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Clinical implications of the intrinsic molecular subtypes in hormone receptor-positive and HER2-negative metastatic breast cancer

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ABSTRACT

Traditionally, the classification of breast cancer relies on the expression of immunohistochemical (IHC) biomarkers readily available in clinical practice. Using highly standardized and reproducible assays across patient cohorts, intrinsic molecular subtypes of breast cancer - also called "intrinsic subtypes" (IS) - have been identified based on the expression of 50 genes. Although IHC-based subgroups and IS moderately correlate to each other, they are not superimposable. In fact, non-luminal biology has been detected in a substantial proportion (5–20%) of hormone receptor-positive (HoR+) tumors, has prognostic value, and identifies reduced and increased sensitivity to endocrine therapy and chemotherapy, respectively. During tumor progression, a shift toward a non-luminal estrogen-independent and more aggressive phenotype has been demonstrated. Intrinsic genomic instability and cell plasticity, alone or combined with external constraints deriving from treatment selective pressure or interplay with the tumor microenvironment, may represent the determinants of such biological diversity between primary and metastatic disease, and during metastatic tumor evolution. In this review, we describe the distribution and the clinical behavior of IS as the disease progresses, focusing on HoR+/HER2-negative advanced breast cancer. In addition, we provide an overview of the ongoing clinical trials aiming to validate the predictive and prognostic value of IS towards their incorporation into routine care.

Background

Hormone receptor-positive (HoR+) breast cancer (BC) accounts for 75% of all invasive breast tumors [1]. Endocrine therapy (ET) with estrogen receptor (ER) modulators, aromatase inhibitors (AIs), or ER antagonists, constitutes the backbone of treatment for HoR+/HER2-negative BC, alone or in combination with targeted therapies (i.e., CDK4/6, mTOR or PI3K inhibitors). It also represents a highly successful example of precision oncology, where treatment choices are guided by predictive biomarkers. To date, beyond HoR positivity, *PIK3CA* somatic mutations, HER2-low status, germline *BRCA1/2*, and *PALB2* mutations are the only actionable biomarkers informing patient selection for treatment of HoR+/HER2-negative disease (i.e., alpelisib for *PIK3CA*-mutant tumors, trastuzumab deruxtecan for HER2-low disease, PARP-inhibitors for germline *BRCA1/2*- or *PALB2*-mutant tumors) [2–8]. Recently, a first-in-class AKT inhibitor combined with fulvestrant has demonstrated superior clinical outcomes in comparison with fulvestrant alone in the overall trial population and in pre-specified subgroups identified by alterations in the PIK3CA, AKT1, or PTEN genes, likely providing a new therapeutic option for patients progressing on prior endocrine treatment (with or without CDK4/6 inhibitors), but independently of PI3K pathway-related biomarkers [9]. In addition, no biomarkers predicting mechanisms of resistance to novel drugs are currently available, although recent findings from the prospective PADA-1 trial showed that acquired *ESR1* mutations in patients exposed to prior AIs might be used to detect the rise of resistance and the

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opportunity to switch to a different ET, like fulvestrant [10].

Traditionally, breast tumors have been classified based upon 4 immunohistochemical (IHC) biomarkers readily available in clinical practice: ER, progesterone receptor (PR), HER2, and Ki67 (in some countries). Along with these biomarkers, hierarchical clustering based on the unsupervised analysis of differentially expressed "intrinsic" genes has segregated BC into two main clusters, mostly dominated by ER expression, and 4 sub-clusters referred to as "intrinsic molecular subtypes", also called "intrinsic subtypes" (IS) [i.e., Luminal A, Luminal B, HER2-Enriched (HER2-E) and Basal-like]. Biological heterogeneity and clinically relevant differences in incidence, survival, and response to treatment between subtypes have been demonstrated [11–21]. A Normal-like group showing gene expression features usually expressed by the adipose tissue and clustering with fibroadenoma and normal breast tissue has been also identified [22]. The clinical relevance of this subtype is unclear, and many, including our group, consider it a mere artifact, likely attributable to specimen contamination by normal tissue [11–15,23–25].

We have previously illustrated the clinical significance of the IS [26–28]. Here, we describe the distribution of the IS and their evolution during disease progression, along with their clinical behavior, focusing on the HoR+/HER2-negative advanced disease. Of note, the terms advanced or metastatic breast cancer will be used interchangeably in this review. In addition, we present a summary of the ongoing clinical trials in the advanced setting aiming to define the clinical utility of the IS.

Temporal evolution of the IS during disease progression

There is accumulating evidence on the genomic and phenotypic evolution between primary and metastatic breast tumors [29–31]. For example, in a study analyzing 123 paired primary breast tumors and metastases (of which 70% were HoR+/HER2-negative), high concordance for the Basal-like (100%), and moderate concordance for the HER2-E (76.9%) and Luminal B (70%) subtypes was observed, whereas a substantial rate of conversion of the Luminal A subtype (55.3%) to non-luminal disease was noted. In addition, ~15% of Luminal A and Luminal B primary tumors changed to HER2-E disease in the corresponding metastasis, despite being clinically HER2-negative. Overall, the HER2-E subtype was more frequently detected in metastasis (22%) than in the corresponding primary lesion (11.4%). Moreover, metastatic tissues showed higher expression of proliferation-related genes and lower expression of luminal genes when compared to the primary tumors, except for the Basal-like disease that is characterized by a relatively stable intrinsic gene expression profile [31].

Recent molecular subtype analysis of longitudinal primary tumors and metastases (64% of the tumors were HoR+/HER2-negative) from 152 patients enrolled in the European AURORA study revealed an overall 36% conversion rate of IS. Ninety percent of Luminal A primary tumors switched to Luminal B or HER2-E in the metastatic sample. Overall, 17.9% of the Luminal A or B primary tumors were identified as non-luminal in the metastases. Luminal tumors switching to the HER2-E subtype were more enriched in *TP53* and/or *PI3KCA* mutations in comparison with stable tumors. Gene expression differences between primary tumor and metastasis were larger in HoR+/HER2-negative disease in comparison with other groups. These differences correlated with a longer time to relapse indicating an adaptive transcriptional reprogramming associated with endocrine resistance and/or an accumulation of mutations in late-recurrent tumors [32].

To support the growing body of evidence of subtype changes in early-stage vs advanced-stage BC, we examined studies reporting subtype distribution in both settings in the context of the HoR+/HER2-negative disease. Original research articles published in English in Pubmed or abstracts presented at the main international annual conferences between January 2009 and December 2021 were retrieved and reviewed using the following search terms: "PAM50", "hormone receptor-positive", "HER2-negative", "intrinsic subtype" and "breast cancer". Overall, 27 different studies, for a total of 10,458 primary tumors and 763 metastases, were identified and deemed suitable for the subtype distribution analysis (paired and unpaired; **Supplementary Table A**). Overall, a higher proportion of HER2-E subtypes was detected in metastases compared to primary tumors (18% vs 6%; $p < 0.001$). On the contrary, the proportion of the Luminal A subtype was lower in metastases (34% vs 54%; $p < 0.001$), whereas the proportion of Luminal B and Basal-like subtypes was similar (Fig. 1).

Taken together, these findings support the evidence of a higher prevalence of estrogen-independent and more aggressive IS in metastases as compared to primary tumors. Intrinsic tumor biology and cell plasticity, along with external constraints like selective pressure by adjuvant treatments, the interplay with the tumor microenvironment and undetected cellular sub-clones at the initial diagnosis might be, alone or combined, at the basis of such biological diversity observed between primary and metastatic disease.

Main molecular features of non-luminal IS within HoR+/HER2-negative breast cancer

IS capture most of the biological diversity present in BC, are represented in each IHC-based subgroup, and add prognostic and predictive information beyond classical clinical and pathological parameters (i.e., age, node status, tumor size, and histological grade), in both early and metastatic settings [14,20,23,24,33–41]. Non-luminal IS (i.e., Basal-like and HER2-E) in the context of primary or metastatic HoR+ BC have been associated with poorer outcome, less endocrine sensitivity, and increased responsiveness to chemotherapy (CT) compared to luminal tumors, despite being ER-positive by IHC [18,26,27,31,35–37,42–47]. Intriguingly, a pooled analysis of the MONALEESA trials observed that the HER2-E subtype exhibits the worst prognosis with ET alone but the greatest relative benefit when ribociclib was added to ET for the treatment of HoR+/HER2-negative advanced disease [46]. The hypothesis behind this observation is that effective therapies such as ribociclib can switch an aggressive IS such as the HER2-E to a more estrogen-dependent subtype such as the Luminal A. This has been previously demonstrated in patients with HoR+/HER2-positive BC, where anti-HER2 therapies can turn a HER2-E/HER2-positive tumor into a Luminal A/HER2-positive [48]. Whether this subtype switching is functionally relevant is under investigation.

