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Original Research

Trastuzumab-lapatinib as neoadjuvant therapy for HER2-positive early breast cancer: Survival analyses of the CHER-Lob trial

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KEYWORDS
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Abstract ***Aim:*** The Cher-LOB randomised phase II study showed that the combination of lapatinib-trastuzumab plus chemotherapy increases pathologic complete response (pCR) rate compared with chemotherapy plus either trastuzumab or lapatinib. Here, we report the post hoc survival analysis as per treatment arm, pCR and biomarkers.
Methods: The Cher-LOB study randomised 121 patients with human epidermal growth factor receptor 2–positive, stage II–IIIA breast cancer. A specific protocol to collect recurrence-free survival (RFS) and overall survival (OS) data was designed. Tumour-infiltrating lymphocytes (TILs) and PAM50-intrinsic subtyping were evaluated at baseline.
Results: At 9-year median follow-up, a trend towards RFS improvement with lapatinib-trastuzumab over trastuzumab was observed (hazard ratio [HR] 0.44, 95% confidence interval [CI] 0.18–1.05). Combining treatment arms, pCR was significantly associated with both RFS (HR 0.12, 95% CI 0.03–0.49) and OS (HR 0.12, 95% CI 0.03–0.49). TILs were significantly associated with RFS (HR = 0.978 for each 1% increment). Luminal-A subtype was a significant and independent predictor of improved RFS as compared with other PAM50-based intrinsic subtypes at the multivariate analysis including the most relevant clinicopathologic variables (HR 0.29, 95% CI 0.09–0.94, p = 0.040).
Conclusions: Cher-LOB trial survival analysis confirmed the prognostic role of pCR and TILs and showed a signal for a better outcome with lapatinib-trastuzumab over trastuzumab.
Trial registration: NCT00429299.

1. Introduction

Human epidermal growth factor receptor 2 (HER2) is overexpressed/amplified in around 20% of early breast cancers (BCs) and, in the absence of targeted treatment, represents a poor prognostic factor.

Over the past three decades, the implementation of HER2-targeted treatments has progressively improved the prognosis of HER2-positive BC [1–3]. The neoadjuvant model represents an ideal platform to evaluate new therapeutic strategies, and the adoption of pathologic complete response (pCR) as a surrogate end-point in neoadjuvant trials with regulatory intent is currently endorsed by US Food and Drug Administration (FDA) [4]. Indeed, irrespective of tumour biology and disease stage at diagnosis, patients achieving pCR have a better outcome than patients with residual disease [5]. In the neoadjuvant setting, the addition of trastuzumab to chemotherapy has been associated with a significant increase in pCR rates over chemotherapy alone and also with an improved outcome [6–8]. Therefore, several studies testing different strategies have been set up, with the primary aim of increasing pCR rate. In particular, dual HER2-blockade with trastuzumab and lapatinib provided meaningful clinical results. As compared with chemotherapy and trastuzumab, the combination of trastuzumab-lapatinib and chemotherapy was associated with an increased pCR rate in several randomised trials [9–13], thus providing evidence supporting the incorporation of this escalated approach in the neoadjuvant management of HER2-positive disease. However, the surrogacy of pCR for drug efficacy is still object of extensive debate. Indeed, in the above-mentioned FDA-promoted CTNeoBC meta-analysis [5], authors failed to formally establish a trial-level relationship between pCR differences between treatment arms and survival. Emblematically, although a trend towards increased survival rates has been consistently observed with dual versus single HER2-blockade across escalated neoadjuvant trials, only the CALGB 40601 phase III trial so far reported a significant improvement in survival outcomes [9,10].

We previously reported findings from the phase II non-comparative randomised Cher-LOB trial, showing a relative 80% increase in pCR rates (ypT0is/N0) in patients treated with lapatinib plus trastuzumab plus chemotherapy versus chemotherapy plus either lapatinib or trastuzumab [13].

