Adjuvant paclitaxel and trastuzumab for node-negative, HER2-positive breast cancer: final 10-year analysis of the open-label, single-arm, phase 2 APT trial

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Summary

Background We aimed to report on long-term outcomes of patients with small, node-negative, HER2-positive breast cancer treated with adjuvant paclitaxel and trastuzumab and to establish potential biomarkers to predict prognosis.

Methods In this open-label, single-arm, phase 2 study, patients aged 18 years or older, with small (≤3 cm), node-negative, HER2-positive breast cancer, and an Eastern Cooperative Oncology Group performance status of 0–1, were recruited from 16 institutions in 13 cities in the USA. Eligible patients were given intravenous paclitaxel (80 mg/m²) with intravenous trastuzumab (loading dose of 4 mg/kg, subsequent doses 2 mg/kg) weekly for 12 weeks, followed by trastuzumab (weekly at 2 mg/kg or once every 3 weeks at 6 mg/kg) for 40 weeks to complete a full year of trastuzumab. The primary endpoint was 3-year invasive disease-free survival. Here, we report 10-year survival outcomes, assessed in all participants who received protocol-defined treatment, with exploratory analyses using the HER2DX genomic tool. This study is registered on ClinicalTrials.gov, NCT00542451, and is closed to accrual.

Findings Between Oct 29, 2007, and Sept 3, 2010, 410 patients were enrolled and 406 were given adjuvant paclitaxel and trastuzumab and included in the analysis. Mean age at enrolment was 55 years (SD 10·5), 405 (99·8%) of 406 patients were female and one (0·2%) was male, 350 (86·2%) were White, 28 (6·9%) were Black or African American, and 272 (67·0%) had hormone receptor-positive disease. After a median follow-up of 10·8 years (IQR 7·1–11·4), among 406 patients included in the analysis population, we observed 31 invasive disease-free survival events, of which six (19·4%) were locoregional ipsilateral recurrences, nine (29·0%) were new contralateral breast cancers, six (19·4%) were distant recurrences, and ten (32·3%) were all-cause deaths. 10-year invasive disease-free survival was 91·3% (95% CI 88·3–94·4), 10-year recurrence-free interval was 96·3% (95% CI 94·3–98·3), 10-year overall survival was 94·3% (95% CI 91·8–96·8), and 10-year breast cancer-specific survival was 98·8% (95% CI 97·6–100). HER2DX risk score as a continuous variable was significantly associated with invasive disease-free survival (hazard ratio [HR] per 10-unit increment 1·24 [95% CI 1·00–1·52]; p=0·047) and recurrence-free interval (1·45 [1·09–1·93]; p=0·011).

Interpretation Adjuvant paclitaxel and trastuzumab is a reasonable treatment standard for patients with small, node-negative, HER2-positive breast cancer. The HER2DX genomic tool might help to refine the prognosis for this population.

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Introduction

Patients with early-stage HER2-positive breast cancer derive substantial survival benefits from adjuvant trastuzumab in combination with chemotherapy.[1] Clinical practice guidelines from the National Comprehensive Cancer Network (NCCN) recommend use of docetaxel and carboplatin in combination with trastuzumab (with or without pertuzumab) for patients with stage II–III disease.[2] Few patients with stage I HER2-positive breast cancer were included in the large, randomised, phase 3 trials that reported the benefit of adjuvant trastuzumab with chemotherapy in early-stage, HER2-positive breast cancer.[1] However, patients with untreated, small, node-negative, HER2-positive breast tumours have recurrence rates that range from 5% to 30%,[3–5] and retrospective evidence suggested that adjuvant chemotherapy and trastuzumab might be beneficial in this setting.[6,7]

The Adjuvant Paclitaxel and Trastuzumab (APT) trial[8] prospectively investigated the safety and efficacy of 12 weeks of paclitaxel with trastuzumab, followed by 9 months of trastuzumab monotherapy, in patients with small (≤3 cm), node-negative, HER2-positive breast cancer. After a median follow-up of 4 years, the 3-year invasive disease-free survival was 98·7% (95% CI

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See **Comment** page 200

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See Online for appendix

Research in context

Evidence before this study

HER2-positive breast cancers harbour a particularly poor prognosis compared with HER2-negative tumours if left untreated. However, the blockade of HER2 with trastuzumab, when added to adjuvant multiagent chemotherapy, has been shown to improve outcomes for this population. Few patients with small, node-negative, HER2-positive tumours were included in the pivotal trials of trastuzumab, warranting dedicated trials for this lower risk population. We searched PubMed for articles on the adjuvant treatment of small, node-negative, HER2-positive breast cancer, without language restrictions, from database inception to Nov 29, 2022, using the search terms "small" AND "HER2-positive" AND "adjuvant" AND "clinical trial" AND "breast cancer". We identified 23 separate entries in PubMed, including two (Adjuvant Paclitaxel and Trastuzumab [APT] and ATEMPT) phase 2 adjuvant clinical trials, one testing the activity of paclitaxel and trastuzumab and one testing trastuzumab emtansine, and a subanalysis of the ALTTO phase 3 trial evaluating outcomes for this population when treated with multiagent chemotherapy plus trastuzumab or lapatinib, or both. All trials found good survival outcomes for this population of patients with adjuvant regimens, including trastuzumab or trastuzumab emtansine, but a lower disease-free survival rate was observed in ALTTO for patients who only received lapatinib. Additionally, the previous results of the phase 2 APT study, at a median follow-up of 6·5 years, showed that adjuvant paclitaxel and trastuzumab had a 7-year invasive disease-free survival of 93·3% in patients with small, node-negative, HER2-positive breast cancer. Given that two-thirds of the patients enrolled in this trial had hormone receptor-positive disease, which is associated with late recurrences, longer follow-up was warranted to assess the effectiveness of the APT regimen.

Added value of this study

To our knowledge, this is the first study to report the long-term outcomes of patients with small, node-negative, HER2-positive breast cancers prospectively treated with a de-escalated adjuvant regimen. Our study showed that 12 weeks of treatment with paclitaxel and trastuzumab, followed by 9 months of trastuzumab monotherapy, had very good long-term outcomes for this population, with few distant recurrences and very good tolerability.

Implications of all the available evidence

The APT regimen is currently endorsed by most international guidelines as a standard adjuvant regimen for patients with small, node-negative, HER2-positive breast cancer. This end-of-study analysis supported that paclitaxel and trastuzumab is an appropriate regimen for most patients, and found a small population of patients, identified in exploratory analysis, with a high HER2DX score or a luminal B intrinsic subtype, who might harbour an increased risk of recurrence. If validated in further cohorts, these biomarkers might aid treatment tailoring for patients with small, node-negative, HER2-positive tumours.

97·6–99·8), and the occurrence of serious adverse events was low.[8] A subsequent analysis after a median follow-up of 6·5 years reported a 7-year invasive disease-free survival of 93·3% (95% CI 90·4–96·2) and a 7-year recurrence-free interval (which includes distant recurrence, death from breast cancer, and invasive locoregional recurrence) of 97·5% (95% CI 95·9–99·1).[9] Based on these results, adjuvant paclitaxel with trastuzumab has become a standard treatment option for patients with small, node-negative, HER2-positive breast cancer, and is endorsed by standard international guidelines, including the NCCN guidelines,[2] the European Society of Medical Oncology guidelines,[10] and St Gallen International Consensus Guidelines.[11] Notably, most patients in the APT trial had hormone receptor-positive, HER2-positive breast cancer,[8] which is associated with late recurrences,[12] thus necessitating longer follow-up. In this planned final analysis of the APT trial, we report the 10-year invasive disease-free survival, recurrence-free interval, breast cancer-specific survival, and overall survival. Correlative analyses included 10-year outcomes according to stromal tumour-infiltrating lymphocytes (sTILs), intrinsic subtypes based on the 50-gene set Prediction Analysis of Microarray 50 (PAM50), and according to the HER2DX genomic test.

