Adjuvant letrozole versus tamoxifen according to centrally-assessed ERBB2 status for postmenopausal women with endocrine-responsive early breast cancer: supplementary results from the BIG 1-98 randomised trial

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Summary

Background The Breast International Group (BIG) 1-98 trial (a randomised double-blind phase III trial) has shown that letrozole significantly improves disease-free survival (DFS) compared with tamoxifen in postmenopausal women with endocrine-responsive early breast cancer. Our aim was to establish whether the benefit of letrozole versus tamoxifen differs according to the ERBB2 status of tumours.

Methods The BIG 1-98 trial consists of four treatment groups that compare 5 years of monotherapy with letrozole or tamoxifen, and sequential administration of one drug for 2 years followed by the other drug for 3 years. Our study includes data from the 4922 patients randomly assigned to the two monotherapy treatment groups (letrozole or tamoxifen for 5 years; 51 months median follow-up [range <1 to 90 months]). A central assessment of oestrogen receptor (ER), progesterone receptor (PgR) and ERBB2 status using paraffin-embedded primary tumour material was possible for 3650 (74%) patients. ER, PgR, and ERBB2 expression were measured by immunohistochemistry (IHC) and ERBB2-positivity was confirmed by fluorescence in-situ hybridisation (FISH). Positive staining in at least 1% of cells was considered to show presence of ER or PgR expression. Tumours were deemed ERBB2-positive if amplified by FISH, or, for the few tumours with unassessable or unavailable FISH results, if they were IHC 3+. Hazard ratios (HR) estimated by Cox modelling were used to compare letrozole with tamoxifen for DFS, which was the primary endpoint, and to assess treatment-by-covariate interactions. The BIG 1-98 trial is registered on the clinical trials site of the US National Cancer Institute website http://www.clinicaltrials.gov/ct/show/NCT00004205.

Findings By central assessment 7% (257 of 3650) of tumours were classified as ERBB2-positive. In 3533 patients with tumours confirmed to express ER, DFS was poorer in patients with ERBB2-positive tumours (n=239) than in those with ERBB2-negative tumours (n=3294; HR 2·09 [95% CI 1·59–2·76]; p<0·0001). There was no statistical evidence of heterogeneity in the treatment effect according to ERBB2 status of the tumour (p=0·60 for interaction), thus, letrozole improves DFS compared with tamoxifen regardless of ERBB2 status. The observed HRs were 0·62 (95% CI 0·37–1·03) for ERBB2-positive tumours and 0·72 (0·59–0·87) for ERBB2-negative tumours.

Interpretation A benefit of letrozole over tamoxifen was noted, irrespective of ERBB2 status of the tumour, and, therefore, ERBB2 status does not seem to be a selection criterion for treatment with letrozole versus tamoxifen in postmenopausal women with endocrine-responsive early breast cancer.

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Introduction

In patients with primary breast cancer, the presence of ERBB2, which belongs to the ERBB family of receptor tyrosine kinases, is not only a prognostic factor,[1,2] but also predicts clinical response, mainly by acting as a target for treatment with trastuzumab. However, ERBB2 status also seems to predict clinical outcome in women who receive certain types of chemotherapy[3,4] and tamoxifen,[5] and published reports have shown that tumours that are both oestrogen-receptor (ER) positive and ERBB2 positive are resistant to treatment with tamoxifen.[6] Therefore, patients with such tumours might benefit from treatment with an aromatase inhibitor as an alternative to tamoxifen.

The Breast International Group (BIG) 1-98 study is an international, double-blind, randomised phase III trial that is investigating the aromatase inhibitor, letrozole, compared with tamoxifen in the adjuvant setting, in postmenopausal women with endocrine-responsive early invasive breast cancer. The trial has four treatment groups comparing 5 years of monotherapy with letrozole, 5 years of monotherapy with tamoxifen and the sequential administration of one drug for 2 years followed by the other drug for 3 years. So far, findings have shown that initial treatment with letrozole is better than treatment with tamoxifen in terms of disease-free survival.[7,8]

We did a central pathological assessment of the biological characteristics of the tumours, which included ER and progesterone–receptor (PgR) expression, and ERBB2 status. The results of the ER and PgR review have already been published.[9] In this article, we assess whether

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the beneficial effect of letrozole was especially noted in patients with ERBB2-positive tumours.

