (Supplementary Material). Therefore, as in previous DCIS studies (11, 28), samples with 3+ IHC score were classified as HER2 positive and the remainder of samples, including those with equivocal (2+) HER2 expression were classified as HER2 negative for primary analyses. Assays were performed and scored blinded to the study endpoint and clinicopathological variables.

Clinicopathological variables

Data on clinicopathological variables were derived from the trial database and pathology review of the trial (29). Age, completeness of excision, treatment allocation, tumor size (mm), cytonuclear grade (UK National Pathology Group; ref. 30), presence of necrosis, and periductal inflammation were analyzed.

Statistical analysis

Trial procedures, follow-up (23), and histopathology review (29) have been reported previously. For these analyses, only the first new breast event was considered. Missing data were not imputed. All *P* values are two sided and a *P* value less than or equal to 0.05 was deemed significant. Incremental improvement of models was based on differences in χ^2 values from respective likelihood ratio tests. Statistical analyses were performed using STATA 13.0 (StataCorp LP) and Review Manager 5.3 (The Nordic Cochrane Centre).

Associations between continuous and ordinal variables were assessed by the Kruskal–Wallis test and those between two ordinal variables by Goodman–Kruskal's gamma statistic. For the time-to-recurrence analyses, the Cox proportional hazards model was used to estimate Hazard Ratios (HR). Ten-year estimates and survival plots were produced by the Kaplan–Meier method. IBE was the primary endpoint, and additional analyses with DCIS-IBE and I-IBE as endpoints were also performed.

Sensitivity analyses

Sensitivity analyses categorizing HER2 as true negative (IHC 0, 1+), equivocal (IHC 2+) and positive (IHC 3+) were performed (Supplementary Materials and Methods). In addition, analyses of the prognostic role of HER2 expression restricted to the nested case–control study were undertaken to rule out any residual treatment-related confounding. Cases were matched to controls by age ± 7 years and treatment allocation using a 1:2 case–control ratio (181 cases and 362 controls), and controls had to be followed up for at least as long as in their matching case (full description in the Supplementary Material). In the case–control study, analyses of the risk of recurrence by groups

Table 1. HER2 status as a predictor of recurrence: categorized (negative, equivocal, positive) and binary (negative, positive).

			Reference IHC 0, 1+				Reference IHC 0, 1+, 2+			
			Univariate		Multivariate		Univariate		Multivariate	
Endpoint	**Events**	**HER2**	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
IBE	147[a]	IHC 2+	2.77 (1.70–4.54)	<0.0001	2.76 (1.63–4.66)	0.0001	—	—	—	—
		IHC 3+	2.90 (2.02–4.17)	<0.0001	2.46 (1.64–3.68)	<0.0001	2.27 (1.64–3.14)	<0.0001	1.92 (1.33–2.76)	0.0004
I-IBE	54	IHC 2+	2.08 (0.97–4.47)	0.061	1.99 (0.88–4.52)	0.10	—	—	—	—
		IHC 3+	1.63 (0.91–2.92)	0.10	1.40 (0.73–2.70)	0.31	1.40 (0.81–2.42)	0.23	1.21 (0.65–2.23)	0.55
DCIS-IBE	91	IHC 2+	3.49 (1.82–6.69)	0.0002	3.52 (1.77–6.99)	0.0003	—	—	—	—
		IHC 3+	4.03 (2.47–6.58)	<0.0001	3.28 (1.91–5.61)	<0.0001	2.90 (1.91–4.40)	<0.0001	2.34 (1.47–3.74)	0.0003

[a]Recurrence type not known in two cases. Multivariate analyses are in a smaller number of samples ($n = 612$) due to non-available clinicopathological data in some samples.

were performed by conditional logistic regression model (IBE as primary case definition) to estimate matched Odds Ratio (OR).