Here, we report the post hoc analysis of long-term survival outcomes (recurrence-free survival, RFS; overall survival, OS) from the Cher-LOB trial, as per treatment arms, pCR status and biomarkers.

2. Methods

2.1. Cher-LOB study design, patients and study procedures

The Cher-LOB study design, procedures and primary efficacy results have been previously published and are detailed in Supplementary Methods [13–15]. Briefly, 121 patients with stage II–IIIA HER2-positive BC were centrally randomised (1:1:1) to receive paclitaxel-anthracycline–based neoadjuvant chemotherapy plus trastuzumab (arm A), lapatinib (arm B) or concomitant trastuzumab plus lapatinib (arm C). Ethical Committees of participating sites approved the study, and informed consent was obtained from patients before study entry.

pCR was defined as the absence of residual invasive tumour in breast and axillary nodes. Methodology used to evaluate tumour-infiltrating lymphocytes (TILs) and PAM50 intrinsic subtyping [14,15] is detailed in Supplementary Methods.

2.2. Outcomes

A specific protocol to continue survival data collection of patients enrolled in the Cher-LOB trial was approved by participating centers' institutional review boards.

RFS was calculated from randomisation to BC recurrence (locoregional or distant; contralateral BC excluded) or death from any cause, whichever first. OS was calculated from randomisation to death from any cause or last follow-up. Patients without an event were censored at time of the last clinical assessment.

2.3. Statistical analysis

The Kaplan-Meier method was adopted to estimate survival curves and 5-year rates, whereas the Cox proportional hazard model was used to compare RFS and OS among subgroups. We examined the relationship between survival end-points and treatment arm, pathologic response at surgery (pCR vs no-pCR), oestrogen receptor (ER) status and biomarkers (TILs, PAM50-based intrinsic subtyping). The association between RFS and biomarkers was evaluated at univariate and multivariate analyses. Hazard ratios (HRs) and 95% confidence intervals (CIs) were also reported. All tests were two-sided and significant for $P \leq 0.05$. Statistical analyses were performed using SPSS v.26.

Because the Cher-LOB trial was not designed or powered to detect treatment differences with respect to survival end-points, RFS and OS analyses are to be considered for descriptive purposes only.

3. Results

In the Cher-LOB trial, 36, 39 and 46 patients were randomly assigned to trastuzumab (A), lapatinib (B) and concomitant trastuzumab plus lapatinib (C) arms, respectively. Patients' clinicopathologic features have been previously reported [13].

Survival data were available for 114 of the 121 patients originally included in the Cher-LOB trial (CONSORT chart in Supplementary Fig. S1). Patient characteristics were in line with the overall Cher-LOB study cohort, as shown in Table 1. Distribution per arm was as follows: 36 in arm A, 35 in arm B and 43 in arm C. Patient clinicopathologic features as per treatment arm are summarised in Table 2. pCR was observed in 37 patients (32%).

At a median follow-up of 9 years (95% CI 8.4–9.7), 32 RFS events and 15 deaths were recorded. Patients experiencing an RFS event were 14 (39%), 10 (29%) and 8 (19%) in the trastuzumab, lapatinib or concomitant trastuzumab plus lapatinib arm, respectively. Patterns of first recurrence and causes of death as per treatment arm are reported in Supplementary Table I. The 5-year RFS was 77.8% in the trastuzumab arm, 77.1% in the lapatinib arm and 85.8% in the combination arm (Fig. 1A). RFS was not statistically different among the three treatment arms (log rank $P = 0.162$); however, a trend towards a benefit for the combination as compared with the trastuzumab arm (HR 0.44, 95% CI 0.18–1.05) was observed. Globally, the 5-year RFS was 77.5% for patients treated with chemotherapy plus single HER2-blockade (A + B) and 85.8% for patients treated with chemotherapy plus dual HER2-blockade (C) (HR 0.52, 95% CI 0.23–1.15; log-rank $P = 0.102$; Fig. 1B). There were no significant differences in RFS as per ER expression (5-year RFS 79.8% vs 81.1% for ER-negative and ER-positive tumours, respectively; HR 1.032, 95% CI 0.50–2.11; log-rank $P = 0.932$).

