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Pathological complete response and prognosis after neoadjuvant chemotherapy for *HER2*-positive breast cancers before and after trastuzumab era: results from a real-life cohort

Anne-Sophie Hamy-Petit[1], Lisa Belin[2], Hélène Bonsang-Kitzis[1,3], Caroline Paquet[3], Jean-Yves Pierga[4], Florence Lerebours[4], Paul Cottu[4], Roman Rouzier[3], Alexia Savignoni[2], Marick Lae[5] and Fabien Reyal[*,1,3]

[1]*Institut Curie, PSL Research University, Translational Research Department, INSERM, U932 Immunity and Cancer, Residual Tumor & Response to Treatment Laboratory (RT2Lab), F-75248, Paris, France;* [2]*Biostatistics Department, Institut Curie, Paris 75005, France;* [3]*Department of Surgery, Institut Curie, Paris F-75248, France;* [4]*Department of Medical Oncology, Institut Curie, Paris, France and* [5]*Department of Tumor Biology, Institut Curie, Paris 75005, France*

Background: Trastuzumab was introduced a decade ago and has improved outcomes for *HER2*-positive breast cancer. We investigated the factors predictive of pathological complete response (pCR), prognostic factors for disease-free survival (DFS), and interactions between pCR and DFS after neoadjuvant treatment.

Methods: We identified 287 patients with primary *HER2*-positive breast cancers given neoadjuvant chemotherapy (NAC) between 2002 and 2011. Univariate and multivariate analyses of clinical and pathological factors associated with pCR and DFS were performed.

Results: pCR rates differed between patients receiving neoadjuvant trastuzumab treatment or not (47.7% *versus* 19.3%, $P<0.0001$). DFS also differed significantly between patients receiving adjuvant trastuzumab or not (hazard ratio = 4.84, 95% CI (2.52; 9.31), $P<0.001$). We analysed 199 patients given neoadjuvant and adjuvant trastuzumab. Multivariate analysis identified older age and hormone receptor-negative tumours as independent predictors of pCR. T stage (hazard ratio = 2.55, 95% CI (1.01; 6.48), $P=0.05$) and strict pCR (hazard ratio = 9.15, 95% CI (1.22; 68.83), $P=0.03$) were independent predictors of DFS. The latter association was significant in the HR-negative subgroup ($P=0.02$) but not in the HR-positive subgroup ($P=0.12$).

Conclusions: Major pCR and DFS gains in *HER2*-positive BC were observed since 'trastuzumab' era. Further improvements rely on the enrollment of accurately selected patients into clinical trials.

Breast cancer (BC) is the most frequently diagnosed cancer and the leading cause of cancer-related death in women. *HER2*-positive breast carcinomas display amplification and overexpression of the *HER2* tyrosine kinase receptor gene (17q12). This subgroup is defined by aggressive pathological features and a high rate of early distant metastatic events. Trastuzumab-based treatments have been used for the past decade and have improved outcomes in patients with early or metastatic *HER2*-positive breast cancer.

*Correspondence: Dr F Reyal; E-mail: fabien.reyal@curie.fr

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Neoadjuvant treatment is currently being used in patients with early-stage and advanced disease. Its clinical benefits are: (a) higher rates of breast-conserving surgery, (b) similar prognoses for breast cancer patients receiving a neoadjuvant and for those receiving an adjuvant therapy regimen, and (c) a body of evidence showing that the achievement of a pathological complete response (pCR) after neoadjuvant chemotherapy (NAC) is associated with a good prognosis in specific subgroups (triple-negative, *HER2*-positive). Furthermore, it may serve as a test of *in vivo* chemosensitivity, making it possible to evaluate the efficacy of systemic therapy early and to discontinue ineffective treatment.

In parallel, interest has increased in the use of pCR as a surrogate marker for long-term outcome to accelerate the approval process for new drugs since the publication by the Food and Drug Administration (FDA) of a set of guidelines entitled 'Guidance for Industry. Pathologic Complete Response in Neoadjuvant Treatment of High-Risk Early-Stage Breast Cancer: Use as an Endpoint to Support Accelerated Approval'.

In the past few years, the combination of trastuzumab with NAC has become standard, as two phase III trials comparing a regimen in which trastuzumab was added to NAC and NAC alone reported higher pCR rates (MD Anderson Cancer Center trial: pCR rates: 26.3% *versus* 65.2% with and without trastuzumab, respectively (Buzdar *et al*, 2007); NOAH trial: pCR rates: 19% *versus* 38%, respectively) and longer disease-free survival (DFS) for the combined treatment (NOAH trial: 3-year EFS, 71% *versus* 56% with and without trastuzumab, respectively (Gianni *et al*, 2010)). In patients with *HER2*-positive breast tumours for whom neoadjuvant treatment is indicated, trastuzumab is generally added to chemotherapy, and the patient then receives 1 year of adjuvant trastuzumab treatment.