Role of the funding source

Funders had no role in study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the article for publication. M.A. Thorat had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

HER2 expression in DCIS and correlation with clinicopathological variables

HER2 expression was evaluable in 713 of 755 (94.4%) cases, of which 245 (34.4%) were HER2 positive (IHC 3+), 80 (11.2%) equivocal (IHC 2+), and 388 (54.4%) negative (IHC 0 or 1+). HER2 overexpression (Supplementary Table S2) was associated with larger lesion size ($P = 0.0001$), higher cytonuclear grade ($P < 0.0001$), presence of necrosis ($P < 0.0001$), and periductal inflammation ($P < 0.0001$).

HER2 status and the risk of recurrence: univariate analyses

HER2-positive DCIS had a greater than 2-fold risk of ipsilateral recurrence compared with HER2-negative DCIS, mainly driven by a 3-fold higher risk of *in situ* recurrence (**Table 1**); invasive recurrence risk was nonsignificantly elevated (HR = 1.40; 95% CI, 0.81–2.42; $P = 0.23$). Kaplan–Meier survival plots by HER2 status are displayed in **Fig. 1** ($P_{\text{heterogeneity}} = 0.04$). Analyses using HER2 H-score as a continuous variable showed similar results (Supplementary Table S3). Analyses with equivocal HER2 expression (IHC 2+) as a separate category showed that the increase in recurrence risk (**Table 1**) in HER2-equivocal DCIS (IHC 2+) was similar to that in HER2-positive DCIS (IHC 3+). The risk of DCIS-IBE was 4-fold higher in HER2-positive DCIS (HR, 4.03; 95% CI, 2.47–6.58) compared with pure HER2-negative (IHC 0 or 1+) DCIS.

HER2 status and type of recurrence

IBE were less frequent in HER2-negative DCIS compared with HER2-positive DCIS (15.2% vs. 30.2%, respectively) but almost half (46.5%) were invasive in HER2-negative DCIS (Supplementary Table S4) compared with 28.4% in HER2-positive DCIS. The odds of invasive as opposed to an *in situ* event were lower in HER2-positive DCIS (OR = 0.46; 95% CI, 0.23–0.91) compared with HER2-negative DCIS.

Multivariate analyses

Inclusion of HER2 in the model of clinicopathological and treatment variables significantly improved prediction of recurrence [$\Delta\chi^2$ (1d.f.) 12.25; $P = 0.0005$] and the HR of HER2-positive status [1.92 (1.33–2.76)] changed very little from its univariate value

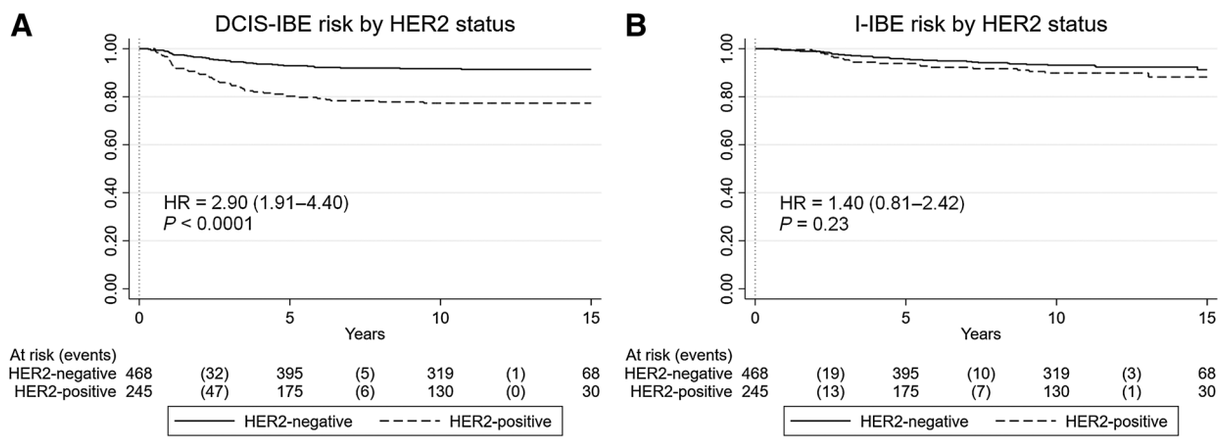

Figure 1.
Ipsilateral recurrence risk by HER2 status. Kaplan–Meier survival plots of DCIS-IBE (**A**) and I-IBE (**B**) risk.