Patients experiencing an OS event were 5 (14%), 4 (11%) and 6 (14%) in the trastuzumab, lapatinib or concomitant trastuzumab plus lapatinib arm, respectively. No significant difference was observed in OS between the combination and the trastuzumab arm (HR 1.00, 95% CI 0.31–3.27).

Combining treatment groups, a significant association between pCR and RFS was observed. Patients achieving pCR had a significantly higher 5-year RFS (97.3%) than those without pCR (72.7%) (HR 0.12, 95% CI 0.03–0.49; log-rank $P < 0.001$; Fig. 2A). Results were consistent in the ER-negative (5-year RFS in pCR vs no-pCR: 94.7% vs 69.2%, HR 0.11, 95% CI 0.01–0.85; log-rank $P = 0.010$) and ER-positive (5-year RFS in pCR vs no-pCR: 100% vs 74.5%, HR 0.12, 95% CI 0.02–0.90; log-rank $P = 0.014$) cohorts separately.

Patients achieving pCR also presented a significantly higher 5-year OS (97.2% vs 89.5%) than those without

Table 1
Clinicopathological characteristics of patients included in the survival analysis compared with the overall CherLOB population.

Variable	Overall CherLOB population			Survival analysis population			
	N (%)	Mean (range)	IQ (Q1; Q3)	N (%)	Mean (range)	IQ (Q1; Q3)	p
Age							
Available	121 (100)	49 (26–68)	12 (44–56)	114 (100)	49 (26–68)	13 (44–57)	ns
Missing	0 (0)						
Clinical stage							
IIA	38 (31.4)			37 (32.5)			ns
IIB	61 (50.4)			57 (50.0)			
IIIA	22 (18.2)			20 (17.5)			
Missing	0 (0)			0 (0)			
Histotype							
Ductal	115 (95)			108 (94.7)			ns
Lobular	6 (5)			6 (5.3)			
NA	0 (0)			0 (0)			
Grade							
1–2	23 (19)			19 (16.7)			ns
3	77 (63.9)			74 (64.9)			
Missing	21 (17.4)			21 (18.4)			
ER							
ER-	50 (41.3)			45 (39.5)			ns
ER+	71 (58.7)			69 (60.5)			
Missing	0 (0)			0 (0)			
Ki67							
Available	113 (93.4)	29.6 (4–90)	15 (20–35)	107 (93.8)	29.5 (4–90)	15 (20–35)	ns
Missing	8 (6.6)			7 (6.2)			
Arm							
A (CT + T)	36 (29.8)			36 (31.5)			ns
B (CT + L)	39 (32.2)			35 (30.7)			
C (CT + T + L)	46 (38)			43 (37.8)			
Missing	0 (0)			0 (0)			
strTILs							
Available	105 (86.8)	26.8 (0–100)	31 (9–40)	102 (89.5)	26.8 (0–100)	30.5 (9.5–40)	ns
Missing	16 (13.2)			12 (10.5)			
PAM50							
Luminal A	23 (19.0)			21 (18.5)			ns
Luminal B	14 (11.6)			14 (12.3)			
HER2-enriched	22 (18.2)			22 (19.3)			
Basal-like	12 (9.9)			12 (10.5)			
Normal-like	15 (12.4)			15 (13.1)			
Missing	35 (28.9)			30 (26.3)			

N, number; IQ, interquartile; Q, quartile; P, P-value; ER, oestrogen receptor; CT, chemotherapy; T, trastuzumab; L, lapatinib; strTILs, stromal tumour infiltrating lymphocytes; ns, non-significant.

pCR (HR 0.12, 95% CI 0.03–0.49; log-rank $P = 0.028$; Fig. 2B).