However, factors predictive of pCR and prognostic factors for survival have yet to be identified, and there is still no robust demonstration of the correlation between pCR and outcome in patients treated with optimal therapy. The aim of this study was to identify factors predictive of pCR and prognostic factors in a large cohort of *HER2*-positive breast cancer patients treated by NAC plus trastuzumab.

MATERIALS AND METHODS

Patients. We analysed a cohort of 287 T1–3NxM0 patients with *HER2*-positive invasive breast carcinoma (NEOREP Cohort, CNIL declaration number 1547270) treated at Institut Curie between 2002 and 2012. We included only unilateral, non-recurrent, non-inflammatory, non-metastatic tumours, excluding T4 and lobular tumours. All patients received NAC, followed by surgery and radiotherapy. The study was approved by the Breast Cancer Study Group of Institut Curie and was conducted according to institutional and ethical rules concerning research on tissue specimens and patients. Informed consent from the patients was not required.

Tumour samples. The following histological features were retrieved: tumour type, initial tumour size and nodal status, grade (Elston and Ellis), oestrogen receptor (ER) and progesterone receptor (PR) status, *HER2* status, number of metastatic nodes, and total sentinel and non-sentinel nodes. ER and PR status were determined as follows. Tissue sections were rehydrated and antigen retrieval was carried out in citrate buffer (10 mM, pH 6.1). The sections were then incubated with antibodies against for ER (clone 6F11, Novocastra, Leica Biosystems, Newcastle, UK; 1/200) and PR (clone 1A6, Novocastra, 1/200). The antibodies were then detected with the Vectastain Elite ABC peroxidase-conjugated mouse IgG kit (Vector, Burlingame, CA, USA), with diaminobenzidine (Dako A/S, Glostrup, Denmark) as the chromogen. Positive and negative controls were included in each run. Cases were considered positive for ER and PR if at least 10% of the tumour nuclei were stained, in accordance with standard guidelines used in France (Harvey *et al*, 1999; Gligorov and Namer, 2007). Tumours were considered to be hormone receptor (HR)-positive if they were positive for either ER or PR, and HR-negative if they were negative for both ER and PR. HER2 overexpression status was determined according to the American Society of Clinical Oncology (ASCO) guidelines (Wolff *et al*, 2007).

Treatments. Patients were treated according to national guidelines. NAC regimens changed over time (anthracycline-based regimen or sequential anthracycline–taxane regimen), with trastuzumab used in an adjuvant and/or neoadjuvant setting since the middle of the past decade. Endocrine therapy (tamoxifen, aromatase inhibitor, or GnRH agonists) was prescribed when indicated. Surgery was performed 4–6 weeks after the end of chemotherapy. All patients received adjuvant radiotherapy. Trastuzumab treatments changed over time, and the whole cohort was split into three distinct groups according to trastuzumab use. Patients who did not receive any trastuzumab were indicated as cohort A ($n = 35$); patients who received only adjuvant trastuzumab were indicated as cohort B ($n = 53$); and patients who received both neoadjuvant and adjuvant trastuzumab were indicated as cohort C ($n = 199$).

Pathology assessment at NAC completion. A pCR was defined as the absence of residual invasive cancer cells in the breast and axillary lymph nodes (ypT0/is + /ypN0). Strict pCR (spCR) was defined as an absence of invasive and non-invasive residuals in the breast and invasive disease in the axillary nodes (ypT0 ypN0).

DSF and overall survival (OS). DFS was defined as the time from surgery to death, loco-regional recurrence, or distant recurrence, whichever occurred first, and OS was defined as the time from surgery to death. Patients for whom none of these events were recorded were censored at the date of their last known contact. Survival probabilities were estimated by the Kaplan–Meier method, and survival curves were compared with log-rank tests.

Descriptive analysis of pCR and DFS rates according to the three cohorts. For the pCR rate descriptive analysis, because of the known major impact on trastuzumab use on pCR rates, we chose to pool cohorts A and B (in both of which patients did not receive neaoadjuvant trastuzumab) and compared the resulting pooled cohort with cohort C (in which patients received neoadjuvant trastuzumab).

For the DFS and OS descriptive analysis, because of the known major impact on trastuzumab on DFS rates, we chose to pool cohorts B and C (in both of which patients received trastuzumab) and compared the resulting pooled cohort with cohort A (in which patients did not receive any trastuzumab).

Statistical analysis. The study population was described in terms of frequencies for qualitative variables or medians and associated ranges for quantitative variables. The cutoff date for the analysis was 13 March 2013.

The statistical analyses of the factors predictive of pCR and prognostic for DFS were performed in the cohort C only, as neoadjuvant trastuzumab in association with chemotherapy followed by adjuvant trastuzumab represents the gold standard treatment in 2015.

Factors predictive of pCR were introduced into a univariate logistic regression model. A multivariate logistic model was then implemented. The covariates selected for the multivariate analysis were those with a likelihood ratio test P-value < 0.10 in univariate analysis. A backward stepwise selection procedure was used.