Table 2. Multivariate analysis of HER2, clinicopathological, and treatment variables with IBE as endpoint.

			Univariate		Multivariate	
Variable	**Subgroup**	***n***	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
HER2	Negative	398	1 (reference)	—	1 (reference)	—
	Positive (IHC 3+)	214	2.03 (1.45–2.86)	<0.0001	1.92 (1.33–2.76)	0.0004
Age	Years (mean)	57.4	0.99 (0.96–1.02)	0.42	0.98 (0.95–1.01)	0.27
Excision	Complete	415	1 (reference)	—	1 (reference)	—
	Uncertain	100	1.49 (0.96–2.31)	0.074	1.65 (1.06–2.56)	0.027
	Incomplete	97	1.68 (1.09–2.58)	0.019	1.94 (1.25–3.02)	0.0031
	Trend test			*0.0095*		*0.0012*
Size	mm (mean)	15.7	1.03 (1.02–1.05)	0.0002	1.02 (1.01–1.04)	0.012
Grade	Low	34	1 (reference)	—	1 (reference)	—
	Intermediate	102	1.43 (0.48–4.25)	0.52	0.89 (0.28–2.83)	0.84
	High	476	2.15 (0.79–5.84)	0.13	1.01 (0.32–3.22)	0.99
	Trend test			*0.036*		*0.74*
Necrosis	No	45	1 (reference)	—	1 (reference)	—
	Yes	567	1.85 (0.81–4.19)	0.14	1.17 (0.45–3.05)	0.75
Inflammation	No	123	1 (reference)	—	1 (reference)	—
	Yes	489	1.96 (1.16–3.31)	0.011	1.39 (0.77–2.50)	0.27
Radiotherapy	No	388	1 (reference)	—	1 (reference)	—
	Yes	224	0.33 (0.21–0.51)	<0.0001	0.30 (0.19–0.47)	<0.0001
Tamoxifen	No	288	1 (reference)	—	1 (reference)	—
	Yes	324	0.65 (0.46–0.92)	0.015	0.71 (0.50–1.01)	0.056

Note: Endpoint: IBE; $n = 612$; events = 133; univariate analyses restricted to the same sample size as available for multivariate analyses.

[2.27 (1.64–3.14)], further supporting its role as an independent predictor of recurrence (**Table 2**). The improvement in the multivariate model (Supplementary Table S5) was greater [$\Delta\chi^2$ (2 d.f.) 24.96; $P < 0.0001$] if HER2 was included as a three-level variable (with IHC 2+/equivocal as a separate category).

Similar to univariate analyses, the multivariate analyses with equivocal HER2 expression (IHC 2+) as a separate category also showed that the increase in recurrence risk (**Table 1**) in HER2-equivocal DCIS (IHC 2+; HR, 2.76; 95% CI, 1.63–4.66) was similar to that in HER2-positive DCIS (IHC 3+; HR, 2.46; 95% CI, 1.64–3.68). The risk of DCIS-IBE was more than 3-fold higher in HER2-positive DCIS (HR, 3.28; 95% CI, 1.91–5.61) compared with pure HER2-negative (IHC 0 or 1+) DCIS in multivariate analyses.

Sensitivity analyses of the prognostic role of HER2 expression restricted to the case–control study showed similar results (Supplementary Tables S7–S11) ruling out any residual treatment-related confounding.

HER2 status and radiotherapy effect

The magnitude of radiotherapy effect in preventing DCIS-IBE was significantly greater (I^2, 77%; $P_{\text{heterogeneity}} = 0.04$) in HER2-positive DCIS (HR = 0.16; 95% CI, 0.07–0.41) versus HER2-negative DCIS (HR = 0.58; 95% CI, 0.28–1.19). The effect of radiotherapy in preventing I-IBE (**Table 3**; **Fig. 3**) did not however differ by HER2 status (I^2, 0%; $P_{\text{heterogeneity}} = 0.98$).

