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ORIGINAL RESEARCH

Prognostic value of intrinsic subtypes in hormone-receptor-positive metastatic breast cancer: systematic review and meta-analysis☆

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Background: In hormone receptor-positive (HoR+) breast cancer (BC), gene expression analysis identifies luminal A (LumA), luminal B (LumB), human epidermal growth factor receptor 2 (HER2)-enriched (HER2-E), basal-like (BL) intrinsic subtypes and a normal-like group. This classification has an established prognostic value in early-stage HoR+ BC. Here, we carried out a trial-level meta-analysis to determine the prognostic ability of subtypes in metastatic BC (MBC).

Materials and methods: We systematically reviewed all the available prospective phase II/III trials in HoR+ MBC where subtype was assessed. The primary endpoint was progression-free survival (PFS)/time to progression (TTP) of the LumA subtype compared to non-LumA. Secondary endpoints were PFS/TTP of each individual subtype, according to treatment, menopausal and HER2 status and overall survival (OS). The random-effect model was applied, and heterogeneity assessed through Cochran's Q and I^2. Threshold for significance was set at $P < 0.05$. The study was registered in PROSPERO (ID: CRD42021255769).

Results: Seven studies were included (2536 patients). Non-LumA represented 55.2% and was associated with worse PFS/TTP than LumA [hazard ratio (HR) 1.77, $P < 0.001$, $I^2 = 61\%$], independently of clinical HER2 status [$P_{subgroup\ difference}$ (P_{sub}) = 0.16], systemic treatment ($P_{sub} = 0.96$) and menopausal status ($P_{sub} = 0.12$). Non-LumA tumors also showed worse OS (HR 2.00, $P < 0.001$, $I^2 = 65\%$), with significantly different outcomes for LumB (PFS/TTP HR 1.46; OS HR 1.41), HER2-E (PFS/TTP HR 2.39; OS HR 2.08) and BL (PFS/TTP HR 2.67; OS HR 3.26), separately (PFS/TTP $P_{sub} = 0.01$; OS $P_{sub} = 0.005$). Sensitivity analyses supported the main result. No publication bias was observed.

Conclusions: In HoR+ MBC, non-LumA disease is associated with poorer PFS/TTP and OS than LumA, independently of HER2, treatment and menopausal status. Future trials in HoR+ MBC should consider this clinically relevant biological classification.

Key words: intrinsic subtypes, breast cancer, metastatic, prognosis, hormone receptors

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☆Note: This study was previously originally presented as a poster presentation at the San Antonio Breast Cancer Symposium 2021 (poster ID: P4-07-08). Abstract reference: Schettini F, Martínez-Sáez O, Chic N, et al. Prognostic value of intrinsic subtypes (IS) in hormone receptor-positive (HoR+) metastatic breast cancer (MBC): a systematic review and meta-analysis of prospective trials. *Cancer Res.* 2022;82(suppl 4):P4-07-08. https://doi.org/10.1158/1538-7445.SABCS21-P4-07-08.

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INTRODUCTION

Metastatic breast cancer (MBC) is a major cause of cancer death with 5-year overall survival (OS) rates of ∼38%.[1,2] Similar to early-stage BC, MBC is a heterogeneous disease characterized by the presence/absence of estrogen and progesterone receptor expression and human epidermal growth factor receptor 2 (HER2) overexpression/amplification, all of which define four clinically relevant subgroups, namely hormone receptor-positive (HoR+)/HER2-negative, HoR+/HER2-positive (HER2+), HoR-negative/HER2+ and triple-negative breast cancer (TNBC) with different therapeutic implications.[3] However, HoR and HER2 status cannot capture the entire biological complexity of MBC.

Gene expression profiling has allowed a better understanding of the biological heterogeneity of MBC with the identification of four molecular 'intrinsic subtypes' (IS), namely luminal A (LumA), luminal B (LumB), HER2-enriched (HER2-E), basal-like (BL) BC, and a normal-like (NL) group.[4-7] These entities significantly differ in growth drivers, incidence, prognosis and treatment response.[4,5,8-12] The most validated assay to identify IS in the clinic is Prosigna®, which is a gene expression assay based on the PAM50 algorithm developed by Parker et al.[10,13] Through 50 key genes, the test is able to detect the BC IS and provide a risk of recurrence score that integrates and weighs IS correlations, a subset of proliferation genes, tumor size and nodal status.[10] The PAM50 assay is prognostic and predictive of (neo) adjuvant chemotherapy (CT) response.[14-16]

In the last decade, several studies demonstrated that all IS can be detected within each immunohistochemistry-based subtype.[8,17] While 90%-95% of early-stage HoR+/HER2-negative tumors are LumA or B, 5%-10% are non-luminal (i.e. HER2-E and BL), a proportion that is usually much higher (20%-30%) in MBC.[7,12,17,18] Furthermore, the proportion of non-luminal subtypes in HoR+/HER2+ disease is usually >30%.[12,17,18] In contrast, the vast majority of tumors in HoR-negative/HER2+ disease and TNBC are non-luminal, with the HER2-E being the most frequently observed subtype in the HER2+ subgroup (∼70%) and BL being the most frequent tumor type in TNBC (∼80%).[8,17,18]

In early-stage BC, IS are now an established prognostic biomarker.[19-24] However, the prognostic value of IS in MBC is not fully recognized despite several retrospective analyses of this biological classification in completed prospective trials in HoR+ MBC (i.e. MONALEESA 2/3/7, BOLERO2, EGF30008, DBCG and SOLTI-PATRICIA).[25-35] For these reasons, we conducted a systematic literature review and meta-analysis to assess the clinical validity of IS in HoR+ MBC, regardless of HER2 clinical status.

