Breast Cancer Research and Treatment (2019) 177:103–114
https://doi.org/10.1007/s10549-019-05284-y

CLINICAL TRIAL

Dissecting the effect of hormone receptor status in patients with HER2-positive early breast cancer: exploratory analysis from the ALTTO (BIG 2-06) randomized clinical trial

Matteo Lambertini[1,2] · Christine Campbell[3] · Richard D. Gelber[4] · Giuseppe Viale[5] · Ann McCullough[6] · Florentine Hilbers[7] · Larissa A. Korde[8] · Olena Werner[9] · Saranya Chumsri[10] · Christian Jackisch[11] · Antonio C. Wolff[12] · Ines Vaz-Luis[13] · Arlindo R. Ferreira[14] · Aleix Prat[15] · Alvaro Moreno-Aspitia[10] · Martine Piccart[16] · Sherene Loi[17] · Evandro de Azambuja[16]

Received: 13 April 2019 / Accepted: 16 May 2019 / Published online: 27 May 2019

Abstract

Purpose Limited evidence exists on the impact of hormone receptor (HR) status to counsel HER2-positive early breast cancer patients receiving adjuvant anti-HER2 therapy.

Methods ALTTO (BIG 2-06) was an international, intergroup, open-label, randomized phase III trial in HER2-positive early breast cancer patients randomized to receive 1 year of trastuzumab and/or lapatinib. HER2, estrogen and progesterone receptors were centrally tested for all patients. We investigated the impact of HR status on prognosis, risk of disease-free survival (DFS) events over time, patterns of first DFS events, and factors associated with risk of DFS events overall, in years 0–5 and 6–8.

Results Out of 6273 patients included in this analysis, 3603 (57.4%) had HR-positive tumors. Median follow-up was 6.93 years. Five-year and 8-year DFS were 86% and 80% in patients with HR-positive disease, and 83% and 79% in those with HR-negative tumors, respectively. Mean annual hazards of recurrence in years 0–5 were 3% in patients with HR-positive disease and 4% in those with HR-negative tumors, while in years 6–8 they were 3% and 2%, respectively. Distribution of first DFS event in years 6–8 ($P=0.005$) and type of first distant recurrence ($P<0.001$) were significantly different between the two groups. Risk factors for DFS events overall, in years 0–5, and 6–8 were different in patients with HR-positive and HR-negative tumors.

Conclusions HER2-positive early breast cancer is characterized by the presence of two diseases with distinct natural history based on HR status requiring the development of different follow-up strategies and future de-escalation and escalation clinical trials.

Keywords Breast cancer · HER2 · Estrogen receptor · Progesterone receptor

Introduction

Hormone receptors (HRs; estrogen [ER] and progesterone [PR] receptors) and human epidermal growth factor receptor 2 (HER2) are key prognostic and predictive markers in breast cancer [1]. In approximately 50% of HER2-positive tumors, the overexpression and/or amplification of HER2 co-exists with the presence of HRs; their functional cross-talk may modify natural history, response to therapy, and outcomes of patients affected by this breast cancer subtype [2, 3]. Indeed, presence or absence of ER and/or PR may define two distinct biologic subsets within the group of HER2-positive tumors [4–6].

Although several studies in the metastatic and neoadjuvant settings have shown the impact of HR status on clinical outcomes and behavior of HER2-positive tumors [2, 3], limited evidence exists to counsel patients with HER2-positive early breast cancer who are candidates to adjuvant

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10549-019-05284-y) contains supplementary material, which is available to authorized users.

✉ Matteo Lambertini
matteo.lambertini@unige.it

Extended author information available on the last page of the article

anti-HER2 therapy. Exploratory analyses from the adjuvant trials showed that patients with HR-positive/HER2-positive tumors experienced a different clinical behavior in terms of type and patterns of recurrence over time as compared to those with HR-negative/HER2-positive disease [7–9]. However, in these studies, most patients received chemotherapy alone without anti-HER2 therapy, HR status was based only on local assessment without quantification of ER and/or PR expression, and limited data beyond 5 years of follow-up were available. Therefore, these results cannot be translated and adequately applied to the modern era in which almost all patients receive chemotherapy plus trastuzumab-based therapy, level of ER and/or PR expression is known to provide valuable prognostic information, and long-term follow-up data are crucial to properly assess the behavior of HR-positive tumors. Hence, current recommendations on the management of patients with HER2-positive early breast cancer do not differ according to HR status [10, 11].

The Adjuvant Lapatinib and/or Trastuzumab Treatment Optimization (ALTTO) trial is the largest trial in HER2-positive early breast cancer [12]; results at approximately 7 years of median follow-up are now available [13]. HER2, ER, and PR status were centrally tested and quantified for all included patients. Therefore, this provided a unique setting for a comprehensive investigation of clinical outcomes and behavior of HER2-positive tumors according to HR status in patients treated in the modern era with chemotherapy and trastuzumab-based treatment. A better understanding of how HR status influences patients' outcomes and treatment response over time would contribute to improve clinical recommendations for patients and trial design exploring tailored treatment strategies within the HER2-positive population according to the presence or absence of HRs.

Methods

Study design and patients

Details of the ALTTO trial were previously reported [12]. Briefly, ALTTO (Breast International Group [BIG] 2-06/EGF106708 and North Central Cancer Treatment Group [Alliance] N063D) is an international, intergroup, open-label, randomized phase III trial investigating chemotherapy plus adjuvant trastuzumab and/or lapatinib in HER2-positive early breast cancer patients.

Eligibility criteria were histologically confirmed and completely excised invasive non-metastatic HER2-positive breast cancer, either node-positive or node-negative with pathologic tumor size of ≥ 1 cm.

