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## HER2-enriched subtype and pathological complete response in HER2-positive breast cancer: a systematic review and meta-analysis

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Abstract

**Background:** HER2-positive (HER2+) breast cancer (BC) comprises all the four PAM50 molecular subtypes. Among these, the HER2-E appear to be associated with higher pathological complete response (pCR) rates following anti-HER2-based regimens. Here, we present a meta-analysis to validate the association of the HER2-E subtype with pCR following anti-HER2-based neoadjuvant treatments with or without chemotherapy (CT).

**Methods:** A systematic literature search was performed in February 2019. The primary objective was to compare the association between HER2-E subtype (versus others) and pCR. Selected secondary objectives were to compare the association between 1) HER2-E subtype and pCR in CT-free studies, 2) HER2-E subtype within hormone receptor (HR)-negative and HR+ disease and 3) HR-negative disease (versus HR+) and pCR in all patients and within HER2-E subtype. A random-effect model was applied. The Higgins'  $I^2$  was used to quantify heterogeneity.

**Results:** Sixteen studies were included, 5 of which tested CT-free regimens. HER2-E subtype was significantly associated with pCR in all patients (odds ratio [OR]=3.50,  $p<0.001$ ,  $I^2=33\%$ ), in HR+ (OR=3.61,  $p<0.001$ ,  $I^2=1\%$ ) and HR-negative tumors (OR=2.28,  $p=0.01$ ,  $I^2=47\%$ ). In CT-free studies, HER2-E subtype was associated with pCR in all patients (OR=5.52,  $p<0.001$ ,  $I^2=0\%$ ) and in HR+ disease (OR=4.08,  $p=0.001$ ,  $I^2=0\%$ ). HR-negative status was significantly associated with pCR compared to HR+ status in all patients (OR=2.41,  $p<0.001$ ,  $I^2=30\%$ ) and within the HER2-E subtype (OR=1.76,  $p<0.001$ ,  $I^2=0\%$ ).

**Conclusions:** The HER2-E biomarker identifies patients with a higher likelihood of achieving a pCR following neoadjuvant anti-HER2-based therapy beyond HR status and CT use. Future trial designs to escalate or de-escalate systemic therapy in HER2+ disease should consider this genomic biomarker.

## Keywords

PAM50; breast cancer; HER2-positive; HER2-enriched; biomarker; pathologic complete response

## INTRODUCTION

Breast cancer (BC) with overexpression and/or amplification of the Human Epidermal Growth Factor Receptor 2 (HER2-positive) represents 11-30% of all breast tumors<sup>1</sup>. HER2 positivity is defined today by immunohistochemistry (IHC) as complete and strong membrane staining (i.e. score of 3+) in 10% of cancer cells, and/or by *in situ* immunofluorescence (ISH) techniques as amplified using a HER2/CEP17 ratio cutoff of 2.0 and an average HER2 gene copy number 4.0 signals per cell<sup>2</sup>. This consensus definition is based on the methods and cutoffs used over the years in pivotal trials that led to the approval of trastuzumab<sup>3</sup>, pertuzumab<sup>4</sup>, neratinib<sup>5</sup>, lapatinib<sup>6</sup> and T-DM1<sup>7</sup> in HER2+ breast cancer.

The current HER2 definition do not sufficiently consider HER2+ disease's clinical and biological heterogeneity. On one hand, high variability in patient's response and survival outcomes following anti-HER2-based therapy is common<sup>8,9</sup>. On the other hand, high biological variability exists within HER2+ disease<sup>10-12</sup>. For example, all the BC intrinsic subtypes [i.e. Luminal A, Luminal B, HER2-enriched (HER2-E) and Basal-like] can be identified through gene expression profiling<sup>9,10,13</sup>. Among them, the HER2-E subtype is the most frequent (31-76%), shows the highest levels of *ERBB2* mRNA and protein and appears to be the subtype with the highest activation of the EGFR-HER2 signaling pathway<sup>11,14-31</sup>. Importantly, these biological entities within HER2+ disease are not fully recapitulated by hormone receptor (HR) status since 40% of HER2+/HR+ tumors are HER2-E and 15% of HER2+/HR-negative tumors are Basal-like<sup>10,11,32</sup>.

To date, no biomarker has demonstrated clinical utility in HER2+ early disease beyond HER2 and HR status<sup>33</sup>. However, accumulating evidence supports the clinical validity of two biomarkers: intrinsic subtyping and stromal tumor infiltrating-lymphocytes (TILs). In particular, either the HER2-E subtype or high TILs appears to be associated with high response to anti-HER2-based treatments in the neoadjuvant setting<sup>14-31,34,35</sup>. From a prognostic point of view, however, HER2-E subtype is associated with a worse prognosis<sup>10,36</sup> whereas TILs are associated with a better survival outcome<sup>34,37,38</sup>. Unfortunately, the majority of these data were derived from retrospective analyses from individual clinical trials using baseline tumor samples. In addition, most analyses were exploratory and unplanned, and limited by relatively small sample sizes.

To increase the level of evidence of the association of the HER2-E subtype with the response to anti-HER2 based neoadjuvant regimens, we decided to review the literature and perform a meta-analysis.

## MATERIALS AND METHODS

### Search strategy and selection criteria

A systematic literature search was performed on 12/February/2019 to identify published observational, phase II and phase III (randomized and non-randomized) neoadjuvant clinical studies involving anti-HER2-based treatments in HER2+ BC, where the association between pathological complete response (pCR) and BC molecular intrinsic subtypes was evaluated. The literature search had no time nor language restriction, however, only clinical studies involving anti-HER2-based neoadjuvant regimens were included, with or without chemotherapy. Additional studies particularly relevant to the topic, for which molecular data had not been published but were available at the Translational Genomic and Targeted Therapeutics in Solid Tumors laboratory of the IDIBAPS (Barcelona, Spain), were also included in the analysis. All pre-clinical studies, phase I trials, non-neoadjuvant trials and neoadjuvant trials without anti-HER2 agents were excluded. The recommendations of the Cochrane Collaboration<sup>44</sup> were followed to identify all relevant studies. For our query, we used a combination of disease characteristics, study design, treatment setting and strategies or drugs. The full query is reported in the Suppl. Materials. Both full articles and studies published in the abstract form were included in the analysis, if odds ratios (OR) data were directly available or computable. The search was conducted on the electronic databases

Pubmed and Web of Science®, as well as on San Antonio Breast Cancer Symposiums (SABCS)'s, American Society of Clinical Oncology (ASCO)'s and European Society of Medical Oncology (ESMO)'s annual meetings online archives. Four reviewers (FS, TP, NC and CR) independently evaluated whether each selected randomized clinical trials (RCT) respected the predetermined criteria, and another reviewer (AP) was consulted in case of controversy.