MATERIALS AND METHODS

Search strategy and selection criteria

A systematic literature search and trial-level meta-analysis were carried out to identify published prospective, phase II and phase III (randomized and non-randomized) clinical studies concerning HoR+ MBC, where the prognostic role of IS was evaluated in terms of progression-free survival (PFS) or time to progression (TTP), if the former was not available. Three independent reviewers (FS, OMS and CF) carried out the literature research. In case of controversy a fourth reviewer (AP) was consulted. The literature search had no restrictions based on language or time of publication; however, only clinical studies enrolling patients affected by HoR+ MBC were included. The recommendations of the Cochrane Collaboration were followed to identify all relevant studies.[36] We used the following query for the literature search: breast AND (tumor OR tumour OR cancer) AND intrinsic AND (subtype OR subtypes) AND (metastatic OR metastasic OR advanced OR inoperable). Given the expected scarcity of evidence on the topic we did not include further restrictions regarding HoR status and prognosis/prediction-related terms, so as to maximize data retrieval. Both full articles and studies published in abstract form were included in the analysis, if PFS or TTP data were directly available or computable. The search was conducted on the electronic databases PubMed and the Cochrane Controlled Register of Trials (CENTRAL). Cross-references between studies and online archives of main international congresses, including European Society for Medical Oncology (ESMO) and American Society of Clinical Oncology (ASCO) Congresses, as well as the San Antonio Breast Cancer Symposium (SABCS), were also consulted, including 2022 editions.

Data extraction and objectives

Data and details on patients and tumor characteristics, study design, interventions and outcome were extracted from each paper. Only the most recent and complete reports were included when duplicate publications were identified. The primary objective was to demonstrate a PFS/TTP advantage for the LumA subtype compared to the other IS in HoR+ MBC. Secondary objectives were to demonstrate the better PFS/TTP for LumA compared to other IS according to treatment, menopausal and HER2 status. An exploratory OS analysis of non-LumA versus LumA disease was also carried out. Hazard ratios (HRs) and 95% confidence intervals (CIs) for PFS/TTP and OS had to be available or computable.

Statistical analyses

Since a certain degree of heterogeneity was expected, analyses were carried out under the random-effect model of DerSimonian and Laird. Heterogeneity was assessed through Cochran's Q and I^2. Publication bias was explored through funnel plot visual inspection and Egger's and Begg's linear regression tests for funnel plot asymmetry.[37] Multiple sensitivity analyses were also conducted.[37,38] Subgroup analyses with prespecified subgroups of interest (i.e. treatment type, HER2 status, separate IS, menopausal status) were carried out. 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.[36] All tests were two-sided and the threshold for significance was set at $P < 0.05$. RevMan version 5.4 (The Cochrane Collaboration,

London, UK) and R version 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria) for MacOSX were used for statistical analyses. The study was registered in the PROSPERO online database with ID: CRD42021255769.

RESULTS

Summary of studies and patient characteristics

The selection process is summarized in the PRISMA diagram (Supplementary Figure S1, available at https://doi.org/10.1016/j.esmoop.2023.101214). From PubMed and Cochrane CENTRAL, 360 studies were extracted, 11 of which reported information on BC IS in HoR+ MBC. Preliminary results were reported as poster presentation at the SABCS 2021 (see Note). Then, two more references were assessed for inclusion in 2022, including a late-breaking abstract from the 2022 ESMO Congress. Therefore, a literature search with the same query was again carried out on 12 September 2022 (details not reported), but no new studies were identified. Overall, 13 prospective trials [12 randomized controlled trials (RCTs) and 1 non-randomized] assessed retrospectively IS in HoR+ MBC.[25,32-35,39-44] Ten of 13 identified studies carried out a prognostic assessment, although only 7 provided sufficient data to be included in this meta-analysis (Table 1).[25,32-35] Of these, the primary endpoint was PFS or TTP, while OS was a secondary endpoint, available for five (71%) studies. However, for the MONALEESA 2/3/7 trials, only pooled OS results according to IS and treatment arm were available.

Six (85%) randomized phase III trials and one (15%) single-arm phase II trial were included. Endocrine therapy (ET) with or without targeted therapy (TT) was used in five of six (83.3%) phase III trials, while CT was the backbone of treatment in one of six (16.7%) phase III trials. The non-randomized phase II study involved treatment with ET + TT. TT administered in the included studies was represented by anti-HER2 lapatinib and trastuzumab, mammalian target of rapamycin inhibitor everolimus and cyclin-dependent kinase 4/6 (CDK4/6) inhibitors palbociclib or ribociclib. All the original research articles were published between 2014 and 2021. A total of 2536 patients with IS data were included. Seventy-two percent (min-max: 54.2%-80.6%) of samples were from primary tumors and 28% (min-max: 19.2%-42.4%) were metastatic. Overall, 1401 (55.2%) non-LumA subtypes and 1135 (44.8%) LumA subtypes were identified. Among non-LumA diseases, 574 (41.0%) were LumB, 280 (20.0%) HER2-E and 62 (4.4%) BL, while 299 (21.3%) were classified as NL and 186 (13.3%) were only reported to be non-LumA, without providing detailed IS classification and after excluding NL. In all included studies, PAM50 subtyping was carried out under the nCounter platform (NanoString Technologies, Seattle, WA), although different nCounter gene panels were used.[13,25,32-35] All studies were conducted in patients with HoR+ MBC, with two (28.6%) of them also including HoR+/HER2+ disease. One (i.e. PATRICIA) included 55 (77.5%) HoR+/HER2+ and 16 (22.5%) HoR-negative/HER2+ patients, and PAM50 subtyping could be carried