Hazard ratios and their associated 95% confidence intervals were calculated with the Cox proportional hazard model. Variables

with a *P*-value for the likelihood ratio test <0.10 in univariate analysis were included in the multivariate model. Backward selection was used to establish the final multivariate model. The proportional hazards hypothesis was tested for each factor, with Schoenfeld's residuals test and plotting. The significance threshold was 5%. Analyses were performed with the R software, version 2.13.2 (R Development Core Team, 2011).

RESULTS

Overall, 287 patients were identified in our database. The baseline characteristics of these patients are summarised in Table 1. All 287 patients received NAC and underwent surgery followed by radiotherapy. The median age of the patients was 48 years (27–79); 193 patients had T2 tumours (67.2%), and 169 had clinically involved nodes (58.9%). In total, 129 patients had HR-negative breast cancer (44.9%). Trastuzumab treatments changed over time and the characteristics of the patients are presented by treatment (cohort A, $n=35$, no trastuzumab at all; cohort B, $n=53$, adjuvant trastuzumab only; cohort C, $n=199$, both neoadjuvant and adjuvant trastuzumab) in Table 1. There were significant differences between cohorts A, B, and C for treatment period, number of nodes involved (patients in cohort C were less likely to have nodes involved), and median follow-up. Strict pCR (spCR) and pCR rates differed significantly between the three cohorts ($P<0.0001$). These rates were higher in patients given neoadjuvant trastuzumab (cohort C, pCR rate: 47.7%) than in patients who did not receive this treatment (cohorts A and B, pCR rate: 19.3% $P<0.0001$) and in the HR-negative subgroups than in the HR-positive subgroups (pCR rate: 48.8% *versus* 31.2%, $P=0.003$) (Table 2).

DFS (Figure 1A) also differed significantly between cohorts ($P<0.001$). Patients treated without trastuzumab (cohort A) had a higher risk of relapse (hazard ratio = 4.84 95% CI (2.52; 9.31)) than patients receiving adjuvant trastuzumab with or without neoadjuvant trastuzumab (cohorts B and C pooled). Five-year DFS rates were 48.6% (95% CI (34.5–68.3), cohort A) *versus* 83.5% (95% CI (77.6–89.9), cohorts B and C pooled) and were not different between cohorts B and C (cohort B: 80.0%, 95% CI (69.5–92.0) *versus* cohort C 85.8%, 95% CI (79.0–93.3)).

OS (Figure 1B) was also significantly lower in cohort A (hazard ratio = 9.01, 95% CI (2.95–27.52)) than in cohorts B and C pooled ($P<0.001$; 5-year OS rates: 76.9%, 95% CI (64.1–92.3)) *versus* 96.9; 95% CI (94.2–99.7) respectively).

pCR was predicted and prognostic analysis was performed for cohort C only (patients who received optimal neoadjuvant and adjuvant treatment, $n=199$). After neoadjuvant treatment, 66 patients had no residual disease on the surgical specimen, and 29 patients had residual carcinoma *in situ* only (spCR rate: 33.2% (66 out of 199); pCR: 47.7% (95 out of 199)). The following results are given for spCR. Univariate logistic regression analysis identified two factors correlated with spCR: age at diagnosis and HR expression. Both factors remained significant in the multivariate logistic regression model (Table 3). spCR rates increased with age in both HR-positive tumours (12.5, 18.6, and 28.6% for patients <45 years, 45–55, and >55 years, respectively), and in HR-negative ones (27.3, 36.0 and 50.0%, respectively) (Figure 2).

After a median of 33 months of follow-up (range: 6–92), 18 patients experienced relapses (8 local, 3 regional, 7 distant). Two of these patients died. In univariate analysis, the factors associated with DFS were age at diagnosis, spCR and pCR, menopausal status and initial tumour stage. Tumour stage (T3: HR = 2.55, 95% CI (1.01–6.48) *versus* T1–T2: HR = 1, reference class) and spCR (No pCR: HR = 9.15, 95% CI (1.22–68.83) *versus* spCR (reference class), $P=0.03$) remained significantly associated with DFS in multivariate analysis (Table 4), though the number of events was very low in patients whose tumour achieved pCR after NAC. Five-year DFS rates were 78% (95% CI (66.9–90.9); no pCR group) *versus* 95% (95% CI (89.4–100); spCR group).

The persistence of *in situ* carcinoma after chemotherapy was not associated with shorter DFS than the absence of any residual disease ($P=0.17$) or invasive disease only ($P=0.32$).

pCR was positively associated with DFS in patients with HR-negative tumours (Figure 3A) but not in those with HR-positive tumours (Figure 3B).

DISCUSSION

Our retrospective longitudinal study highlights the major impact of the introduction of trastuzumab on *HER2*-positive tumours, with a dramatic improvement in pCR (19.3 to 47.7%), DFS (5-year DFS: 48.6 to 83.5%), and OS rates (5-year OS: 76.9 to 96.9%) between 'pre-trastuzumab' and 'trastuzumab' eras. In patients treated by NAC plus trastuzumab, we identified age at diagnosis and HR status as predictive factors for spCR and pCR and tumour stage at diagnosis as prognostic factors for DFS.