### Data extraction and objectives

Details on study design, patient/tumor characteristics, interventions and outcome were extracted from each paper. Only the most recent and complete reports were included when duplicate publications were identified. Crude odds ratio (OR) for pCR with their 95% confidence intervals (CI) were extracted or calculated, when necessary, from each published paper or internal datasets. The definition of pCR varied across studies. In 12/16 (75%) studies (2,176/2,703 patients with known PAM50 subtype), pCR was defined as the absence of invasive neoplastic cells at microscopic examination of the primary tumor at surgery in breast and axilla (pCR in-breast and axilla), with remaining in-situ lesions allowed. In 4/16 (25%) studies (527/2,703 patients with known molecular subtype), pCR was defined as the absence of tumor cells only in breast, without considering tumor response in axillary lymph nodes (pCR in-breast).

The primary objective was to compare the association between HER2-E subtype (versus others) and pCR in all patients. Secondary objectives were to:

1. compare the association between HER2-E subtype (versus others) and pCR in CT-free studies;
2. compare the association between HR-negative disease (versus HR+) and pCR in all patients;
3. compare the association between HR-negative disease (versus HR+) and pCR within HER2-E subtype;
4. compare the association between HER2-E subtype (versus others) and pCR within HR+ and HR-negative disease;
5. compare the association between each intrinsic subtype (versus the others) and pCR.

### Statistical analyses

Since a certain degree of heterogeneity was expected, analyses were performed under the Random-Effect Model of DerSimonian and Laird<sup>45</sup>. Heterogeneity was assessed with Higgin's  $I^2$  index<sup>46</sup>. Pre-planned exploratory subgroup analyses for the primary endpoint were conducted, even if heterogeneity was not relevant. Subgroup analyses of interest were: 1) phase II vs phase III trials, 2) randomized vs. non-randomized trials 3) CT-containing vs. CT-free studies 4) pCR in-breast vs pCR in-breast and axilla. For the primary endpoint, to assess whether the pooled OR estimates were stable or strongly dependent on one or few studies, sensitivity analyses were conducted by interactively recalculating the pooled OR estimates after exclusion of each single study. Publication bias was explored through funnel

plot visual inspection and the Egger's linear regression test for funnel plot asymmetry<sup>47,48</sup>. All reported *p* values were two-sided. All statistical analyses and the generation of forest plots were conducted using R and RevMan<sup>49,50</sup>. The Cochrane risk of bias assessment tool was employed to assess the quality of the data obtained and the risk of bias in each study. Significance was set at  $p < 0.05$ , except for Egger's test, for which significance was set as  $p < 0.1$ , as usual. The project was registered in the PROSPERO online database<sup>51</sup>, with registration number: CRD42019140902.

### Assessment of risk of bias

The risk of bias for each trial was assessed by using the criteria outlined in the Cochrane Handbook for Systematic Reviews of Interventions<sup>44</sup>. Each domain related to a risk of bias was assessed in each included trial, since there is evidence that these issues are associated with biased estimates of treatment effect. The domains were the following: 1) random sequence generation; 2) allocation concealment; 3) blinding of participants and personnel; 4) blinding of outcome assessment; 5) incomplete outcome data; 6) selective reporting; 7) other bias. Review authors' judgments were categorized as "low risk", "high risk" or "unclear risk" of bias. Internal validity of eligible studies was assessed according to the Cochrane Collaboration's 'Risk of Bias' tool in Review Manager<sup>50</sup>.

## RESULTS

### Summary of studies and patient characteristics

A total of 16 studies were included (Tables 1 and 2; Supplementary Tables 1 and 2)<sup>14–31</sup>. From Pubmed and Web of Science® online databases, 2,207 studies were extracted and 10 were included<sup>14–18,20–22,24,25,28</sup>. From ASCO, ESMO and SABCS online abstracts books, 4 studies were included<sup>19,26,27,30,31</sup>. Finally, data from 2 studies (ICO-CLINIC, LPT109096) were available at the Translational Genomic and Targeted Therapeutics in Solid Tumors laboratory at IDIBAPS (Barcelona, Spain)<sup>14,26,31</sup>. Some data were also retrieved from later-published full articles<sup>11,52</sup>. The selection process is resumed in the PRISMA diagram (Fig. 1). Overall 5 (31.25%) phase III RCT, 5 (31.25%) phase II RCT, 5 (31.25%) non-randomized phase II trials and 1 (6.25%) retrospective observational study were included. All the articles/abstracts containing molecular results have been published between 2014 and 2019.

From a total of 3,733 patients, PAM50 intrinsic subtype was available for 2,703 (72.4%) patients, while HRs status was known for 3,373 (90.3%) patients. Except for one trial (i.e. PerELISA) which enrolled HR+ tumors-only<sup>28</sup>, the others included both HR+ and HR-negative tumors. All studies included evaluated anti-HER2-based neoadjuvant regimens with or without CT<sup>14,23,28,29,53</sup>, and included tumor stages II or III, except for the PAMELA trial and the retrospective observational study from the Catalan Institute of Oncology and the Hospital Clinic of Barcelona (ICO-CLINIC), which allowed stage I disease<sup>14,26</sup>. Various methods for assessing the PAM50 BC intrinsic subtypes were used across all trials (Tables 1–2), but all were based upon gene expression data<sup>14–31</sup>.