Table 1. Characteristics of included studies

Study	Trial name	Population	Menopausal status	Trial type	Phase	Treatment	Line	Non-luminal A	Luminal A	Normal-like	Adjusted HR	Primary samples	Metastatic samples
Prat et al. *Oncologist.* 2019[32]	BOLERO2	HoR+/HER2-neg	Postmenopausal	Randomized	III	Everolimus + exemestane versus exemestane	≥1st	139	122	Included	No	80.7%	19.3%
Jørgensen et al. *Acta Oncol.* 2014[35]	DBCG	Mainly HoR+/HER2-neg	Mixed	Randomized	III	Docetaxel + gemcitabine versus docetaxel	1st-2nd	186	84	Not included	No	100%	0%
Prat et al. *JAMA Oncol.* 2016[16]	EGF30008	HoR+/HER2+ and neg	Postmenopausal	Randomized	III	Letrozole + lapatinib versus letrozole	1st	424	377	Included	No	80.6%	19.4%
Prat et al. *J Clin Oncol.* 2021[34]	MONALEESA 2	HoR+/HER2-neg	Postmenopausal	Randomized	III	Ribociclib + letrozole versus letrozole	1st	618	542	Included	Yes	72.0%	28.0%
Prat et al. *J Clin Oncol.* 2021[34]	MONALEESA 3	HoR+/HER2-neg	Postmenopausal	Randomized	III	Ribociclib + fulvestrant versus fulvestrant	1st-2nd			Included	Yes		
Prat et al. *J Clin Oncol.* 2021[34]	MONALEESA 7	HoR+/HER2-neg	Premenopausal	Randomized	III	Ribociclib + AI/TAM + GnRHa versus AI/TAM + GnRHa	1st			Included	Yes		
Ciruelos et al. *Clin Can Res.* 2020[25]	PATRICIA	HoR+/HER2+[a]	Postmenopausal	Non-randomized	II	Palbociclib ± letrozole + trastuzumab	3rd-5th	34	10	Included	No	54.2%	42.4%

+, positive; AI, aromatase inhibitors; ER, estrogen receptor; GnRHa, gonadotropin-releasing hormone analogue; HoR, hormone receptor; HR, hazard ratio; neg, negative; TAM, tamoxifen.
[a]The trial included HER2+ patients, regardless of HoR status. In this meta-analysis only HoR+/HER2+ were included; all studies defined HoR positivity as ER or PR ≥1% staining and used NanoString nCounter for subtyping.

out on 59 samples (83.1%). In this pooled analysis, results specific to the HoR-negative/HER2+ subpopulation were excluded. Another trial (i.e. EGF30008) included 157 (19.6%) patients with HoR+/HER2+ tumors and 644 (80.4%) patients with HoR+/HER2-negative disease.[25,33] Separate results for the two subpopulations were considered. Five (71.4%) studies included postmenopausal patients only, one (14.3%) study enrolled both pre- and postmenopausal women and one (14.3%) study enrolled only premenopausal patients. Treatment details and main study characteristics are reported in Table 1.

Primary objective and subgroup analysis according to individual intrinsic subtypes

Non-LumA disease was associated with worse PFS/TTP compared to the LumA subtype (HR 1.77, 95% CI 1.54-2.05, $P < 0.001$). Moderate heterogeneity was observed [$I^2 =$