As per study protocol, HER2, ER, and PR were centrally tested for all patients before random assignment in one of the three central laboratories: European Institute of Oncology (Italy), Mayo Clinic (United States), and Peking Union Medical College Hospital (China). The 2007 American Society of Clinical Oncology/College of American Pathologists guidelines were used to define HER2 positivity [14]. Tumors with $\geq 1\%$ tumor cells expressing ER and/or PR receptors were defined as HR positive.

Study procedures

An interactive voice response system was used to randomize (1:1:1:1) patients to one of the following 1-year duration adjuvant anti-HER2 treatment arms: trastuzumab alone, lapatinib alone, trastuzumab for 12 weeks followed by lapatinib for 34 weeks after a 6-week washout period, and trastuzumab plus lapatinib. As per physician's choice, anti-HER2 treatment could be administered at the completion of chemotherapy (design 1), after anthracycline-based chemotherapy and concomitantly with a taxane (design 2), or concurrently with an anthracycline-free regimen (6 cycles of docetaxel and carboplatin [i.e., TCH]; design 2B).

Permuted blocks stratified for timing of chemotherapy, lymph node, and HR status were used to prepare randomization lists.

After the first interim analysis in 2011, the lapatinib arm was prematurely closed; adjuvant commercial trastuzumab was offered to 1087 out of 2100 (51.8%) patients [12]. All patients randomized in the lapatinib alone arm were excluded from the present analysis.

As per study protocol, adjuvant endocrine therapy had to be administered to patients with HR-positive disease unless contraindicated and prescribed as per local guidelines according to menopausal status for at least 5 years.

Ethics Committees/Independent Review Boards of all participating institutions approved the ALTTO trial and written informed consent was obtained from all patients before study entry. The ALTTO Executive and Steering Committees approved the present analysis.

Outcomes

The ALTTO trial was designed to compare disease-free survival (DFS) in each of the three lapatinib-containing arms separately with the trastuzumab alone arm [12]. Distant DFS (DDFS) and overall survival (OS) were secondary endpoints.

The aims of the current analysis were to investigate the impact of HR status on prognosis (in terms of DFS, DDFS, and OS) and the risk of DFS events over time in patients with HER2-positive early breast cancer. In addition, patterns of first DFS events and factors associated with risk of DFS events overall, in years 0–5, and 6–8 were assessed separately in patients with HR-positive and HR-negative disease.

Statistical methods

Details on sample size calculations and statistical assumptions of the ALTTO trial were previously described [12]. The present analysis was not pre-planned in the study protocol, and the power of the statistical analyses was not pre-specified.

DFS, DDFS, and OS were computed using the original definitions of the main ALTTO analysis [12]. The study cut-off date used for all time-to-event analyses was December 14, 2016.

All survival analyses comparing patients with HR-positive and negative disease were tested in a Cox proportional hazards model. Patients still in follow-up after 5 years were censored at 5 years in the analysis of early DFS events (years 0–5). A conditional landmark analysis was conducted to investigate late DFS events in patients who were disease-free after 5 years from randomization (years 6–8).

Univariate and multivariate Cox proportional hazards models were used to compare hazard rates within the HR subgroups; the multivariate model was fitted using factors significantly associated with outcome in the univariate model. The final multivariate model was achieved by stepwise selection.

Hazard rates were fitted in an Epanechnikov kernel-smoothed hazard function with bandwidth selected by minimizing the mean integrated squared error. Data are also presented using the Kaplan–Meier survival plots. Patterns of first DFS events were tabulated by HR status.

All statistical tests were two sided; P values < 0.05 were considered statistically significant.

Results

Between June 2007 and July 2011, 8381 patients with HER2-positive early breast cancer were randomized. After excluding patients in the lapatinib alone arm or without centrally tested HR status, 6273 were included in the present analysis: 3603 (57.4%) and 2670 (42.6%) had HR-positive and HR-negative tumors, respectively (Supplementary Figure S1).

Patients with HR-positive disease were younger, more often premenopausal at diagnosis, of white ethnicity, had higher rate of breast-conserving surgery, node-negative, smaller, well or moderately differentiated tumors, and HER2 FISH ratio < 5 (all $P < 0.001$; Table 1). A lower number of patients with HR-positive disease received anthracycline- and taxane-based regimens ($P < 0.04$).

Survival outcomes (DFS, DDFS, and OS)

Overall median follow-up was 6.93 years (95% CI 6.80 to 6.96).

Five-year DFS was 86% (95% CI 85% to 87%) in patients with HR-positive disease and 83% (95% CI 82% to 85%) in those with HR-negative tumors, while 8-year DFS was 80% (95% CI 78% to 82%) and 79% (95% CI 77% to 81%), respectively. Adjusted hazard ratios for HR status (reference group = HR negative) were 0.93 (95% CI 0.82 to 1.05; $P = 0.21$) overall (Fig. 1a), 0.83 (95% CI 0.73 to 0.95; $P = 0.006$) for years 0–5 (Fig. 1b), and 1.67 (95% CI 1.21 to 2.31; $P = 0.002$) for years 6–8 (Fig. 1c). Mean annual hazards of recurrence in years 0–5 were 3% in patients with HR-positive disease and 4% in those with HR-negative tumors, while in years 6–8 they were 3% and 2%, respectively (Fig. 1d, e).

Five-year DDFS was 90% (95% CI 89% to 91%) in patients with HR-positive disease and 87% (95% CI 85% to 88%) in those with HR-negative tumors, while 8-year DDFS was 85% (95% CI 83% to 87%) and 85% (95% CI 83% to 86%), respectively. Adjusted hazard ratios for HR status (reference group = HR-negative) were 0.93 (95% CI 0.81 to 1.07; $P = 0.31$) overall (Fig. 2a), 0.81 (95% CI 0.69 to 0.94; $P = 0.007$) for years 0–5 (Fig. 2b), and 2.61 (95% CI 1.64 to 4.15; $P < 0.001$) for years 6–8 (Fig. 2c).