Among the studies included, only the PAMELA single arm phase II trial was specifically designed to prospectively assess PAM50 intrinsic subtypes and test whether patients with the HER2-E subtype benefited more than the other subtypes from a neoadjuvant anti-HER2-based CT-free regimen<sup>14</sup>. The other studies evaluated PAM50 as an exploratory retrospective analysis; therefore, tumor samples were not always available for all patients included. However, samples were always available for at least half of the population enrolled within each study (Tables 1–2). pCR rates in HER2-E subtype were higher than nonHER2-E subtypes in each study, except in the LPT109096 trial. Individual trials' results are reported in Tables 1 and 2.

### pCR and HER2-E subtype

The HER2-E subtype was significantly associated with pCR compared to others (OR=3.50, 95% CI 2.79 – 4.39,  $p<0.001$ ,  $I^2=33\%$ , Fig. 2). The funnel plot suggested the absence of publication bias (Suppl. Fig. 1), confirmed by a non-significant Egger's test ( $p=0.48$ ). The influential analysis showed consistent results when omitting a single trial with an  $I^2$  range varying from 3.4% (omitting the NeoSphere trial)<sup>20</sup> to 37.7% (omitting the TBCRC023 trial)<sup>31</sup>. Full results of the influential analysis are reported in Table 3. Considering the absence of significant heterogeneity, an exploratory, non-preplanned analysis done with the fixed-effect model<sup>54</sup> was performed with a similar result (OR=3.51, 95% CI: 2.96 – 4.16,  $p<0.001$ ,  $I^2=33\%$ ).

There were no statistically significant differences in terms of association with pCR for all the subgroups considered for the preplanned sensitivity analyses, namely randomized vs. non-randomized studies ( $p=0.46$ ), phase II vs. phase III studies ( $p=0.13$ ), CT-containing vs. CT-free studies ( $p=0.30$ ), pCR in-breast vs pCR in-breast+axilla ( $p=0.32$ ). Compared to other intrinsic subtypes, the HER2-E subtype was significantly associated with pCR compared to Basal-like (OR=2.50, 95% CI 1.78 – 3.52,  $p<0.001$ ,  $I^2=0\%$ , Suppl. Fig. 2A), Luminal A (OR=4.81, 95% CI 3.16 – 7.33,  $p<0.001$ ,  $I^2=55\%$ , Suppl. Fig. 2B), Luminal B (OR=3.82, 95% CI 2.97 – 4.91,  $p<0.001$ ,  $I^2=0\%$ , Suppl. Fig. 2C) and Luminal A/B (OR=4.36, 95% CI 3.17 – 6.00,  $p<0.001$ ,  $I^2=52\%$ , Suppl. fig. 2D) subtypes. Other comparisons among intrinsic subtypes can be found in the Suppl. Materials.

### pCR, HR status and HER2-E subtype

Fifteen of the 16 trials were used to assess the association between HR status and pCR. HR-negative disease was significantly associated with pCR compared to HR+ disease (OR=2.41, 95% CI 2.00 – 2.92,  $p<0.001$ ,  $I^2=30\%$ , Fig. 3A). The inspection of the funnel plot (Suppl. Fig. 3), as well as the result of the Egger's test ( $p=0.68$ ), did not reveal a significant publication bias. The HER2-E subtype was significantly associated with pCR within both HR-negative disease (OR=2.28, 95% CI 1.21 – 4.29,  $p=0.01$ ,  $I^2=47\%$ , Fig. 3B) and HR+ disease (OR=3.61, 95% CI 2.61 – 5.00,  $p<0.001$ ,  $I^2=1\%$ , Fig. 3C). Similar to what was observed for the general population, HR-negative disease was significantly associated with pCR compared to HR+ disease within the HER2-E subtype (OR=1.76, 95% CI 1.30 – 2.38,  $p<0.001$ ,  $I^2=0\%$ , Fig. 3D).

### pCR, HR status and HER2-E subtype in the absence of CT

A total of 5 studies evaluated dual HER2 blockade in the absence of CT<sup>14,20,28,29,31</sup>, although for one of these (i.e. NeoSphere), data for the CT-free arm were not available separately from the other CT-containing arms' data<sup>20</sup>. In CT-free regimens, HER2-E subtype was significantly associated with pCR compared to the other subtypes (OR=5.52, 95% CI 2.89 – 10.54,  $p<0.001$ ,  $I^2=0\%$ , Fig. 4A), while there was no apparent difference between HR-negative vs. HR+ disease (OR=1.49, 95% CI 0.44 – 5.03,  $p=0.52$ ,  $I^2=76\%$ , Fig. 4B). When considering HR status, the HER2-E subtype was found to be significantly associated with pCR within HR+ disease (OR=4.08, 95% CI: 1.76 – 9.46,  $p=0.001$ ,  $I^2=0\%$ , Suppl. Fig. 4A), but not within HR-negative disease (OR=2.18, 95% CI: 0.66 – 7.26,  $p=0.20$ ,  $I^2=0\%$ , Suppl. Fig. 4B). Conversely, in patients with HER2-E subtype, HR status was not significantly associated with pCR (OR=1.30, 95% CI 0.67 – 2.52,  $p=0.44$ ,  $I^2=0\%$ , Suppl. Fig. 5).

### Risk of bias analysis

With respect to the risk of bias, as defined by the Cochrane's manual for systematic reviews<sup>44</sup>, the risk of selection bias for random sequence generation and allocation concealments was present in the 6/16 (37.5%) of the studies in both cases, with an unclear risk in 1/16 (6.25%) studies included, concerning the random sequence generation selection bias (Fig. 5 and Suppl. Fig. 6). The performance bias due to blinding of participants and personnel was present in 12/16 (75%) of cases, with an unclear risk in 3/16 (18.75%) of the studies included. No detection bias related to the blinding of outcome assessment, attrition bias due to incomplete outcome data and selective reporting bias were observed. Concerning the last two, an unclear risk was present in 1/16 (6.25%) cases. Finally, we accounted for a 6.25% high risk of other bias related to the ICO-CLINIC study, due to its retrospective and non-trial design.