Methods

Study design and participants

The APT study was a multicentre, single-arm, investigator-initiated, phase 2 trial. Details of the trial design and study population have been previously reported, as well as 3-year[8] and 7-year[9] interim results; here, we report the final 10-year analysis. Patients were recruited from 16 institutions in 13 cities in the USA (appendix p 17). Eligible patients were aged 18 years or older, with HER2-positive breast cancer, pathologically confirmed by local testing through immunohistochemical staining for the HER2 protein of 3+ intensity or amplification of the *HER2* (also known as *ERBB2*) gene of 2·0 or higher on fluorescence in-situ hybridisation. The primary invasive tumour was to measure 3·0 cm or smaller in the greatest dimension. Initially, participants were required to have node-negative disease (as defined by the American Joint Committee on Cancer 7th edition); however, the protocol was amended on June 2, 2009, after 188 patients had been enrolled, to allow inclusion of patients with a single micrometastatic node. Patients were required to have a left ventricular ejection fraction (LVEF) of a least 50%, an Eastern Cooperative Oncology Group performance status of 0 or 1, no previous myocardial infarction or uncontrolled hypertension, no neuropathy worse than grade 1, no

history of invasive breast cancer, and no previous malignancy within 5 years (except for early-stage tumours of the skin or cervix treated with curative intent) before study entry. Patients were required to have had surgery with modified radical mastectomy or lumpectomy, with either a sentinel node biopsy or axillary dissection. Up to 4 weeks of adjuvant endocrine treatment were permitted before enrolment, whereas no previous chemotherapy or trastuzumab therapy were permitted. Full eligibility criteria are listed in the protocol (appendix).

This study was conducted in accordance with the International Conference on Harmonization Good Clinical Practice Standards and the Declaration of Helsinki. All patients provided written informed consent before participating in this study. The study protocol, informed consent document, and relevant supporting information were approved by the Institutional Review Board (IRB) at Dana-Farber Cancer Institute (Boston, MA, USA) and at all participating institutions before the study was initiated. The principal investigator was responsible for providing an annual report to the Dana-Farber Cancer Institute IRB and informing the IRB of any changes made to the protocol as deemed appropriate. Investigators immediately notified their respective IRB about any serious, unexpected adverse events. Monitoring of safety and efficacy was also done on a semiannual basis by the Dana-Farber Cancer Institute Data Safety Monitoring Board (DSMB). The exact timing of interim analyses was determined by the DSMB meeting schedule.

Procedures

Patients were given weekly intravenous paclitaxel 80 mg/m² plus weekly intravenous trastuzumab (loading dose of 4 mg/kg, subsequent doses 2 mg/kg) for 12 weeks. Thereafter, patients were given either weekly trastuzumab (2 mg/kg) or trastuzumab once every 3 weeks (6 mg/kg) for 40 weeks to complete 1 year of adjuvant therapy per investigator choice. Patients who had breast-conserving surgery received either partial breast irradiation before the start of protocol therapy or whole breast irradiation after the completion of paclitaxel. Patients with hormone receptor-positive disease were allowed to receive adjuvant endocrine therapy after the completion of paclitaxel.

All patients adhered to the same follow-up schedule. LVEF was assessed at baseline and at 12 weeks, 6 months, and 1 year after the start of protocol therapy by echocardiography or multitargeted acquisition scanning. Clinical assessments (physical examination, vital signs, and appropriate breast screening) after completing trastuzumab monotherapy were scheduled every 6 months for the first 4 years and then annually during years 5–10 after study enrolment or after the primary end point was reached.

The standardised HER2DX genomic test[13] was done at the Hospital Clinic (Barcelona, Spain) on available RNA

	All patients (N=406)	PAM50 (n=278)*	sTILs (n=284)	HER2DX (n=284)
Age, years				
Mean (SD)	55 (10·5)	56 (10·7)	56 (10·7)	56 (10·6)
<50	134 (33·0%)	79 (28·4%)	80 (28·2%)	83 (29·2%)
50–59	136 (33·5%)	101 (36·3%)	99 (34·9%)	102 (35·9%)
60–69	96 (23·6%)	65 (23·4%)	72 (25·4%)	69 (24·3%)
≥70	40 (9·9%)	33 (11·9%)	33 (11·6%)	30 (10·6%)
Sex				
Female	405 (99·8%)	277 (99·6%)	283 (99·6%)	283 (99·6%)
Male	1 (0·2%)	1 (0·4%)	1 (0·4%)	1 (0·4%)
Race				
White	350 (86·2%)	242 (87·1%)	240 (84·5%)	243 (85·6%)
Black or African American	28 (6·9%)	17 (6·1%)	24 (8·5%)	21 (7·4%)
Asian	11 (2·7%)	8 (2·9%)	10 (3·5%)	10 (3·5%)
Other	17 (4·2%)	11 (4·0%)	10 (3·5%)	10 (3·5%)
Ethnicity				
Non-Hispanic	371 (91·4%)	253 (91·0%)	260 (91·5%)	260 (91·5%)
Hispanic or Latino	8 (2·0%)	5 (1·8%)	6 (2·1%)	5 (1·8%)
Not known	27 (6·7%)	20 (7·2%)	18 (6·3%)	19 (6·7%)
Cancer type				
Invasive ductal	381 (93·8%)	258 (92·8%)	265 (93·3%)	265 (93·3%)
Invasive lobular	13 (3·2%)	10 (3·6%)	9 (3·2%)	9 (3·2%)
Both	12 (3·0%)	10 (3·6%)	10 (3·5%)	10 (3·5%)
Grade				
I	44 (10·9%)	26 (9·4%)	27 (9·5%)	29 (10·2%)
II	131 (32·3%)	88 (31·7%)	91 (32·0%)	91 (32·0%)
III	228 (56·1%)	164 (59·0%)	166 (58·5%)	164 (57·7%)
Unknown	3 (0·7%)	0	0	0
Disease stage				
1	355 (87·4%)	241 (86·7%)	246 (86·6%)	242 (85·2%)
2	51 (12·6%)	37 (13·3%)	38 (13·4%)	42 (14·8%)
ECOG performance status				
0	362 (89·2%)	249 (89·6%)	252 (88·7%)	250 (88·0%)
1	44 (10·8%)	29 (10·4%)	32 (11·3%)	34 (12·0%)
Hormone receptor status				
Positive	272 (67·0%)	196 (70·5%)	193 (68·0%)	200 (70·4%)
Negative	134 (33·0%)	82 (29·5%)	91 (32·0%)	84 (29·6%)
HER2 status				
Positive	403 (99·3%)	276 (99·3%)	281 (98·9%)	281 (98·9%)
Negative	3 (0·7%)	2 (0·7%)	3 (1·1%)	3 (1·1%)
Premenopausal				
Yes	76 (18·7%)	45 (16·2%)	49 (17·3%)	48 (16·9%)
No	330 (81·3%)	233 (83·8%)	235 (82·7%)	236 (83·1%)
TNM stage				
T stage				
T1mi	10 (2·5%)	1 (0·4%)	3 (1·1%)	1 (0·4%)
T1a	68 (16·7%)	29 (10·4%)	38 (13·4%)	32 (11·3%)
T1b	123 (30·3%)	81 (29·1%)	83 (29·2%)	88 (31·0%)
T1c	169 (41·6%)	137 (49·3%)	132 (46·5%)	132 (46·5%)
T2	36 (8·9%)	30 (10·8%)	28 (9·9%)	31 (10·9%)

(Table 1 continues on next page)

	All patients (N=406)	PAM50 (n=278)*	sTILs (n=284)	HER2DX (n=284)
(Continued from previous page)				
N stage				
N0	400 (98·5%)	274 (98·6%)	280 (98·6%)	280 (98·6%)
N1mi	6 (1·5%)	4 (1·4%)	4 (1·4%)	4 (1·4%)
PAM50 subtype†				
Basal-like	22 (5·9%)	22 (7·9%)	17 (7·3%)	20 (7·9%)
HER2 enriched	183 (65·8%)	183 (65·8%)	153 (65·7%)	166 (65·4%)
Luminal A	35 (12·6%)	35 (12·6%)	28 (12·0%)	34 (13·4%)
Luminal B	38 (13·7%)	38 (13·7%)	35 (15·0%)	34 (13·4%)
Missing or unknown	128	0	51	30
sTILs†				
Low (1 to ≤10%)	184 (64·8%)	151 (64·8%)	184 (64·8%)	152 (65·5%)
Medium (>10 to 50%)	81 (28·5%)	69 (29·6%)	81 (28·5%)	68 (29·3%)
High (>50%)	19 (6·7%)	13 (5·6%)	19 (6·7%)	12 (5·2%)
Missing or unknown	122	45	0	52

Data are n, n (%), or mean (SD). ECOG=Eastern Cooperative Oncology Group. PAM50=Prediction Analysis of Microarray 50. sTIL=stromal tumour-infiltrating lymphocyte. *Patient characteristics of the non-PAM50-tested group were previously published.[9] †Percentages shown are with respect to the number non-missing.