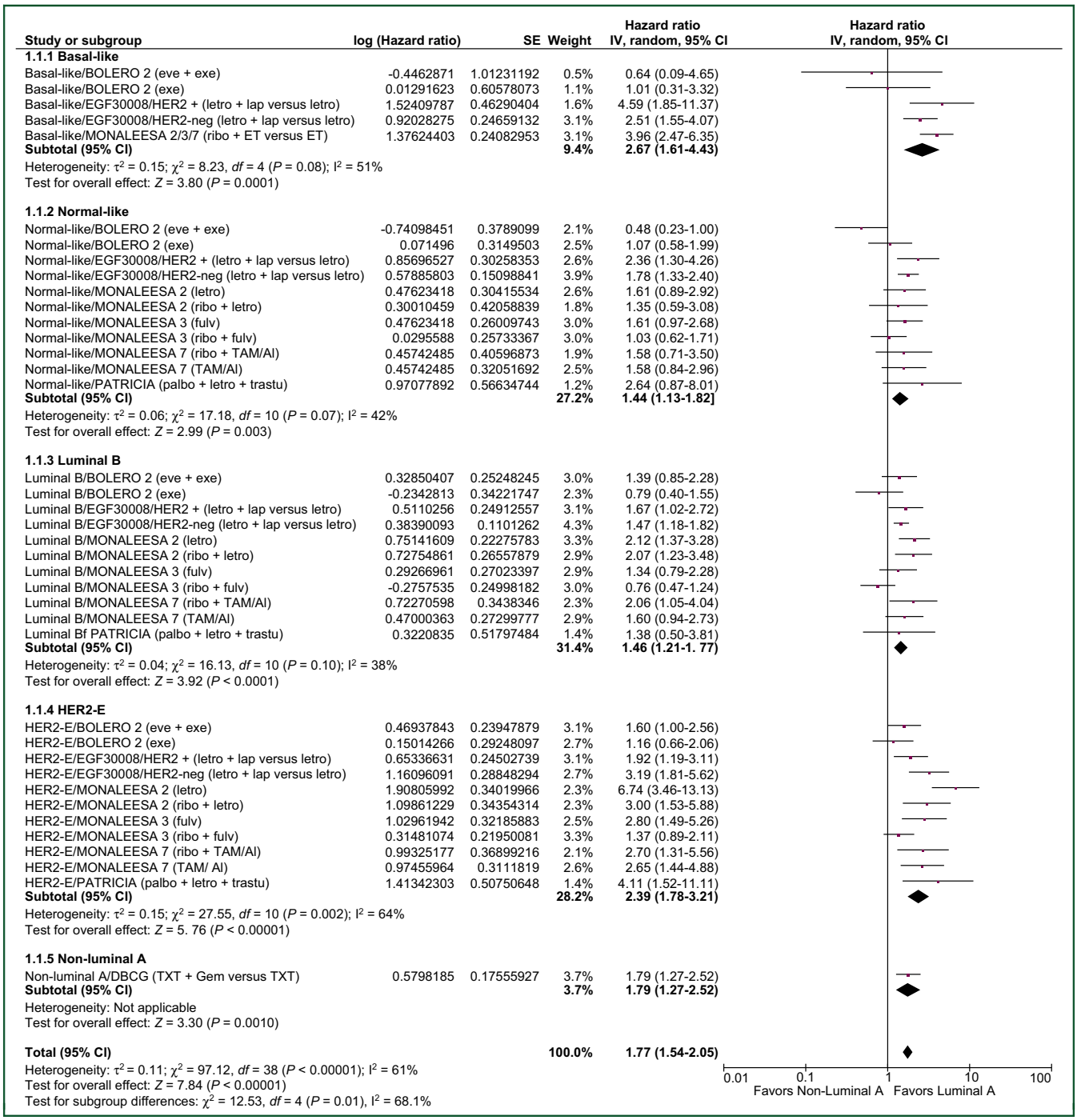

Figure 1. Progression-free survival of non-luminal A versus luminal A breast tumors and subgroup analysis according to separate intrinsic subtypes.
AI, aromatase inhibitor; CI, confidence interval; ET, endocrine therapy; eve, everolimus; exe, exemestane; fulv, fulvestrant; gem, gemcitabine; HER2-E, human epidermal growth factor receptor 2-enriched; IV, inverse variance; lap, lapatinib; letro, letrozole; neg, negative; rib, ribociclib; SE, standard error; TAM, tamoxifen; trastu, trastuzumab; TXT, docetaxel.

61%, $P_{heterogeneity}$ (P_H) < 0.001] (Figure 1). Considering that NL tumors are usually considered as an artifact instead of an independent subtype,[13] we repeated the analysis after removing NL. The results did not substantially change (HR of non-LumA versus LumA 1.93, 95% CI 1.62-2.29, $P < 0.001$; $I^2 = 64\%$, $P_H < 0.001$). The test for subgroup differences according to each individual IS (versus LumA) was also statistically significant [P value for subgroup differences (P_{sub}) = 0.01], with LumB (HR 1.46, 95% CI 1.21-1.77, $P < 0.001$), HER2-E (HR 2.39, 95% CI 1.78-3.21, $P < 0.001$), BL (HR 2.67, 95% CI 1.61-4.43, $P < 0.001$) and NL (HR 1.44, 95% CI 1.13-1.82, $P = 0.003$) all performing worse than LumA. Results were confirmed also when removing the NL subtype ($P_{sub} = 0.02$).

Worse prognosis for non-LumA subtypes versus LumA was observed in all prespecified subgroups, namely HoR+/HER2+ (HR 2.19, 95% CI 1.61-2.97, $P < 0.001$; Figure 2) and HER2-negative (HR 1.71, 95% CI 1.46-2.00, $P < 0.001$; Figure 2), patients treated with ET alone (HR 1.72, 95% CI 1.31-2.26, $P < 0.001$; Figure 3), ET + TT (HR 1.80, 95% CI 1.50-2.16, $P < 0.001$; Figure 3) or CT (HR 1.79, 95% CI 1.127-2.52, $P < 0.001$; Figure 3) and in premenopausal (HR 2.15, 95% CI 1.67-2.76, $P < 0.001$; Figure 4) and postmenopausal (HR 1.69, 95% CI 1.43-1.99, $P < 0.001$; Figure 4) disease. Additionally, there were no significant subgroup differences based on HER2 status ($P_{sub} = 0.16$), type of systemic treatment ($P_{sub} = 0.96$) and menopausal status ($P_{sub} = 0.12$) (Figures 2-4).

Non-LumA tumors showed an association with worse OS in comparison to LumA disease, as well (HR 2.00, 95% CI 1.63-2.45, $P < 0.001$) with similar heterogeneity to that observed for the primary endpoint ($I^2 = 65\%$, $P_H < 0.001$) (Supplementary Figure S2, available at https://doi.org/10.1016/j.esmoop.2023.101214). LumB (HR 1.41, 95% CI 1.20-1.66, $P < 0.001$), HER2-E (HR 2.08, 95% CI 1.62-2.69, $P < 0.001$), BL (HR 3.26, 95% CI 1.84-5.77, $P < 0.001$) and NL (HR 2.30, 95% CI 1.37-3.86, $P = 0.002$) were confirmed to be associated with worse OS than LumA ($P_{sub} = 0.005$)