## DISCUSSION

The development of effective drugs against HER2+ BC has been particularly successful in the last few years<sup>3-7</sup>. Since the introduction of trastuzumab<sup>3</sup>, other effective and tolerable anti-HER2 drugs (i.e. lapatinib, pertuzumab, neratinib and T-DM1) have been introduced in the metastatic and/or early disease settings, contributing to important improvements in survival outcomes<sup>8,55</sup>. However, HER2+ disease is clinically and biologically heterogeneous and not all patients benefit to the same extent from current treatments. Thus, better identification of patients using biomarkers should allow the design of prospective trials aiming to improve precision medicine in HER2+ BC.

Among the different biomarkers evaluated in HER2+ disease over the last decade<sup>10,14,21,31,34,35,37,39,40,56</sup>, the HER2-E subtype has been proposed to identify patients whose HER2+ tumors are HER2 “addicted” (meaning driven primarily by signaling via the HER2 pathway). Retrospective analysis of the HER2-E subtype, mostly exploratory and unplanned, using baseline tumor samples from individual clinical trials have linked this phenotype with high rates of pathological complete response following neoadjuvant anti-HER2-based therapies<sup>14-31</sup>. However, to date, no combined analysis or meta-analysis has

been performed and analyses within all of those studies were limited by relatively small sample sizes. Here, we performed a trial-level meta-analysis of 16 neoadjuvant studies and 2,703 patients to evaluate the association of the HER2-E subtype with pCR. In particular, we confirmed that the HER2-E subtype is a consistent biomarker to identify patients with a higher likelihood of achieving a pCR following anti-HER2-based therapy with or without cytotoxic therapy. Importantly, the association of the HER2-E subtype with pCR appeared to be independent of HR status, which is the only biomarker with clinical utility in HER2+ disease. Additionally, our results confirm the ability of HR status to predict pCR by itself and within the HER2-E subtype, although we could not demonstrate this in the CT-free setting, which had substantially fewer contributing trials.

We adopted pCR as our clinical endpoint for this meta-analysis. This is because numerous studies have demonstrated a favorable prognostic role in early stage HER2+ BC<sup>56–60</sup> so its use as primary endpoint in neoadjuvant trials has been increasing over the years and has also been endorsed for regulatory purposes by regulatory agencies such as US Food and Drug Administration (FDA), for accelerated approval of neoadjuvant treatments in high risk early-stage BC<sup>61</sup>. Furthermore, the FDA recently approved the use of adjuvant T-DM1 (in HER2+ BC) or capecitabine (in HER2-negative BC) in case of no achievement of pCR following standard neoadjuvant systemic therapy and surgery, making of pCR a fundamental tool in therapeutic decision-making in non-metastatic BC for escalating treatment strategies. At the same time, there is also an increasing use of pCR as a tool to identify potentially effective and safe de-escalating therapeutic approaches in HER2+ BC<sup>14,28,29,62</sup>. In fact, identification of effective de-escalating treatment strategies to spare toxicity and financial costs to patients is a main focus of the research community nowadays<sup>63,64</sup>. In adjuvant setting, several prospective trials of early stage HER2+ BC have attempted to demonstrate that de-escalating strategies based on a shorter duration of adjuvant trastuzumab provided similar benefits as the conventional 1 year; however, the results using non-inferiority trial designs were mixed<sup>65</sup>. On the contrary, a single-arm trial from a single institution (i.e. the APT trial) evaluating 12 doses of adjuvant weekly paclitaxel and 1-year of trastuzumab in largely HR+ stage I disease significantly impacted on daily clinical practice after showing extraordinary DFS and OS rates at 7-years<sup>66</sup>. In this scenario, at least 3 critical questions remain to be answered regarding de-escalation of systemic therapy in early HER2+ disease: 1) who can be treated with less or even no adjuvant trastuzumab after surgery? 2) who does not need (neo)adjuvant pertuzumab in stage II and III disease? 3) can we decrease the amount of chemotherapy? In fact, immunohistochemically HER2+/non-HER2-E tumors might be poorly dependent, if not totally independent, from the HER2-signaling pathway and not gain any benefit from adjuvant trastuzumab following previous neoadjuvant therapy and surgery. At the same time some HER2+ tumors might be “HER2 addicted” enough not to need chemotherapy at all or to require a shortened adjuvant trastuzumab duration and/or no adjuvant dual blockade therapy. To address these questions, well-designed clinical trials integrating clinical variables (such as tumor dimension and axillary nodes involvement), response data and biomarkers such as the HER2-E subtype, TILs, intra-tumor heterogeneity<sup>67</sup> and PIK3CA status are needed.

This meta-analysis has several limitations. First, some secondary end-points were affected by discrete levels of heterogeneity ( $I^2 > 75\%$  and  $p_{\text{heterogeneity}} < 0.05$ , results in Fig. 4B and



Suppl. Materials). This was mostly attributable to the paucity of molecular data from some trials and differences in the effects observed, preventing them from being fully reliable, regardless of the analytical model applied. However, this consideration doesn't apply to the main result of the study. Second, although several studies correlated pCR with long-term survival outcomes (EFS/DFS and OS) in the context of HER2+ BC<sup>56–60</sup>, others failed to demonstrate its role as an efficient surrogate endpoint for survival<sup>68,69</sup>. Additionally, there is a specific lack of survival data related to intrinsic subtypes within the clinical trials included in this study. Therefore, no claims regarding the association of HER2-E with patient's survival outcome can be made based on this meta-analysis. Moreover, 4/16 trials reported data regarding in-breast pCR, which has not been recognized by the FDA as a validated endpoint for drug approval in neoadjuvant setting<sup>61</sup>. Third, the methods used to apply the PAM50 algorithm varied across trials. For example, 13 studies used the nCounter platform<sup>14,17,20,22–29,31</sup>, 2 studies used RNA-seq data<sup>15,18</sup> and 2 studies used microarray-based data<sup>16,21</sup>. Finally, we were only able to perform a study-level meta-analysis instead of a patient-level meta-analysis, which would have increased precision and homogeneity and enabled thorough exploration of potential effect moderators.