Our study confirms that patients with pCR have excellent DFS and OS. Several studies documented trastuzumab benefits in real-world practice in the adjuvant (Vici *et al*, 2014; Matos *et al*, 2014; Inwald *et al*, 2014; Bonifazi *et al*, 2014; Seferina *et al*, 2015; Jackisch *et al*, 2014) and in the metastatic setting (Olson *et al*, 2013; Karam *et al*, 2013; Park *et al*, 2009; Jackisch *et al*, 2014). Most of these authors found that the magnitude of trastuzumab benefits was equivalent to what was observed in clinical trials (improvement the relative risk for DFS by approximately 50% and OS by 30%). Few – if any – observational studies focussed on the neoadjuvant setting. Our results suggest an even higher magnitude of trastuzumab benefits in a population of *HER2*-positive breast tumours treated by NAC. Because of the retrospective, non-randomised design of the study, we cannot conclude to the single role of trastuzumab effect. Indeed, there were significant differences in the three cohorts in the number of nodes involved as node-negative patients represented 77.4% of cohort C, *versus* 60% and 47.2% of cohorts A and B respectively. As it is known that the prognostic of breast carcinoma following NAC is largely driven by nodal status (Hennessy *et al*, 2005), we can assume that the dramatic differences in DFS between the three cohorts are not only explained by the trastuzumab treatment but also by post-NAC nodal status.

As expected from previous studies of neoadjuvant treatment (Untch *et al*, 2012; Baselga *et al*, 2012; Gianni *et al*, 2012), the absence of HR expression was an important predictor of pCR. This relationship may be quantitative, as some authors have reported an inverse correlation between the level of HR expression and pCR (Bhargava *et al*, 2011). Trastuzumab emtansine (T-DM1) is an antibody–drug conjugate composed of the cytotoxic agent DM1 and trastuzumab, connected by a stable thioether linker. The ADAPT trial (NCT01745965) is currently investigating if the concomitant adjunction of endocrine therapy to T-DM1 neoadjuvant therapy would increase pCR rates in *HER2*+/HR+ operable breast cancers.

In our cohort, older age was significantly associated with spCR. These finding are consistent with those of a retrospective study of 229 *HER2*-positive tumours treated by NAC plus trastuzumab, in which both being young and premenopausal status were significantly associated with lower pCR rates (Kim *et al*, 2013). By contrast, Huober *et al* (2010) found no difference in pCR rates between two age groups (<40 years *versus* $\geqslant 40$ years) for 475 *HER2*-positive tumours. However, none of these patients were treated with neoadjuvant trastuzumab. Similarly, the German

Table 1. Patients, tumours, and treatment characteristics, by cohort (cohort A, $n=35$, no trastuzumab; cohort B, $n=53$, adjuvant trastuzumab only; cohort C, $n=199$, both neadjuvant and adjuvant trastuzumab)