Methods

Patients

The BIG 1-98 patient population was defined as postmenopausal women with early breast cancer whose tumours were assessed by local pathologists as either ER-positive or PgR-positive, or both.[7] Between March 18, 1998, and March 23, 2000, patients were randomly assigned to a monotherapy group, and from April 21, 1999, to May 12, 2003, to all four groups. The trial enrolled 8010 patients into the 2-group or 4-group randomisation option. Ethics committees and health authorities for each participating centre approved the study protocol, and all patients provided written informed consent. The primary efficacy analysis[7] was updated as specified by the protocol and reported for the 4922 patients who were randomly assigned to the monotherapy groups at a median follow-up time of 51 months (figure 1).[8] This updated analysis of patients assigned to 5 years' monotherapy with either tamoxifen or letrozole, is used for the current report.

Central pathology review

Retrospective tissue collection at the different centres was carried out in accordance with institutional guidelines and national laws. Funding was provided by the trial's pharmaceutical partner, Novartis (Basel, Switzerland), to cover the costs associated with the central review. The International Breast Cancer Study Group (IBCSG) Central Pathology Laboratory received material from 6549 (82%) of 8010 patients. The material was assessed for histopathological features and expression of tumour markers (ie, ER, PgR, ERBB2, and Ki67) without knowledge of the patients' treatment assignments or outcomes. Assessable data were obtained for 6291 (79%) of 8010 patients. Material was submitted for 3807 (77%) of 4922 patients who were randomly assigned to the monotherapy groups and was assessable for 3650 (74%).

Tumours were assessed for ER and PgR expression by immunohistochemistry (IHC) as previously described.[9,10] ER and PgR expression were recorded as the percentage of stained cells and categorised by dichotomising IHC expression into present (≥1% stained cells) or absent (<1%).[9,10] ERBB2 immunoreactivity was assessed for all tumours by use of the US Food and Drug Administration (FDA)-approved HercepTest kit (Dako, Glostrup, Denmark), as recommended by the manufacturer. Tumours were scored for intensity of immunostaining, completeness of cell-membrane staining, and percentage of immunoreactive neoplastic cells, by use of a four-tier scale from 0 to 3+ as recommended.[11] Only tumours showing 3+ staining (ie, circumferential and intense membrane staining of >10% invasive tumour cells) were considered to be positive for ERBB2 overexpression. Fluorescence in-situ hybridisation (FISH) was subsequently done using the FDA-approved PathVysion HER-2 DNA Probe Kit according to the recommendations of the manufacturer (Abbott Molecular-Vysis, Chicago, IL), to confirm IHC data with a second unrelated assay or to avoid false-positive IHC results, on 1006 tumours that were scored as IHC 2+ or 3+, IHC 1+ with at least 50% immunoreactive cells, or that were not assessable with IHC for technical reasons (eg, staining failure or detachment of the tissue section from slides during the assay). An *ERBB2* gene-to-chromosome 17 ratio of at least 2·0 was deemed as *ERBB2* amplification. Tumours were considered ERBB2-positive if amplified by FISH, or, in the few tumours with unassessable or unavailable FISH results, if IHC was 3+ (18 of 3650 [0·5%]).

To ensure intraobserver and interobserver reliability of the central assessment, all of the samples were assessed by one of two pathologists (MGM and GV). Each reviewed 5% of their own assessments (intraobserver control) and 10% of the other pathologist's assessments (interobserver control). In the case of discrepancies, collegial reassessment by use of a multi-headed microscope was done by the two pathologists until an agreement was reached; this reassessment was required for about 22 (0·6%) tumours.