To conclude, our results demonstrate that the HER2-E subtype is a consistent biomarker of response following neoadjuvant anti-HER2-based regimens, with and without CT and beyond HR status. This biomarker, along with TILs and other biomarkers, such as *PIK3CA* mutations<sup>39–42</sup>, either alone or in combination<sup>43</sup>, should be routinely incorporated in future prospective clinical trials designed to implement strategies to escalate and/or de-escalate systemic therapies<sup>11,14–31</sup>.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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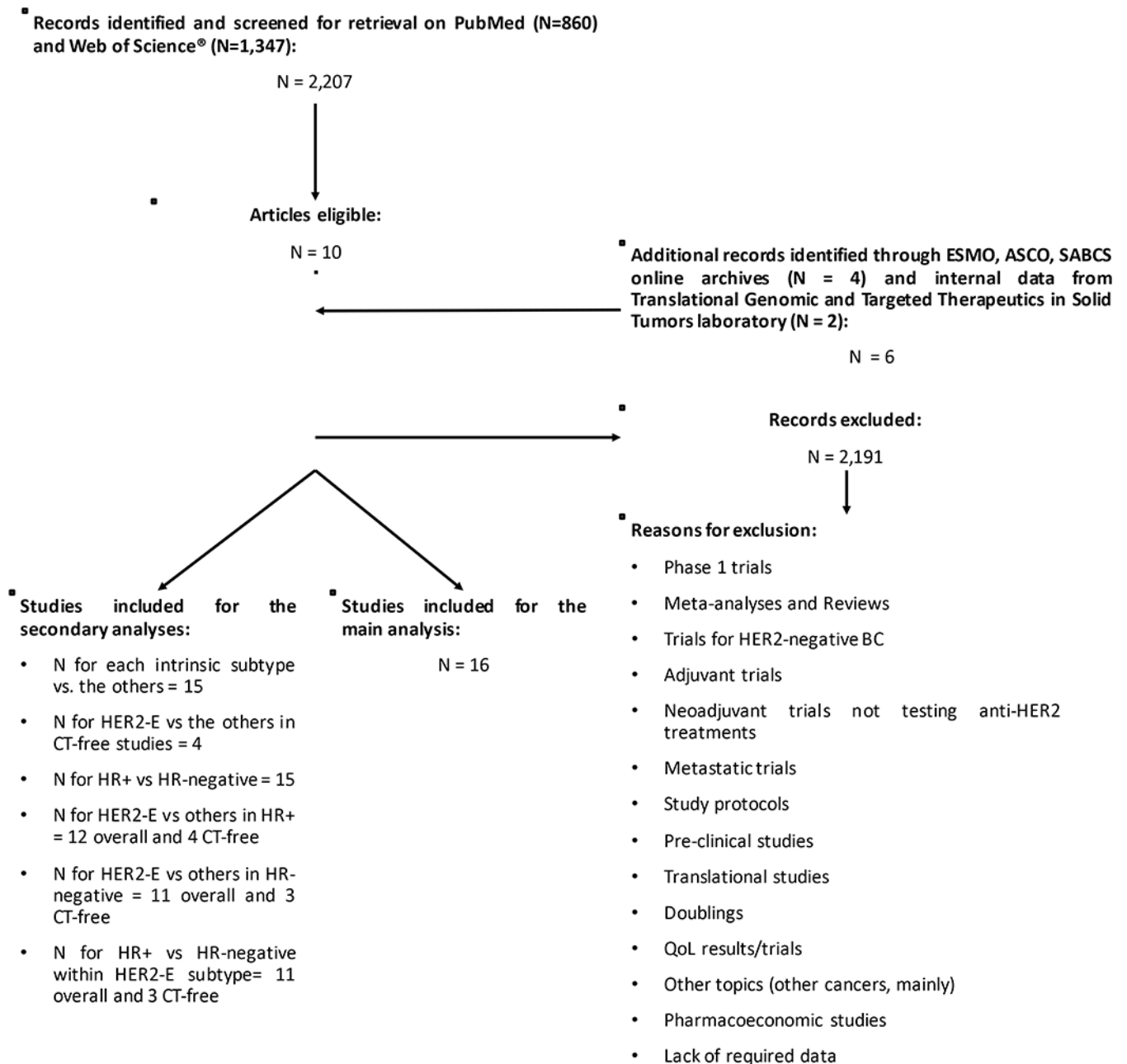
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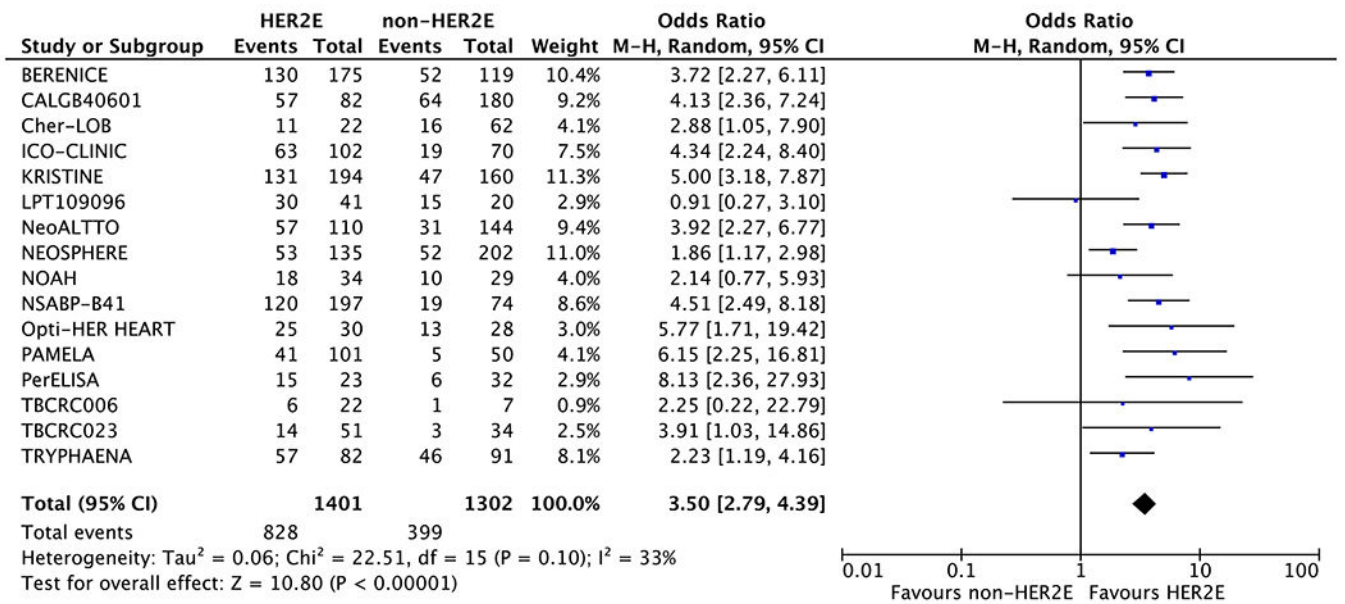
### Highlights