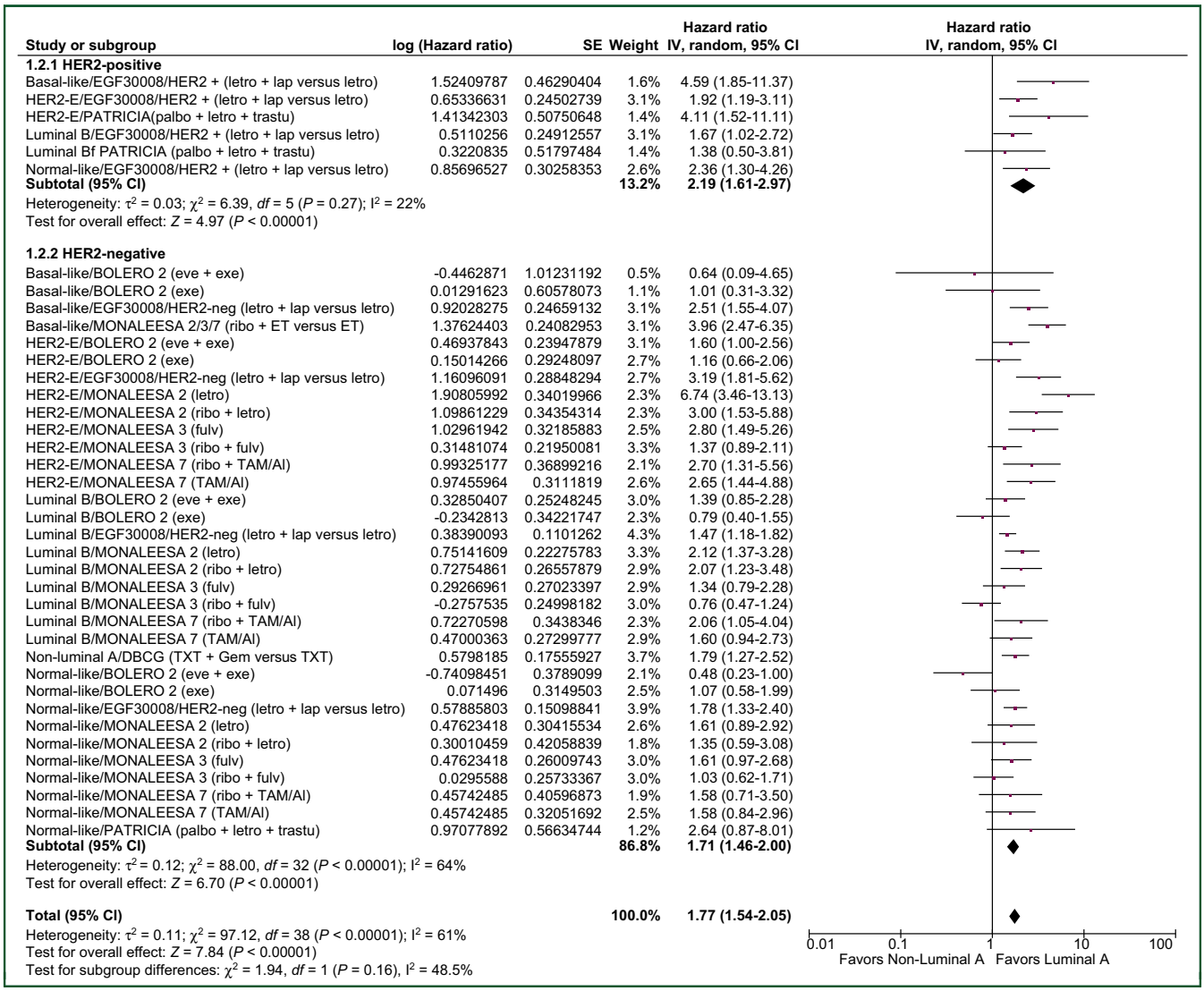

Figure 2. Subgroup analysis according to HER2 status.

AI, aromatase inhibitor; CI, confidence interval; ET, endocrine therapy; eve, everolimus; exe, exemestane; fulv, fulvestrant; gem, gemcitabine; HER2-E, human epidermal growth factor receptor 2-enriched; IV, inverse variance; lap, lapatinib; neg, negative; rib, ribociclib; SE, standard error; TAM, tamoxifen; trastu, trastuzumab; TXT, docetaxel.