Five-year OS was 95% (95% CI 94% to 95%) in patients with HR-positive disease and 91% (95% CI 90% to 92%) in those with HR-negative tumors, while 8-year OS was 89% (95% CI 88% to 91%) and 88% (95% CI 87% to 90%), respectively. Adjusted hazard ratios for HR status (reference group = HR-negative) were 0.78 (95% CI 0.66 to 0.92; $P = 0.004$) overall (Fig. 2d), 0.65 (95% CI 0.54 to 0.80; $P < 0.001$) for years 0–5 (Fig. 2e), and 1.37 (95% CI 0.95 to 1.97; $P = 0.088$) for years 6–8 (Fig. 2f).

Patterns of DFS events and factors associated with risk of DFS events

No difference in type of first DFS event was observed between the two groups overall ($P = 0.10$) and in years 0–5 ($P = 0.07$); patients with HR-positive tumors developing DFS event in years 6–8 had higher incidence of distant recurrence and lower incidence of contralateral breast cancer and death without recurrence ($P = 0.005$; Table 2 and Supplementary Table S1). Among patients who developed a distant recurrence as first DFS event, those with HR-positive tumors had lower incidence of visceral metastasis (58.1% vs. 69.2%; $P = 0.004$) and more frequent bone-only involvement (35.3% vs. 21.3%; $P < 0.001$).

Type of first distant recurrence was significantly different ($P < 0.001$) with bone (31.7% vs. 18.7%) and liver (21.0% vs. 16.3%) metastases being more common in patients with

Table 1 Patient, tumor, and treatment characteristics according to hormone receptor status

	Hormone receptor-positive ($n=3603$) No. (%)	Hormone receptor-negative ($n=2670$) No. (%)	*P* value
Age at diagnosis (median)	50 (43 to 58)	52 (45 to 59)	< 0.001
Age at diagnosis			
≤ 40 years	657 (18.2)	399 (14.9)	< 0.001
41–64 years	2610 (72.4)	1963 (73.5)	
≥ 65 years	336 (9.3)	308 (11.5)	
Menopausal status			
Premenopausal	1715 (47.6)	1027 (38.5)	< 0.001
Post-menopausal	1888 (52.4)	1643 (61.5)	
BMI, kg/m^2			
Underweight (BMI < 18.5)	83 (2.3)	52 (2.0)	0.35
Normal (BMI = 18.5–24.9)	1635 (45.4)	1178 (44.1)	
Overweight (BMI = 25–29.9)	1143 (31.7)	899 (33.7)	
Obese (BMI ≥ 30)	737 (20.5)	539 (20.2)	
Missing	5 (0.1)	2 (0.1)	
Ethnicity			
White	2685 (74.5)	1659 (62.1)	< 0.001
Black	54 (1.5)	39 (1.5)	
Asian	767 (21.3)	876 (32.8)	
Other/missing	97 (2.7)	96 (3.6)	
Surgery			
Breast-conserving surgery	1776 (49.3)	1055 (39.5)	< 0.001
Radical surgery	1827 (50.7)	1613 (60.4)	
Missing	0 (0.0)	2 (0.1)	
Tumor size			
pT1	1556 (43.2)	1018 (38.1)	< 0.001
pT2	1590 (44.1)	1219 (45.7)	
pT3–4	168 (4.7)	179 (6.7)	
Not applicable (NACT)	276 (7.7)	242 (9.1)	
Missing	13 (0.4)	12 (0.5)	
Nodal status			
pN0	1520 (42.2)	1010 (37.8)	< 0.001
pN1	1088 (30.2)	745 (27.9)	
pN2–3	719 (20.0)	673 (25.2)	
Not applicable (NACT)	276 (7.7)	242 (9.1)	
Tumor grade			
G1	116 (3.2)	42 (1.6)	< 0.001
G2	1479 (41.1)	828 (31.0)	
G3	1893 (52.5)	1690 (63.3)	
GX	103 (2.9)	96 (3.6)	
Missing	12 (0.3)	14 (0.5)	
Type of expression of hormone receptors			
ER positive and PR positive	2665 (74.0)	Not applicable	Not applicable
ER positive and PR negative	896 (24.9)		
ER negative and PR positive	42 (1.2)		
Level of expression of ER			
ER < 10%	281 (7.8)	Not applicable	Not applicable
ER ≥ 10% and < 30%	307 (8.5)		
ER ≥ 30%	3014 (83.7)		
Missing	1 (0.0)		

Table 1 (continued)

	Hormone receptor-positive ($n=3603$) No. (%)	Hormone receptor-negative ($n=2670$) No. (%)	*P* value
Level of expression of PR			
PR < 10%	1315 (36.5)	Not applicable	Not applicable
PR ≥ 10%– < 30%	515 (14.3)		
PR ≥ 30% (reference)	1772 (49.2)		
Missing	1 (0.0)		
HER2 FISH ratio			
< 2	68 (1.9)	17 (0.6)	< 0.001
≥ 2 to < 5	1793 (49.8)	1169 (43.8)	
≥ 5	1714 (47.6)	1456 (54.5)	
Missing	28 (0.8)	28 (1.1)	
Type of chemotherapy			
Anthracycline and taxane based	2470 (68.6)	1909 (71.5)	0.04
Anthracycline based	933 (25.9)	629 (23.6)	
Taxane based	196 (5.4)	128 (4.8)	
Missing	4 (0.1)	4 (0.2)	
Timing of chemotherapy			
Sequential (design 1)	1971 (54.7)	1470 (55.1)	0.56
Concurrent non-TCH (design 2)	1436 (39.9)	1071 (40.1)	
Concurrent TCH (design 2B)	196 (5.4)	129 (4.8)	
Adjuvant endocrine therapy[a]			
Administered	3237 (89.8)	Not applicable	Not applicable
Not administered	366 (10.2)		
Type of adjuvant endocrine therapy[b]			
AI	1141 (35.3)	Not applicable	Not applicable
Tamoxifen and AI	525 (16.2)		
Tamoxifen	1547 (47.8)		
LHRHa	24 (0.7)		