Table 1: **Baseline patient and disease characteristics**

from baseline archival formalin-fixed paraffin-embedded (FFPE) tumour tissue. This is a single 27-gene expression and clinical feature-based classifier developed for early-stage, HER2-positive breast cancer. The HER2DX risk score is based on the expression of three gene signatures tracking immune or immunoglobulin (IgG) features, tumour cell proliferation, and luminal differentiation (appendix p 5), and provides a score (from 0 to 100, with a higher score indicating a higher risk of relapse) to predict long-term risk of relapse. Genomic analyses were done by investigators who were masked to clinical outcome data. The main objective of the HER2DX analyses was to evaluate the association of the HER2DX risk score with invasive disease-free survival and recurrence-free interval when assessed as a continuous variable. As secondary objectives, we assessed a pre-established HER2DX risk score to define low risk (ie, a score of <50) and high risk (ie, a score of ≥50).[13]

Details regarding the PAM50 and sTILs analyses have been previously reported.[9] For PAM50 determinations, total RNA was isolated from 5 mm-thick FFPE slides for each available tumour sample using the Qiagen FFPE kit for RNA isolation from FFPE tissue (Qiagen; Hilde, Germany). PAM50 gene expression analysis was done on the nCounter gene expression platform (NanoString Technologies; Seattle, WA, USA). Data were analysed using the Prosigna algorithm (NanoString Technologies) to determine the intrinsic subtype calls (luminal A, luminal B, HER2 enriched, or basal-like).[14,15] Quality assessment and normalisation were done in nSolver (version 4.0), as per the manufacturer's instructions. We assessed sTILs using available haematoxylin and eosin-stained sections according to the International TIL Working Group guidelines.[16] sTILs were scored as the proportion of stroma within the invasive area covered by mononuclear cells divided by total intratumoural stromal area (0, 1, 5, 10, 15, 20, or >20 in 10% increments). All mononuclear cells, including lymphocytes and plasma cells, were quantified, with the exclusion of granulocytes and polymorphonuclear leucocytes. sTILs expression was assessed as a continuous measure, and as three ordinal levels of low (≤10%), medium (>10 to 50%), and high expression (>50%). The analysis was done by two pathologists masked to tumour T stage and clinical outcome for the first 77 samples, followed by a single pathologist masked to the tumour stage and clinical outcome for the remaining samples, to assess the intra-class correlation between pathologists. The intra-class correlation coefficient between the first two pathologists was 0·79 (p<0·001).

Whole-exome sequencing (WES) was conducted for five available tumour samples (based on availability of the recurrent samples) from three patients who had distant recurrence in the trial, including matched primary and recurrent samples for two patients, and primary only for the third patient (full methods for the WES analyses are provided in the appendix [pp 1–4]). The rate of genomic alterations identified at WES was descriptively evaluated in The Cancer Genome Atlas (TCGA) publicly available database.

Outcomes

The primary endpoint was invasive disease-free survival, defined as the time from study enrolment to the first of the following events: locoregional ipsilateral invasive recurrence (or ipsilateral invasive new primary), contralateral invasive breast cancer, distant recurrence, or death from any cause.[17] Participants who were alive and free from recurrence were censored at the date of the last follow-up, or at the date of second primary cancer diagnosis if applicable. Patients who did not consent to additional follow-up were censored at their off-study date.

Statistical analysis

Details of the statistical design have been published previously.[8] In the primary analysis, we used a group-sequential Poisson test to assess the occurrence of invasive disease-free survival events at 3 years against a null hypothesis of 9·2% using a one-sided type I error of 0·05. The planned sample size was 400 patients, with interim futility analyses after 225 and 800 patient-years of follow-up, and a final analysis of the primary endpoint after 1600 patient-years of follow-up. Under this design, the probability of rejecting the null was 0·95 if the true 3-year rate of invasive disease-free survival events was 5%. All analyses were conducted among patients that received protocol-defined treatment (hereafter referred

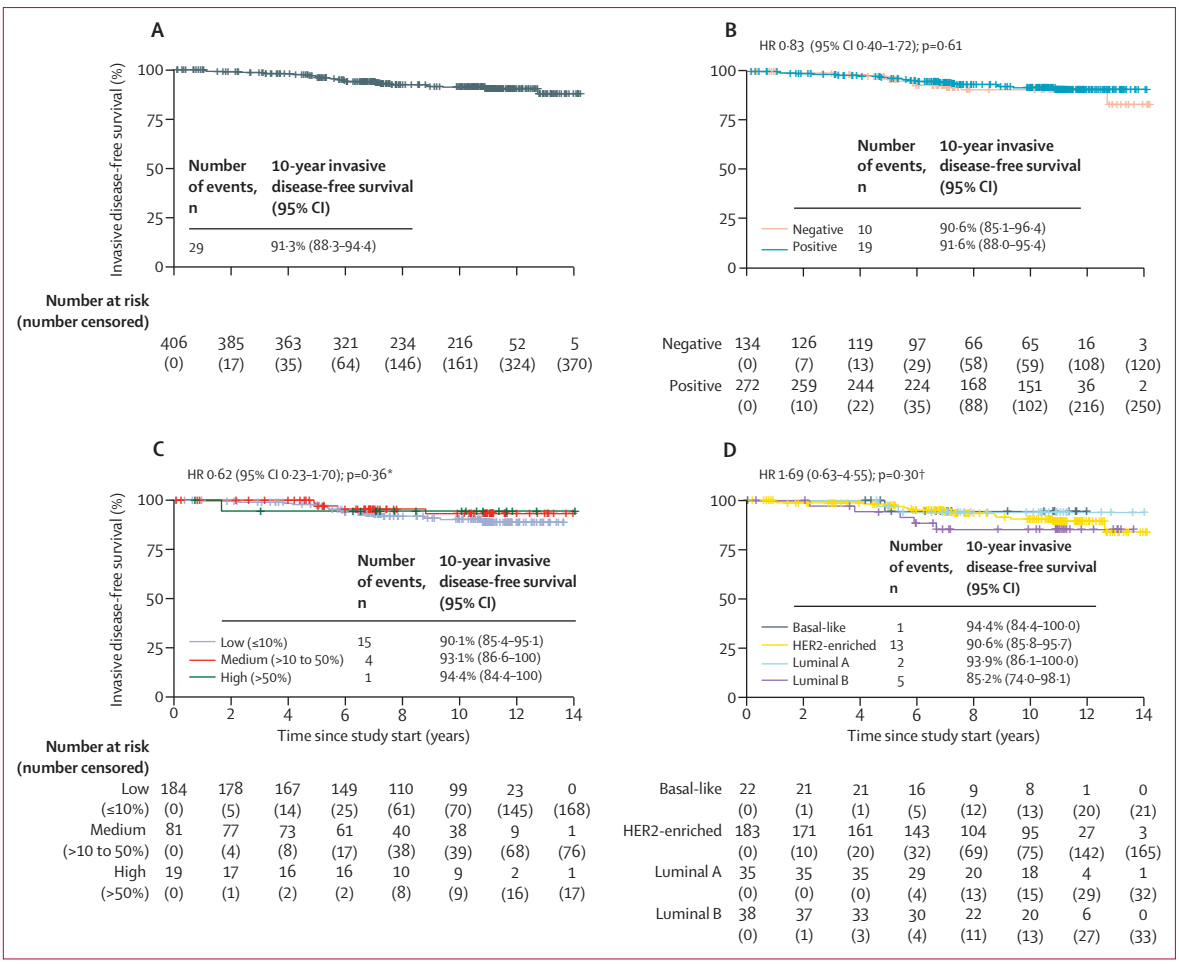

Figure 1: **10-year invasive disease-free survival, overall (A) and by hormone receptor status (B), sTIL expression (C), and PAM50 subtype (D)**
Reported number of events are those that occurred by 10 years of follow-up. HR=hazard ratio. PAM50=Prediction Analysis of Microarray 50. sTIL=stromal tumour-infiltrating lymphocyte. *Parameterised as high (>50%) versus low (≤10%)/medium (>10–50%) (ref). †Parameterised as luminal B versus basal-like/HER2-enriched/luminal A (ref).

to as the analysis population); patients who did not receive protocol-defined treatment were excluded.