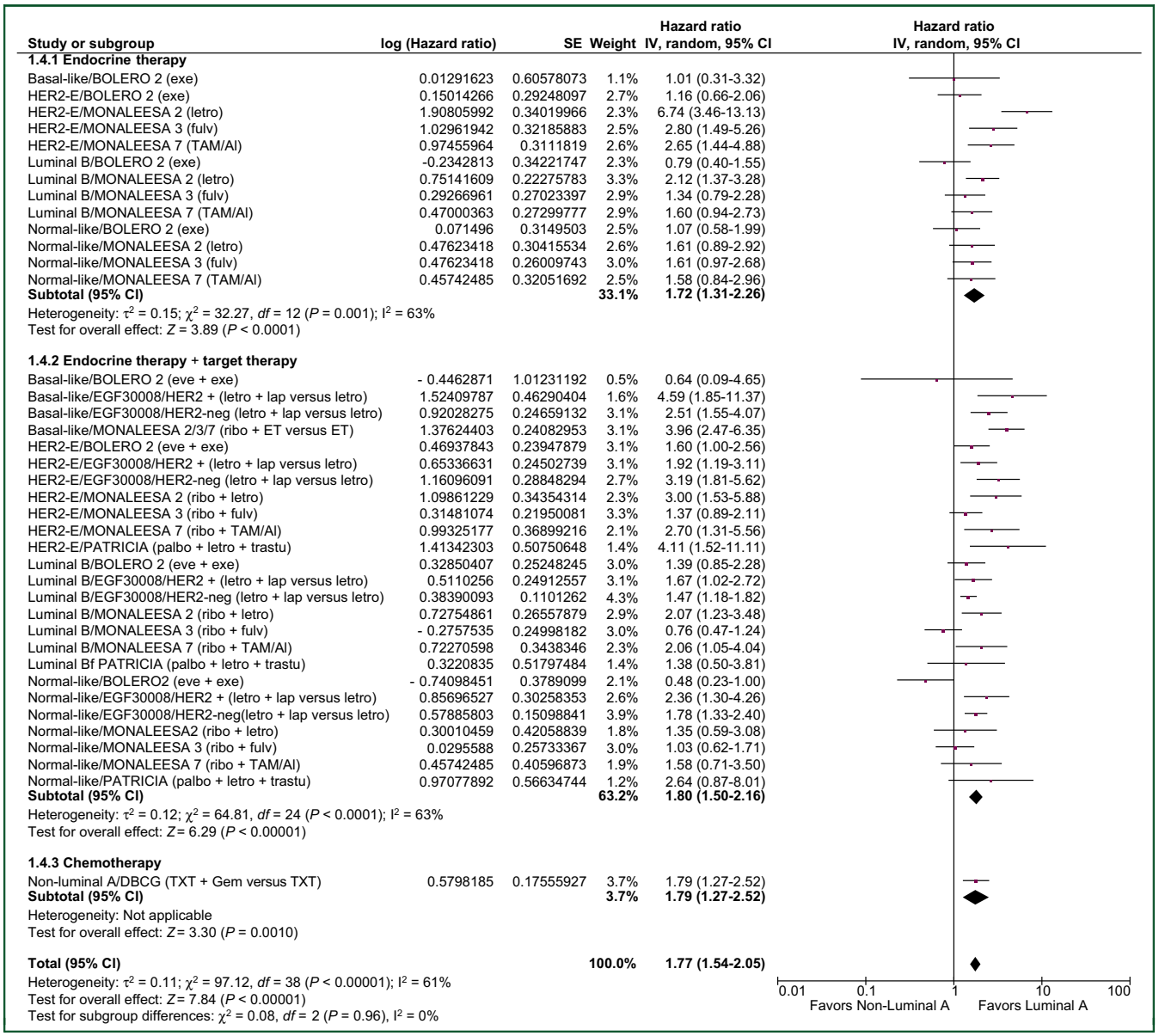

Figure 3. Subgroup analysis according to systemic treatment.
AI, aromatase inhibitor; CI, confidence interval; ET, endocrine therapy; eve, everolimus; exe, exemestane; fulv, fulvestrant; gem, gemcitabine; HER2-E, human epidermal growth factor receptor 2-enriched; IV, inverse variance; lap, lapatinib; neg, negative; rib, ribociclib; SE, standard error; TAM, tamoxifen; trastu, trastuzumab; TXT, docetaxel.

(Supplementary Figure S2, available at https://doi.org/10.1016/j.esmoop.2023.101214). Further subgroup analyses of OS according to HER2 status, treatment type and menopausal status were not conducted for the paucity of available data.

Sensitivity analyses, publication bias and risk of bias analysis

To explore the potential causes of the heterogeneity observed for the primary analysis, we first conducted a leave-one-out analysis. After sequentially removing each single study and each IS comparison, and re-running the analyses multiple times, we observed that no single comparison influenced the overall result; leave-one-out HR ranged from 1.73 to 1.82, all being statistically significant (i.e. $P < 0.001$). The residual I^2 ranged from 54.2% to 61.9% ($P_H < 0.001$ for all analyses) (Supplementary Table S1, available at https://doi.org/10.1016/j.esmoop.2023.101214).

Regarding the influential analysis, we observed that the most influential comparisons were the following: NL versus LumA (BOLERO2 everolimus plus exemestane arm), HER2-E versus LumA (MONALEESA 2 letrozole arm), LumB versus LumA (MONALEESA 3 ribociclib plus fulvestrant arm) and BL versus LumA comparison (MONALEESA 2/3/7 overall population) (Supplementary Figure S3, available at https://doi.org/10.1016/j.esmoop.2023.101214).

After excluding all these four comparisons, non-LumA disease was still found significantly associated with poorer PFS/TTP than LumA subtype (HR 1.79, 95% CI 1.50-2.24, $P < 0.001$), without significant heterogeneity ($I^2 = 27.4\%$, $P_H = 0.07$). Therefore, the primary result was not significantly