Ten-year ipsilateral recurrence rates (**Fig. 2**; Supplementary Table S6) were similar ($P = 0.69$) in HER2-positive (11.0%) and HER2-negative DCIS (9.6%) if patients received radiotherapy, but were much higher ($P = 0.0002$) in HER2-positive DCIS (42.1%) as compared with HER2-negative DCIS (17.5%) in patients allocated to no adjuvant radiotherapy.

Discussion

We investigated both the prognostic value of HER2 expression in DCIS and its role in predicting radiotherapy response. To the best of our knowledge, this is the first such study and the largest biomarker study conducted within a randomized trial. Treatment-related confounding is a key problem in evaluating prognostic role of a biomarker, and particularly relevant for HER2 as discussed before. Although it can be eliminated by excluding patients who have received any adjuvant therapy as done by Visser and colleagues (12), implications of such

Table 3. Effect of radiotherapy by HER2 status.

Endpoint	**Subgroup**	***n***	**Events[a]**	**HR (95% CI)**	***P***	P_{het}
IBE	HER2 negative	468	71	0.47 (0.27–0.82)	0.0048	0.07
	HER2 positive	245	76	0.21 (0.10–0.42)	<0.0001	
I-IBE	HER2 negative	468	33	0.36 (0.15–0.87)	0.023	0.98
	HER2 positive	245	21	0.35 (0.12–1.05)	0.062	
DCIS-IBE	HER2 negative	468	38	0.58 (0.28–1.19)	0.14	0.04
	HER2 positive	245	53	0.16 (0.07–0.41)	0.0001	

Note: Subgroups: HER2 positive (3+) and HER2 negative (0, 1+, and 2+).
[a]Recurrence type not known in two cases.

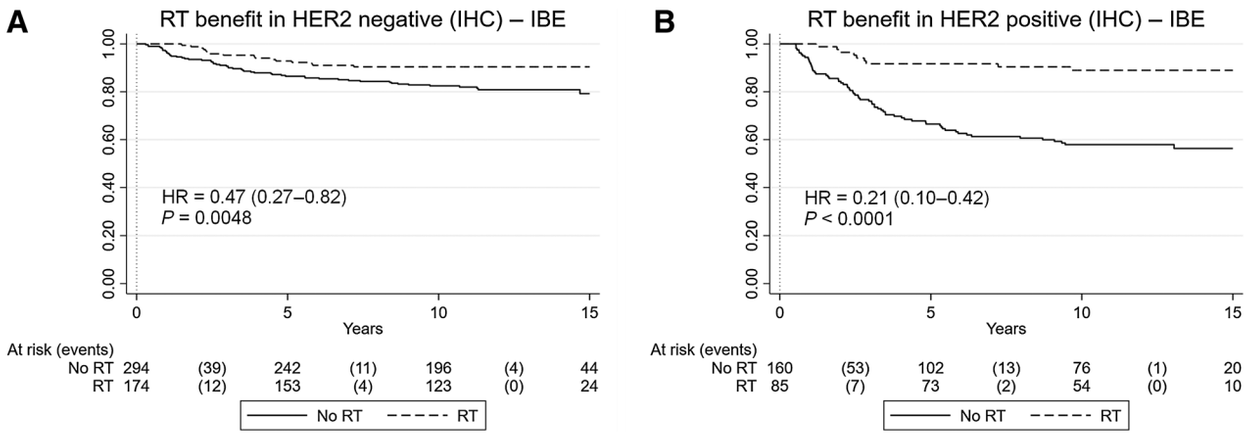

Figure 2.
Benefit of radiotherapy by HER2 status. Kaplan–Meier survival plots in HER2-negative DCIS (**A**) and HER2-positive DCIS (**B**).