Biologically, the HER2-E subtype is characterized by high expression of growth factor receptor-related genes, such as *ERBB2* and *FGFR4*, and of cell cycle-related genes, by low expression of luminal-related genes, such as ER and PR, and Basal-related genes (e.g., keratin 5 and *FOXC1*). This subtype presents high aneuploidy and the highest number of mutations across the genome. Overall, 72% and 39% of HER2-E tumors are *TP53* and *PIK3CA* mutated, respectively, and show amplification of FGFR, HER1/EGFR, CDK4, and Cyclin D1 genes [14]. *APOBEC3B*, a subclass of APOBEC cytidine deaminase that converts cytosine to uracil, is also frequently mutated in the HER2-E subtype [49]. Among HoR+/HER2-negative BCs by IHC, *ERBB2* gene expression (and the expression

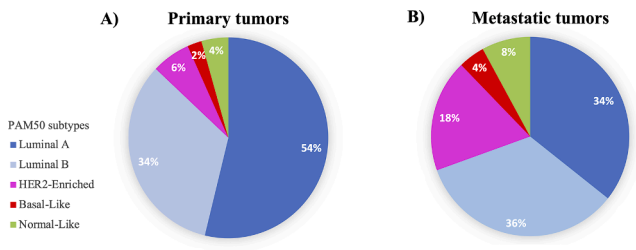


Fig. 1. PAM50 subtype distribution in hormone receptor-positive (HoR+) and HER2-negative breast cancer. (**A**) primary disease (N = 10,458) and (**B**) metastatic disease (N = 763).

of other 17q12 amplicon genes) is higher in HER2-E than in the other subtypes but lower in comparison to HoR+/HER2-positive disease (Fig. 2A).

The HER2-E subtype has a similar copy number alteration profile, higher *TP53,* and *ERBB2* and lower *PIK3CA* mutation frequency, lower *ESR1* expression (Fig. 2B), and higher immune genes expression in comparison with luminal tumors but similar proliferative characteristics compared to the Luminal B subtype [14,50]. Globally, the HER2-E/HER2-negative subtype is almost molecularly indistinguishable from its counterpart within the HER2-positive disease. In fact, HER2-E/HER2-positive tumors share most genomic and genetic alterations with HER2-E/HER2-negative tumors, except for the upregulation of genes located in, or near, the HER2 amplicon on chromosome 17 [18].

The Basal-like subtype is characterized by high expression of proliferation-, tyrosine kinase, and cell cycle-related genes as well as keratins (e.g., keratins 5, 14, and 17), intermediate expression of HER2-related genes, and low expression of luminal-related genes (Fig. 2B and Fig. 3). At the DNA level, these tumors present a high mutation rate, *TP53* mutation in 80% of the cases while most frequently mutated genes of the luminal repertoire, except for *PIK3CA* (9%), are nearly absent [14]. Although the Basal-like subtype, given its lower frequency, has been less extensively characterized than the HER2-E subtype in the context of the HoR+/HER2-negative disease, evidence suggests that the genomic profile of Basal-like tumors is well-preserved across IHC-based subgroups [14,51]. Basal-like tumors share considerable genomic commonalities (i.e. high expression of *CCNE1*, *EGFR*, and keratins genes, among others, and low expression of luminal-related genes, such as *ESR1* and *FOXA1*) with the triple-negative tumors or the prototypical PAM50 Basal-like subtype (Fig. 3) [46].

HER2-low tumors, defined as those with HER2 IHC score 1+ or 2+ with negative ISH assays [52], represent most of the HER2-negative BCs, regardless of the HoR status [53,54]. Results from a comprehensive retrospective analysis of more than 3,500 patients revealed that the luminal biology predominates among HoR+/HER2-low primary tumors, whereas the HER2-E subtype is infrequent (<5%) and equally distributed between HER2 0 and HER2-low. This subtype distribution was consistent with a pattern of differential *ERBB2* gene expression between HER2 0 and HER2-low BCs, especially in the HoR+ group. Conversely, no relevant differences in subtype distribution were observed among triple-negative BCs when subdivided by HER2 IHC levels [53]. Importantly, HER2-low status did not show any prognostic effect [54]. Overall, there is no evidence supporting the HoR+/HER2-low tumor subgroup being an independent nosological entity [53,55–57]. It might represent a subset of HoR+ tumors with some activation of the HER2 pathway, potentially representing a proxy for the identification of tyrosine kinase receptor-based endocrine-resistant BC [58], with low or absent association with the IS, deserving special attention due to its potential therapeutical implications [59].

Clinical relevance of the IS in HoR+/HER2-negative metastatic breast cancer

Prognostic and predictive role in patients treated with chemotherapy

PAM50-based analysis from 2 randomized clinical trials has previously investigated the value of the IS in patients receiving CT. The first trial led by Nielsen D.L. and colleagues [60] tested the efficacy of docetaxel alone or combined with gemcitabine for the 1st or 2nd-line of treatment in 337 patients with HER2-positive or negative advanced BC. PAM50 IS distribution from 181 (67%) HoR+/HER2-negative primary tumors, among the 270 samples that were profiled, is reported in Table 1. The Luminal A subtype was associated with a significantly lower risk of progression, measured as time to progression (TTP) [hazard ratio (HR) 0.56], and better overall survival (OS) (HR 0.71) compared to non-luminal subtypes, whereas the Basal-like IS was associated with poorer survival outcomes compared to non-Basal-like disease (TTP: HR 1.80; OS: HR 1.65). The associations remained significant when adjusted for the main clinical-pathological variables including HER2 status. Overall, the IS were not predictive of TTP ($p_{interaction} = 0.50$). Nevertheless, patients with Basal-like tumors receiving the combination of gemcitabine plus docetaxel achieved a 10-month longer median OS vs those treated with docetaxel alone (18.7 months vs 8.5 months; $p_{interaction} = 0.0006$), reaching similar OS estimates as the non-Basal-like disease [61].

The TEX study was a Swedish multicenter trial that assigned 287 patients with metastatic BC to 1st-line CT with epirubicin and paclitaxel with or without capecitabine [69]. Tumor samples from 120 locoregional or distant relapses were profiled using the research-based PAM50 classifier (Table 1). ER+ and PR+ tumors represented 62.5% and 48.5% of the whole cohort, respectively. As a limitation, information on HER2 status was not available. The PAM50 subtypes were significantly associated with post-relapse BC-specific survival, with the shortest survival seen in the non-luminal subgroups. In a model adjusted for clinical variables, including study treatments, a more than 3-fold increased risk for death from BC was found in patients whose tumors were Basal-like or HER2-E compared to Luminal A (HR of 3.70 and 4.40, respectively) [62]. These findings were validated in a subsequent post hoc analysis [70]. Interestingly, IS in this trial displayed preferential

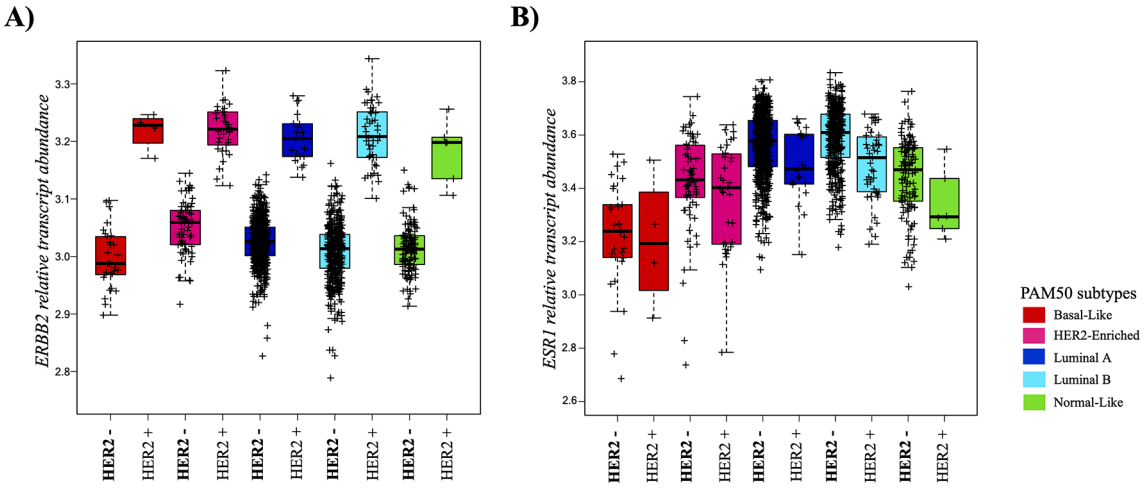


Fig. 2. Relative transcript abundance of *ERBB2* and *ESR1* among hormone receptor-positive (HoR+) tumors according to clinical HER2 status. (**A**) *ERBB2* and (**B**) *ESR1* gene expression across the PAM50 intrinsic subtypes in 1434 tumor samples. Data was obtained from the Molecular Taxonomy of Breast Cancer International Consortium (METABRIC) publicly available dataset *(Curtis C. et al., Nature 2012)*.