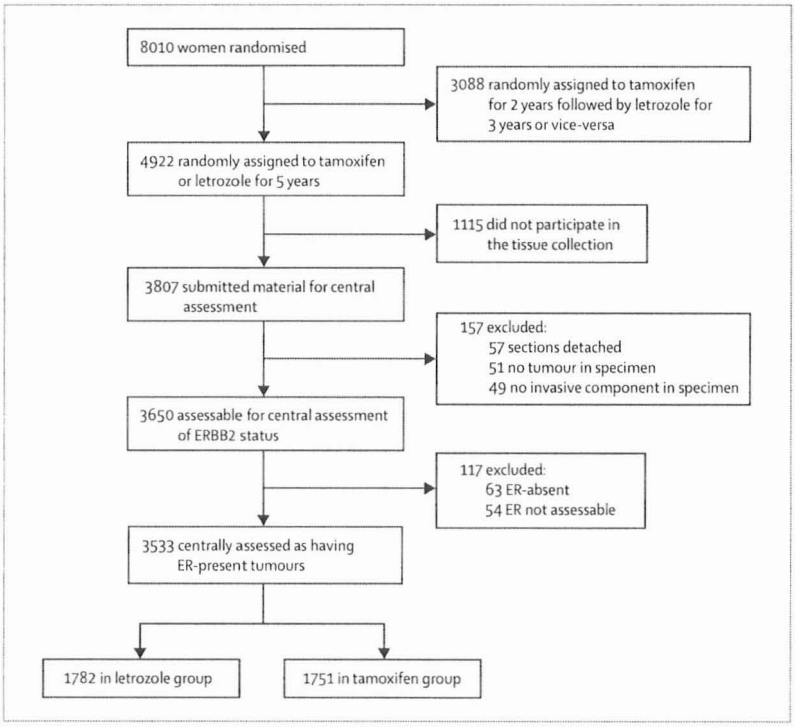

Figure 1: **Patients from the BIG 1-98 trial included and excluded in this study according to treatment group and availability of tumour material**
ER=oestrogen receptor.

Statistical analysis

A comparison of patients for whom material was and was not available for central review has been reported.[9]

Those with available material (around three-quarters of the total) had been enrolled more recently at centres with a more accurate local identification of ER-positive status, had a better overall disease-free survival, and had a larger treatment effect favouring letrozole than those without material. Therefore, the assessment of patients with available material is likely to be a more accurate indication of modern practice than the assessment of all patients, including those without available material.

The association of ERBB2-positivity with patient and tumour characteristics was assessed using Fisher's exact test and Wilcoxon rank sum tests. The protocol specified primary endpoint was disease-free survival (DFS), which was defined as the time from randomisation to the earliest time of invasive recurrence; a new invasive breast cancer in the contralateral breast; any second (non-breast) malignancy; or death from any cause.[7] The distribution of DFS and 4-year DFS percentages were estimated by use of the Kaplan-Meier method. Cox proportional hazards regression (stratified for randomisation option [ie, two-group or four-group] and chemotherapy use) was used to estimate hazard ratios (HR) and 95% confidence intervals (CIs), and to assess interactions of the treatment effect according to subgroups defined by ERBB2 and PgR status. The non-parametric Subpopulation Treatment Effect Pattern Plot (STEPP) method[12] was used to investigate trends in the difference of treatment effects across the continuum of PgR expression. The STEPP uses a sliding-window to define several overlapping subpopulations of fixed numbers of patients on the basis of the covariate of interest (in this case PgR expression) and to study the resulting pattern of the treatment effects estimated within each subpopulation. The plot's x-axis shows the median PgR value and the y-axis shows the treatment effects—measured here as 4-year DFS—from data of patients in each subpopulation. In patients with ERBB2-negative tumours, the analysis used subpopulations of about 350 patients with subsequent subpopulations changing by 50 patients; the analysis of patients having ERBB2-positive tumours used subpopulations of about 50 patients with subsequent subpopulations changing by ten patients. Statistical analyses used SAS version 9·1 and S-PLUS version 6·1. All statistical tests provided two-sided p values and p-values less than or equal to 0·05 were considered statistically significant. The BIG 1-98 trial is registered on the clinical trials site of the US National Cancer Institute website http://www.clinicaltrials.gov/ct/show/NCT00004205.