results to most patients with DCIS who receive adjuvant treatment will remain unclear and such study cannot investigate the predictive role of HER2. Therefore, prognostic and predictive roles of HER2 are best evaluated in patients where adjuvant treatment allocation was random and ours is the only study investigating the role of HER2 in DCIS to eliminate treatment-related confounding in patients treated by breast conservation surgery (BCS) with/without radiotherapy. Sensitivity analyses in the case–control series, in which treatment allocation was one of the matching variables, further allowed us to rule out any residual treatment-related confounding adding to the robustness of our study. As in other studies (11, 28), only IHC 3+ DCIS was assigned as HER2 positive for the primary analyses. Analyses with HER2 expression categorized as negative, equivocal (IHC 2+), and positive were also performed to allow comparison of pure HER2-negative and pure HER2-positive DCIS in the absence of gene amplification data. The proportion of HER2-positive (34.4%) DCIS in our study was consistent with other cohorts (11, 31) including the NSABP-B43 trial (32) screening cohort (34.9% HER2 positive). Consistent with the literature, HER2 expression correlated positively with DCIS size (18), cytonuclear grade (18, 19), presence of necrosis (19, 20), and presence of periductal inflammation (20).

HER2-positive DCIS had a significantly higher overall ipsilateral recurrence risk (**Table 1**), driven largely by a 3-fold increase in DCIS-IBE risk. Although I-IBE risk was higher, the increase was not statistically significant. These findings are consistent with recent reports which suggest that HER2 overexpression is mainly associated

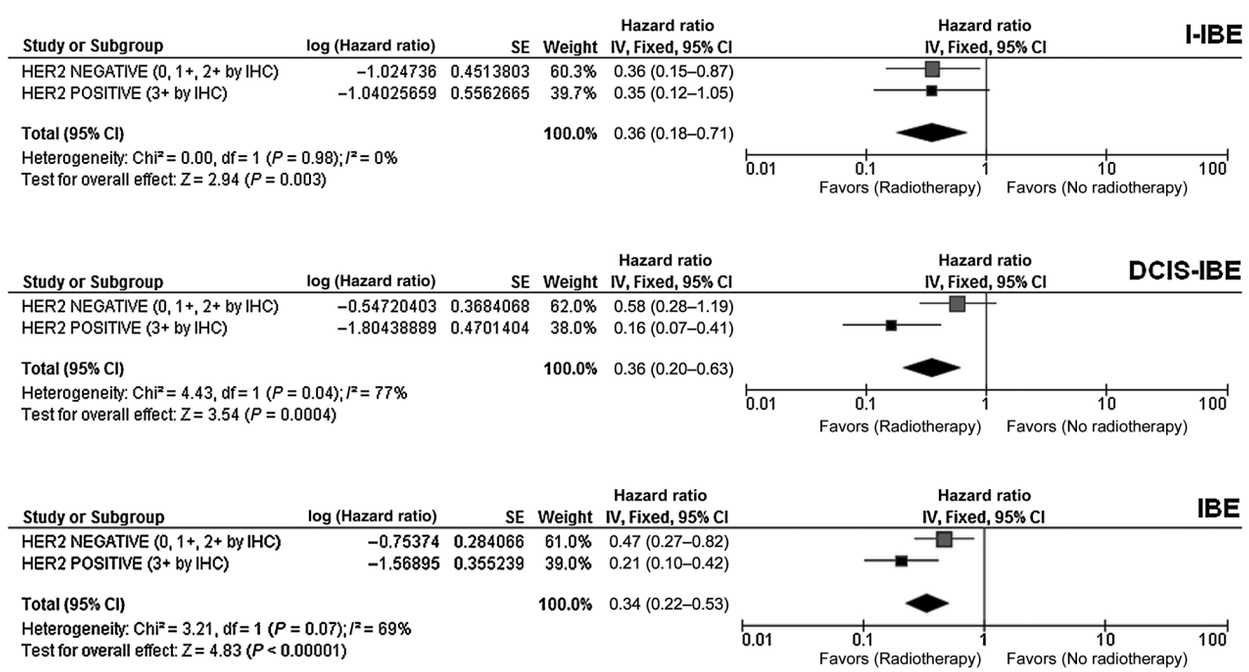

Figure 3.
Effect of radiotherapy by HER2 status.

with *in situ* recurrences (7, 9, 11). Preponderance of *in situ* recurrences (two thirds of all IBEs) in the preliminary results of the NSABP-B43 trial (33) is also consistent with our findings. HER2-equivocal DCIS also showed an increased risk of DCIS-IBE, but the increase was the largest for HER2-positive (IHC 3+) DCIS (Supplementary Table S4). HER2 status was an independent predictor in multivariate analyses (**Table 2**) and inclusion of HER2 improved the multivariate model significantly ($P = 0.0005$).