	Whole population ($n=287$)		Cohort A ($n=35$), no trastuzumab		Cohort B ($n=53$), adjuvant tz* only		Cohort C ($n=199$), neoadjuvant and adjuvant tz*		
	n	%	*n*	%	*n*	%	*n*	%	*P*-value
Time period									
2002–2005	70	24.4%	34	97.1%	25	47.2%	11	5.5%	<0.01
2006–2011	217	75.6%	1	2.9%	28	52.8%	188	94.5%	
Age									
<45 y.o.	115	40.1%	12	34.3%	24	45.3%	79	39.7%	0.70
45–55 y.o	94	32.8%	11	31.4%	18	34.0%	65	32.7%	
>55 y.o.	78	27.2%	12	34.3%	11	20.8%	55	27.6%	
Menopausal status									
Premenopausal	176	61.3%	20	57.1%	36	67.9%	120	60.3%	0.42
Postmenopausal	110	38.3%	15	42.9%	16	30.2%	79	39.7%	
T stage									
T1	18	6.3%	3	8.6%	2	3.8%	13	6.5%	0.89
T2	193	67.2%	24	68.6%	35	66.0%	134	67.3%	
T3	75	26.1%	8	22.9%	15	28.3%	52	26.1%	
N stage									
N0	118	41.1%	20	57.1%	17	32.1%	81	40.7%	0.06
N1, N2, N3	169	58.9%	15	42.9%	36	67.9%	118	59.3%	
BMI									
BMI⩽30	253	88.2%	31	88.6%	49	92.5%	173	86.9%	0.59
BMI>30	33	11.5%	4	11.4%	4	7.5%	25	12.6%	
Histological type									
Ductal	271	94.4%	29	82.9%	50	94.3%	192	96.5%	0.84
Others	7	2.4%	1	2.9%	1	1.9%	5	2.5%	
Grade (EE)									
Grade I	2	0.7%	1	2.9%	0	0.0%	1	0.5%	0.23
Grade II	93	32.4%	12	34.3%	20	37.7%	61	30.7%	
Grade III	179	62.4%	17	48.6%	29	54.7%	133	66.8%	
Hormone receptor (HR)									
Positive	157	54.7%	18	51.4%	28	52.8%	111	55.8%	0.88
Negative	129	44.9%	17	48.6%	24	45.3%	88	44.2%	
Oestrogen receptor (ER)									
Negative	138	48.1%	17	48.6%	27	50.9%	94	47.2%	0.89
Positive	149	51.9%	18	51.4%	26	49.1%	105	52.8%	
Progesterone receptor (PR)									
Negative	183	63.8%	25	71.4%	33	62.3%	125	62.8%	0.51
Positive	100	34.8%	9	25.7%	19	35.8%	72	36.2%	
Surgery									
Lumpectomy	199	69.3%	26	74.3%	34	64.2%	139	69.8%	0.61
Mastectomy	88	30.7%	9	25.7%	19	35.8%	60	30.2%	
Sentinel node Biopsy	8	2.8%	0	0.0%	0	0.0%	8	4.0%	0.32
Axillary node dissection	279	97.2%	35	100.0%	53	100.0%	191	96.0%	
Number of nodes involved									
None	200	69.7%	21	60.0%	25	47.2%	154	77.4%	<0.01
1–3	67	23.3%	11	31.4%	21	39.6%	35	17.6%	
⩾4	20	7.0%	3	8.6%	7	13.2%	10	5.0%	
Strict pCR									
No	211	73.5%	29	82.9%	49	92.5%	133	66.8%	<0.01
Yes	76	26.5%	6	17.1%	4	7.5%	66	33.2%	
pCR									
No	175	61.0%	26	74.3%	45	84.9%	104	52.3%	<0.01
Yes	112	39.0%	9	25.7%	8	15.1%	95	47.7%	
Adjuvant chemotherapy									
No	254	88.5%	33	94.3%	37	69.8%	184	92.5%	<0.01
Yes	33	11.5%	2	5.7%	16	30.2%	15	7.5%	
Trastuzumab									
No	35	12.2%	35	100.0%	0	0.0%	0	0.0%	
Adjuvant only	53	18.5%	0	0.0%	53	100.0%	0	0.0%	
Neoadjuvant and adjuvant	199	69.3%	0	0.0%	0	0.0%	199	100.0%	

Table 1. (Continued)

	Whole population ($n=287$)		Cohort A ($n=35$), no trastuzumab		Cohort B ($n=53$), adjuvant tz* only		Cohort C ($n=199$), neoadjuvant and adjuvant tz*		
	n	%	*n*	%	*n*	%	*n*	%	*P*-value
Endocrine therapy									
No	150	52.3%	19	54.3%	32	60.4%	99	49.7%	0.38
Yes	137	47.7%	16	45.7%	21	39.6%	100	50.3%	
Tamoxifen	65	22.6%	14	40.0%	7	13.2%	44	22.1%	
Aromatase inhibitor	50	17.4%	1	2.9%	5	9.4%	44	22.1%	
Others	22	7.7%	1	2.9%	9	17.0%	12	6.0%	
Follow-up									
Median (range)	46	(6–122)	96	(40–122)	67	(25–95)	33	(6–92)	<0.01

Abbreviations: EE = Elston Ellis; pCR = pathological complete response; tz* = trastuzumab; y.o. = years old. Missing values: menopausal status $n=1$; T stage $n=1$; BMI $n=1$; histological type $n=9$; grade $n=13$; HR, $n=1$; PR: $n=4$; radiotherapy $n=2$; adjuvant chemotherapy $n=34$.

Table 2. Pathological response rates by definition, by cohort, and by hormone receptor status

	Whole population ($n=287$)	*P*	Cohort A ($n=35$)	Cohort B ($n=53$)	Cohort C ($n=199$)	*P*
pCR						
No	175/287 (60.9%)		26/35 (74.3%)	45/53 (84.9%)	104/199 (52.3%)	
Yes	112/287 (39.0%)		9/35 (25.7%)	8/53 (15.1%)	95/199 (47.7%)	<0.0001
			17/88 (19.3%)		95/199 (47.7%)	<0.0001
HR positive	49/157 (31.2%)		2/18 (11.1%)	5/28 (17.9%)	42/111 (37.8%)	
HR negative	63/129 (48.8%)	0.0035	7/17 (41.2%)	3/24 (12.5%)	53/88 (60.2%)	
Strict pCR						
No	211/287 (73.5%)		29/35 (82.8%)	49/53 (92.5%)	133/199 (66.8%)	
Yes	76/287 (26.5%)		6/35 (17.1%)	4/53 (7.5%)	66/199 (33.1%)	0.0002
			10/88 (11.4%)		66/199 (33.1%)	<0.00001
HR positive	29/157 (18.5%)		1/18 (5.6%)	2/28 (7.1%)[a]	26/111 (23.4%)	
HR negative	47/129 (36.4%)	0.001	5/17 (29.4%)	2/24 (8.3%)	40/88 (45.5%)	

Abbreviations: HR = hormone receptor; pCR = pathological complete response.
[a]HR not available, $n=1$.