Role of the funding source

The IBCSG is responsible for the study design and coordination, data collection and management, tissue management and central pathology assessment, data analysis, and reporting of the findings (including the decision to publish). Novartis (Basel, Switzerland), the manufacturer of letrozole, provided financial support for the collection of pathology material and imposed no restrictions on the investigators in terms of trial data. The manuscript was prepared by all authors, who had full access to the data (MMR had access to raw data) and who made final decisions on content. The Steering Committee (including a minority membership of Novartis employees) reviewed the paper and offered changes.

	ERBB2-negative (N=3393)	ERBB2-positive (N=257)	p value*
Tumour size			0·007
≤2 cm	2126 (62·7%)	139 (54·1%)	
>2 cm	1250 (36·8)	117 (45·5)	
Unknown	17 (0·5)	1 (0·4)	
Tumour grade, n (%)			<0·0001
1	965 (28·4)	15 (5·8)	
2	1635 (48·2)	118 (45·9)	
3	461 (13·6)	96 (37·4)	
Unknown	332 (9·8)	28 (10·9)	
Lymph-node status, n (%)			0·10
Not assessed/unknown	199 (5·9)	18 (7·0)	
Negative	1770 (52·2)	118 (45·9)	
Positive	1424 (42·0)	121 (47·1)	
ER expression†			<0·0001
Median % (interquartile range)	90 (90–99)	85 (50–95)	
0%, n (%)	50 (1·5)	13 (5·1)	
≥1%, n (%)	3294 (97·1)	239 (93·0)	
Not assessable, n (%)	49 (1·4)	5 (1·9)	
PgR expression†			<0·0001
Median % (interquartile range)	70 (15–90)	10 (0–68)	
0%, n (%)	363 (10·7)	71 (27·6)	
≥1%, n (%)	2981 (87·9)	181 (70·4)	
Not assessable, n (%)	49 (1·4)	5 (1·9)	

*Two-sided p values from Fisher's exact test (tumour size, tumour grade, and lymph-node status) or Wilcoxon rank sum tests (oestrogen receptor [ER]% and progesterone receptor [PgR]%). †ER expression and PgR expression assessed centrally and recorded as percentage of stained cells.

Table 1: **Tumour characteristics of patient population according to ERBB2 status**

Results

A total of 3650 (74%) of 4922 patients who were randomly assigned to the monotherapy groups had assessable material for central review, and in 7% of patients (257 of 3650) tumours were identified to be ERBB2-positive. Patients with ERBB2-positive tumours were younger than those with ERBB2-negative tumours (median age 60 years *vs* 61 years respectively, p=0·04) and were treated more frequently with mastectomy (138 of 257 [54%] *vs* 1485 of 3393 [44%] respectively; p=0·005) and chemotherapy (83 of 257 [32%] *vs* 700 of 3393 [21%]; p<0·0001). ERBB2-positivity was associated with larger tumour size and higher tumour grade (both p<0·01), but not with positive lymph-node status (p=0·10), and was associated with lower ER and PgR expression (both p<0·0001; table 1).