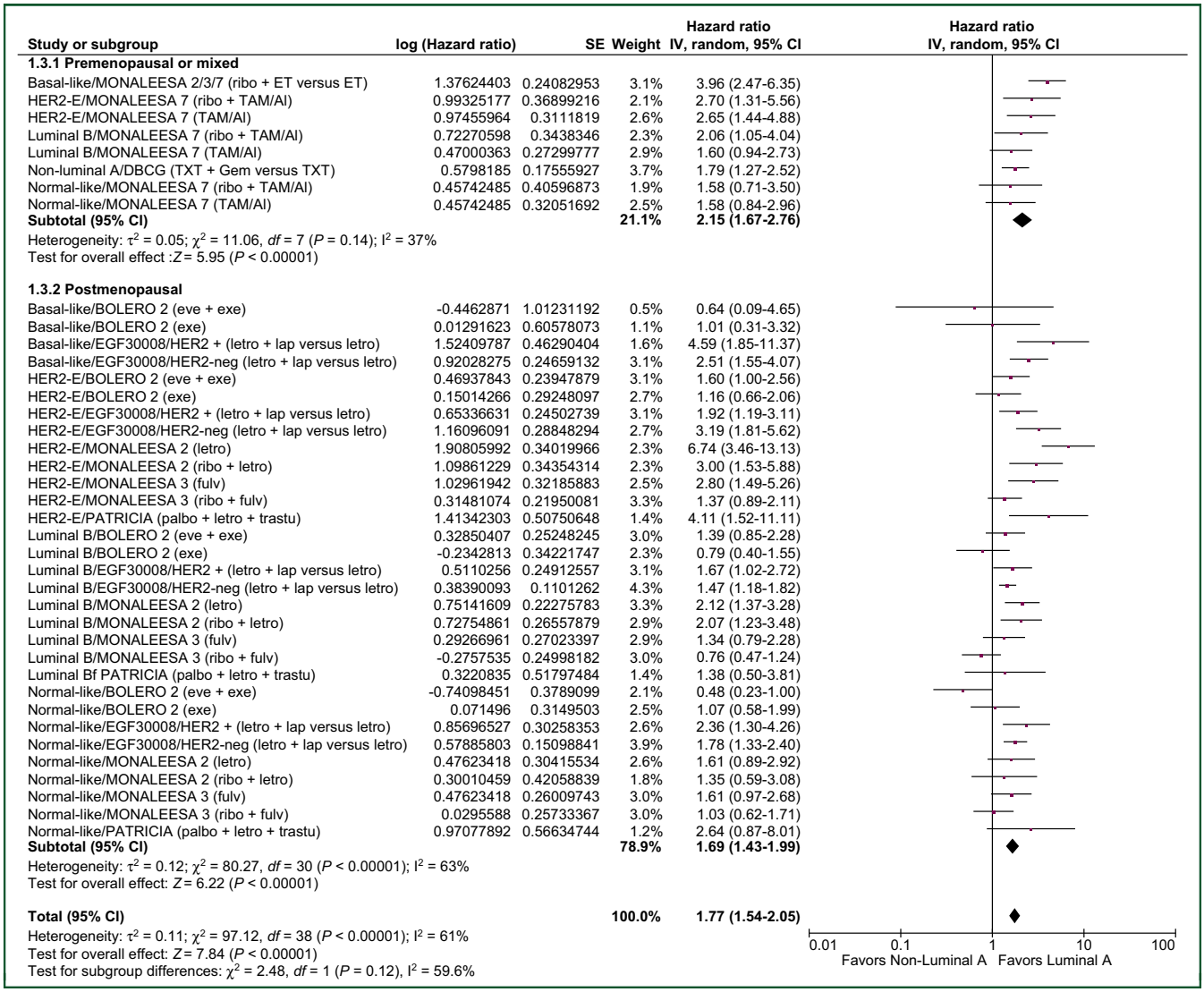

Figure 4. Subgroup analysis according to menopausal status.
AI, aromatase inhibitor; CI, confidence interval; ET, endocrine therapy; eve, everolimus; exe, exemestane; fulv, fulvestrant; gem, gemcitabine; HER2-E, human epidermal growth factor receptor 2-enriched; IV, inverse variance; lap, lapatinib; neg, negative; rib, ribociclib; SE, standard error; TAM, tamoxifen; trastu, trastuzumab; TXT, docetaxel.

affected by the most influential cases. No publication bias was observed (Egger's $P = 0.492$; Begg's $P = 0.719$; Supplementary Figure S4A, available at https://doi.org/10.1016/j.esmoop.2023.101214). Finally, the analysis of the risk of bias did not show substantial concerns with respect to studies' internal validity (Supplementary Figures S4B and S5, available at https://doi.org/10.1016/j.esmoop.2023.101214).

DISCUSSION

In our trial-level meta-analysis, the prognostic role of PAM50 IS in the context of HoR+ MBC was confirmed, with LumA being the subtype with better prognosis, compared to all others, either separately or taken together, and independently of treatment type, HER2 and menopausal status. Conversely, HER2-E and BL were the subtypes at worst prognosis. These findings are in line with what has been demonstrated in the last couple of decades in early-stage disease, after the discovery of IS by Perou and colleagues in 2000 and subsequent development of the PAM50 assay to detect them in individual patient's tumors.[4,8-12,45-48] PAM50 IS detection has not been broadly implemented in the clinic, because the standardized PAM50 assay Prosigna® was approved for clinical use only in early-stage HoR+/HER2-negative disease. Our results in HoR+ metastatic disease point out that PAM50 IS adds important biological information in this context and may be helpful in accurately identifying expected clinical evolution of the disease, providing more accurate prognostication beyond standard clinicopathological features.

Noteworthy, from some of the 13 prospective trials in HoR+ MBC where retrospective correlative biomarker analyses were conducted to assess IS clinical value, a potential therapeutic predictive role also seemed to emerge. For example, Prat et al. showed that identifying BL tumors with the PAM50 assay could be useful to identify a small subgroup of HoR+/HER2-negative MBC substantially resistant to either ET or ET plus CDK4/6 inhibitors, the current first/second-line standard of care.[34,49] These tumors might gain

benefit from alternative CT-based therapeutic options. Moreover, the HER2-E subtype within HoR+/HER2-negative MBC may be particularly sensitive to ribociclib added to ET,[34] or even sensitive to anti-HER2-directed therapies, despite lack of HER2 amplification.[33] However, there were no sufficient and homogeneous data to assess the predictive role of PAM50 IS in a meaningful pooled analysis.