- We correlated the breast cancer intrinsic subtypes with pCR in HER2+ disease
- The HER2-E signature was significantly and consistently associated with pCR after anti-HER2-based therapy
- The HER2-E subtype was associated with pCR irrespective of hormone receptor status
- The HER2-E subtype was associated with pCR also with chemo-free neoadjuvant schemes



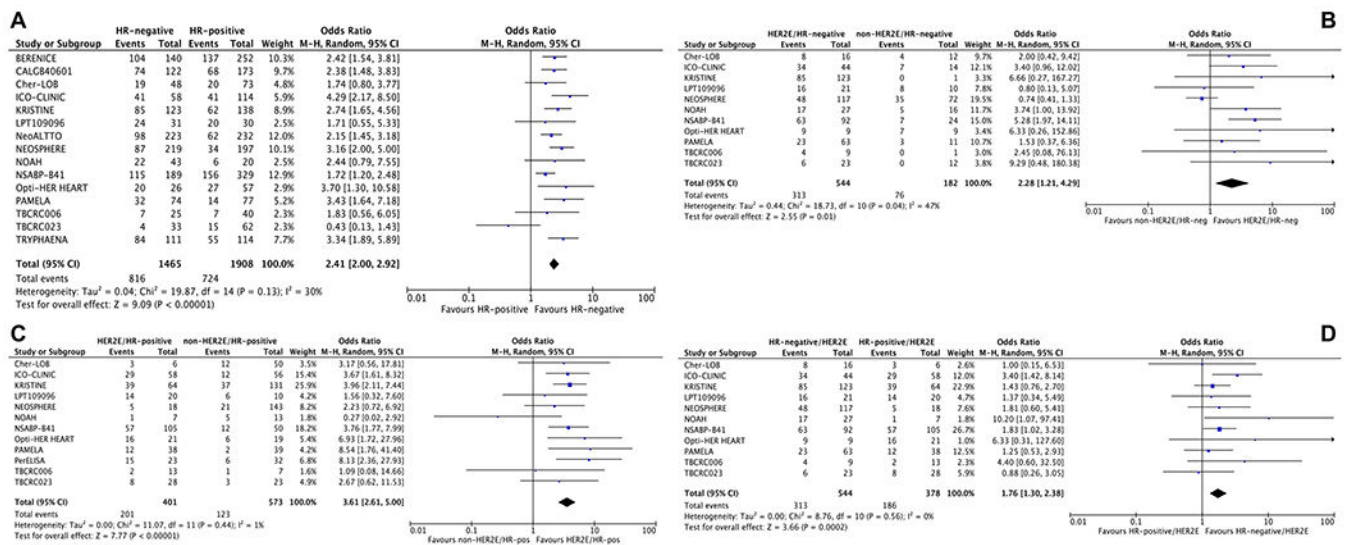
**Fig. 1.**  
PRISMA diagram.



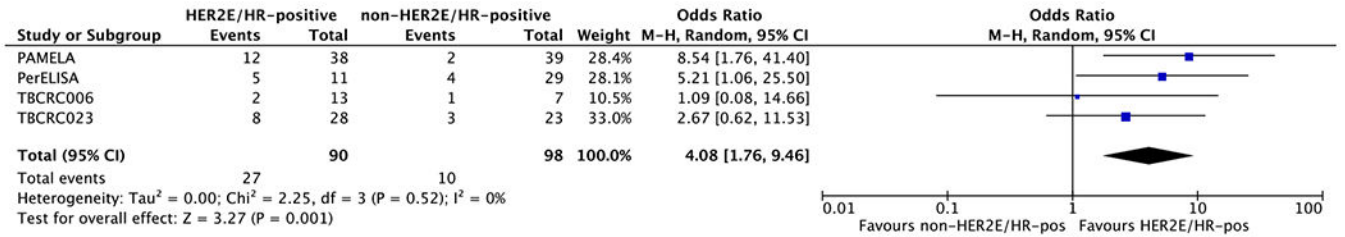
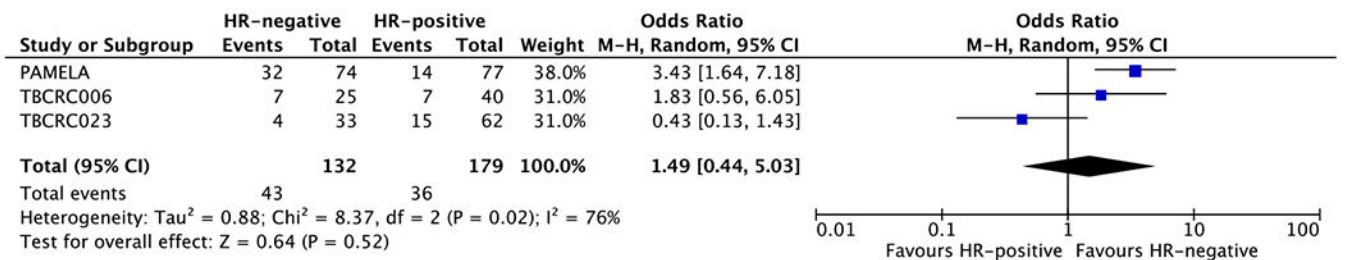