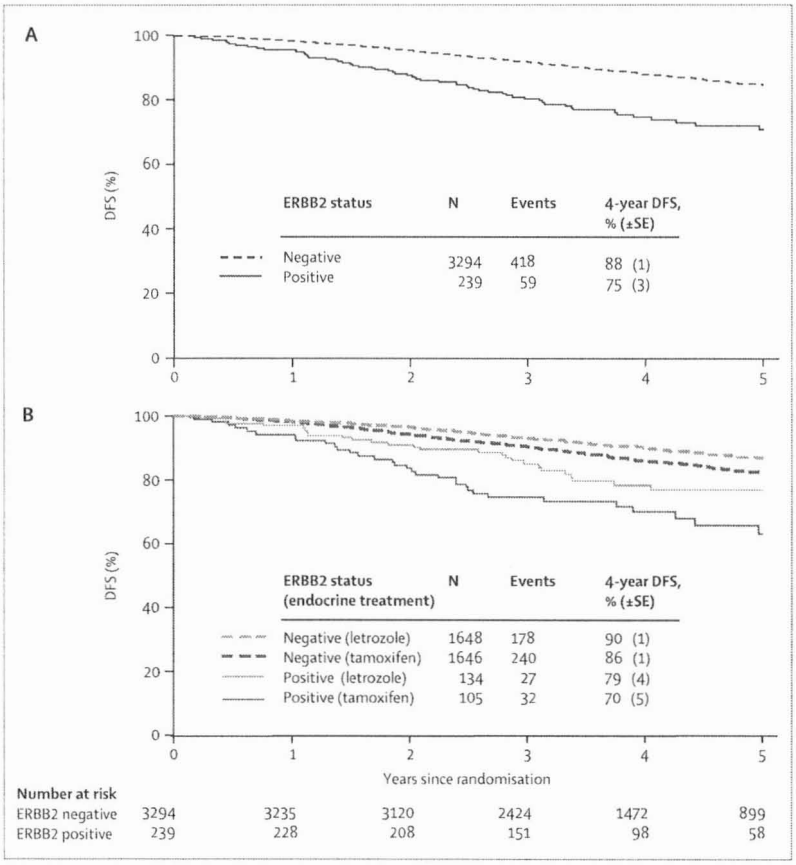

Figure 2: Disease-free survival (DFS) according to ERBB2 status in all patients with tumours confirmed to be oestrogen-receptor present (A) and the same group according to treatment (B)

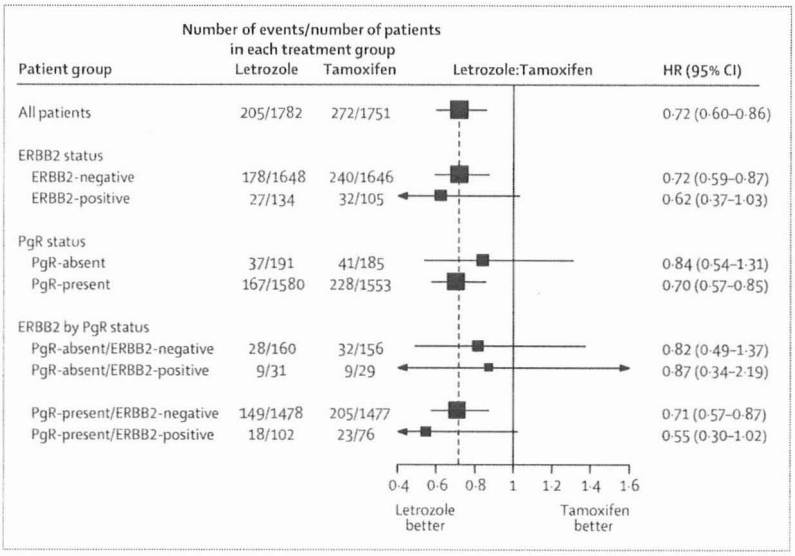

Figure 3: Hazard ratios (HR) and 95% CIs for disease-free survival when comparing the efficacy of letrozole versus tamoxifen

Box size is inversely proportional to standard error of HR and extending horizontal lines indicate 95% CI. The HR for letrozole versus tamoxifen was 0·82 (95% CI 0·71–0·95) for all 4922 patients randomly assigned to the monotherapy groups.[8] PgR=progesterone receptor.

To investigate the association of ERBB2 status and treatment with DFS in endocrine-responsive disease, analyses were restricted to the 3533 patients who were confirmed by central assessment as having ER-expressing tumours, as previously described.[9] ERBB2 status was shown to be associated with DFS; the 239 (6·8%) patients whose tumours were ERBB2-positive had a poorer outcome than those whose tumours were ERBB2-negative (HR 2·09 [95% CI 1·59–2·76]; p<0·0001), with an estimated 4-year DFS of 75% (95% CI 68–80) and 88% (87–89) in the two groups, respectively (figure 2A). No statistical evidence of heterogeneity in the treatment effect according to ERBB2 status of the tumour existed (p=0·60 for interaction). Letrozole improved DFS compared with tamoxifen regardless of ERBB2 status (ERBB2-positive tumours: HR 0·62 [95% CI 0·37–1·03]; ERBB2-negative tumours: HR 0·72 [0·59–0·87]; figure 2B). These findings were consistent in models that controlled for other prognostic tumour characteristics (ie, tumour size, tumour grade, and ER and PgR expression) with an adjusted HR for ERBB2 status of 1·69 (95% CI 1·27–2·25).