Finally, the impact of tumour biology and microenvironment on RFS was assessed.

Baseline stromal TIL (strTIL) evaluation was available for 102 of 114 patients assessable for the long-term outcome and significantly correlated with RFS at univariate analysis: an HR of 0.978 for each 1% TIL increment was observed (95% CI 0.957–0.999, $P = 0.041$). At multivariate analysis including strTILs, pCR, clinical stage, treatment arm (single versus dual HER2-blockade), ER status and age, only pCR maintained a significant association with RFS, as shown in Supplementary Table 2.

PAM50 intrinsic subtyping was assessable for 84 of 114 patients included in the long-term outcome analysis. Subtype distribution was as follows: HER2-enriched = 26.2% (N = 22), luminal A = 25.0% (N = 21), luminal B = 16.7% (N = 14), basal-like = 14.3% (N = 12) and normal-like = 17.9% (N = 15). Excluding normal-like cases, no significant difference in RFS as per PAM50 intrinsic subtyping was observed (evaluable N = 69; Supplementary Fig. S2); however, at multivariate analysis including PAM50 subtypes (luminal A versus other subtypes), pCR, clinical stage, treatment arm (single versus dual HER2-blockade), ER status and age, both pCR and luminal A subtype (as compared with other PAM50 subtypes) were confirmed to retain an independent prognostic value, as shown in Table 3.

Table 2
Clinicopathological characteristics of patients included in the survival analysis as per the treatment arm.

Variable	Arm A (CT + T)			Arm B (CT + L)			Arm C (CT + T + L)		
	N (%)	Mean (range)	IQ (Q1; Q3)	N (%)	Mean (range)	IQ (Q1; Q3)	N (%)	Mean (range)	IQ (Q1; Q3)
Number of patients	36			35			43		
Age									
Available	36 (100)	50 (34–65)	13 (44–57)	35 (100)	49 (34–68)	13 (43–56)	43 (100)	48 (26–65)	13 (43–56)
Missing	0 (0)			0 (0)			0 (0)		
Clinical stage									
IIA	11 (30.6)			13 (37.1)			13 (30.2)		
IIB	19 (52.8)			17 (48.6)			21 (48.8)		
IIIA	6 (16.7)			5 (14.3)			9 (20.9)		
Missing	0 (0)			0 (0)			0 (0)		
Histotype									
Ductal	32 (88.9)			33 (94.3)			43 (100)		
Lobular	4 (11.1)			2 (5.7)			0 (0)		
NA	0 (0)			0 (0)			0 (0)		
Grade									
1–2	8 (22.3)			4 (11.4)			7 (16.3)		
3	24 (66.7)			23 (65.7)			27 (62.8)		
Missing	4 (11.1)			8 (22.9)			9 (20.9)		
ER									
ER-	16 (44.4)			12 (34.3)			17 (39.5)		
ER+	20 (55.6)			23 (65.7)			26 (60.5)		
Missing	0 (0)			0 (0)			0 (0)		
Ki67									
Available	34 (94.4)	26.8 (10–60)	13.5 (17.7–31.2)	33 (94.3)	29.3 (12–65)	16.5 (20–36.5)	40 (93)	32.1 (4–90)	20 (20–40)
Missing	2 (5.6)			2 (5.7)			3 (7)		
pCR									
Yes	9 (25)			9 (25.7)			19 (44.2)		
No	27 (750,029)			26 (74.3)			24 (55.8)		

N, number; IQ, interquartile; Q, quartile; ER, oestrogen receptor; CT, chemotherapy; T, trastuzumab; L, lapatinib; pCR, pathological complete response.