In contrast to the meta-analysis by Zhang and colleagues (17), Borgquist and colleagues (18) observed that HER2-positive DCIS was associated with a nonsignificantly lower I-IBE risk. Treatment-related confounding may have contributed to such a result, because a greater proportion of HER2-positive (50%) patients in their study received radiotherapy when compared with HER2-negative patients (43%). Visser and colleagues (12) in their well-designed case–control study observed a significantly higher risk of I-IBE (OR = 1.56; 95% CI, 1.05–2.31) in HER2-positive DCIS, an effect size similar to our results (HR = 1.40; 95% CI, 0.81–2.42). In the context of existing evidence, our study provides the necessary confirmatory evidence to now merit use of HER2 as a prognostic biomarker in DCIS.

We found that although HER2-negative DCIS had a much lower risk of any recurrence, the odds of invasive recurrence were actually higher (Supplementary Table S4) similar to a report by Zhou and colleagues (34). Therefore, if HER2-negative DCIS recurs, it has a higher likelihood of being an invasive recurrence than a relapse in HER2-positive DCIS. This finding is congruent with the observation that DCIS adjacent to invasive breast cancer is less often HER2 positive (with gene amplification) than pure DCIS (3, 31, 35), and it could be hypothesized that the majority of invasive breast cancers develop from a HER2-negative precursor, either a purely HER2-negative lesion or from progression and expansion of HER2-negative subclone of DCIS with heterogenous HER2 expression. Although some studies (36, 37) associate HER2 overexpression with invasive foci in DCIS with microinvasion, designs of these studies and potential biases do not support their inference that HER2 overexpression is associated with progression to an invasive stage. Furthermore, a recent study by Visser and colleagues (38) reported that 36% of the HER2-positive DCIS was followed by a HER2-negative invasive recurrence, possibly through progression and expansion of HER2-negative subclone. Therefore, even though HER2 overexpression is associated with increased cell proliferation, the role of HER2 in DCIS progression to invasive breast cancer is unclear. It is likely that in the majority of DCIS, progression to invasive cancer occurs through mechanisms independent of HER2 and that HER2 plays a more important role in initiation of DCIS than its progression (35, 39). An almost 3-fold increase in the risk of DCIS-IBE in HER2-positive DCIS could possibly mean that HER2 overexpression is an early event in the temporal sequence of the development of DCIS and therefore has a higher likelihood of "reoccurring" within the sick lobe (40) or the entire breast, thus leading to the development of what appear to be "new" DCIS lesion/s. The benefit of whole-breast radiotherapy in reducing DCIS-IBE in HER2-positive DCIS could result from eradication of majority of these potential foci. The preliminary results of the NSABP-B43 trial (33) also support the above hypothesis where two doses of trastuzumab resulted in a nonsignificant 32% reduction (HR = 0.68; 95% CI, 0.43–1.08; $P = 0.10$) in the risk of *in situ* recurrences but did not reduce the risk of invasive IBE (HR = 1.11; 95% CI, 0.59–2.10; $P = 0.74$).