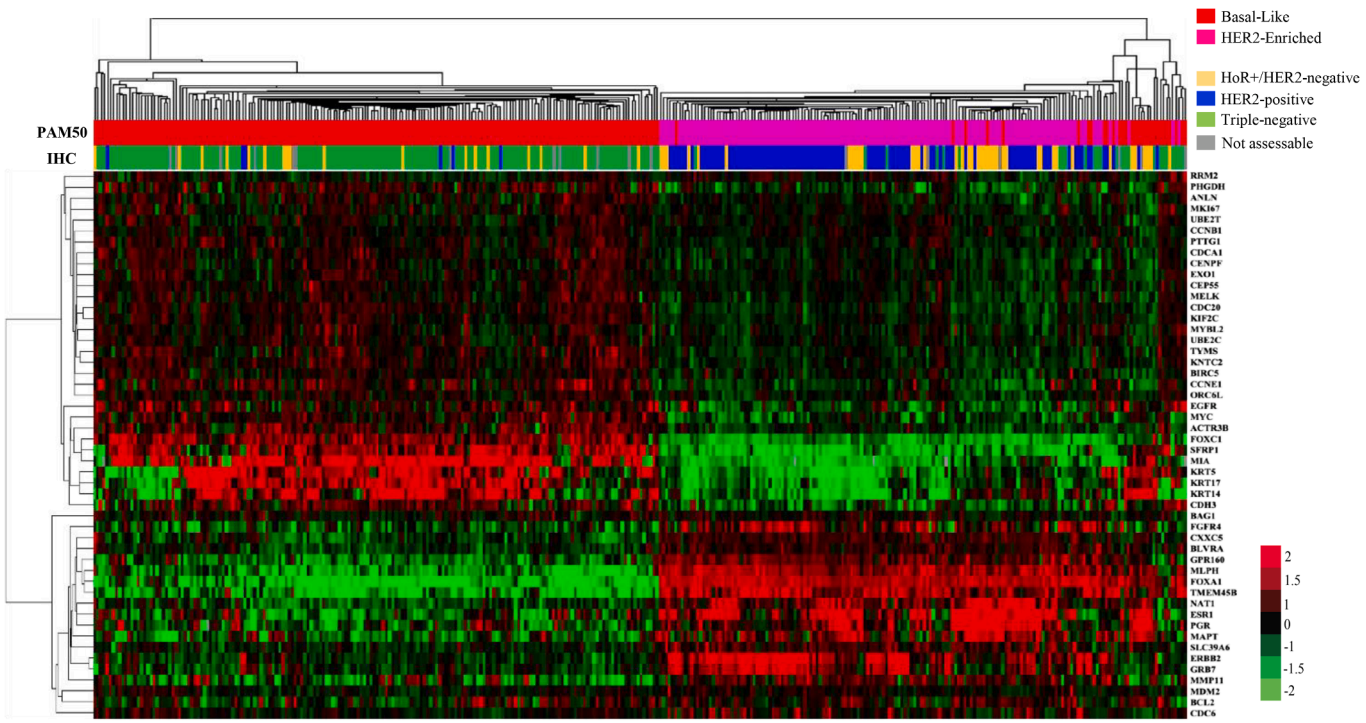


Fig. 3. Heatmap from an unsupervised analysis of the PAM50 genes in 348 breast tumors representing the PAM50 HER2-E (N = 138) and Basal-like (N = 210) subtypes. The three immunohistochemical (IHC)-based subgroups (i.e., HoR+/HER2-negative, HER2-positive, and Triple-negative) are shown below the array tree. Data was obtained from The Cancer Genome Atlas (TCGA) publicly available dataset *(Koboldt D.C. et al., Nature 2012)*.

Table 1
PAM50 subtype distribution across studies in the setting of hormone receptor-positive and HER2-negative metastatic breast cancer.

	Source of biopsy	N	Luminal A (%)	Luminal B (%)	HER2-Enriched (%)	Basal-Like (%)	Normal-Like (%)	Reference
Jorgensen C.L.T., 2014	Primary tumor	181	38.7	49.2	7.2	4.9		[61]
*Tobin N.P., 2015**	Metastasis	120	10.0	28.0	32.0	25.0	5.0	[62]
*Falato C., 2015**	Primary tumor	187	21.9	23.5	26.2	21.9	6.5	[35]
Prat A., 2016	Primary tumor or metastasis	644	52.0	30.0	3.0	3.0	12.0	[37]
Prat A., 2019	Primary tumor or metastasis	261	46.7	15.7	21.5	1.9	14.2	[63]
*Turner N.C., 2019***	Primary tumor or metastasis	285	44.0	30.7	20.9	1.7	2.7	[64]
*Finn R.S., 2020***	Primary tumor or metastasis	455	50.0	30.0	19.0	1.0		[65]
Prat A., 2021	Primary tumor or metastasis	1160	46.7	24.0	12.7	2.6	14.0	[46]
Martinez Saez O., 2021	Primary tumor or metastasis	114	33.0	37.0	17.0	5.0	8.0	[66]
*Martin M., 2021***	Primary tumor or metastasis	455	51.0	42.0	6.4	0.6		[67]
Lee S., 2022	Primary tumor or metastasis	165	38.0	36.0	12.0	8.0	7.0	[68]

* Mixed ER-positive and ER-negative tumors.
** Profiled using the Absolute Intrinsic Molecular Subtyping (AIMS) algorythm.

sites of metastasis and no liver metastasis was classified as Basal-like. Consistently, a recent retrospective study from our group demonstrated that the Basal-like subtype was detected only in 6.5% of liver metastases (while Luminal A, Luminal B, and HER2-E tumors were found in 25.8%, 32.3%, and 35.5% of cases, respectively). Basal-like tumors showed the highest expression of immune genes, whereas liver metastases (together with brain metastases) had the lowest expression of immune genes [38]. These findings are in line with previous evidence showing that site-specific tumor colonization is regulated by the expression of specific genes and support a potential role of the tumor microenvironment in metastasis organotropism [71,72].

Prognostic and predictive role in patients treated with endocrine therapy with or without targeted therapy

In 2015, the prognostic value of PAM50 BC subtypes was explored in a population-based retrospective cohort identified within the Stockholm Breast Cancer Registry (Table 1). Among patients treated with ET in the 1st-line metastatic setting, Luminal A IS was associated with a clinically relevant but non-statistically significant 14-month longer median post-relapse survival compared to Luminal B. In an analysis restricted to HoR+ tumors, the results were unchanged. Although purely explorative and not meant to provide any treatment recommendations, these findings represented an early signal of the ability of the PAM50 classifier to identify a subgroup of patients with metastatic disease with a better prognosis than others [35].

The EGF3008 phase III clinical trial randomized 1,286 patients with HoR+ loco-regionally relapsed or metastatic BC to letrozole with or without lapatinib [73]. Overall, 821 tumor samples (86% from the primary tumor) were PAM50 profiled, and 644 were HoR+/HER2-negative (Table 1). Luminal A tumors showed the longest median progression-free survival (PFS) and OS, while Basal-like biology was associated with the worst outcome. Luminal B, HER2-E, and Basal-like IS presented 1.46-, 2.88-, and 2.26-times higher risk of progression in comparison with Luminal A, respectively. The IS conferred the strongest prognostic information in terms of PFS and OS (second to prior ET), and this information was independent of clinical and pathological parameters such as number of metastases, performance status, and visceral

disease. Of interest, this study showed a statistically significant benefit deriving from lapatinib, a reversible EGFR and HER2 tyrosine kinase inhibitor, for the HER2-E ($p_{interaction} = 0.020$) but not for the other subtypes, in both univariate and multivariable analyses (median PFS of 6.5 months with lapatinib vs 2.6 months with placebo; $p = 0.030$) [37].