BMI body mass index, *T* tumor size, *NACT* neoadjuvant chemotherapy, *N* nodal status, *G* grade, *ER* estrogen receptor, *PR* progesterone receptor, *FISH* fluorescence in situ hybridization, *TCH* docetaxel, carboplatin, *AI* aromatase inhibitor, *LHRHa* luteinizing hormone-releasing hormone agonist

[a]Calculated on the total number of patients with ER positive and/or PR positive disease

[b]Calculated on the total number of patients with ER positive and/or PR positive disease who received adjuvant endocrine therapy (a total of 17 patients [< 1%] in the tamoxifen group received a selective estrogen receptor modulator other than tamoxifen)

HR-positive tumors, while lung (28.0% vs. 17.4%) and brain (22.8% vs. 18.0%) in those with HR-negative disease.

Results of univariate analyses for factors associated with risk of DFS events are reported in Supplementary Tables S2 and S3.

In patients with HR-positive disease, at the multivariate analysis (Table 3), factors that remained statistically significantly associated with higher risk of DFS events overall were age ≥ 65 ($P=0.044$), nodal status pN1–pN3 ($P<0.001$), tumor grade 3 ($P=0.004$), no use of aromatase inhibitors ($P<0.001$), and estrogen receptor positivity < 10% ($P=0.003$). In years 0–5, risk factors for DFS events were tumor size pT2-pT4 ($P<0.001$), nodal status pN1-pN3 ($P<0.001$), tumor grade 3 ($P=0.019$), administration of anthracycline only-based chemotherapy ($P<0.001$), and no use of aromatase inhibitors ($P<0.001$). In years 6–8, risk factors for DFS events were nodal status pN2-pN3 ($P=0.044$), and HER2 FISH ratio < 5 ($P=0.018$).

In patients with HR-negative disease, at the multivariate analysis (Table 3), factors that remained statistically significantly associated with higher risk of DFS events overall were BMI (obesity; $P=0.012$), tumor size pT3–4 ($P<0.001$), nodal status pN1–3 ($P<0.001$), and administration of anthracycline only-based chemotherapy ($P<0.001$). In years 0–5, risk factors for DFS events were tumor size pT2–4 ($P<0.001$), nodal status pN1–3 ($P<0.001$), and administration of anthracycline only-based chemotherapy