In this final analysis, we reassessed the primary endpoint of invasive disease-free survival, and examined exploratory post-hoc survival endpoints, including recurrence-free interval, breast cancer-specific survival, and overall survival. Recurrence-free interval was defined as the time from study enrolment to disease recurrence, including invasive locoregional recurrence and distant recurrence, or death due to breast cancer. Breast cancer-specific survival was defined as the time from study enrolment until death from breast cancer, and overall survival was defined as the time from study enrolment until death from any cause. We used the Kaplan-Meier method to estimate the survival function for primary and exploratory endpoints, and we report point estimates with two-sided 95% CIs, and Kaplan-Meier plots. We also used univariable and multivariable Cox proportional hazards regression to assess the associations between prognostic factors of interest and invasive disease-free survival, and recurrence-free interval. Prespecified prognostic factors assessed were tumour size and hormone receptor status; post-hoc prognostic factors assessed were tumour T stage, tumour grade, N stage, sTIL expression, and PAM50 subtype and HER2DX score was included as an exploratory post-hoc variable; all multivariable analyses were post hoc. In all Cox models, the proportional hazards assumption was tested and inspected visually using Schoenfeld residuals; mention of such testing was included alongside results only if the assumption was rejected with a p value of less than 0·01. Additionally, to account for competing risks of death in the analysis of breast cancer-specific survival, we obtained estimates of the cumulative incidence functions of death from breast cancer and death from other causes using Gray's subdistribution hazard technique.

In post-hoc analyses, to identify an exploratory optimal HER2DX risk score cutoff, we used the approach proposed by Contal and O'Quigley[18] to assess all potential cutoffs and select the value that maximised the log-rank statistic for the

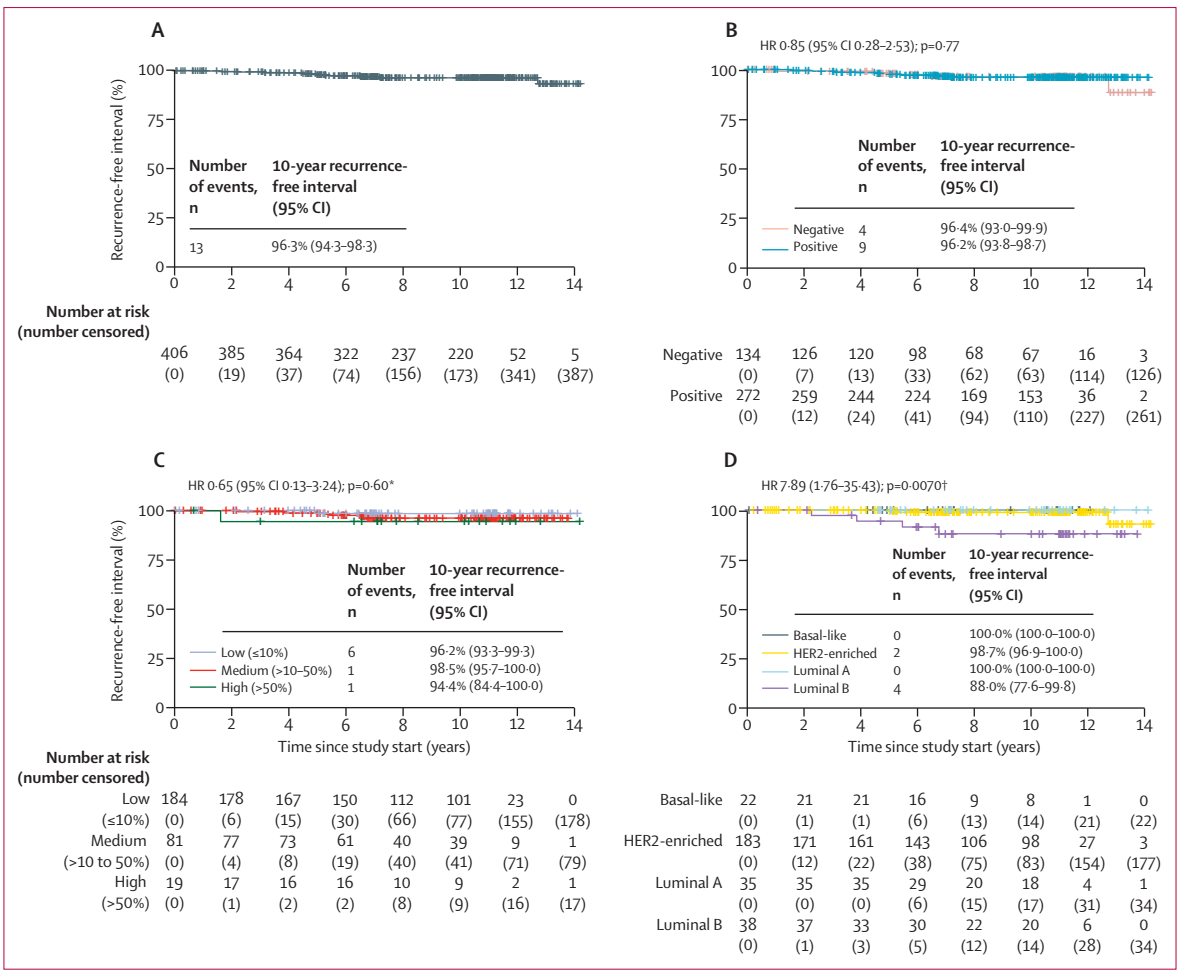

Figure 2: **10-year recurrence-free interval overall (A) and by hormone receptor status (B), sTIL expression (C), and PAM50 subtype (D)**
Reported number of events are those that occurred by 10 years of follow-up. HR=hazard ratio. PAM50=Prediction Analysis of Microarray 50. sTIL=stromal tumour-infiltrating lymphocyte. *Parameterised as high (>50%) versus low (≤10%)/medium (>10–50%) (ref). †Parameterised as luminal B versus basal-like/HER2-enriched/luminal A (ref).

recurrence-free interval endpoint. The reported p values associated with the optimal cutoff were adjusted for the multiple comparisons done to identify the cutoff point.[18] We summarised the diagnostic performance of HER2DX risk groups in terms of sensitivity, specificity, positive and negative predictive value, and area under the receiver operating characteristic curve using a time-dependent approach to predict the recurrence-free interval endpoint at 10-years.[19] We examined the correlation between the IgG signature of the HER2DX assay and sTILs using the Spearman's rank correlation as a post-hoc analysis. P values of less than 0·05 were considered to be significant. All analyses were done using R version 4.0.5. This study is registered with ClinicalTrials.gov, NCT00542451.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Between Oct 29, 2007, and Sept 3, 2010, 410 patients were enrolled, of whom 406 started study treatment and were included in the analysis population; four patients were excluded due to having never started study treatment.[8] Baseline patient and disease characteristics are included in table 1, with additional patient and disease characteristics in the appendix (pp 6–9). In the primary analysis population, the mean age at enrolment was 55 years (SD 10·5), 405 (99·8%) of 406 participants were female, one (0·2%) was male, 350 (86·2%) were White, 28 (6·9%) were Black or African American, 11 (2·7%) were Asian, and 17 (4·2%) were other races. 76 (18·7%) of 406 patients were self-reported to be premenopausal at baseline, of whom 15 (20%) had surgically induced menopause during the trial and three (4%) received ovarian suppressing medications.[20] After a median follow-up among the analysis population of 10·8 years (IQR 7·1–11·4; 3744 total patient-years),