The BOLERO-2 trial was an international randomized phase III study in which endocrine pre-treated patients with HoR+/HER2-negative advanced disease were randomized to the mTOR-inhibitor everolimus or placebo, in combination with exemestane [74]. The study led to the approval of everolimus for the 2nd-line treatment of HoR+/HER2-negative metastatic BC. A retrospective PAM50 subtype analysis on 261 primary tumors or metastases (Table 1) showed that metastases were enriched in the HER2-E subtype (32% in metastasis vs 19% in the primary tumor). Overall, a 1.5-month median PFS difference was registered between non-luminal and luminal tumors (5.2 months vs 6.7 months; adjusted HR 0.66). Everolimus and exemestane significantly improved median PFS in patients with HER2-E (5.8 vs 4.1 months), non-HER2-E (8.7 vs 4.1 months), non-luminal (5.8 vs 4.1 months) and luminal (8.7 vs 4.1 months; adjusted HR 0.39) disease, compared to exemestane alone. No interaction was observed between subtype and treatment. Luminal A IS ($N = 122$) derived statistically significant PFS benefit from everolimus compared to placebo (8.3 vs 4.1 months; $p < 0.001$). A non-statistically significant 4-month longer median PFS was reached in the Luminal B subgroup ($N = 41$) when everolimus was administered [63].

Several clinical trials have demonstrated that CDK4/6 inhibitors, namely palbociclib, ribociclib, or abemaciclib, in combination with an AI or fulvestrant significantly prolong PFS and OS of HoR+/HER2-negative metastatic BC [75–84]. Predictive biomarkers of response for optimal patient selection, beyond IHC, are currently under investigation. For example, the PALOMA-3 study randomly assigned 521 patients with endocrine pre-treated metastatic BC to receive fulvestrant with or without palbociclib [76]. Overall, 302 tumor samples from primary tumor or metastasis were profiled (Table 1) using the EdgeSeq Oncology BM Panel which includes 42 genes among the 100 genes of the Absolute Intrinsic Molecular Subtyping (AIMS) algorithm [85]. Consistently with the previous literature, a 76% agreement with the PAM50 classifier was ascertained. Both AIMS-based luminal and non-luminal tumors benefitted from the addition of palbociclib. In the luminal subgroup, only Luminal A showed a significant benefit with the addition of palbociclib although no significant interaction was found between Luminal A and Luminal B biology. Palbociclib significantly prolonged median PFS (9.5 vs 5.5 months; HR 0.58) of non-luminal tumors but the limited number of Basal-like IS ($N = 5$) and the presence of Normal-like tumors ($N = 8$) precluded any solid conclusion [64].

Finn et al. performed a comprehensive biomarkers analysis using tumor samples from the PALOMA-2 randomized phase III trial of 1st-line palbociclib plus letrozole vs letrozole in postmenopausal women with HoR+/HER2-negative advanced BC [75]. The AIMS algorithm was adopted for the subtype classification of tumor samples from 455 patients (Table 1) and a 75% concordance with the PAM50 IS was detected. Patients with luminal tumors significantly benefited from the addition of palbociclib to letrozole (Luminal A: HR 0.55; Luminal B: HR 0.51), while HER2-E tumors derived a smaller absolute benefit. Yet, the number of patients with the HER2-E ($N = 85$), Basal-like ($N = 2$), and Normal-like ($N = 2$) IS was very limited and no formal statistical comparison was performed [65]. It is worth noting that only a moderate agreement (54%) between the validated research-use only (ruo) PAM50 assay and the AIMS algorithm-based method for the IS detection was observed in another PALOMA-2 trial sub-analysis. Overall, 46% of Luminal B and 67% of Basal-like tumors were classified as Luminal A and HER2-E, respectively, using the AIMS method. This analysis suggests that the lack of interchangeability of distinct subtyping assays may lead to clinically relevant tumor misclassifications [28].

In 2021, a large correlative analysis assessing the prognostic and predictive role of the PAM50 IS across the MONALEESA phase III pivotal trials of ribociclib and ET was reported [46]. Overall, 1,160 tumor samples were evaluable for analysis (Table 1). The Luminal A subtype was associated with the best PFS rates in comparison with the other subtypes, in the overall population as well as in each treatment arm. In the overall population and compared to Luminal A, adjusted HRs for risk of progression were 1.44, 2.31, 3.96, and 1.28 for Luminal B, HER2-E, Basal-like, and Normal-like IS, respectively. This pattern was similar in both treatment arms. Except for Basal-like, each IS exhibited a consistent PFS benefit when ribociclib was administered. Notably, HER2-E showed the greatest relative benefit from the addition of ribociclib to ET (HR = 0.39). Adjusted HRs were 0.47, 0.52, and 0.63 for Normal-like, Luminal B, and Luminal A IS, respectively [46]. More recently, comparable OS results were presented and the IS remained prognostic for OS in both arms, also after adjusting for clinical covariates [86]. In summary, ribociclib combined with ET demonstrated clinical activity in all subtypes, except for the Basal-like, with the greatest relative PFS and OS benefit observed in the HER2-E disease.

The prognostic and predictive value of the HER2-E subtype was also explored outside clinical trials in a cohort of 141 consecutive patients diagnosed with HoR+/HER2-negative metastatic BC and treated with CDK4/6 inhibitors and ET in the real-world setting between 2014 and 2020. Data on PAM50 subtypes was available from 114 patients (Table 1) and the IS were evaluated in primary (50%) or metastatic (50%) tumors. Median PFS was 7.4 months in the HER2-E subgroup vs 21.1 months in the non-HER2-E subgroup ($p = 0.010$). Median OS was 30.9 months in the HER2-E subgroup and was not reached in the non-HER2-E subgroup at a median follow-up time of 22.5 months ($p = 0.010$). In an exploratory analysis comparing ribociclib to palbociclib and abemaciclib, the HRs for PFS were in favor of ribociclib in all subtypes. Intriguingly, in the group of HER2-E tumors, a 6.8-month longer median PFS was observed for ribociclib vs palbociclib/abemaciclib, although it did not reach the statistical significance (median PFS 13.6 vs 6.8 months). Finally, the objective response rate (ORR) of Luminal A/B and HER2-E disease was 40.5% and 42.9% with ribociclib vs 36.8% and 25.0% with palbociclib [66].

A systematic review and trial-level metanalysis, including most of the previous studies and an additional phase II single-arm trial in the setting of HoR+/HER2-positive disease, ultimately validated the prognostic role of the IS in HoR+ advanced BC. In this metanalysis, all other IS, compared to Luminal A, were significantly associated with worse PFS, regardless of HER2 status, type of systemic treatment, and menopausal status. Moreover, the Luminal B subtype (HR = 1.46) was associated with a better prognosis than HER2-E (HR 2.39) and Basal-like (HR = 2.67) tumors [87].

Predictive role in patients treated with ET-based therapy vs chemotherapy

The efficacy of CDK4/6 inhibitors in combination with ET has been compared to CT in 2 clinical trials. The PEARL study was a multicenter phase III study where 601 patients with AI-resistant metastatic BC were randomized to palbociclib plus ET (cohort 1: exemestane; cohort 2: fulvestrant) or capecitabine [67]. Co-primary endpoints were PFS in ESR1-wild type tumors (cohort 1 + cohort 2) and cohort 2. The superiority of the palbociclib arm could not be demonstrated but, overall, the treatment was better tolerated than CT. As pre-specified explorative objectives, the predictive role of AIMS-based IS from 455 primary tumors or metastases was investigated (Table 1). In the group of wild-type *ESR1* luminal tumors, PFS did not differ significantly between patients treated with palbociclib + ET or capecitabine (HR = 1.01). Conversely,

non-luminal tumors (N = 25) showed a significantly worse PFS with palbociclib + ET compared to capecitabine (2.3 vs 13.7 months of median PFS, respectively; HR = 7.36). Similarly, in cohort 2, PFS did not differ according to treatment arm in the luminal subgroup (HR 1.07), while in non-luminal disease (N = 20, all HER2-E) PFS was significantly worse with palbociclib + fulvestrant vs capecitabine (3.3 vs 13.7 months of median PFS, respectively; HR = 5.87) [67].