PgR status of the tumour was associated with DFS (p<0·0001),[9] but no statistical evidence of heterogeneity in the treatment effect existed (p=0·47 for interaction; figure 3), which was also apparent in the STEPP analysis,[9] suggesting a consistent benefit of letrozole over tamoxifen regardless of PgR expression. We investigated—with the caveat of smaller numbers of patients in subgroups—whether evidence existed of heterogeneity of the treatment effect according to PgR and ERBB2 status of the tumour. There was no statistically significant interaction (p=0·63 for interaction; figure 3), suggesting improved DFS with letrozole compared with tamoxifen, regardless of PgR or ERBB2 status. These subgroup analyses focused attention on the estimated HRs and CIs to assess whether any patient cohorts had results that were greatly different from the overall treatment outcome (as shown by the dashed line in figure 3). The STEPP method further shows the benefit of letrozole versus tamoxifen across the continuum of PgR expression (ie, from low to high expression), both in patients with ERBB2-negative tumours (figure 4A) and those with ERBB2-positive tumours (figure 4B).

Discussion

This study consists of 3650 patients who were randomly assigned to adjuvant endocrine treatment with letrozole or tamoxifen for 5 years in the BIG 1-98 trial and on whom we have done a central review of ERBB2, ER, and PgR status by assessment of primary tumour tissue. Positive hormone-receptor status as assessed by institutional pathology was mandatory for trial entry and was confirmed for most patients.[7] We identified only 257 (7·0%) patients with tumours positive for ERBB2,

which is consistent with the well-documented inverse relation between ERBB2-positivity and a positive hormone-receptor status.[13] After central pathological review, 63 tumours were reclassified as ER, and also PgR, absent and 13 (20·6%) of these were ERBB2-positive, which is consistent with the published work for an absent hormone-receptor cohort.[13]

Despite the low prevalence of ERBB2 amplification or overexpression in the centrally confirmed ER-present tumours, we have been able to confirm the prognostic significance of a positive ERBB2 status. Patients with ERBB2-positive tumours had a significantly worse DFS than those who had ERBB2-negative tumours. A significant relation existed between positive ERBB2 status and other poor prognostic factors, such as large tumour size, high tumour grade, and low ER and PgR expression, but the association of ERBB2 status with DFS was independent of these factors.

We did not find any evidence for heterogeneity of the treatment effect according to ERBB2 status, with letrozole being better than tamoxifen in patients with ERBB2-positive tumours and with ERBB2-negative tumours. Although the confidence interval for the treatment effect in the ERBB2-positive cohort is wide and crosses 1·0, there is no evidence that the effect for this cohort differs from that noted for the ERBB2-negative cohort (interaction p=0·60). Subgroup analyses should focus on differences in treatment effects around the overall result (vertical dashed line in figure 3), and not on rejecting the null hypothesis tests relative to 1·0 within each subgroup. In view of the small number of ERBB2-positive tumours, the power to detect an interaction between treatment effect and ERBB2 status was about 80% for the scenario in which the treatment HRs were 0·8 for ERBB2-negative and 0·4 for ERBB2-positive tumours (given the average observed effect was 0·72).

Both in laboratory tests and in clinical trials, the decreased efficacy of tamoxifen in relation to ERBB2-positivity has been reported to be confined to the group of patients with PgR-negative tumours.[14,15] Our findings do not confirm this differential effect, because the benefit of letrozole over tamoxifen was evident not only in patients with ERBB2-positive, PgR-absent tumours, but also in the group of patients that had PgR-present tumours (figures 3 and 4). In a previous article, we reported that patients with tumours that expressed both ER and PgR and patients with tumours that expressed ER only benefited from treatment with letrozole.[9] A central analysis of samples from about a third of the patients in the Arimidex, Tamoxifen, Alone, or in Combination (ATAC) trial is consistent with our findings: no significant difference was noted in the relative benefit of anastrozole versus tamoxifen according to PgR status (Mitch Dowsett, The Royal Marsden NHS Trust, London, UK, personal communication), which is in contrast to the findings originally reported for the ATAC study.[16] Our study, based on a central pathological review of three-quarters of the patients enrolled in the BIG 1-98 trial, noted that the benefit of letrozole over tamoxifen in patients with ER-present tumours is consistent, regardless of PgR and ERBB2 status, and neither PgR nor ERBB2 status alone or together should be used as discriminators in selecting initial adjuvant endocrine treatment for postmenopausal women with endocrine-responsive primary breast cancer.