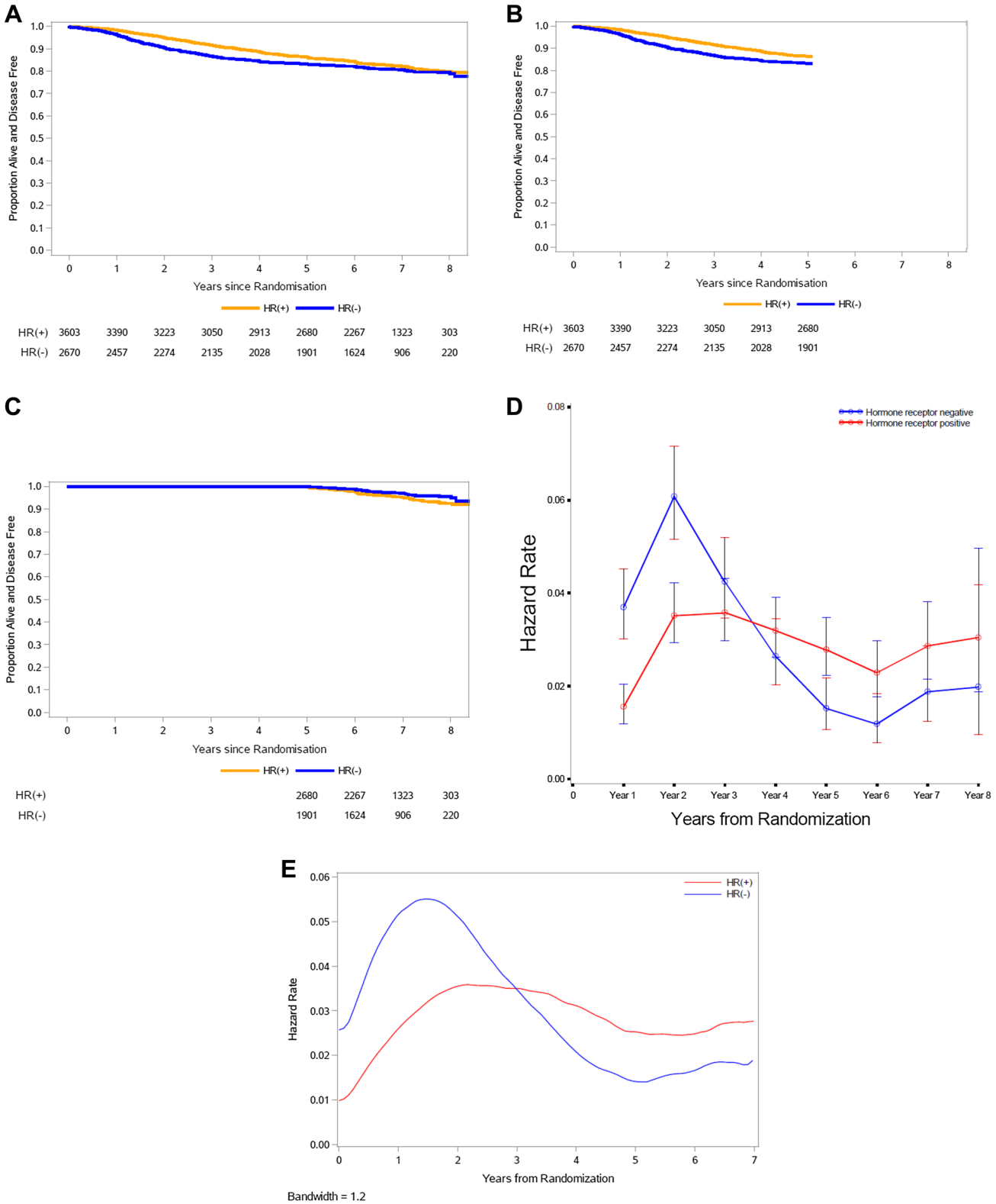

Fig. 1 Disease-free survival in patients with hormone receptor-positive and negative/HER2-positive early breast cancer: **a** Overall; **b** Years 0–5; **c** Years 6–8; **d** Mean annual hazards of recurrence; **e** Epanechnikov Kernel-Smoothed annual hazards of recurrence. *HR+* hormone receptor positive, *HR−* hormone receptor negative

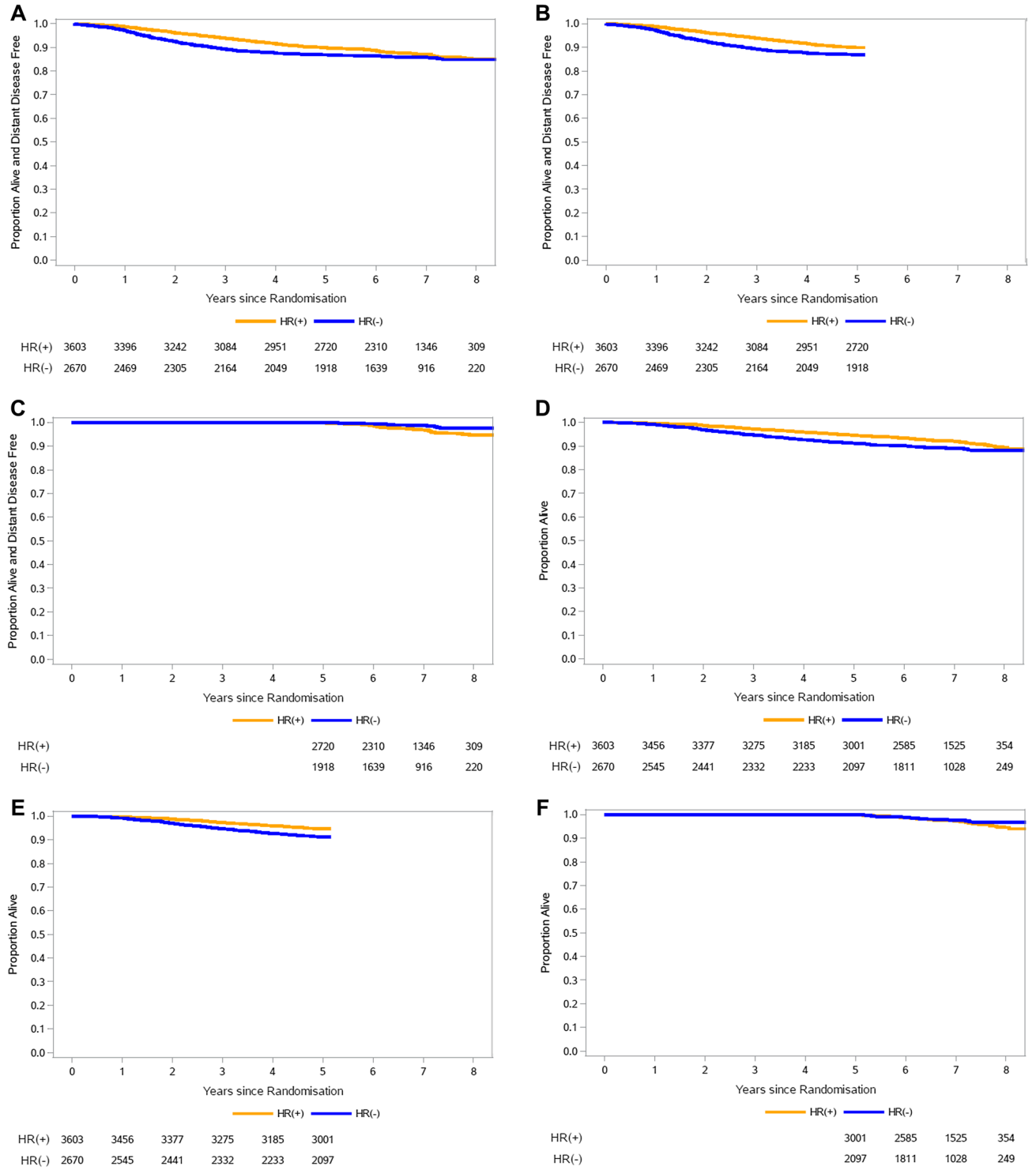

Fig. 2 Distant disease-free survival in patients with hormone receptor-positive and negative/HER2-positive early breast cancer: **a** Overall; **b** Years 0–5; **c** Years 6–8. Overall survival in patients with hormone receptor-positive and negative/HER2-positive early breast cancer: **d** Overall; **e** Years 0–5; **f** Years 6–8. *HR*+ hormone receptor positive, *HR*− hormone receptor negative

Table 2 Patterns of first disease-free survival event according to hormone receptor status