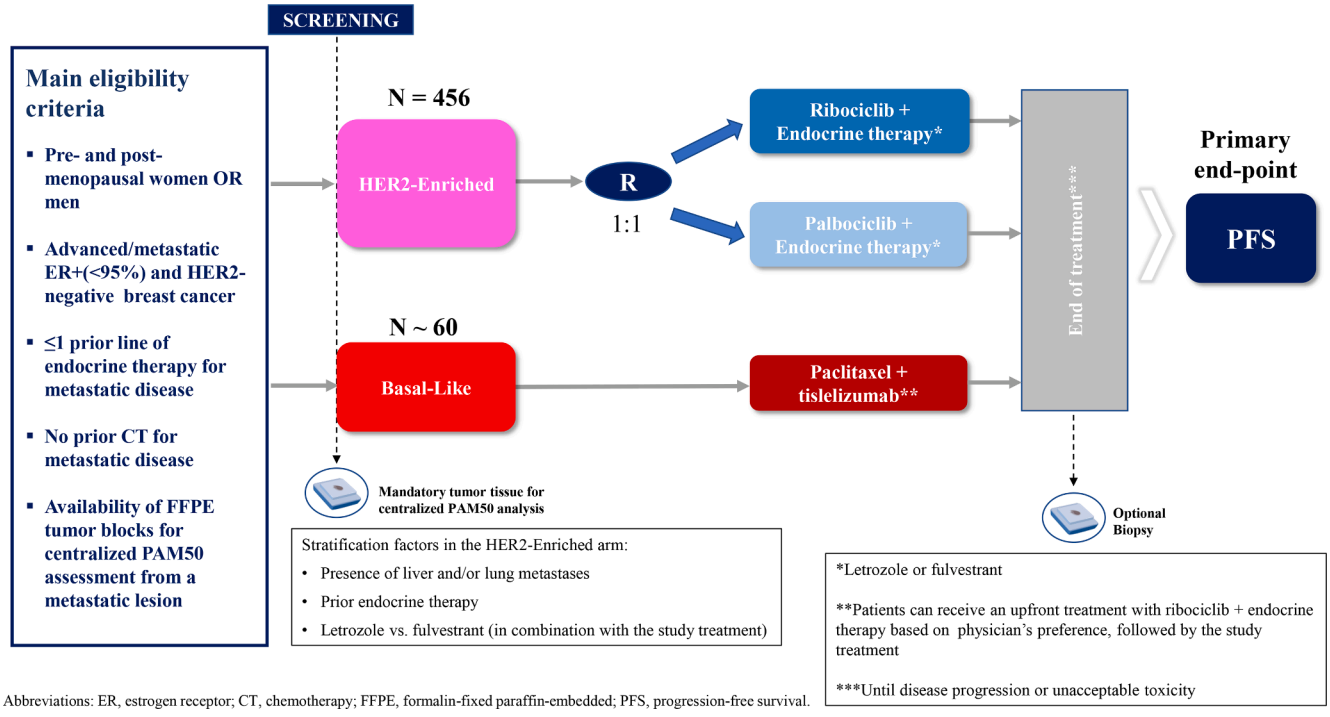
The phase II Young-PEARL study randomized 184 pre-menopausal women to palbociclib and exemestane (with a GnRH agonist) or capecitabine and demonstrated a significantly longer PFS for palbociclib and exemestane [88]. Using a research-based PAM50 assay, 73% of tumors were classified as Luminal A or Luminal B (Table 1). Luminal A/B tumors had significantly longer PFS in the whole population (p = 0.006) and in the palbociclib arm (p = 0.002), but not in the capecitabine arm, in comparison to non-luminal disease. Palbociclib was associated with improved PFS when compared to chemotherapy for the treatment of Luminal A tumors (p = 0.004), while no significantly different outcome was demonstrated for the Luminal B or non-luminal disease. Overall, these findings provide evidence in support of the potential predictive value of the IS in the pre-menopausal setting [68].

Toward the clinical implementation of the IS in advanced HoR+/HER2-negative breast cancer

Determinants of response to CDK4/6 inhibitors and their validation

Considering the relevance of CDK4/6 inhibitors in the current therapeutic scenario of the HoR+/HER2-negative metastatic disease and the consistent predictive role of the IS emerged from retrospective analyses of prospective trials, intensive research initiatives to validate the clinical utility of PAM50 subtypes as predictive biomarkers are ongoing. To date, evidence suggests that the HER2-E biology is associated with high sensitivity to ribociclib and, potentially, more limited sensitivity to palbociclib [46,64,65,86,89,90]. To elucidate the mechanistic determinants of such differential effect, we recently carried out a gene expression analysis with and without ribociclib in BC patient-derived xenografts (PDXs) [91]. HER2-E and Luminal B IS showed statistically significantly higher response (measured as mean change in volume) than Basal-like tumors. Ribociclib induced a luminal phenotype with upregulation of estrogen-related genes and downregulation of proliferation genes, which might explain the finding of the high efficacy of ribociclib in estrogen-resistant HER2-E tumors described in the clinical setting [91]. More research is ongoing to confirm this hypothesis. Furthermore, an increase in cell cycle-related gene signatures, p16 overexpression, and specific *RB1* loss-of-function events have been recently associated with reduced anti-tumor activity of CDK4/6 inhibitors plus ET in ER-positive BC, suggesting a potential role in predicting primary/acquired drug resistance and high tumor plasticity in response to treatment [92,93]. The multicenter, biomarker platform SOLTI-1801-CDK-PREDICT prospectively collected archival metastatic tumor tissue from 114 patients across five sites in Spain with the scope to investigate mechanisms of resistance to 1st-line CDK4/6 inhibitors (plus ET) and associations between PAM50 IS (along with other biomarkers) and clinical outcome [94].

The multicenter, randomized phase III trial SOLTI-2101-HARMONIA (NCT05207709) trial will test the hypothesis of the superiority of ribociclib over palbociclib in terms of PFS for the 1st-line or 2nd-line treatment of patients with histologically confirmed HoR+/HER2-negative metastatic BC with PAM50 HER2-E biology (Fig. 4) . The study, which will enroll 456 patients across Spain, Portugal, and United States, represents the first-of-kind, large randomized comparative trial selecting patients based on the intrinsic BC biology to optimize the clinical management of non-luminal HoR+/HER2-negative disease while elucidating the biological mechanisms of such differential activity between the two molecules. For this purpose, an extensive translational research plan has been designed to shed light on the determinants of the response to CDK4/6 inhibition and to detect molecular alterations in circulating tumor DNA. Moreover, an exploratory cohort of patients with Basal-like disease will be included in the HARMONIA trial and treated with paclitaxel and tislelizumab, a humanized IgG4 anti-PD-1 monoclonal antibody being developed as a monotherapy or in combination with other therapies for the treatment of different tumor types [95].



Abbreviations: ER, estrogen receptor; CT, chemotherapy; FFPE, formalin-fixed paraffin-embedded; PFS, progression-free survival.

Fig. 4. HARMONIA trial. Study workflow of the SOLTI-2101-HARMONIA trial.

New therapeutic opportunities for the IS

Despite evidence on distinct therapeutic effects of different classes of drugs in non-luminal compared to luminal HoR+/HER2-negative disease coming from retrospective analyses of prospective trials, there is no specific therapeutic recommendation based on the IS, at present. Several trials are currently underway to try to fill this gap.

In advanced HoR+/HER2-negative disease, the HER2-E subtype showed a significant therapeutic benefit deriving from the addition of lapatinib to letrozole [37]. Grounded on this, the SOLTI-1718-NEREA open-label, single-arm, multicenter phase II study (NCT04460430) aimed to evaluate the efficacy of combined neratinib, a pan-HER irreversible inhibitor, and ET, in patients with HER2-E/HoR+/HER2-negative advanced BC resistant to ET. The primary outcome was PFS at 24 weeks [96]. Unfortunately, the NEREA trial closed prematurely due to reasons independent of safety and efficacy.