The effect sizes of radiotherapy benefit were larger in HER2-positive DCIS compared with HER2-negative DCIS for preventing *in situ* but not invasive recurrence (**Table 3**; **Fig. 3**). Therefore, HER2 status is a predictor of radiotherapy benefit for DCIS-IBE but not I-IBE. The above hypothesis of HER2 not being mechanistically involved in progression to invasive breast cancer is also consistent with absence of differential radiotherapy benefit by HER2 subgroups in reducing I-IBE. The absolute difference in 10-year DCIS-IBE rates with and without radiotherapy in this study was 25.9% versus 3.5% in HER2-positive and HER2-negative DCIS, respectively (Supplementary Table S7). Our findings are consistent with the report by Curigliano and colleagues (11), which indicated that HER2 overexpression had a higher risk of DCIS-IBE and IBE only in patients not receiving radiotherapy. We found that the 10-year DCIS-IBE rates did not differ between HER2-positive (6.0%) and HER2-negative (6.1%) in patients allocated to radiotherapy, but the difference was 22.3% (31.9% vs. 9.6%) in those allocated to no radiotherapy. It is unlikely that another radiotherapy RCT with stratification by HER2 status could be launched to prospectively validate this predictive role of HER2 expression; it could however be considered indirectly externally validated if risk-weighted event rate in the control arm of the NSABP-B43 trial (32) is suggestive of a radiotherapy benefit much larger than the 54% reduction reported in pooled analyses of RCTs (22).

Crosstalk between estrogen receptor (ER) and growth factor pathways and enhanced HER2 signaling has been shown to be associated with tamoxifen resistance in preclinical settings (41). We, therefore explored the interaction between tamoxifen benefit and HER2 expression in ER-positive patients as well. Unlike radiotherapy benefit, tamoxifen benefit did not differ by HER2 status ($P_{\text{heterogeneity}} = 0.58$). This lack of interaction however needs to be interpreted with caution because ER status was available only for the nested case–control component of this cohort thus limiting the power for such analyses. Furthermore, stringent matching by individual treatment allocation strata in the nested case–control component limits the robustness of predictive inferences that can be drawn from this subgroup.

Our analyses show that HER2 status provides independent prognostic information which can prove very valuable in surgical decisions. For example, a patient with a high baseline recurrence risk due to a high-grade HER2-positive DCIS lesion requiring level II oncoplastic procedure with complex localization and a high chance of margin involvement requiring further revision may instead opt for a mastectomy with immediate breast reconstruction and avoid radiotherapy altogether.

Prognostic and predictive information HER2 status provides can also be used to avoid overtreatment as well as undertreatment. Current European Society of Medical Oncology (ESMO) guidelines (42) recommend whole-breast radiotherapy for the majority of women with DCIS treated with BCS. Guidelines also state that in patients with low-risk DCIS (tumor size <10 mm, low/intermediate nuclear grade, adequate surgical margins), omitting radiation can be an option, without an explicit recommendation to omit radiotherapy. Our data of lower IBE risk and less radiotherapy benefit in HER2-negative DCIS clearly identify a large subgroup of these low-risk cases (88% in our study) where radiotherapy can now be safely omitted. Cohort 1 of E5194 trial (43) comprised of low/intermediate grade DCIS measuring up to 25 mm and 12-year IBE rate without radiotherapy was 14.4%. A substantial proportion of these (>10 mm) should receive radiotherapy according to the ESMO guidelines (42). Given that the IBE rate in this E5194 cohort is neither high nor low, adjuvant radiotherapy decisions are difficult. Indeed, due to uncertainty regarding absolute radiotherapy benefit, many centers do not routinely offer radiotherapy in these patients contrary to the ESMO guidelines. HER2 status would help simplify these decisions with radiotherapy offered to HER2-positive patients, and others being spared overtreatment. HER2 status will also help avoid undertreatment in a small proportion of these patients. Cohort 2 of E5194 trial (43) comprised of high-grade DCIS measuring

up to 10 mm with 12-year IBE rate without radiotherapy of 24.6%. A substantial proportion of these events may be driven by HER2-positive lesions as 42% of high-grade DCIS lesions were HER2 positive in our study, with a further 12% being HER2 equivocal. It may be reasonable to consider omitting radiotherapy in HER2-negative patients in this subgroup when other patient factors like smoking exist (44).