Importantly, prospective clinical trials led by the Spanish Cooperative Research Group SOLTI are ongoing and specifically designed to assess the clinical utility of PAM50 IS as a predictive tool for the personalized treatment of patients with HoR+/HER2-negative MBC (NCT04460430, NCT04251169, NCT05207709, NCT04142060). Paradigmatic in this perspective is the HARMONIA randomized phase III trial, which is randomizing patients with HoR+/HER2-negative/HER2-E MBC to receive either palbociclib or ribociclib plus ET. Additionally, an exploratory cohort of patients with HoR+/HER2-negative/BL tumors will be treated with upfront CT plus immunotherapy, instead of ET.[50] To note, in our retrospective joint analysis, a proportion of ∼12% of cases had been classified as NL. For this reason, some might consider PAM50 subtyping for inclusion in clinical trials as potentially impaired by the identification of NL breast tumors, providing that this subset is more of a technical artifact rather than a true subtype.[7] Nevertheless, in a prospective clinical trial context, with a careful selection of the most appropriate pathology samples, along with centralized sample processing for RNA extraction and subtyping, the experience coming from the same SOLTI group suggests that it is extremely rare to observe tumors classified as NL.

Our study has some limitations to consider. Firstly, no sufficient data were available to carry out a more detailed OS assessment with meaningful subgroup analyses, although what observed for PFS/TTP result, overall and according to each IS, seemed to be confirmed at the OS level. Secondly, three studies with prognostic information could not be included in this pooled analysis because: (1) the data provided were insufficient (i.e. only *P* values without HR and 95% CI in the Young-PEARL trial of first-line palbociclib plus ET versus capecitabine in premenopausal women); (2) a different comparison was realized (i.e. luminal A + B versus non-luminal in the Young-PEARL trial and within the MONARCHER trial of late-line abemaciclib plus trastuzumab with/without fulvestrant versus CT plus trastuzumab); (3) a different survival endpoint was assessed (i.e. long- and short-term post-relapse survival in the CT-based TEX trial).[39,41,42] Different outcomes were also observed in other three RCTs, namely the pivotal trials of palbociclib plus letrozole or fulvestrant PALOMA 2 and 3, respectively, and the PEARL trial of palbociclib plus ET versus capecitabine in postmenopausal women.[40,43,44] However, no formal comparison was carried out because the main focus of the biomarker analysis was to demonstrate a potential predictive role.[40,43,44] Thirdly, the BL versus LumA comparison for the MONALEESA trials included in our analysis was not provided for each study separately, because of the low number of cases. Thus, the pooled MONALEESA 2/3/7 result was considered. Similarly, but for the entire OS exploratory analysis, only pooled MONALEESA 2/3/7 data for each IS were available, limiting the possibility to carry out subgroup analyses. Fourthly, the result from the non-randomized PATRICIA trial included both ET-treated and ET-untreated patients. Reassuringly, the recently presented updated analyses of the MONARCHER trial in a pure HoR+/HER2+ population showed that luminal A + B disease was associated with longer PFS (8.6 versus 5.4 months, HR 0.54, 95% CI 0.38-0.79) and OS (31.7 versus 19.7 months, HR 0.68, 95% CI 0.46-1.00) versus non-luminal metastatic disease, though a separate comparison against LumA-only tumors was not provided.[42] Finally, the sub-analysis regarding CT might have been influenced by the low number of cases and should be taken with caution. Moreover, no separate result according to subtype was provided in the DBCG trial, where all IS were regrouped in non-LumA and LumA. Nevertheless, in support of our analysis, a second CT-based trial, the TEX of first-line paclitaxel plus epirubicin plus capecitabine versus paclitaxel plus epirubicin, showed poorer prognosis for HER2-E and BL IS compared to LumA in terms of short- and long-term post-relapse survival.[41]

Despite some limitations, our meta-analysis is the first to have grouped all available evidence on the topic; sensitivity and risk of bias analyses were reassuring that results are robust. Furthermore, in all included studies, IS were detected through a NanoString nCounter® platform with a PAM50 algorithm, thus results were not biased by the potential lack of interchangeability among different subtyping methodologies and technological platforms, as we have elsewhere pointed out.[13] What remains to be addressed is the relevance of tracking the molecular evolution of the disease through treatment lines, provided that a subtype shift promoted by different treatments has been demonstrated in several translational and preclinical studies,[18,51-53] as well as heterogeneity between different metastatic tumor sites.[54] In all studies assessing IS prognostic value, mixed primary and metastatic biopsies were analyzed. In addition, no comparison of the prognostic role at different time points of the natural history of the disease has been carried out so far. Moreover, a change in tumor biology has been demonstrated to occur between primary and metastatic disease, with a tendency to shift from less to more aggressive IS while more aggressive disease, particularly BL, showing a tendency to remain stable over time.[18,55] It is currently unknown whether a subtype change might truly have an impact on prognosis beyond the original IS identified either in the primary or the first metastatic assessment. This implies that the best timepoint to detect IS in the metastatic setting is yet to be defined, provided that a single assessment is sufficient for an optimal prognostic evaluation.

In conclusion, this trial-level meta-analysis established the prognostic value of PAM50 IS in HoR+ MBC, beyond treatment, menopausal and HER2 status, with LumA being the most favored subtype and HER2-E and BL showing the worst outcomes. Nevertheless, before entering the routine clinical setting, further implementation of intrinsic subtyping should be encouraged in the context of prospective

clinical trials, either for prognostic stratification of the population enrolled or for selecting patients to receive more tailored therapeutic strategies (escalation versus de-escalation, targeted approaches). Finally, more efforts should be done to clarify potential clinical implications of assessing the PAM50 IS in different tumor sites (primary versus metastatic) and at multiple timepoints during the natural history of the disease.

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DATA SHARING

Data were collected from already published papers.

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