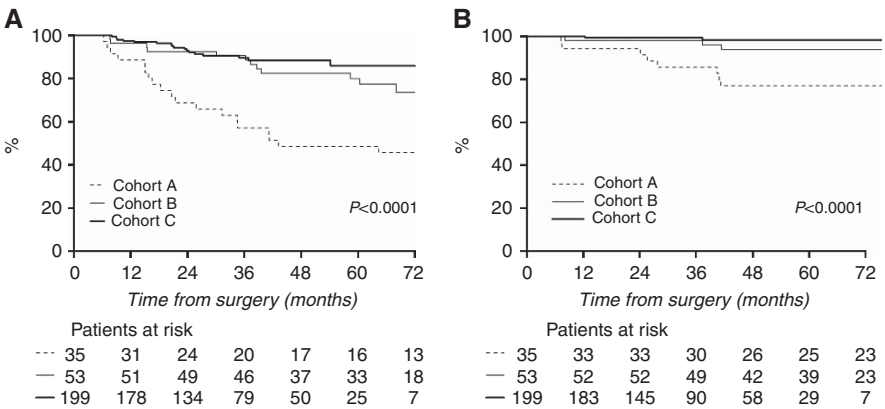

Figure 1. (**A**) DFS and (**B**) OS by cohort (cohort A: no trastuzumab; cohort B: adjuvant trastuzumab only; cohort C: neoadjuvant and adjuvant trastuzumab).

Breast Group (GBG) and the AGO-B study group published a meta-analysis focussing on the impact of age on NAC outcomes. In 1820 patients with *HER2*-positive tumours, pCR rates did not differ significantly with age in either HR-positive or HR-negative tumours (Loibl *et al*, 2015). Patients with *HER2*-positive disease received anti-*HER2* treatment as part of the neoadjuvant treatment in three of the eight trials of this meta-analysis.

Initial T stage remained a significant prognostic factor. This finding is consistent with those of several other studies (Kim *et al*, 2013; Takada *et al*, 2014; Tanioka *et al*, 2014), although similar results were obtained only for the HR-negative subgroup in the study by Takada *et al* (2014).

In a sub-study of EORTC 10994/BIG 1-00 phase III trial (Fei *et al*, 2015) on 283 patients with pCR achievement after NAC, only clinical tumour size independently predicted relapse. Several hypotheses can be drawn to explain the independent impact of tumoral size. The first one is that large tumours may be more likely to present intrinsic or acquired chemoresistance. Causal factors

Table 3. Odds ratios for predicting strict pCR (univariate and multivariate analyses)

		Univariate analysis			Multivariate analysis		
	pCR (%)	OR	95% CI	*P*-value	OR	95% CI	*P*-value
BMI, kg m^{-2}							
BMI ⩽ 30	33.5	1		0.88			
BMI > 30	32.0	0.93	(0.36; 2.23)				
Age							
< 45 y.o.	24.1	1		0.02	1	—	0.03
45–55 y.o	35.4	1.73	(0.84; 3.60)		1.44	(0.68; 3.05)	
> 55 y.o.	43.6	2.44	(1.17; 5.19)		2.74	(1.27; 5.93)	
Menopausal status							
Premenopausal	29.2	1		0.14			
Postmenopausal	39.2	1.57	(0.86; 2.86)				
T stage							
T1–T2	34.0	1		0.67			
T3	30.8	0.86	(0.43; 1.68)				
N stage							
N0	37.0	1		0.34			
N1, N2, N3	30.5	0.75	(0.41; 1.36)				
Grade (EE)							
Grade I–II	35.5	1		0.66			
Grade III	32.3	0.87	(0.46; 1.65)				
ER							
Positive	21.9	1		< 0.01			
Negative	45.7	3.01	(1.64; 5.56)				
PR							
Positive	19.4	1		< 0.01			
Negative	41.6	2.95	(1.51; 5.88)				
HR							
Positive	23.4	1		< 0.01	1	—	< 0.01
Negative	45.5	2.72	(1.49; 5.05)		2.97	(1.57; 5.63)	

Abbreviations: BMI = body mass index; CI = confidence interval; EE = Elston Ellis; ER = oestrogen receptor; HR = hormone receptor; OR = odds ratio; pCR = pathological complete response; PR = progesterone receptor; y.o. = years old.