	Hormone receptor-positive ($n=3603$) No. (%)	Hormone receptor-negative ($n=2670$) No. (%)	*P* value
Type of DFS event			
Local recurrence	54 (9.3)	55 (11.6)	0.10
Regional recurrence	14 (2.4)	15 (3.2)	
Distant recurrence	334 (57.5)	289 (60.7)	
Contralateral breast cancer	39 (6.7)	36 (7.6)	
Second primary malignancy	106 (18.2)	57 (12.0)	
Death, no evidence of disease	34 (5.9)	24 (5.0)	
Type of metastatic presentation (only for distant recurrences)[a]			
Visceral	194 (58.1)	200 (69.2)	
Non-visceral	140 (41.9)	89 (30.8)	0.004
Metastatic site (only for distant recurrences)[b]			
Brain	60 (18.0)	66 (22.8)	< 0.001
Liver	70 (21.0)	47 (16.3)	
Lung	58 (17.4)	81 (28.0)	
Bone	106 (31.7)	54 (18.7)	
Others	40 (12.0)	41 (14.2)	
Type of metastatic presentation (only for distant recurrences)[a]			
Visceral (visceral with no bone or visceral + bone)	194 (64.7)	200 (78.7)	< 0.001
Bone-only	106 (35.3)	54 (21.3)	

DFS disease-free survival

[a]Patients with relapses in bone, skin, lymph node, and soft tissue were considered as non-visceral; all the others were considered as visceral

[b]For patients who developed relapse in more than one organ, the first site of distant metastasis were defined by pre-specified importance in the following order: brain, liver, lung, bone, and others. The category "others" includes skin, lymph node, soft tissue, pleura, and other rarer sites of relapse

($P < 0.001$). No significant risk factors for DFS events in years 6–8 were identified.

Discussion

This exploratory analysis of the ALTTO trial investigated clinical outcomes and behavior of HER2-positive tumors according to HR status in patients treated with chemotherapy and adjuvant trastuzumab-based treatment. We observed similar survival outcomes at 8 years between HR-positive and negative/HER2-positive early breast cancer patients but with significantly distinct hazards of DFS events over time. HR status was shown to strongly impact on the type of first DFS events and the risk factors for the development of DFS events overall, in years 0–5 and 6–8.

While the prognostic value of HR status and their different DFS patterns over time have been clearly established in patients with HER2-negative early breast cancer [15, 16], this important information remains uncertain in the HER2-positive population. In the TEACH trial, among patients exposed to chemotherapy without anti-HER2 therapy, a different hazard of DFS events over time according to HR expression was observed and patients with HR-positive/HER2-positive disease showed superior 4-year outcomes [8]. Our analysis adds to this information long-term evidence for patients uniformly treated with chemotherapy and adjuvant trastuzumab-based treatment. We observed that patients with HR-positive/HER2-positive disease had better survival outcomes in the first 5 years as a consequence of the high risk of DFS events in those with HR-negative/HER2-positive tumors. Nevertheless, survival outcomes at 8 years were similar between the two groups given that the annual hazard of DFS events in the HR-positive/HER2-positive group, albeit lower during years 0–5, remained constant over time. These findings may indirectly partially explain the different results observed in the APHINITY and ExteNET trials in HER2-positive early breast cancer patients according to HR status [17, 18]. A more profound upfront HER2-blockade obtained by combining trastuzumab and pertuzumab was particularly beneficial in patients with HR-negative/HER2-positive disease [17]. This could be related to the high dependence on HER2 signaling and the more aggressive clinical behavior of these tumors at short-term follow-up.

Table 3 Factors associated with risk of disease-free survival events in patients with hormone receptor-positive disease and hormone receptor-negative disease: multivariate analyses

	Hormone receptor-positive			Hormone receptor-negative		
	Overall (HR; 95% CI)	**Years 0-5 (HR; 95% CI)**	**Years 6-8 (HR; 95% CI)**	**Overall (HR; 95% CI)**	**Years 0-5 (HR; 95% CI)**	**Years 6-8 (HR; 95% CI)**
Age at diagnosis: ≤ 40 years 41-64 years ≥ 65 years	*P*=.044 1.06 (0.84 to 1.33) REFERENCE 1.45 (1.08 to 1.94)					
BMI, kg/m2: Underweight (BMI < 18.5) Normal (BMI = 18.5-24.9) Overweight (BMI = 25-29.9) Obese (BMI ≥ 30)				*P*=.012 1.80 (1.00 to 3.23) REFERENCE 1.16 (0.94 to 1.44) 1.42 (1.12 to 1.79)		
Tumor size: pT1 (reference) pT2 pT3-4 Not applicable (NACT)		*P*<.001 REFERENCE 1.33 (1.04 to 1.70) 1.76 (1.16 to 2.67) 5.77 (4.04 to 8.25)		*P*<.001 REFERENCE 1.22 (0.99 to 1.52) 1.68 (1.20 to 2.34) 4.08 (2.92 to 5.69)	*P*<.001 REFERENCE 1.30 (1.03 to 1.64) 1.93 (1.37 to 2.73) 4.66 (3.28 to 6.63)	
Nodal status: pN0 pN1 pN2-3 Not applicable (NACT)	*P*<.001 REFERENCE 1.29 (1.01 to 1.64) 2.92 (2.33 to 3.67) 3.39 (2.53 to 4.54)	*P*<.001 REFERENCE 1.45 (1.08 to 1.96) 3.64 (2.72 to 4.86) -	*P*=.038 REFERENCE 1.24 (0.80 to 1.92) 1.97 (1.24 to 3.12) 1.29 (0.63 to 2.65)	*P*<.001 REFERENCE 1.89 (1.44 to 2.48) 3.14 (2.41 to 4.07) -	*P*<.001 REFERENCE 1.87 (1.40 to 2.51) 3.33 (2.52 to 4.39) -	
Tumor grade: G1 G2 G3 GX	*P*=.004 0.44 (0.19 to 0.98) REFERENCE 1.23 (1.02 to 1.48) 0.91 (0.54 to 1.55)	*P*=.019 0.40 (0.15 to 1.07) REFERENCE 1.29 (1.05 to 1.60) 0.91 (0.49 to 1.68)				
Type of chemotherapy: Anthracycline- and taxane-based Anthracycline-based Taxane-based		*P*<.001 0.62 (0.49 to 0.80) REFERENCE 0.57 (0.34 to 0.98)		*P*<.001 0.54 (0.44 to 0.68) REFERENCE 0.64 (0.39 to 1.05)	*P*<.001 0.49 (0.39 to 0.62) REFERENCE 0.65 (0.40 to 1.08)	
Type of adjuvant endocrine therapy[a]:	*P*<.001	*P*<.001				
AI Tamoxifen and AI Tamoxifen LHRHa	0.70 (0.57 to 0.87) 0.45 (0.33 to 0.61) REFERENCE 0.81 (0.30 to 2.19)	0.81 (0.65 to 1.01) 0.36 (0.25 to 0.52) REFERENCE 0.98 (0.36 to 2.66)				
Level of expression of ER: ER <10% ER ≥10% - <30% ER ≥30%	*P*=.003 1.76 (1.27 to 2.45) 1.15 (0.83 to 1.59) REFERENCE					
HER2 FISH ratio: <2 ≥2 to <5 ≥5			*P*=.018 0.70 (0.17 to 2.86) 0.58 (0.40 to 0.85) REFERENCE			