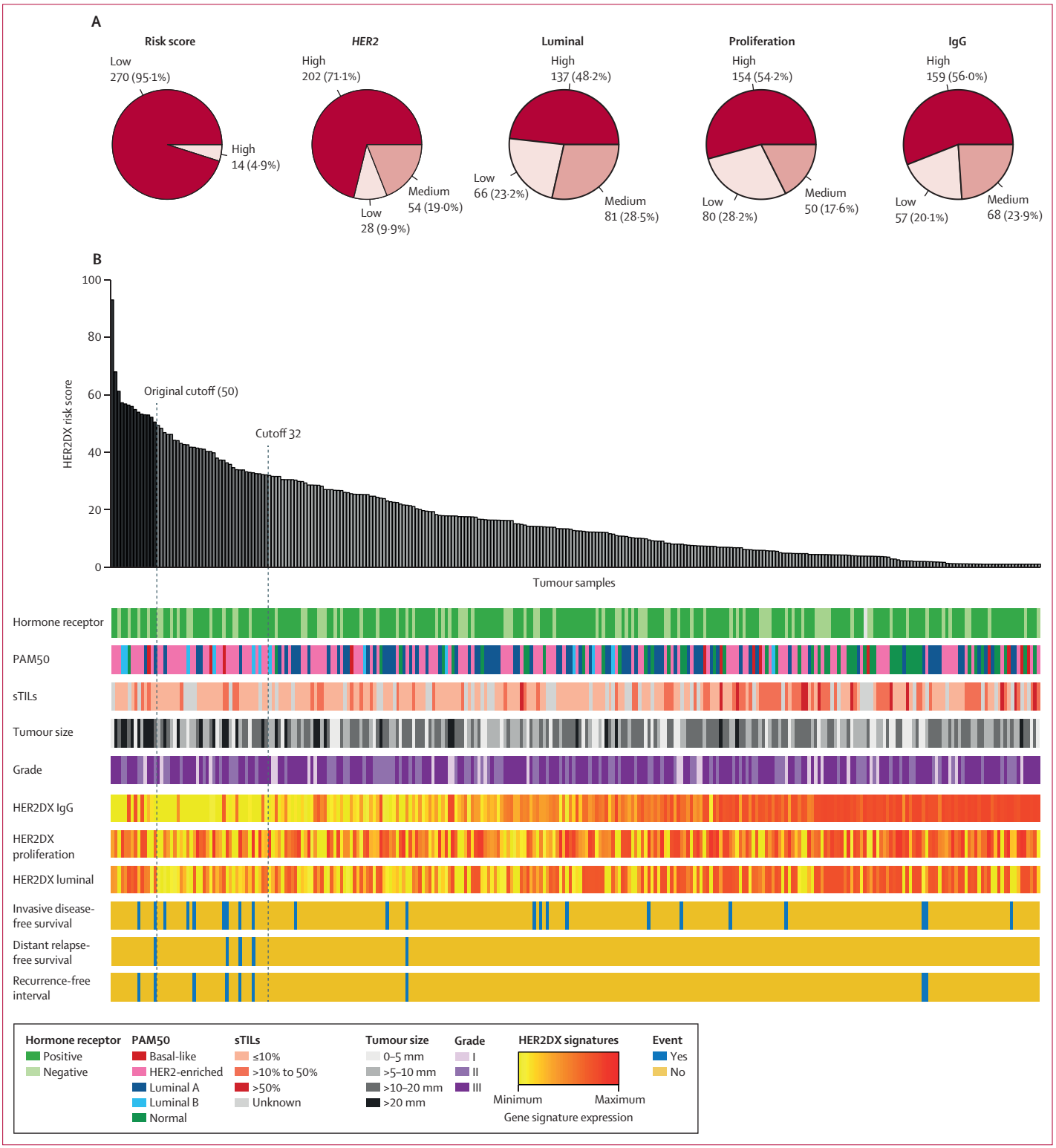

Figure 3: **HER2DX risk score**

(A) Distribution of the HER2DX risk score and main biological features captured by HER2DX in 284 available tumour samples, including the expression of *HER2* mRNA, the luminal signature, the proliferation signature, and the IgG signature. (B) HER2DX risk score ranking and association with hormone receptor status, PAM50 subtype, sTILs level, tumor size, tumor grade, HER2DX signatures, invasive disease-free survival events, distant relapse-free survival, and recurrence-free interval events. PAM50=Prediction Analysis of Microarray 50. sTIL=stromal tumour-infiltrating lymphocyte.

	Number of samples	Invasive disease-free survival		Recurrence-free interval	
		HR (95% CI)	p value	HR (95% CI)	p value
HER2DX risk score (10-unit increment)	284	1·24 (1·00–1·52)	0·047	1·45 (1·09–1·93)	0·011
HER2DX risk score groups (original cutoff)					
Low	270	1 (ref)	..	1 (ref)	..
High	14	1·94 (0·45–8·27)	0·37	5·71 (1·18–27·67)	0·030
HER2DX risk score groups (cutoff of 32)					
Low	236	1 (ref)	..	1 (ref)	..
High	48	3·72 (1·61–8·60)	0·0021	11·08 (2·76–44·39)	<0·001
T stage					
T1	253	1 (ref)	..	1 (ref)	..
T2	31	1·73 (0·59–5·10)	0·32	2·34 (0·48–11·30)	0·29
N stage					
N0	280	1 (ref)	..	1 (ref)	..
N1	4	Not estimable	..	Not estimable	..
Hormone receptor status					
Negative	84	1 (ref)	..	1 (ref)	..
Positive	200	0·71 (0·30–1·67)	0·43	0·78 (0·20–3·14)	0·73
sTIL expression (per 10-unit increment)	284	0·71 (0·43–1·19)	0·19	0·66 (0·27–1·66)	0·38
sTIL expression					
Low (≤10%)	152	1 (ref)	..	1 (ref)	..
Intermediate/high (>10%)	80	0·48 (0·14–1·68)	0·25	0·41 (0·05–3·52)	0·42
Grade					
I–II	120	1 (ref)	..	1 (ref)	..
III	164	0·90 (0·40–2·04)	0·80	2·90 (0·60–14·01)	0·19
PAM50 subtype					
Basal-like, HER2-enriched, or luminal A	220	1 (ref)	..	1 (ref)	..
Luminal B	34	2·12 (0·77–5·83)	0·15	8·20 (1·83–36·83)	0·0061

HR=hazard ratio. sTIL=stromal tumour-infiltrating lymphocyte.

Table 2: **Univariable association of pretreatment baseline variables with invasive disease-free survival and recurrence-free interval in patients with HER2DX data (n=284)**

31 invasive disease-free survival events were observed. Six (19·4%) of 31 events were locoregional ipsilateral recurrences, nine (29·0%) were new contralateral breast cancers (eight were HER2-negative and one was HER2-positive), six (19·4%) were distant recurrences, and ten (32·3%) were all-cause deaths (appendix p 10). Baseline demographics of patients who had invasive disease-free survival events, by recurrence type and HER2DX score, are in the appendix (pp 11–14).

10-year invasive disease-free survival for the analysis population was 91·3% (95% CI 88·3–94·4; figure 1A). 10-year invasive disease-free survival by hormone-receptor status, sTIL expression (post hoc), and PAM50 subtype (post hoc) are shown in figure 1B–D, and by tumour size are shown in the appendix (p 18).

10-year recurrence-free interval in the analysis population was 96·3% (95% CI 94·3–98·3; figure 2A). 13 recurrence-free interval events occurred, including six locoregional ipsilateral recurrences, six distant recurrences, and one breast-cancer-related death. 10-year recurrence-free interval by hormone receptor status, sTIL expression (post hoc), and PAM50 subtype (post hoc) are shown in figure 2A–D, and by tumour size are shown in the appendix (p 19).