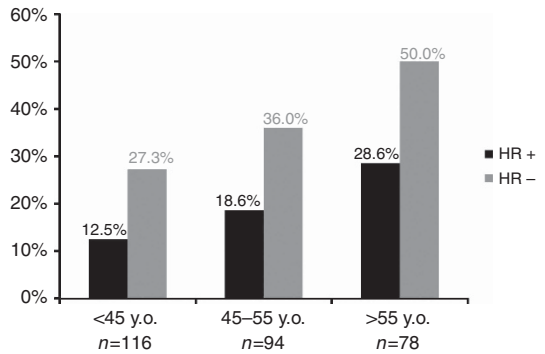

Figure 2. pCR rates by age and HR status.

may first include a variety of physical and mechanical effects (e.g., inefficient distribution of the drug, central necrosis and hypoxia, anarchic neoangiogenesis). Second, the immune reaction appears to evolve with tumour progression, and it is known that immune subpopulations densities change with increasing stage (Bindea *et al*, 2013; Fridman *et al*, 2012) potentially impairing the response to chemotherapy. Third, tumoral heterogeneity increases with tumour size, leading to the potential emergence of drug-multiresistant clones.

A second hypothesis considers the kinetics of the tumour growth. Mathematical modellings (Hartung *et al*, 2014) validate the link between primary tumour size and emission rate, that is, metastatic spreading. In clinical practice, this relation between a large tumour size and the presence of circulating tumour cells in peripheral blood has also been identified (Liao *et al*, 2014). Considering initial exponential growth phase of the Gompertz model (Benzekry *et al*, 2014) and the high proliferation rate of *HER2*-positive breast cancers, it seems plausible that these tumours may rapidly toggle from localised breast cancers to a micrometastatic disease. The subsequent pivotal transition between micrometastases and macrometastases (namely the metastatic colonisation) is still poorly understood. It remains unknown if tumoral size may impact this process. Both phenomena (chemoresistance and micrometastic spreading) may coexist and share pathways by complex homing interactions.

It remains a matter of debate whether pCR can be used as a surrogate for DFS in *HER2*-positive breast carcinomas, particularly those that are HR-positive. In our cohort, residual disease was associated with a hazard ratio for relapse of 9 relative to patients with spCR. This effect was limited to HR-negative tumours. In a large meta-analysis of 6377 patients with primary breast cancer receiving neoadjuvant anthracycline-taxane-based chemotherapy in seven randomised trials, Von Minckwitz *et al* (2012) identified pCR as a surrogate marker for both DFS and OS in *HER2*-positive subgroups. In patients with HER2-positive tumours treated with trastuzumab ($n = 662$), pCR was associated with a hazard ratio of 2.85 ((1.69–4.83), $P < 0.001$) for DFS and of 14.11 ((1.93–103.03), $P < 0.009$) for OS. However, the prognostic impact of pCR was restricted to HR-negative tumours. It was not observed in the luminal B/HER2-positive subgroup. In a recent pooled analysis of 12 international trials and 11955 patients (CTNeoBC), Cortazar *et al* (2014) found a significant association between pCR and

Table 4. Hazard ratios for predicting DFS (univariate and multivariate analyses)

	Univariate analysis			Multivariate analysis		
	HR	**95% CI**	***P***	**HR**	**95% CI**	***P***
BMI, kg m^{-2}						
BMI ⩽ 30	1	—	0.24			
BMI > 30	2.05	(0.67; 6.26)				
Age						
< 45 y.o.	1	—	0.04			
45–55 y.o	1.32	(0.51; 3.43)				
> 55 y.o.	0.17	(0.02; 1.36)				
Menopausal status						
Premenopausal	1	—	0.08			
Postmenopausal	0.40	(0.13; 1.21)				
T stage						
T1–T2	1	—	0.07	1	—	0.05
T3	2.44	(0.96; 6.19)		2.55	(1.01–6.48)	
N stage						
N0	1	—	0.45			
N1, N2, N3	1.45	(0.55; 3.87)				
Grade (EE)						
Grade I–II	1	—	0.39			
Grade III	0.65	(0.25; 1.71)				
HR						
Positive	1	—	0.41			
Negative	1.48	(0.59; 3.76)				
pCR						
Yes	1	—	< 0.01			
No	4.68	(1.36–16.18)				
Strict pCR						
Yes	1	—	< 0.01	1	—	0.03
No	8.81	(1.17–66.25)		9.15	(1.22; 68.83)	

Abbreviations: BMI = body mass index; CI = confidence interval; DFS = disease-free survival; EE = Elston Ellis; HR = hazard ratio; HR = hormone receptor; pCR = pathological complete response; y.o. = years old.

event-free survival in both the HR-positive and HR-negative subgroups, although the magnitude of this effect was greater in HR-negative tumours (HR-positive, 0.58 (0.42–0.82); HR-negative: 0.25 (0.18–0.34)). However, a subset of *HER2*-positive breast cancer did not receive adjuvant trastuzumab. When the analysis was restricted to patients who received trastuzumab, the association between pCR and OS was not significant in HR-positive tumours (0.56 (0.23–1.37)). In addition, three multicenter retrospective studies on *HER2*-positive breast tumours treated with NAC and trastuzumab identified pCR as a surrogate marker for DFS in HR-negative disease (Tanioka *et al*, 2014; Takada *et al*, 2014; Kim *et al*, 2013), but the results for the HR-positive group were discordant, with a positive association retrieved by some authors (Kim *et al*, 2013) but not by others (Takada *et al*, 2014; Tanioka *et al*, 2014).