4. Discussion

Findings from the post hoc survival analysis of the phase II randomised Cher-LOB trial regarding the prognostic role of pCR in patients with HER2-positive BC receiving neoadjuvant chemotherapy plus anti-HER2 treatment are consistent with previous reports. By combining treatment arms, we showed that achieving pCR was highly and significantly associated with long-term survival. Patients with pCR experienced a 78% relative decrease in the risk of an RFS or OS event at 5 years as compared with those with residual invasive disease. This supports the strong prognostic role that the achievement of pCR retains in HER2-positive BC at a single-patient level, as already highlighted in the context of the CtNeoBC meta-analysis [5] and in studies testing dual HER2-blockade as the escalated neoadjuvant approach [7,9,16–18]. Importantly, the positive prognostic impact of pCR observed in our analysis was

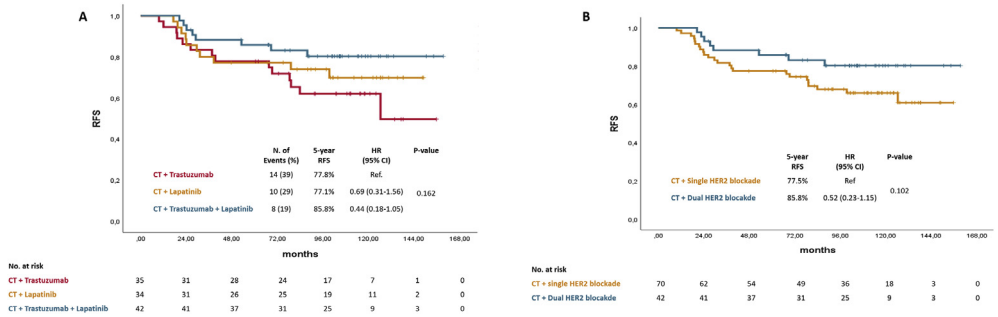

Fig. 1. Kaplan-Meier RFS curves as per A. treatment arm (chemotherapy + trastuzumab vs chemotherapy + lapatinib vs chemotherapy + trastuzumab + lapatinib) and B. dual HER2 blockade vs single HER2 blockade (CT + trastuzumab and CT + lapatinib arms combined). RFS, recurrence-free survival; CI, confidence interval; HR, hazard ratio; CT, chemotherapy; HER2, human epidermal growth factor receptor 2.

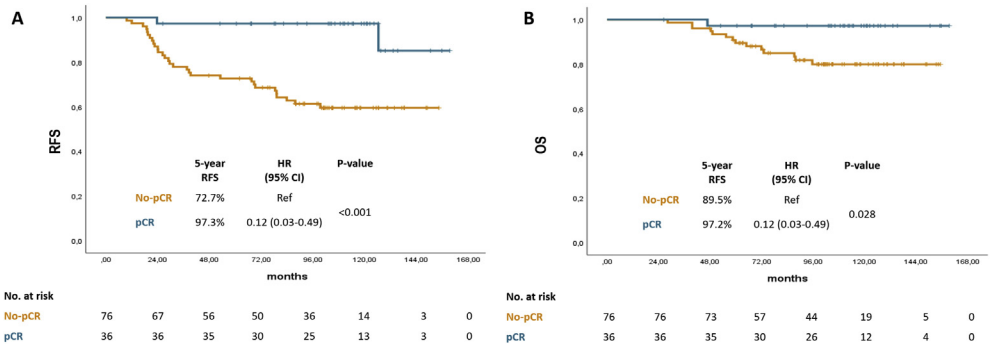

Fig. 2. Kaplan-Meier curves by pCR. A. RFS curve; B. OS curve. RFS, recurrence-free survival; CI, confidence interval; HR, hazard ratio; pCR, pathological complete response; OS, overall survival.

consistent in both ER-positive and ER-negative subgroups, in line with previous evidence [5,16–19].

Although the Cher-LOB trial was not powered to formally detect survival differences across treatment arms, we confirmed the trend towards improved survival with the combination of lapatinib plus trastuzumab over single HER2-blockade. In the CALGB 40601 phase III trial, a notable 68% and 66% relative improvement in the 7-year RFS and OS rates, respectively, was reported with lapatinib plus trastuzumab as compared with the trastuzumab single agent [9]. In addition, consistent with our findings, in both the NSABP B41 and NeoALTTO trials, a numerical increase in long-term survival rates with dual HER2-targeting was observed [16,17].