10-year overall survival for the analysis population was 94·3% (95% CI 91·8–96·8), with 19 deaths being recorded (appendix p 21). 10-year breast cancer-specific survival was 98·8% (95% CI 97·6–100), with four deaths due to breast cancer recorded. The 10-year breast cancer-specific survival accounting for competing risks of death was 98·8% (95% CI 97·7–100; appendix pp 22–23). The 10-year overall survival by hormone receptor status, tumour size, sTIL expression (post hoc) and PAM50 subtype (post hoc) are shown in the appendix (pp 20–21).

284 (70·0%) of 406 samples were available and adequate for HER2DX genomic testing. The proportion of patients with HER2DX high-risk disease (according to the pre-established cutoff of a score of 50) was 4·9% (14 of 284; figure 3A). The proportion of tumours with high expression of IgG signatures was 56·0% (n=159), proliferation signatures was 54·2% (n=154), and luminal signatures was 48·2% (n=137), and 202 (71·1%) of 284 tumour samples had high *HER2* expression. In a post-hoc univariable analysis, no association between HER2DX score and hormone receptor status was observed (p=0·71; figure 3B).

In post-hoc univariable analyses, HER2DX risk score as a continuous variable was significantly associated with invasive disease-free survival (HR per 10-unit increment 1·24 [95% CI 1·00–1·52], p=0·047) and recurrence-free interval (HR per 10-unit increment 1·45 [95% CI 1·09–1·93], p=0·011; table 2). In prespecified univariable analyses, hormone receptor status and T stage (T2 *vs* T1) were not significantly associated with invasive disease-free survival and recurrence-free interval. Using the pre-established HER2DX risk score cutoff of 50, HER2DX high-risk disease was significantly associated with an increase in the risk of recurrence but not in invasive disease-free survival (figure 4A–B). A HER2DX risk score of 32 was identified as the optimal cutoff to separate patients in the APT trial with low-risk disease from those with high-risk disease (figure 4C–D). Post-hoc multivariable analyses showed no significant associations between survival endpoints and any of the explored prognostic factors (appendix p 15). Diagnostic performance of HER2DX risk group thresholds is shown in the appendix (p 16), as well as the correlation between the IgG motif and sTILs (post hoc; appendix p 24).

Post hoc, WES and tumour-only variant calling and copy number analyses were done on five tumour

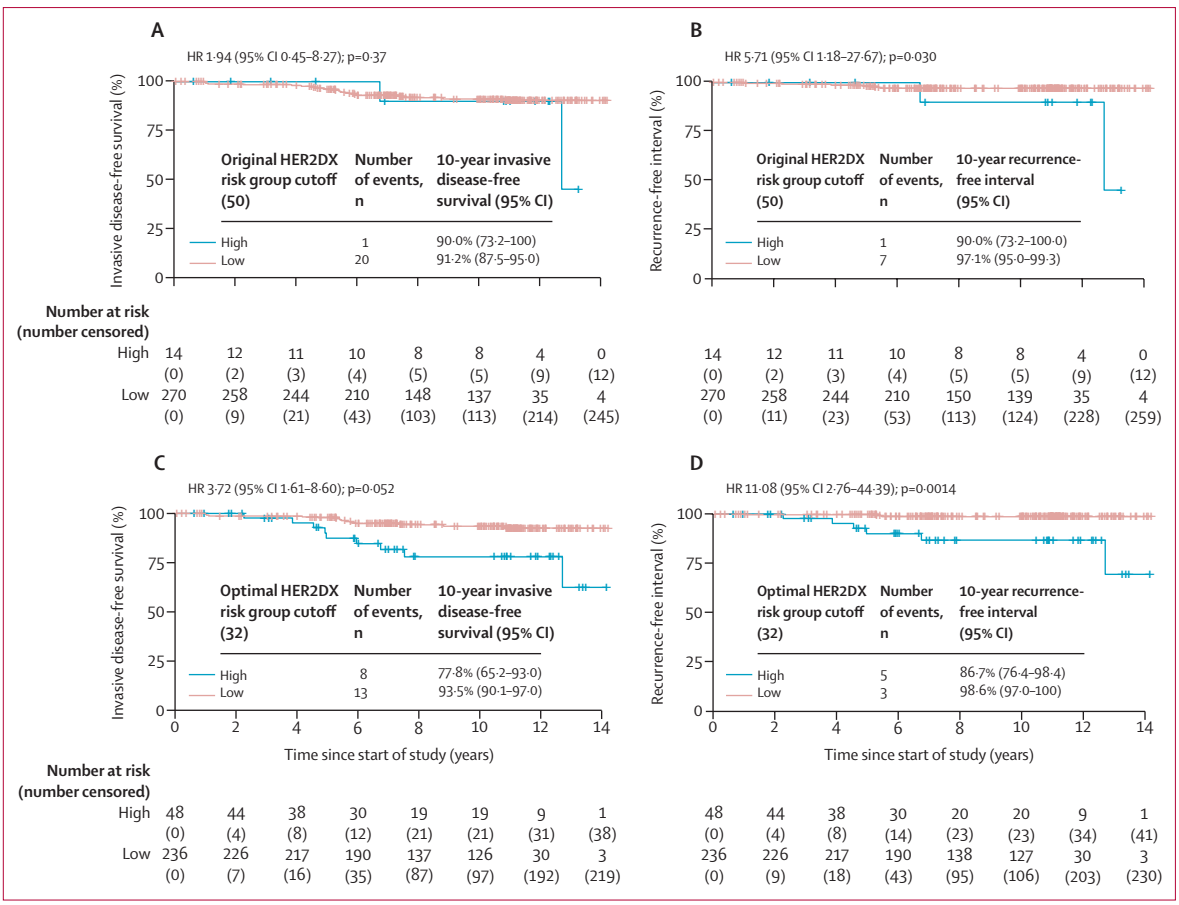

Figure 4: **10-year invasive disease-free survival (A, C) and 10-year recurrence free survival (B, D), stratified by HER2DX risk group**
Panels A and B show survival outcomes stratified by the original HER2DX cutoff of a score of 50, and panels C and D show survival outcomes stratified by the optimal cutoff of a score of 32. HR=hazard ratio.

samples from three patients who had distant recurrence. For two patients, paired baseline and recurrent tumour samples could be sequenced, whereas the recurrent sample for the remaining patient was not sufficient for conducting WES, thus we only sequenced the baseline tumour sample (appendix pp 25–26). All three patients had stage 1, HER2-positive, poorly differentiated, invasive ductal carcinomas, two of whom coexpressed hormone receptors. The two patients with hormone receptor-positive tumours had recurrence in bone and mediastinum, respectively, whereas the patient with a hormone receptor-negative tumour had pleural and mediastinal recurrence. On recurrence, all tumours retained the baseline hormone receptor status; two of three tumours retained HER2-positive status, whereas one converted to HER2-negative.

All five tumours showed an increase in *HER2* copy numbers relative to ploidy, with four showing a focal high amplification or high amplification of *HER2*. Evolutionary analysis from the patients who had paired baseline and recurrent samples identified clonal oncogenic mutations in chromatin remodelling genes: truncal *ARID1A* and *EP300* oncogenic mutations in one patient, and an acquired *KMT2A* oncogenic mutation in the recurrent sample from the other. Other notable alterations are described in the appendix (pp 25–26). No oncogenic alterations in *TP53* or *PIK3CA* were detected among these samples.

When evaluating the rate of the alterations identified in the publicly available TCGA database, 0% of stage 1 HER2-positive TCGA samples were found to have an oncogenic alteration in either ARID1A, EP300, or KMT2A, compared with 5·5% of stage 1–4 HER2-positive TCGA samples (appendix pp 25–26).