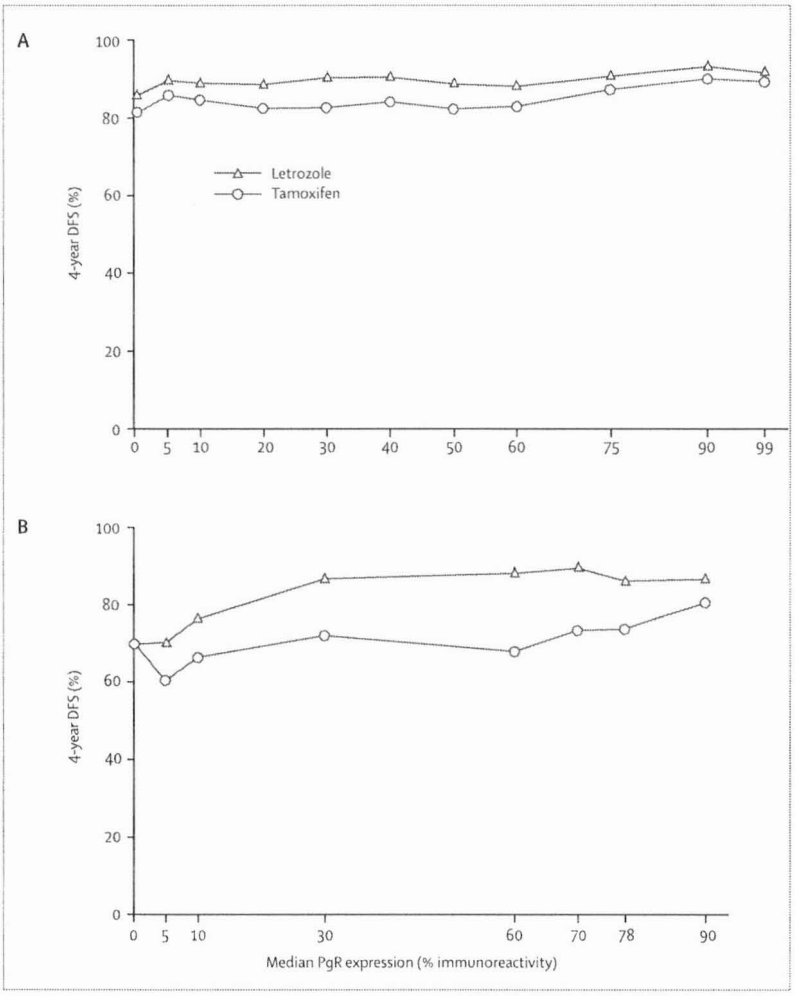

Figure 4: Subpopulation Treatment Effect Pattern Plot analysis of 4-year disease-free survival (DFS) according to progesterone-receptor (PgR) expression in tumours confirmed to express oestrogen receptor for ERBB2-negative (A) and ERBB2-positive (B) tumours

Contributors

BBR, AEL, GV, AG, ASC, RDG, and MCG were responsible for the design of the study. BBR, AEL, KLH, MGM, EM, MLT, SB, HJA, BT, AG, and GV provided patient material and patient data. GV, PDO, BDC, MGM and BAG were responsible for the central pathology review and MMR, RDG, and KNP did the data analysis. MCG and KNP were responsible for administration and BBR, MMR, GV, and ASC formed the primary writing team. All authors were involved in the final review and provided approval.

Conflicts of interest

BT is the owner of shares with Novartis (Basel, Switzerland) and ASC has been paid travel expenses by Novartis for reporting findings of the BIG 1-98 trial. All other authors declared no conflicts of interest.

Acknowledgments

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See Online for webappendix

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