Correlative studies regarding the association between survival and tumour microenvironment and biology in the context of the Cher-LOB study were also performed. We previously reported a positive association between higher baseline TIL levels and pCR in the Cher-LOB trial. Here, we found baseline TILs to be prognostic in the overall Cher-LOB study population. In particular, similar to the findings of the NeoALTTO translational analysis [20], each 1% TIL increase corresponded to a 2.2% decrease in the risk of an RFS event across treatment groups. These results are further supported by a pooled analysis assessing the prognostic role of TILs as per BC subtype [21]. Indeed, among 1379 patients with HER2-positive BC undergoing neoadjuvant chemotherapy (the majority also receiving anti-HER2 treatment), a positive association between TILs and disease-free survival was detected. Our findings therefore add further evidence in support to the clinical validity of TILs as a prognostic biomarker in HER2-positive early BC [20–25]. Importantly, TIL evaluation is currently endorsed by both European Society for Medical Oncology guidelines [26] and World Health Organisation Classification of Tumours, 5th edition, (http://publications.iarc.fr) in this setting.

Besides TILs, PAM50-based intrinsic subtyping is gaining increasing clinical relevance in HER2-positive disease. We previously reported that HER2-enriched and luminal A subtypes account for most of the Cher-LOB study population (HER2-enriched = 26.2%, luminal A = 25%), with the first being associated with the highest (50%) and the second with the lowest (21%) pCR rate [14]. In the present work, no significant association was identified between RFS and intrinsic subtypes. However, the luminal A phenotype was significantly and independently associated with longer 5-year RFS than other PAM50 subtypes, and this is consistent with the CALGB 40601 translational analysis, where, similarly, patients with luminal A BC showed the lowest pCR rate (14.3%) while experiencing the most favourable long-term outcome as compared with other subtypes (no RFS events at 7 years) [9]. The notion that the luminal A phenotype exhibits a lower degree of sensitivity to chemotherapy plus HER2-blockade while retaining a positive impact on survival apparently challenges the prognostic value of pCR. However, this is an example of so-called Simpson's paradox [27,28], describing the confounding impact of unmeasured, but causal, variables on associations, which translates into an inverse relationship

Table 3
Multivariate analysis for RFS including PAM50 subtypes (luminal-A versus other subtypes), pCR, clinical stage (IIA vs IIB vs IIIA), treatment arm (single versus dual HER2-blockade), ER status (positive versus negative) and age (continuous variable).

Variable	HR (95% CI)	p
PAM50		
Luminal-A vs other	0.29 (0.09–0.94)	**0.040**
Pathologic response	0.09 (0.01–0.77)	**0.027**
pCR vs no pCR		
Clinical stage		
Stage IIA	0.65 (0.16–2.70)	0.554
Stage IIB	0.84 (0.22–3.27)	0.806
Stage IIIA	ref	0.810
Treatment arm		
Dual vs single HER2-blockade	0.83 (0.32–2.18)	0.711
ER status		
Positive vs negative	1.69 (0.61–4.73)	0.314
Age (cont.)	0.99 (0.95–1.04)	0.863

p values < 0.05 are highlighted in bold. RFS, recurrence-free survival; CI, confidence interval; HR, hazard ratio; HER2, human epidermal growth factor receptor 2; cont, continuous; vs, versus; pCR, pathologic complete response; ER, oestrogen receptor.

between chemosensitiveness and prognosis in case of residual disease after neoadjuvant treatment. Consistently, although pCR is prognostic in all traditionally defined BC subtypes (hormone receptor–positive/HER2-negative, HER2-positive and triple-negative), this association is stronger in most chemosensitive subgroups, namely triple-negative and HER2-positive. These observations [7,9,16,29,30] suggest that even among patients with HER2-positive BC, PAM50 analysis may add a further layer of stratification, beyond hormone receptor status [31]. Indeed, PAM50 analysis may allow controlling for those causal variables potentially affecting the strength of the association between pCR and prognosis and which would otherwise dilute the observed prognostic impact of pCR itself. Our findings therefore emphasise that this source of heterogeneity within HER2-positive BC should be taken into consideration when designing future neoadjuvant trials.