BMI body mass index, *T* tumor size, *NACT* neoadjuvant chemotherapy, *N* nodal status, *G* grade, *AI* aromatase inhibitor, *LHRHa* luteinizing hormone-releasing hormone agonist, *ER* estrogen receptor, *FISH* fluorescence in situ hybridization

[a]Calculated on the total number of patients with ER positive and/or PR positive disease who received adjuvant endocrine therapy (a total of 17 patients [< 1%] in the tamoxifen group received a selective estrogen receptor modulator other than tamoxifen)

On the contrary, patients with HR-positive/HER2-positive tumors derived particular benefit with the use of 1-year neratinib following standard trastuzumab-based treatment [18]. HR-positive/HER2-positive tumors are less dependent on HER2 signaling and the upregulation of ER signaling may lead to partial anti-HER2 treatment resistance [19]. Together with the continuous risk of DFS events, this may explain the benefit observed in this patient population by prolonging anti-HER2 blockade beyond 1 year using non-cross-resistant agents such as the sequence of trastuzumab and neratinib. Biomarker analyses from the APHINITY and ExteNET trials are awaited to further clarify the impact of tumor biology on treatment effect and the need to individualize anti-HER2 therapy recommendations in the early setting according to HR status.

In patients with HER2-positive disease, HR status strongly influences not only the timing for DFS event development but also the pattern of metastatic spread [20–23]. Our analysis showed that type of first DFS events became significantly different between the two populations only after 5 years. In addition, among distant recurrences, visceral metastases were clearly shown to be the most common events overall, but they were less frequent in patients with HR-positive/HER2-positive tumors. The two most frequent distant sites of recurrence were bone and liver in patients with HR-positive/HER2-positive disease, contrasting with lung and brain in those with HR-negative/HER2-positive tumors. While organ-specific signatures of recurrence in breast cancer have been identified [24–27], more research is needed to further investigate the mechanisms behind site

specificity in HER2-positive disease according to HR status. In terms of potential clinical implications, our findings raise awareness on the need to investigate personalized follow-up strategies in early breast cancer patients that would also take into account tumor biology (i.e., HER2 and HR status) and the time-dependent variation in risk of DFS events [28, 29].

By exploring the effect of several baseline patients' tumor and treatment characteristics, our analysis provides important insights on risk factors for the development of DFS events overall, in years 0–5 and 6–8.

In patients with HR-positive/HER2-positive disease, older age was the only baseline patients' characteristic significantly associated with risk of DFS events overall. Nodal status was an important risk factor in all the analyses including for DFS events in the long-term, while tumor size remained associated with increased risk only during years 0–5. Among tumor biologic characteristics, risk factors for DFS events were grade both overall and during years 0–5, ER expression < 10% overall (a feature known to be associated with significantly less benefit from the use of endocrine therapy) [30], and HER2 FISH ratio ≥ 5 during years 6–8. Regarding the impact of anticancer treatments, type of chemotherapy with use of taxanes appeared to be associated with reduced risk of DFS events during years 0–5. Use of aromatase inhibitors was associated with reduced risk of DFS events overall and during years 0–5. This is in line with biomarker analysis showing a more effective inhibition of tumor proliferation with aromatase inhibitors than tamoxifen independently of HER2 [31]. However, recent clinical data from randomized trials conducted mostly in the pre-trastuzumab era showed that both premenopausal [32] and post-menopausal [33] patients with HR-positive/HER2-positive disease appear to derive less benefit with the use of aromatase inhibitors over tamoxifen as compared to those with HER2-negative tumors. Further research is needed to define the optimal endocrine therapy approach in this setting.

In patients with HR-negative/HER2-positive disease, no risk factors for DFS events in years 6–8 were identified. Notably, except for BMI (obesity), all the other risk factors for DFS events overall were also associated with risk of DFS events in years 0–5. Indeed, obesity appears to influence the dynamic of DFS events in patients with HR-negative tumors by increasing the risk of recurrence also at later follow-up times [34]. Tumor stage (tumor size and nodal status) was the strongest prognostic factor. Type of chemotherapy appeared to retain a crucial role with the use of anthracycline- and taxane-based chemotherapy halving the risk of DFS events; this information may need to be considered in designing future chemotherapy de-escalation trials particularly in patients with HR-negative/HER2-positive disease [35].