Discussion

The 3-year[8] and 7-year[9] invasive disease-free survival results from the APT trial established adjuvant paclitaxel and trastuzumab as a standard-of-care treatment for patients with small, node-negative, HER2-positive breast cancer.[2] This 10-year follow-up of APT shows few additional recurrences and supports the adequateness of the paclitaxel and trastuzumab regimen for this patient population. The invasive disease-free survival rate at 3 years was 98·7%,[8] at 7 years was 93·3%,[9] and at 10 years was 91·3%, with only six (1·5%) of 406 patients

having reported distant recurrences after more than 10 years of follow-up. Many of the invasive disease-free survival events were not related to the original breast cancer diagnosis, including new contralateral breast cancers (nine [29·0%] of 31 events, with all but one occurrence being HER2 negative) and deaths from non-breast cancer causes (ten [32·3%]). The 10-year recurrence-free interval, which includes invasive locoregional and distant recurrences, and deaths due to breast cancer, was 96·3%.

Although adjuvant paclitaxel and trastuzumab is associated with excellent long-term outcomes, unmet needs remain for the population of patients with small, HER2-positive tumours. Indeed, HER2-positive breast cancer is a biologically heterogenous disease, with all the intrinsic subtypes represented and with extensive variability in the composition of the tumour micro-environment, aspects that can lead to heterogenous long-term outcomes.[21] In line with this knowledge, we found numerically worse 10-year invasive disease-free survival in patients with PAM50 luminal B tumours than in those with basal-like, HER2-enrched, or luminal A tumours and with lower sTIL expression (ie, ≤10%) than those with higher expression (>10%), although these differences were not significant. Notably, luminal B disease remained associated with a numerically higher risk of recurrence-free interval events compared with other PAM50 subtypes, whereas the prognostic role of sTILs was unclear in the recurrence-free interval analysis. As a consolidation of elements relevant for intrinsic subtype, immune activation, and tumour features, the HER2DX genomic tool was recently developed, and showed encouraging results in prognostication for HER2-positive breast cancer.[13] In this 10-year analysis of APT, we found that the HER2DX risk score as a continuous variable was significantly associated with both invasive disease-free survival and recurrence-free interval (post hoc univariable analyses). Additionally, in post-hoc univariable analyses, HER2DX was associated with recurrence-free interval when using a previously established cutoff (risk score of 50) and when using an exploratory cutoff optimised for the APT population (risk score of 32). In the post-hoc multivariable analysis, the HER2DX score did not remain significantly associated with invasive disease-free survival or recurrence-free interval after adjustment for hormone receptor status, tumour stage, or sTILs expression, although the statistical power in this analysis was low due to the small number of events (16 invasive disease-free survival events and six recurrence-free interval events). The probability of experiencing recurrence by 10 years in patients with tumours with a HER2DX risk- score of less than 32 was only 1·4%, compared with a 13·3% probability of recurrence among those who had a HER2DX score of 32 or higher. These observations are consistent with a previous study that evaluated the performance of HER2DX and found that the HER2DX risk score was significantly associated with survival outcomes in an in-silico analysis involving 273 patients with small HER2-positive tumours.[13] Nonetheless, our optimised threshold of a score of 32 will require further validation in additional cohorts of patients with small HER2-positive tumours. The availability of an accurate prognostic tool for HER2-positive tumours has particular relevance given the rapid expansion in the pipeline of highly effective anti-HER2 agents, offering opportunities for improved tailoring of treatments. Pertuzumab, trastuzumab emtansine, and neratinib are already approved anti-HER2 agents for high-risk, early-stage, HER2-positive tumours, whereas trastuzumab deruxtecan, tucatinib, and margetuximab are being studied in the early-stage setting after showing improved outcomes in the metastatic setting. Concomitantly, studies have evaluated shorter durations of adjuvant HER2 blockade, with suggestion of non-inferiority in at least one large phase 3 trial,[22] offering additional opportunities for treatment tailoring. Overall, if further validated, the HER2DX genomic tool might aid in optimising adjuvant and neoadjuvant treatments, and ideally be used to identify subsets of patients warranting further de-escalation or possibly omission of systemic treatment, which would need to be confirmed within prospective trials.

In this study, we also report the results of WES on five tumour samples from three patients who had distant recurrences, and identified the presence of mutations in the chromatin remodelling genes *ARID1A*, *EP300*, and *KMT2A*. Although we have not identified a clear biological mechanism of recurrence or resistance, it is intriguing that two recurrent samples had oncogenic alterations in chromatin remodelling genes, because these alterations are not common in early-stage, HER2-positive, primary breast cancer. Interestingly, loss-of-function alterations of *ARID1A* have been associated with resistance to trastuzumab[23] and endocrine treatment.[24] None of the tumours had oncogenic alterations in *TP53* or *PIK3CA*, which are frequently altered in published cohorts of HER2-positive primary breast cancer.[25]

Together with developing useful prognostic and predictive tools, attempts are ongoing to further improve the tolerability of adjuvant treatment strategies through the identification of alternative regimens with equal or improved efficacy and reduced toxicity compared with standard of care. In the randomised phase 2 ATEMPT trial (adjuvant trastuzumab emtansine *vs* paclitaxel and trastuzumab),[26,27] 497 patients with stage 1, HER2-positive breast cancer were randomly assigned (3:1) to receive adjuvant trastuzumab emtansine or paclitaxel plus trastuzumab. The study found no significant difference in the rate of clinically relevant toxicities between the two groups, but a better quality of life was noted for patients given trastuzumab emtansine, who had less neuropathy, less hair loss, and better work productivity than did those given paclitaxel and trastuzumab.[26] 5-year invasive disease-free survival with adjuvant trastuzumab

emtansine was 97% (95% CI 95·3–98·8),[27] showing the efficacy of this drug at preventing recurrences among patients with small HER2-positive tumours. The ongoing ATEMPT 2.0 randomised trial (NCT04893109) is designed to assess whether a shorter course of of trastuzumab emtansine (six cycles) could allow for continued efficacy with reduced toxicity in a similar population of patients. Further de-escalation studies ongoing for this population include the non-randomised phase 2 IRIS trial (NCT04383275) evaluating the performance of oral chemotherapy (capecitabine or vinorelbine) or endocrine therapy when combined with adjuvant trastuzumab, and the ADEPT trial (NCT04569747) evaluating the activity of adjuvant trastuzumab, pertuzumab, and endocrine treatment for patients with stage 1, HER2-positive and oestrogen receptor-positive tumours. These trials could potentially allow for the expansion of well tolerated adjuvant treatments for patients with small, node-negative HER2-positive tumours.

The primary limitation of the APT trial is its single-arm design. Although a randomised trial would have been preferable, there was no standard treatment in patients with small, node-negative, HER2-positive breast cancer at the time the trial was designed, and a study randomised to compare efficacy of two treatments would require several thousand patients given the low event rate with therapy. Nonetheless, the large sample size and outstanding outcomes after more than 10 years of follow-up support that adjuvant paclitaxel and trastuzumab is a reasonable standard of care treatment for patients with small, node-negative, HER2-positive breast cancer. Notably, these long-term outcomes were observed in APT regardless of hormone receptor expression or tumour size at baseline. In this disease setting, a multidimensional risk assessment appears to provide more prognostic information, with the HER2DX genomic tool identifying a population of patients with a significantly increased risk of recurrence. Given the small number of events observed in the trial, the post-hoc nature of HER2DX analyses, and that these could only be performed in a subset of the study population, further validation is recommended before using this tool for escalation or omission of treatment for patients with stage 1 HER2-positive breast cancer. Other limitations of this study were that data were not collected regarding types of endocrine treatment administered, which could have been informative for interpreting outcomes among patients with triple-positive tumours, or regarding lymphovascular invasion.

In conclusion, this end-of-study, 10-year analysis of the APT trial supported that adjuvant treatment with paclitaxel and trastuzumab produces very good long-term outcomes for patients with small, node-negative, HER2-positive breast cancer. Further efforts in optimisation of therapy are ongoing and might provide additional effective regimens to prevent recurrences with the least effect on quality of life. HER2DX testing proved promising in the identification of a subset of tumours with a relatively high risk of recurrence, and, if validated, might aid in the tailoring of adjuvant treatments for patients with stage 1 HER2-positive breast cancer.