**Fig. 2.**

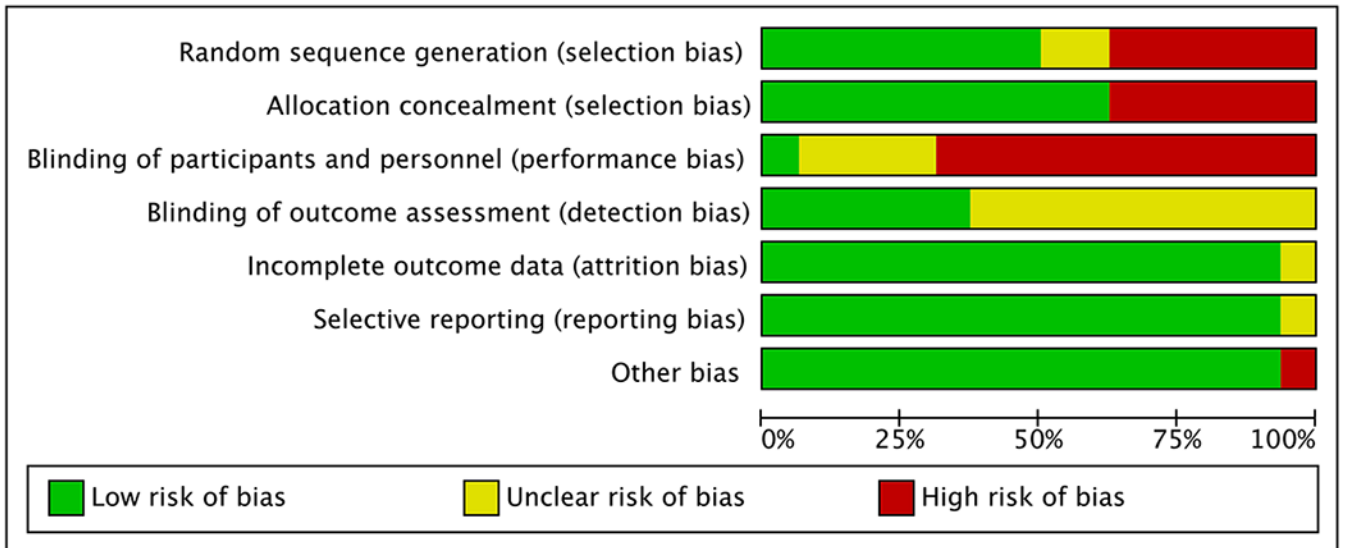
Forest Plots comparing the association with pCR between the HER2-E and the other intrinsic subtypes in the overall population.



**Fig. 3.** A-D. Forest Plots comparing the association with pCR between HR-positive and HR-negative tumors (A) in the overall population; the association with pCR between the HER2-E and the other intrinsic subtypes within the HR-negative (B) and HR-positive (C) disease, and the association of pCR between HR-positive and HR-negative tumors within the HER2-E subtype (D).

**A****B****Fig. 4.**

Forest Plots comparing the association with pCR between the HER2-E and the other subtypes (A), and between HR-negative and HR-positive tumors (B) in CT-free trials.



**Fig. 5.**  
Risk of bias analysis.

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**Table 1.**

II and III trials

RANDOMIZED TRIALS																
LPT109096	TRYPHAENA			NeoSphere			Cher-LOB			KRISTINE		CALGB40601		NeoALTTO		TBCRC023
	T +FECT +P	L +FECT +P	T+L +FECT +L+P	T +D	Pe +T +D	Pe +D	L +T +P +FEC	L +T +P +FEC	T +P +FEC	T DM1+Pe	TCH +Pe	L +P	L +T +P	T +P	L +T +P	
anti-HER2+CT															anti-HER2 w/o CT	
85/97	173/225			337/417			84/121			354/444		262/305		254/451		85/97
61/100#	173/225			337/417			84/121			354/444		262/305		254/451		85/97
II and III	II and III			II and III			II and IIIA			II and III		II and III		II and III		II and III
Pos and neg	Pos and neg			Pos and neg			Pos and neg			Pos and neg		Pos and neg		Pos and neg		Pos and neg
51 (60.0)	82 (47.4)			135 (40.1)			22 (26.2)			194 (54.8)		82 (31.3)		110 (43.8)		51 (60.0)
34 (40.0)	91 (52.6)			202 (59.9)			62 (73.8)			160 (45.2)		180 (68.7)		144 (56.5)		34 (40.0)
14 (27.5)	57 (69.5)			53 (39.3)			11 (50.0)			131 (67.5)		57 (69.5)		57 (51.8)		14 (27.5)
3 (8.8)	46 (50.5)			52 (25.7)			16 (25.8)			47 (29.4)		64 (35.6)		31 (21.5)		3 (8.8)
ypT0/is	ypT0/is ypN0			ypT0/is ypN0			ypT0/is ypN0			ypT0/is ypN0		ypT0/is		ypT0/is ypN0		ypT0/is
nCounter	nCounter			nCounter			Microarray-based			nCounter		RNA seq.		RNA seq.		nCounter
Published material	Published material			Published material			Published material			Published material		Published material		Published material		Published material

Cancer Treatment Reviews Author manuscript available in PMC 2021 March 01.