Limitations of the current study include that this is an exploratory analysis and follow-up data beyond 8 years are not yet available. However, a major strength is that the population investigated comes from a large phase III trial. All patients received chemotherapy plus adjuvant trastuzumab-based treatment and were carefully followed according to study protocol with a median follow-up of approximately 7 years. HR status was a stratification factor in the trial and, importantly, HER2, ER, and PR status were centrally tested and quantified for all included patients.

In conclusions, this large exploratory analysis of the ALTTO trial provides clear evidence that HER2-positive early breast cancer is characterized by the presence of two diseases with distinct natural history based on HR status and adds important prognostic information for counseling patients receiving chemotherapy plus adjuvant trastuzumab-based treatment. Different follow-up strategies and ad hoc future de-escalation and escalation clinical trials should be separately developed for patients with HR-positive or negative/HER2-positive early breast cancer.

Acknowledgements Matteo Lambertini acknowledges the support from the European Society for Medical Oncology (ESMO) for a Translational Research Fellowship at the Institut Jules Bordet in Brussels (Belgium) during the conduction of this analysis. We also acknowledge the ALTTO staff of the BrEAST Data Centre at Institut Jules Bordet in Brussels (Belgium), for clinical record online management, and Sebastien Guillaume of BrEAST Data Centre at Institut Jules Bordet in Brussels (Belgium) for administrative support. None of the individuals named in the acknowledgment received any compensation for their contributions.

Funding The ALTTO trial received financial support from GlaxoSmithKline (until January 2015), Novartis Pharma AG (as of January 2015), and the National Cancer Institute of the National Institutes of Health (NCI-NIH; Grant No. U10CA180821 and U10CA180882 to the Alliance for Clinical Trials in Oncology and Grant No. CA025224 to the legacy North Central Cancer Treatment Group). The present analysis did not receive additional funding. The funders and sponsors had no role in the design or conduct of the study, in the collection, analysis, or interpretation of the data, neither in the preparation, review, or approval of the manuscript.

Compliance with ethical standards

Conflict of interest Dr. Lambertini received honoraria from Teva and speaker's bureau from Theramex outside the submitted work. Dr. Gelber received research grants from Novartis Pharma AG, Pfizer, Roche-Genentech, Ipsen, Celgene, Merck, and Ferring (to the institution). Dr. Werner reports employment at Novartis Pharma AG. Dr. Vaz-Luis received honoraria from Novartis Pharma AG, AstraZeneca, and Ipsen/Kephren outside the submitted work, and research grants from Susan Komen for Cure and Odyssea. Dr. Piccart is a board member of Radius, received honoraria from AstraZeneca, Lilly, MSD, Novartis Pharma AG, Odonate, Pfizer, Roche-Genentech, Camel-IDS, Crescendo Biologics, Periphagen, Huya, Debiopharm, PharmaMar, G1 Therapeutics, Menarini, Seattle Genetics, Immunomedics, Oncolytics outside the submitted work, and research grants from AstraZeneca, Lilly, MSD, Novartis Pharma AG, Pfizer, Roche-Genentech, Synthon, Radius, Servier (to the institution). Dr. Loi acted as consultant (not compensated) to Seattle Genetics, Pfizer, Novartis Pharma AG, BMS, Merck, and Roche-Genentech outside the submitted work, and received research

grants from Novartis Pharma AG, Bristol Meyers Squibb, Merck, Roche-Genentech, Puma Biotechnology, and Pfizer (to the institution). Dr. de Azambuja received honoraria from Roche-Genentech, research grants from Roche–Genentech (to the institution), and travel grants from Roche-Genentech and GlaxoSmithKline outside the submitted work. All the other authors declare no competing interests.

Ethical approval All procedures performed in this trial involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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Affiliations

Matteo Lambertini[1,2] · Christine Campbell[3] · Richard D. Gelber[4] · Giuseppe Viale[5] · Ann McCullough[6] · Florentine Hilbers[7] · Larissa A. Korde[8] · Olena Werner[9] · Saranya Chumsri[10] · Christian Jackisch[11] · Antonio C. Wolff[12] · Ines Vaz-Luis[13] · Arlindo R. Ferreira[14] · Aleix Prat[15] · Alvaro Moreno-Aspitia[10] · Martine Piccart[16] · Sherene Loi[17] · Evandro de Azambuja[16]

1 Department of Medical Oncology, U.O.C. Clinica di Oncologia Medica, IRCCS Ospedale Policlinico San Martino, Largo Rosanna Benzi 10, 16132 Genova, Italy

2 Department of Internal Medicine and Medical Specialties (DiMI), School of Medicine, University of Genova, Genova, Italy

3 Frontier Science, Kingussie, UK

4 Dana-Farber Cancer Institute, Harvard Medical School, Harvard TH Chan School of Public Health and Frontier Science and Technology Research Foundation, Boston, MA, USA

5 IEO, European Institute of Oncology IRCCS, University of Milan, Milan, Italy

6 Mayo Clinic, Phoenix, AZ, USA

7 Breast International Group, Brussels, Belgium

8 National Cancer Institute, Bethesda, MD, USA

9 Novartis Pharma AG, Basel, Switzerland

10 Mayo Clinic, Jacksonville, FL, USA

11 Sana Klinikum Offenbach, Offenbach, Germany

12 Johns Hopkins Sidney Kimmel Comprehensive Cancer Center, Baltimore, MD, USA

13 Institut Gustave Roussy, Villejuif, France

14 Champalimaud Clinical Center, Champalimaud Foundation and Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal

15 Department of Medical Oncology, Hospital Clínic of Barcelona, Barcelona, Spain

16 Institut Jules Bordet and Université Libre de Bruxelles (U.L.B.), Brussels, Belgium

17 Peter MacCallum Cancer Centre, University of Melbourne, Melbourne, Australia