Our study adds weight to the findings of previous investigations, because it focusses on a particular breast cancer subtype and reports results for a large population treated with NAC and trastuzumab, the gold standard treatment in 2015. This study provides a better representation of real-life experience than previous meta-analyses of clinical trials, because, although meta-analysis provide an effective means of acquiring large amounts of data, the patients included in clinical trials differ from the general population. Our data confirm the association of pCR with DFS in *HER2*-positive, HR-negative breast cancers and provide new insight that could improve prognostic prediction. The absence of a significant effect in the HR-positive subgroup might be due to biological differences though we cannot exclude a lack of statistical power.

The confirmation of a quantitative correlation between increments in pCR and gains in survival in large data sets is of paramount importance for accelerated drug approval for the neoadjuvant model. It is particularly important because the *HER2*-targeting drug pipeline contains many candidates. The novel anti-*HER2* antibody pertuzumab has obtained accelerated approval from the US FDA (Prowell and Pazdur, 2012) for use in the neoadjuvant setting for *HER2*-positive breast cancer, based on the results of the NEOSPHERE trial (Gianni *et al*, 2012). Definitive approval for pertuzumab will depend on the results of the APHINITY trial evaluating the addition of pertuzumab to adjuvant trastuzumab-based chemotherapy. Controversy concerning the legitimacy of pCR as a surrogate re-emerged with the results of the ALTTO trial in ASCO 2014 (Piccart-Gebhart *et al*, 2014). In this study, addition of lapatinib to standard trastuzumab adjuvant therapy was not found to improve survival in women with *HER2*-positive early breast cancer. This result was unexpected, because the combination of lapatinib and trastuzumab was associated with higher rates of pCR rates in the neoALTTO trial (Baselga *et al*, 2012). Improving pCR rates may theoretically: (i) increase conservative treatment probabilities; and (ii) identify a population at higher risk of relapse and thus help selecting patients likely to benefit from new therapies. Accurate and sharp patient selection may avoid failure of all-comers trials such as ALTTO and MARIANNE (NCT01120184). The KATHERINE trial (NCT01772472) is currently investigating TDM-1 as alternative adjuvant treatment to trastuzumab in *HER2*-positive patients with residual disease following NAC. Our study supports this design for new drug testing, bearing in mind that even in patients with residual disease DFS rates were high in our cohort.

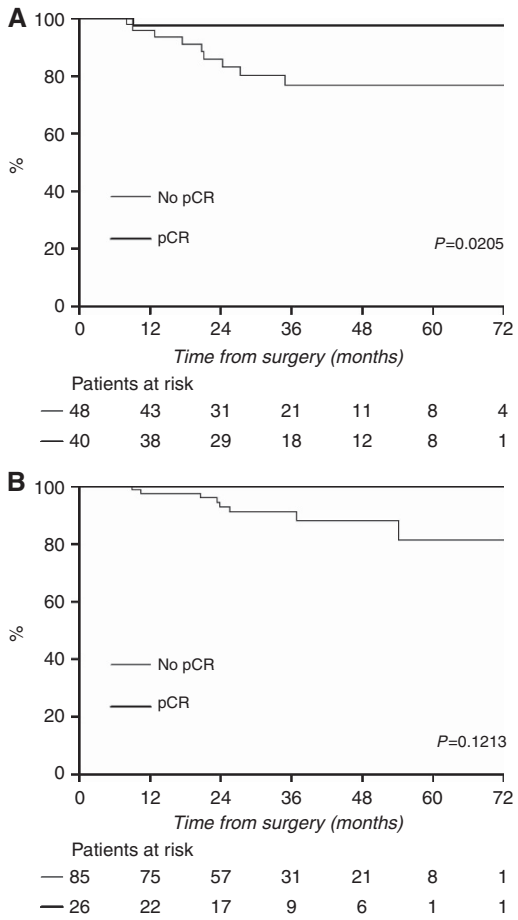

Figure 3. Association of pCR and DFS in (**A**) patients with HR-negative tumours and (**B**) patients with HR-positive tumours.

CONCLUSION

Trastuzumab considerably modifies the prognosis of *HER2*-positive breast carcinomas. These tumours have an excellent prognosis when pCR is achieved. However, it remains unclear whether second-line *HER2*-targeted treatments with pertuzumab, lapatinib or TDM1 following NAC improve survival in selected patients. Our findings suggest that patients with *HER2*-positive tumours of a large initial size, for which pCR is not achieved at the end of NAC, remain at risk of relapse despite adjuvant trastuzumab treatments. Such patients could be studied in second-line treatment trials. However, there is a need to rethink future clinical trial designs bearing in mind several pitfalls: (i) sufficient recruitment of patients despite the scarcity of trastuzumab-resistant patients; (ii) consider a different disease setting with possibly already micrometastatic populations and thus consider new therapeutic targets to investigate (Mina and Sledge, 2011); and (iii) finally, expected events may be low, and only international collaborative works will allow sufficient population size. The challenge still needs to be overcome.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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