The neoadjuvant platform, albeit endorsed by the FDA [32] for the evaluation of investigational interventions given the more favourable cost-effective balance as compared with the adjuvant setting, can be underpowered to demonstrate significant survival differences across treatment arms. Indeed, the sample size required to capture meaningful pCR differences is significantly smaller than that required to detect significant survival differences. Indeed, although nearly all the trials comparing the combination of trastuzumab + lapatinib versus trastuzumab alone (in association with chemotherapy) for the neoadjuvant management of HER2-positive breast cancer reported a substantial improvement in pCR rates with the dual HER2 targeting strategy as compared with single HER2-blockade [9–13], many of them, with the exception of the CALGB 40601 trial [9], failed to demonstrate a statistically significant improvement in survival end-points. However, they were all consistent in confirming the positive prognostic impact of pCR at a single-patient level [5,7,9,16–18]. Overall, these observations suggest that such lack of survival improvement with dual over single HER2-blockade may reflect a statistical limitation, rather than a real absence of long-term advantage.

For this reason, although possibly limited by the relatively small sample size, long-term survival results from the Cher-LOB study are worthy of attention because they add further evidence in the same direction as that provided by other neoadjuvant studies of dual HER2-blockade.

Author contributions

Concept and design: Guarneri, Conte. Acquisition, analysis, or interpretation of data: All authors. Statistical analysis: Dieci. Drafting of the manuscript: Guarneri, Griguolo, Miglietta. Critical revision of the manuscript: All authors.

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Role of the funder/sponsor

The funders had no role in the design and conduct of the study; collection, management, analysis and interpretation of the data; preparation, review or approval of the manuscript and decision to submit the manuscript for publication.

Data sharing statement

The CHER-Lob study data sets are not publicly available to protect patient privacy. Gene expression data from the CHER-Lob study have been previously deposited (GEO database, GSE66399).

Conflict of interest statement

V.G. reports personal fees from Roche, Novartis, Eli Lilly and MSD outside the submitted work. D.G.G. reports personal fees from Roche, Novartis, Eli Lilly and Pierre Fabre, all outside the submitted work. A.F. reports personal fees from Roche, Novartis, Pfizer, Lilly, Daiichi and Seagen, all outside the submitted work. L.G. reports honoraria for lectures and ad boards from Lilly, Pfizer, Novartis and AstraZeneca, all outside the submitted work, reports travel accommodation and expenses from Novartis and Daiichi Sankyo and is an uncompensated member of the Olympia steering committee. A.M. reports grants and personal fees from Roche and Eisai; personal fees from Lilly, MacroGenics and Novartis and grants from Pfizer, all outside the submitted work. S.C. reports personal fees from Lilly Oncology outside the submitted work. A.P. reports grants and personal fees from Roche, AstraZeneca, Daiichi Sankyo, Merck Sharp & Dohme (MSD), PUMA Biotechnology, Novartis and Nanostring Technologies, personal fees from Seattle Genetics, Lilly, Pfizer, Guardant Health, Oncolytics Biotech and Abbvie, all outside the submitted work, and a patent (WO2018/103834A1) licensed to Nanostring Technologies, a patent (WO/2018/096191) issued. M.V.D. reports personal fees from Lilly, Genomic Health, Novartis and Celgene, all outside the submitted work. P.F.C. reports personal fees from Novartis, Eli Lilly, AstraZeneca,

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Appendix A. Supplementary data

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