Strengths and limitations

A large sample size, random treatment allocation, and long follow-up are the major strengths of this study. These allowed us to assess robustly the prognostic and predictive roles of HER2 expression. Furthermore, sensitivity analyses in a careful case–control design ruled out residual treatment-related confounding. However, selection bias remains a potential limitation of this study; the FFPE blocks included in this study were obtained from 45% of patients enrolled in the trial. Although several characteristics of the participants in this subset were similar to the remaining trial participants, DCIS in the current subset was marginally larger and a higher proportion were high grade compared with the remaining trial participants, perhaps a result of more pathology material being available in these patients to share for research purposes. Furthermore, recruitment in the UK/ANZ DCIS trial started in 1989, almost immediately after initiation of the Breast Screening Programme in the United Kingdom. This resulted in more advanced lesions being found in the prevalence screening round and being enrolled in the first few years of the trial. Unsurprisingly, the proportion of high-grade tumors (68%) in our study is higher compared with other large datasets (11, 29) although not substantially higher than contemporary UK reports (57%; ref. 45). Although such selection bias is likely to affect absolute IBE rates, it is unlikely to distort underpinning biological effects and observed effect sizes. Indeed, the effect sizes observed in our study are consistent with those reported in well-designed studies (12) and meta-analyses (15). The trial investigated the roles of adjuvant treatments in DCIS treated by wide local excision. Although as compared with patients in this trial, the completeness of excision has improved; multivariate analyses including this variable demonstrate independent role of HER2. Therefore, our findings are a robust representation of biological effects and relevant for current practice. Because the UK/ANZ DCIS trial recruited DCIS patients diagnosed through screening, our findings may not be applicable to symptomatically diagnosed patients. *ERBB2* gene-amplification status in DCIS with equivocal HER2 expression was not available to reclassify these tumors. However, our primary analysis approach meant that, if anything, the prognostic effect of HER2 expression may be underestimated. We recently showed prognostic value of estrogen receptor (ER) expression (46) in the nested case–control component with further work planned to evaluate ER expression in the entire BSS1. Therefore, the combined analyses of ER and HER2 in the entire BSS1 are currently not possible and out of the scope of this article. Current lack of ER data in the entire BSS1 also means that absence of interaction between tamoxifen benefit and HER2 expression needs to be interpreted with caution.

Overall, HER2 overexpression in DCIS is associated with adverse histopathologic features and it increases recurrence risk, particularly the risk of *in situ* recurrences. HER2 overexpression is also associated with a greater radiotherapy benefit. Thus, HER2 has a prognostic as well as a predictive role in DCIS.

Conclusions

HER2 status is routinely assessed in invasive breast cancer, but not in DCIS and is not included in pathology minimum datasets for DCIS globally (4, 47). Our results show that the risk of *in situ* recurrence is almost 3-fold higher in HER2-positive DCIS, whereas risk of invasive recurrence is not significantly affected. Furthermore, HER2 is not only an independent prognostic factor in DCIS but it is also predictive of radiotherapy benefit. Radiotherapy reduces recurrence risk by almost 80% in HER2-positive DCIS, whereas the reduction in risk is about 50% in HER2-negative DCIS. Although *in situ* recurrences are not life threatening, they still need surgical intervention, which can be a mastectomy. Therefore, a much higher recurrence risk and a much larger radiotherapy effect in HER2-positive DCIS resulting in an absolute benefit of just over 30% in our study would mean that these patients merit adjuvant radiotherapy. HER2 status can be used to personalize adjuvant radiotherapy decisions. Our results make a strong case for routine evaluation of HER2 expression in DCIS.

Authors' Disclosures

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Authors' Contributions

M.A. Thorat: Conceptualization, formal analysis, funding acquisition, investigation, visualization, methodology, writing–original draft, project administration, writing–review and editing. **P.M. Levey:** Data curation, investigation, methodology. **J.L. Jones:** Data curation, supervision, investigation, visualization, methodology. **S.E. Pinder:** Data curation, investigation, writing–review and editing. **N.J. Bundred:** Data curation, writing–review and editing. **I.S. Fentiman:** Data curation, writing–review and editing. **J. Cuzick:** Data curation, formal analysis, supervision, funding acquisition, methodology, writing–review and editing.

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