Previous studies have shown that HER2-E and Basal-like IS are associated with higher expression of immune-related genes and higher infiltration of stromal tumor-infiltrating lymphocytes (TILs) compared to the luminal subtypes [45,97,98]. In HoR+/HER2-negative metastatic BC, a potential role of APOBEC mutagenesis in promoting clonal evolution and genomic instability with the acquisition of neoantigens and induction of immune response has been described upon progression after prior treatment with CDK4/6 inhibitors [99–101]. Interestingly, a high APOBEC genetic signature and a switch from endocrine-sensitive to HER2-E/triple-negative BC phenotype following treatment with a CDK4/6 inhibitor emerged from the mutational and molecular profile of metastatic tissue of a patient showing an exceptionally durable response (>24 months) to atezolizumab and chemotherapy [102]. Basal-like/HoR+/HER2-negative BC is highly similar to Basal-like/triple-negative BC [51] (see also Fig. 3) and has shown a high frequency of *TP53* mutations (80%) and *RB* loss [14], which are both linked to resistance to CDK4/6 inhibitors [103]. Notably, these features are associated with DNA damage repair defects and high tumor mutational burden, which could potentially sensitize to immunotherapy [99]. Altogether, these findings support additional synergies with immunotherapies and suggest that immune checkpoint blockade might be an interesting strategy to explore in patients with a low response to CDK4/6 inhibition. The SOLTI-1716-TATEN trial (NCT04251169) is an open-label, single-arm, multicenter phase II study currently evaluating the efficacy of pembrolizumab, an anti-PD1 immune checkpoint inhibitor, plus paclitaxel in patients with non-luminal/HoR+/HER2-negative BC not previously treated with CT in the advanced setting and progressing after a prior line with CDK4/6 inhibitors [104].

The androgen receptor (AR), frequently expressed in HoR+ BC, is associated with a well-differentiated tumor phenotype and with a more favorable prognosis in early disease [105–108]. Nevertheless, a high AR/ER cellular ratio was proven to negatively influence BC response to traditional ET [108]. HER2-E/HoR+/HER2-negative BC has been associated with a lower expression of the gene for ER and PR but comparable levels of expression of the AR gene (AR/PR > 1.0) compared to luminal tumors. Interestingly, AR levels remain unchanged within the HER2-E IS across the IHC-based groups [109]. The SOLTI-1502-ARIANNA trial (NCT04142060), grounded on these findings, is an exploratory, prospective phase II trial that was recruiting pre/post-menopausal women or men with HoR+/HER2-negative locally advanced/metastatic BC resistant to ET, to receive enzalutamide (+/- exemestane after 2 weeks-biopsy at the discretion of the treating physician). A tumor biopsy for gene expression analysis was obtained at the baseline and after two weeks of treatment. The primary objective was to evaluate the antiproliferative effect of enzalutamide after 2 weeks of treatment for the PAM50 HER2-E tumors (Cohort A). As a secondary objective, the proliferative effect of enzalutamide after 2 weeks of treatment for Luminal A and Luminal B tumors was planned to be explored (Cohort B) [110]. ARIANNA trial was prematurely closed due to lack of efficacy.

Conclusions

While current guidelines recommend the administration of CDK4/6 inhibitors and ET for the 1st-line treatment of all patients diagnosed with HoR+/HER2-negative advanced BC, the optimal treatment sequence upon progression is still uncertain and should be guided by predictive biomarkers, namely *PIK3CA* and germline *BRCA1/2* mutations (ESCAT I-A) [8]. The assessment of additional genomic biomarkers could be considered depending on the availability of corresponding therapies and the cost-effectiveness of the test [8]. In this perspective, the PAM50 assay is a highly standardized, and decentralized assay, capable of detecting the IS with optimal reproducibility across different laboratories [111–114].

To date, IS are not routinely assessed in the clinic despite compelling evidence in the early and, more recently, in the advanced setting supporting their prognostic and predictive ability beyond IHC biomarkers. In particular, the PAM50 assay detects non-luminal tumors with demonstrated poor sensitivity to endocrine therapy, even among tumors with high expression of hormonal receptors by IHC [46,63]. Moreover, HER2-addicted tumors sensitive to the HER2 blockade have been identified also in the context of HER2-negative disease [37]. Based on these considerations, several scenarios for the validation of PAM50 IS clinical utility in early and advanced disease settings can be envisaged. First, PAM50 IS may be employed for de-escalating treatment of high-burden early hormone-responsive BC. For example, the PAM50 assay accurately selects tumors presenting molecular characteristics of lower endocrine sensibility (i.e., Luminal B subtype), among HoR+/HER2-negative tumors, which could be considered for chemotherapy-free targeted therapeutical approaches (e.g., ET + CDK4/6 inhibitors) [115]. Second, PAM50 could be used to select non-luminal tumors, in the context of the HoR+/HER2-negative disease, that could benefit from novel treatment combinations (e.g., standard chemotherapy in association with immunotherapy for non-luminal subtypes). Third, HER2-E/HER2-positive tumors are highly addicted to HER2 and benefit the most from dual HER2-blockade in absence of chemotherapy [36], indicating that PAM50 IS may help to de-escalate treatment among HER2-positive tumors. Currently, the hypothesis that highly HER2-addicted HER2-E/HER2-positive tumors with limited disease burden and achieving a complete pathological response following standard neoadjuvant therapy may be safely spared from surgery, is being prospectively validated in the ELPIS trial (NCT04301375) [116]. Table 2 provides a comprehensive overview of the ongoing and already completed clinical trials using the PAM50 assay across early and advanced disease settings.

Although PAM50-guided treatment scenarios and their sequence for the treatment of HoR+/HER2-negative advanced disease could be envisioned based on the available data, definitive recommendations cannot be provided in absence of a rigorous validation of IS to demonstrate clinical utility. Several window-of-opportunity trials in selected patient populations are presently ongoing (Table 2). These trials, using paired tumor samples taken at multiple time points during the study treatment, provide relevant mechanistic insights into the biological activity of novel compounds tested in a landscape absent of mutations associated with drug resistance. While also representing the ideal clinical scenario for the discovery and validation of tumor biomarkers, they are not designed to prove their clinical utility. Larger prospective,

Table 2
Overview of the clinical trials using PAM50 for the assessment of the primary and/or secondary study outcomes.

Study title	Study number	Study design	Clinical setting	Primary Outcome	Interventions	PAM50-driven patient selection for primary endpoint assessment	Status	Reference (if results are published)
Palbociclib Plus Letrozole in Hormone Receptor Positive Residual Disease After Neoadjuvant Chemotherapy (SOLTI-1710-PROMETEO II)	NCT04130152	Phase 0, window-of-opportunity	Early, post-neoadjuvant (pre-surgery)	CCCA	Palbociclib; letrozole	No (PAM50 performed retrospectively)	Completed	NA
Neoadjuvant and Adjuvant Ribociclib and ET for Clinically High-risk ER + and HER2- Breast Cancer (SOLTI-1911-RIBOLARIS)	NCT05296746	Phase 2, parallel arm	Early, neoadjuvant and adjuvant	DMFS in ROR-low cohort	Ribociclib; endocrine therapy; chemotherapy	Yes	Ongoing	NA
Phase II Trial of anti-HER2 Treatment in HER2-enriched Early Breast Cancer Identified by PAM50 (HER2E-PAM, PAMILIA Study)	NCT04817540	Phase 2, single arm	Early, neoadjuvant	pCR rate	Trastuzumab biosimilar (Herzuma)	Yes	Ongoing	NA
Prospective Study of the Prosigna Assay on Neoadjuvant Clinical Decision-making in Women With HR+/HER2- Breast Cancer	NCT03749421	Observational	Early, neoadjuvant	Impact of PAM50 on treatment decision-making	Prosigna (routine use)	Yes	Ongoing	NA
A Study With Pembrolizumab in Combination With Dual anti-HER2 Blockade With Trastuzumab and Pertuzumab in Early Breast Cancer Patients With Molecular HER2-enriched Intrinsic Subtype (Keyriched-1)	NCT03988036	Phase 2, single arm	Early, neoadjuvant	pCR rate	Pembrolizumab; trastuzumab ABP 980; pertuzumab	Yes	Ongoing	NA
Effect of Physical Exercise on Tumor Proliferation of Luminal B Breast Cancer Patients (EFIK)	NCT03860740	Observational	Early, neoadjuvant	Proliferation suppression by type of physical exercise prior to surgery	Physical exercise	No (PAM50 performed retrospectively	Ongoing	NA
A Window-of-opportunity Study of U3-1402, a HER3-targeting Antibody-drug Conjugate in Operable Breast Cancer According to ERBB3 Expression (SOLTI-1805-TOT-HER3)	NCT04610528	Phase 0, window-of-opportunity	Early, neoadjuvant	CelTIL score (based on tumor cellularity and TILs)	Patritumab deruxtecan	No (PAM50 performed retrospectively)	Ongoing	NA
PAM50 HER2-enriched Phenotype as a Predictor of Response to Dual HER2 Blockade in HER2-positive Early Breast Cancer (SOLTI-1114-PAMELA)	NCT01973660	Phase 2, parallel arm	Early, neoadjuvant	pCR rate	Lapatinib; trastuzumab; endocrine therapy; paclitaxel	Yes	Completed	[36]
Efficacy of Letrozole + Palbociclib Combination as Neoadjuvant Treatment of Stage II-IIIA PAM 50 ROR-defined Low or Intermediate Risk Luminal Breast Cancer, in Postmenopausal Women (NeoPAL)	NCT02400567	Phase 2, parallel arm	Early, neoadjuvant	RCB 0–1	Chemotherapy; palbociclib; letrozole	Yes	Completed	[117]