Contributors

SMT, HJB, and EPW contributed to study conceptualisation. SMT, PT, CTD, DAY, PKM, HSR, MJE, IS, ACW, LAC, AHP, MDe, MDi, NG, NT, LP, GV, PV, NW, JGTZ, JW, and AP curated the data. SMT, PT, NG, NT, LP, GV, RB-S, PV, CTD, DAY, BM, KSA, HSR, ACW, LAC, AHP, AGW, CAH, IEK, HJB, MJE, NW, JGTZ, JW, AP, and EPW contributed to formal analysis. SMT, IEK, HJB, CTD, CAH, DAY, BM, PKM, KSA, HSR, MJE, IS, LAC, AHP, AGW, ACW, and EPW contributed to funding acquisition. All authors contributed to the investigation, methodology, project administration, data validation, data visualisation, and acquiring resources and software. SMT, NT, AP, and EW supervised the work by all other authors involved in the study. SMT, PT, NG, NT, LP, and GV wrote the original draft of the manuscript. All authors reviewed and edited the manuscript. SMT and NT directly accessed and verified the underlying data reporting in the manuscript, and all authors had final responsibility for the decision to submit for publication.

Declaration of interests

SMT has received grant support (paid to institution) from AstraZeneca, Merck, Nektar, Novartis, Pfizer, Genentech (Roche), Gilead, Exelixis, BMS, Eisai, NanoString, Cyclacel, Sanofi, Seagen, and Eli Lilly; and has received consulting or advisory board fees (paid to self) from AstraZeneca, Eli Lilly, Merck, Novartis, Pfizer, Genentech (Roche), Gilead, BMS, Eisai, Sanofi, SeaGen, Daiichi-Sankyo, Athenex, OncoPep, Kyowa Kirin Pharma, CytomX, Certara, Mersana Therapeutics, Ellipses Pharma, 4D Pharma, OncoSec, Infinity Therapeutics, BeyondSpring Pharma, OncXerna, Zymeworks, Zentalis, ARC Therapeutics, Reveal Genomics, Blueprint Medicines, Myovant, Umoja Biopharma, Stemline (Menarini), and Artios Biopharma. PT has received consulting or advisory board fees from AstraZeneca, Daiichi-Sankyo, and Lilly and has received payment or honoraria for educational events from AstraZeneca and Daiichi-Sankyo. NT has received honoraria for a Clinical Research Workshop at the San Antonio Breast Cancer Symposium. LP is an employee of Reveal Genomics and has a patent (EP 20 382 679.7—in vitro method for the prognosis of patients suffering from HER2-positive breast cancer). GV has received payment or honoraria for lectures, presentations, speakers' bureaus, manuscript writing, or educational events from MSD, GSK, Pierre Fabre, and Pfizer and has participated on a data safety monitoring board or advisory board for AstraZeneca. CTD has received research funding from Genentech (Roche) and Puma and has received consulting fees from Daiichi-Sankyo, Novartis, Pfizer, Gilead, Seagen, and Genentech (Roche). DAY has received funding (paid to institution) for the present manuscript from Dana-Farber Cancer Institute; grant support (paid to institution) from Ambrx, Amgen, BIOMARIN, Biothera Pharmaceuticals, Clovis Pharma, Dana-Farber Cancer Institute, Lilly, Genentech (Roche), G1 Therapeutics, Gilead Sciences, Incyte, Innocrin Pharmaceuticals, Macrogenics, MedImmune, Medivation, Merck, Merrimack Pharmaceuticals, Nektar Therapeutics, Novartis, the National Surgical Adjuvant Breast and Bowel Project, Pfizer, and Polyphor; and has received consulting fees (paid to institution) from AstraZeneca, Athenex, bioTheranostics, G1 Therapeutics, Gilead Sciences, Immunomedics, Merck, Novartis, Pfizer, and Sanofi-Aventis. PKM has received support (paid to institution) for the present manuscript from Genentech; has participated on a data safety monitoring board for Genentech (Roche); has stock or stock options in Veracyte; and was a full-time employee of Veracyte at time of manuscript submission. KSA has received funding (paid to institution) for the present manuscript from Dana-Farber Cancer Institute for data management support. HSR has received grant funding (paid to UC Regents only) from Ambrx, Astellas Pharma, AstraZeneca, Daiichi-Sankyo, Genentech (Roche), Gilead Sciences, GSK, Lilly, Merck & Co, Novartis Pharmaceuticals, OBI Pharma, Pfizer, Pionyr Immunotherapeutics, Seattle Genetics, Sermonix Pharmaceuticals, Taiho Oncology, and Veru; has received consulting fees from Puma, Napo Pharmaceuticals, and Blueprint; and has received support for attending meetings and travel from Merck, AstraZeneca, and Gilead. MJE has

reported a PAM50 Bioinformatics for Prosigna royalty-bearing license to Veracyte; and and has been an employee of AstraZeneca since March 7, 2022 (AstraZeneca had no role in this study and the author's involvement in this study preceded his employment with AstraZeneca). LAC has received research funding (paid to institution) from Syndax, Novartis, NanoString Technologies, Seattle Genetics, Veracyte, and AstraZeneca and declares participation in uncompensated consulting activities for Eisai (to self), Sanofi (to self), Lilly (to self), Seagen (to self), Novartis (to institution), G1 Therapeutics (to institution), Genentech (Roche; to institution), GSK (to institution), AstraZeneca (to institution), and Daiichi-Sankyo (to institution). RBS has received consulting fees from AstraZeneca, Libbs, Daiichi-Sankyo, Eli Lilly, Pfizer, Novartis, MSD, and Roche; has received payment or honoraria for lectures, presentations, speakers' bureaus, manuscript writing, or education events from AstraZeneca, Libbs, Daiichi-Sankyo, Eli Lilly, Pfizer, Novartis, MSD, and Roche; has received support for attending meetings and travel from Daiichi-Sankyo, Eli Lilly, MSD, and Roche; has participated on a data safety monitoring board or advisory board for AstraZeneca, Daiichi-Sankyo, and Roche; and is a member of the Clinical Research Committee of the Brazilian Society of Clinical Oncology. JGTZ has stock or stock options in Loncar Cancer Immunotherapy ETF, iShares Genomics Immunology and Healthcare ETF, iShares Biotechnology ETF, SPDR S&P Biotech ETF, Adaptive Biotechnologies, 2seventy bio, and bluebird bio. NW has received grant support from AstraZeneca; has received consulting fees from Relay Therapeutics, Flare Therapeutics, Eli Lilly, and Section 32; has received payment or honoraria for lectures, presentations, speakers' bureaus, manuscript writing, or educational events from Genentech; and has stock or stock options in Relay Therapeutics and Flare Therapeutics. AHP has received royalties from UpToDate. AGW has received grant support (paid to institution) from Macrogenics, Genentech, and Merck and has received consulting fees (paid to self) from AstraZeneca. CAH is Chief Executive Officer at the American Society of Clinical Oncology, which pays 100% of the author's personal annual earned income. IEK has received support for the present manuscript from Genentech (Roche); has received grant support from Macrogenics and Pfizer; has received consulting fees from Daiichi-Sankyo, Macrogenics, Roche, Seagen, and AstraZeneca; and has participated on a data safety monitoring board or advisory board for Novartis, Merck, Daiichi-Sankyo, Macrogenics, Roche, Seagen, and AstraZeneca. AP has received funding for the present manuscript from Reveal Genomics; has received grants (paid to institution) from Roche, Daiichi-Sankyo, and Reveal Genomics; has received royalties or licenses from Reveal Genomics (paid to institution); has received consulting fees (paid to self) from Roche, Novartis, Daiicki-Sankyo, and AstraZeneca; has patents licensed to Reveal Genomics: HER2DX, ERBB2, and DNADX genomic tools; and has stock or stock options in Reveal Genomics and Oncolytics Biotech. All other authors declare no competing interests.

Data sharing

Deidentified individual-level patient data that support the findings of this study are available from the corresponding author on reasonable request. A detailed proposal for how the data will be used is required and we will assess applications on a case-by-case basis. All proposals should be submitted to the corresponding author.

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