**RANDOMIZED TRIALS**

1	2012/2016	2015	2017	2015/2016	2012/2018	2013	2011	2019/2019
	Baselga J/Fumagalli D	Carey L	Prat A	Guameri V/Dieci MV	Gianni L/Bianchini G	Schneeweiss A	Holmes FA	Rimawi MF/Prat A
	Full article	Full article	Abstract	Full article	Full article/Abstract/Poster	Full article	Full article/internal data	Full article/Full article
Res	Lancet/JAMA Oncol	J Clin Oncol	SABCS	The Oncologist/Ann Oncol	Lancet Oncol/ESMO congress	Ann Oncol	BMC Research Notes/Internal data	Clin Cancer Res/JNCI

Baselga J, Normal-like; Pos=positive; Neg=negative; HR=hormone receptors; CT=chemotherapy; N/A=not assessed; pCR=pathologic complete response; CMF=cyclophosphamide+5-fluorouracil; FEC=5-fluorouracil+epirubicin+cyclophosphamide; TCH=docetaxel+trastuzumab; T=trastuzumab; Pe=pertuzumab; L=lapatinib; Let=letrozole; GnRH= GnRH analogue → = followed by;

negative cohort not considered;

in Society of Clinical Oncology; ESMO=European Society for Medical Oncology

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Table 2.

Characteristics of the included non-randomized studies

NON-RANDOMIZED STUDIES									
Study name	ICO-CLINIC	BERENICE		Opti-HER HEART	Per-ELISA	TBCRC006	PAMELA		
Study type	Retrospective Observational	Non-randomized Phase II		Single Ann Phase II	Non-Randomized Phase II	Single Ann Phase II	Single Ann Phase II		
Regimen	Tax+/-Antra+T	ddAC→P+T+Pe	FEC→D+T+Pe	Pe+T+LD+P	Let+T+Pe	L+T +/- Let+/- GnRH	L+T +/- Let or Tam		
Treatment category	anti-HER2+CT	anti-HER2+CT		anti-HER2+CT	anti-HER2 w/o CT	anti-HER2 w/o CT	anti-HER2 w/o CT		
N. of evaluable patients/Total of the arm	172/173	294/400		58/83	40/44	29/65	151/151		
TNM Stage	I-III	II and III		II and III	II and III	II and III	I-III		
HR status	Pos and neg	Pos and neg		Pos and neg	Pos	Pos and neg	Pos and neg		
HER2E (%)	102 (59.3)	175 (59.5)		30 (51.7)	11 (27.5)	22 (75.9)	101 (66.9)		
non-HER2E (%)	70 (40.7)	119 (40.5)		28 (48.3)	29 (72.5)	7 (24.1)	50 (33.1)		
pCR in HER2E (%)	63 (61.8)	130 (74.2)		25 (83.3)	5 (45.5)	6 (20.7)	41 (40.6)		
pCR in non-HER2E (%)	19 (27.1)	52 (43.7)		13 (46.4)	4 (13.8)	1 (14.3)	5 (10.0)		
pCR definition	ypT0/is ypN0	ypT0/is ypN0		ypT0/is ypN0	ypT0/is ypN0	ypT0/is	ypT0/is		
Gene expression platform	nCounter	nCounter		nCounter	nCounter	nCounter	nCounter		
Data source	Published material/Internal data	Published material		Published material	Published material	Published material	Published material		
Year of publication	2017/2019	2017		2019	2019	2013/2019	2017		
First author	Pernas S	Swain SM		Gavilà J	Guarneri V	Rimawi MF/Prat A	Llombart-Cussac A		
Publication form	Abstract/Full article	Full article		Full article	Full article	Full article	Full article		
Journal/Meeting	SABCS /Front Oncol	Ann Oncol		BMC Medicine	Ann Oncol	J Clin Oncol/JNCI	Lancet Oncol		

HER2E=HER2 enriched; non-HER2E=Basal-Like, Luminal A, Luminal B, Normal-like; Pos=positive; Neg=negative; HR=hormone receptors; CT=chemotherapy; N/A=not assessed; pCR=pathologic complete response; AC=doxorubicin+cyclophosphamide; Dox=doxorubicin; CMF=cyclophosphamide+methotrexate+5-fluorouracil; FEC=5-fluorouracil+epirubicin+cyclophosphamide; TCH=docetaxel+carboplatin+trastuzumab; Tax=taxanes; Antra=anthracyclines; P=paclitaxel; D=docetaxel; LD=liposomal doxorubicin; T=trastuzumab; Pe=pertuzumab; L=lapatinib; Let=letrozole; Tam=tamoxifen; GnRH=GnRH analogue; → = followed by; dd=dose dense.

**Table 3.**

Influential analyses concerning the primary end-point

Study Omitted	OR	95% CI	p	I <sup>2</sup>
BERENICE	3.47	2.70 – 4.47	<0.0001	37.6%
CALGB40601	3.44	2.68 – 4.40	<0.0001	36.7%
Cher-LOB	3.52	2.78 – 4.48	<0.0001	37.4%
ICO-CLINIC	3.43	2.69 – 4.38	<0.0001	36.6%
KRISTINE	3.34	2.63 – 4.23	<0.0001	28.9%
LPT109096	3.62	2.95 – 4.45	<0.0001	21.3%
NeoALTO	3.45	2.69 – 4.43	<0.0001	37.3%
NEOSPHERE	3.85	3.18 – 4.66	<0.0001	3.4%
NOAH	3.57	2.82 – 4.50	<0.0001	35.2%
NSABP-B41	3.41	2.67 – 4.35	<0.0001	35.6%
Opti-HER-HEART	3.44	2.72 – 4.35	<0.0001	35.9%
PAMELA	3.41	2.71 – 4.30	<0.0001	34.1%
Per-ELISA	3.41	2.72 – 4.28	<0.0001	32.3%
TBCRC006	3.51	2.78 – 4.43	<0.0001	37.4%
TBCRC023	3.48	2.75 – 4.42	<0.0001	37.7%
TRYPHAENA	3.64	2.88 – 4.59	<0.0001	31.2%

OR=odds ratio; CI=confidence intervals