(*continued on next page*)

Table 2 (*continued*)

Study title	Study number	Study design	Clinical setting	Primary Outcome	Interventions	PAM50-driven patient selection for primary endpoint assessment	Status	Reference (if results are published)
Phase 0 Study of Metronomic Oral Vinorelbine and Letrozole in HR+/ HER2-negative Early Breast Cancer Patients (SOLTI-1501-VENTANA)	NCT02802748	Phase 0, window-of-opportunity	Early, neoadjuvant	Change in PAM50 proliferation signature	Oral vinorelbine; letrozole	Yes	Completed	[118]
Onapristone as Preoperative Treatment for Postmenopausal Women With Hormone Receptor + and HER2- Breast Cancer (SOLTI-1802-ONAWA)	NCT04142892	Phase 0, window-of-opportunity	Early, neoadjuvant	CCCA	Onapristone	No (PAM50 performed retrospectively)	Completed	[119]
Pharmacogenomic Study of Neoadjuvant Eribulin for HER2 Non-overexpressing Breast Cancer (SOLTI-1007-NeoEribulin)	NCT01669252	Phase 0, window-of-opportunity	Early, neoadjuvant	Genetic determinants of pCR	Eribuline	No (PAM50 performed retrospectively)	Completed	[45]
Elacestrant in Preoperative Setting, a Window of Opportunity Study (SOLTI-1905-ELIPSE)	NCT04797728	Phase 0, window-of-opportunity	Early, neoadjuvant	CCCA	Elacestrant	No (PAM50 performed retrospectively)	Completed	[120]
Phase II Trial of Paclitaxel Combined With Trastuzumab and Pertuzumab as Pre-Operative Therapy for Inflammatory Breast Cancer	NCT01796197	Phase 2, single arm	Early, neoadjuvant	pCR, RCB	Trastuzumab; pertuzumab; chemotherapy	No (PAM50 performed retrospectively)	Completed	NA
Neoadjuvant Response-guided Treatment of Luminal B-type Tumors and Luminal A-type Tumors With Node Metastases (PREDIX LumB)	NCT02603679	Phase 2, parallel arm	Early, neoadjuvant	Radiological ORR	Palbociclib; endocrine therapy; chemotherapy	Yes (optional)	Completed	NA
The PRECISION Trial (Profiling Early Breast Cancer for Radiotherapy Omission): A Phase II Study of Breast-Conserving Surgery Without Adjuvant Radiotherapy for Favorable-Risk Breast Cancer	NCT02653755	Phase 2, parallel arm	Early, adjuvant	5-year risk of loco-regional recurrence in absence of adjuvant radiotherapy	Omission of adjuvant radiotherapy	Yes	Ongoing	NA
EXamining PErsonalised Radiation Therapy for Low-risk Early Breast Cancer (EXPERT)	NCT02889874	Phase 3, non-inferiority	Early, adjuvant	Local recurrence rate after breast-conserving surgery	Omission of adjuvant radiotherapy	Yes	Ongoing	NA
Omission of Surgery and Sentinel Lymph Node Dissection in Clinically Low-risk HER2positive Breast Cancer With High HER2 Addiction and a Complete Response Following Standard anti- HER2-based Neoadjuvant Therapy (ELPIS Trial)	NCT04301375	Phase 2, single arm	Early, adjuvant	Loco-regional recurrence rate in patients achieving pCR	Omission of surgery	Yes	Ongoing	NA

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Table 2 (*continued*)

Study title	Study number	Study design	Clinical setting	Primary Outcome	Interventions	PAM50-driven patient selection for primary endpoint assessment	Status	Reference (if results are published)
Establishment of Molecular Profiling for Individual Clinical Routine Treatment Decision in Early Breast Cancer (EMIT-1)	NCT03904173	Observational	Early, adjuvant	Impact of PAM50 on treatment decision-making	Prosigna (routine use)	Yes	Ongoing	NA
A Prospective Observational Study of Clinical Outcomes for the NanoString® Technologies Prosigna Gene Signature Assay	NCT01899079	Observational	Early, adjuvant	Impact of PAM50 on treatment decision-making	None	Yes	Completed	[113]
Intrinsic Breast Cancer Subtypes and Benefit of Paclitaxel in CALGB 9344 and Dose Dense Therapy in CALGB 9741	NCT00991263	Observational, retrospective	Early, adjuvant	DFS	PAM50	No (PAM50 performed retrospectively)	Completed	[121,122]
A Study of Palbociclib in Addition to Standard Endocrine Treatment in Hormone Receptor Positive HER2 Normal Patients With Residual Disease After Neoadjuvant Chemotherapy and Surgery (PENELOPE-B)	NCT01864746	Phase III, superiority	Early, adjuvant	IDFS	Palbociclib; placebo	No (PAM50 performed retrospectively)	Completed	[123]
Assessment of the Impact of RNA Genomic Profile on Treatment Decision-making in HER2 Equivocal Breast Cancer Patients (EQUIVOK)	NCT03197805	Longitudinal cohort study	Early	Impact of PAM50 on treatment decision-making	PAM50	Yes	Completed	NA
PAveMenT: Palbociclib and Avelumab in Metastatic AR + Triple Negative Breast Cancer	NCT04360941	Phase 1	Advanced, second- or third-line	MTD in all comers; ORR in AR+ TNBC	Palbociclib; avelumab	No (PAM50 performed retrospectively)	Ongoing	NA
Study With Atezolizumab in Combination With Trastuzumab and Vinorelbine in HER2-positive Advanced/ Metastatic Breast Cancer (SOLTI-1907-ATREZZO)	NCT04759248	Phase 2, parallel arm	Advanced, pre-treated	ORR in PD-L1 positive cohort	Atezolizumab; trastuzumab; vinorelbine	Mixed depending on the cohort	Ongoing	NA
Study of Palbociclib and Trastuzumab With Endocrine Therapy in HER2-positive Metastatic Breast Cancer (SOLTI-1303-PATRICIA I and II)	NCT02448420	Phase 2, parallel arm	Advanced, pre-treated	PFS	Palbociclib; trastuzumab; endocrine therapy; chemotherapy; TDM-1	Mixed depending on the cohort	Ongoing	NA
Targeting the PAM50 HER2-Enriched Phenotype With Enzalutamide in Hormone Receptor-Positive/Her2-Negative Metastatic BC (SOLTI-1502-ARIANNA)	NCT04142060	Phase 2, parallel arm	Advanced, pre-treated	PAM50 proliferation score variation	Enzalutamide	Yes	Completed	NA
Ipatasertib + Pertuzumab + Trastuzumab in Advanced HER2 + PI3KCA-mutant Breast Cancer Patients (SOLTI-1507-IPATHER)	NCT04253561	Phase 1b	Advanced, maitenance after first-line	RP2D	Ipatasertib; trastuzumab; pertuzumab	No (PAM50 performed retrospectively)	Ongoing	NA

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Table 2 (*continued*)

Study title	Study number	Study design	Clinical setting	Primary Outcome	Interventions	PAM50-driven patient selection for primary endpoint assessment	Status	Reference (if results are published)
Harnessing Analysis RNA Expression and Molecular Subtype to Optimize Novel TherapY MBCA (HARMONY)	NCT03769415	Longitudinal cohort study	Advanced, first- or second-line	Impact of PAM50 on decision-making about the preferred second-line treatment; concordance rate between clinical and molecular tumor classification	PAM50	Yes	Ongoing	NA
Pembrolizumab and Exemestane/ Leuprolide in Premenopausal HR+/ HER2- Locally Advanced or Metastatic Breast Cancer (PEER)	NCT02990845	Phase 2, single arm	Advanced, first- or second-line	PFS	Pembrolizumab; exemestane; leuprorelide	No (PAM50 performed retrospectively)	Ongoing	NA
Pembrolizumab + Paclitaxel in Hormone Receptor-positive (HR +)/Human Epidermal Growth Factor Receptor 2-negative (HER2-) Non-luminal (by PAM50) Advanced Breast Cancer After Cyclin-dependent Kinase 4/6 (CDK4/6) Inhibitors Progression (SOLTI-1716-TATEN)	NCT04251169	Phase 2, single arm	Advanced, first-line	ORR	Pembrolizumab; paclitaxel	Yes	Ongoing	NA
Ribociclib vs Palbociclib in Patients With Advanced Breast Cancer Within the HER2-Enriched Intrinsic Subtype (SOLTI-2101-HARMONIA)	NCT05207709	Phase 3, superiority	Advanced, first-line	PFS	Ribociclib; palbociclib; letrozole; fulvestrant; paclitaxel	Yes	Ongoing	NA
Paclitaxel Plus Pembrolizumab vs Paclitaxel Weekly in ER + Luminal B Metastatic Breast Cancer (PELICAN)	NCT03841747	Phase 2, parallel arm	Advanced, first-line	PFS, OS	Pembrolizumab; paclitaxel	Yes	Ongoing	NA
Evaluation of Biomarkers Associated With Response to Subsequent Therapies in Subjects With HER2-Positive Metastatic Breast Cancer	NCT02213042	Phase 2, parallel arm	Advanced, beyond	Fold change in gene expression	Lapatinib; trastuzumab; endocrine therapy	Yes	Completed	NA
Targeting EGFR/ERBB2 With Neratinib in Hormone Receptor (HR)-Positive/HER2-negative HER2-enriched Advanced/ Metastatic Breast Cancer (NEREA)	NCT04460430	Phase 2, single arm	Advanced, first- or second-line	PFS	Neratinib; endocrine therapy	Yes	Completed	NA
Trans-RosaLEE Study: a Biomarker-directed, Translational Study	NCT05529862	Biomarker platform	Advanced	Molecular alteration during treatment with ribociclib (with endocrine treatment)	Longitudinal tumor biopsies during treatment	No (PAM50 performed retrospectively)	Ongoing	NA
East Asian Breast Cancer Genome Atlas and Recurrence Risk Prediction (TCGA-Asian)	NCT04344496	Biomarker platform	All stages	Prevalence of genomic alterations in East Asian patients	PAM50	Yes	Ongoing	NA

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Table 2 (*continued*)

Study title	Study number	Study design	Clinical setting	Primary Outcome	Interventions	PAM50-driven patient selection for primary endpoint assessment	Status	Reference (if results are published)
Real-World Data of Clinicopathological Characteristics and Management of Breast Cancer Patients According to HER2 Status (RosHER)	NCT05217381	Observational	All stages	HER2 change during tumor progression	None	No (PAM50 performed retrospectively)	Ongoing	NA

Abbreviations: HR, hormone receptor; pCR, pathological complete response; ORR, objective response rate; PFS, progression-free survival; DFS, disease-free survival; TILs, tumor-infiltrating lymphocytes; OS, overall survival; ROR, Risk-of-Recurrence score; DMFS, distant metastasis-free survival; MTD, maximum tolerated dose; AR, androgen receptor; TNBC, triple-negative breast cancer; RP2D, recommended phase 2 dose; RCB, residual cancer burden; CCCA, complete cell cycle arrest; IDFS, invasive disease-free survival; NA, not applicable.

PAM50-driven clinical trials to establish a higher level of evidence are central for the implementation of the IS in clinical practice, and wider collaboration models to support larger research initiatives in this regard should be strongly advocated. The evolving nature of tumor biology highlights the importance of tumor sample collection at relapse and during metastatic disease to better track and understand the molecular and phenotypic changes occurring during disease progression. A major limitation is the tissue availability in metastatic disease to perform gene expression profiling. Finding new and alternative methods to identify IS-related biology in metastatic BC should be a priority, and more intense research efforts should be promoted in this direction.

CRediT authorship contribution statement

Claudette Falato: Conceptualization, Writing – original draft, Writing – review & editing. **Francesco Schettini:** Writing – review & editing. **Tomás Pascual:** Writing – review & editing. **Fara Brasó-Maristany:** Conceptualization, Writing – review & editing. **Aleix Prat:** Conceptualization, Writing – review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: **CF** declares no competing financial and non-financial interests; **FS** declares no competing financial and non-financial interests but reports personal fees from Novartis for educational activities; **TP** declares no competing financial and non-financial interests; **FBM** declares no competing financial and non-financial interests but reports patent application EP21383165 and patent application on DNA-based predictors of breast tumor phenotypes filed; **AP** declares no competing non-financial interests but reports advisory and consulting fees from Roche, Pfizer, Novartis, Amgen, BMS, Puma, Oncolytics Biotech, MSD, Guardant Health, Peptomyc, and Lilly, lecture fees from Roche, Pfizer, Novartis, Amgen, BMS, Nanostring Technologies and Daiichi Sankyo, institutional financial interests from Boehringer, Novartis, Roche, Nanostring, Sysmex Europa GmbH, Medica Scientia inno. Research, SL, Celgene, Astellas, and Pfizer; a leadership role in Reveal Genomics, SL; and a patent PCT/EP2016/080056.

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Appendix A. Supplementary data

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References

[1] Allison KH, Hammond MEH, Dowsett M, McKernin SE, Carey LA, Fitzgibbons PL, et al. Estrogen and progesterone receptor testing in breast cancer: ASCO/CAP guideline update. J Clin Oncol 2020;38(12):1346–66.
[2] André F, Ciruelos E, Rubovszky G, Campone M, Loibl S, Rugo HS, et al. Alpelisib for PIK3CA -mutated, hormone receptor-positive advanced breast cancer. N Engl J Med 2019;380(20):1929–40.
[3] Modi S, Jacot W, Yamashita T, Sohn J, Vidal M, Tokunaga E, et al. Trastuzumab deruxtecan in previously treated HER2-low advanced breast cancer. N Engl J Med 2022;387:9–20. https://doi.org/10.1056/NEJMOA2203690/SUPPL_FILE/NEJMOA2203690_DATA-SHARING.PDF.
[4] Robson M, Im S-A, Senkus E, Xu B, Domchek SM, Masuda N, et al. Olaparib for metastatic breast cancer in patients with a germline BRCA mutation. N Engl J Med 2017;377:523–33. https://doi.org/10.1056/NEJMOA1706450/SUPPL_FILE/NEJMOA1706450_DISCLOSURES.PDF.
[5] Litton JK, Hurvitz SA, Mina LA, Rugo HS, Lee K-H, Gonçalves A, et al. Talazoparib versus chemotherapy in patients with germline BRCA1/2-mutated HER2-negative advanced breast cancer: final overall survival results from the EMBRACA trial. Ann Oncol 2020;31(11):1526–35.
[6] Diéras V, Han HS, Kaufman B, Wildiers H, Friedlander M, Ayoub J-P, et al. Veliparib with carboplatin and paclitaxel in BRCA-mutated advanced breast cancer (BROCADE3): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol 2020;21(10):1269–82.
[7] Gruber JJ, Afghahi A, Timms K, DeWees A, Gross W, Aushev VN, et al. A phase II study of talazoparib monotherapy in patients with wild-type BRCA1 and BRCA2 with a mutation in other homologous recombination genes. Nat Cancer 2022;3(10):1181–91.
[8] Gennari A, André F, Barrios CH, Cortés J, de Azambuja E, DeMichele A, et al. ESMO Clinical Practice Guideline for the diagnosis, staging and treatment of patients with metastatic breast cancer ☆. Ann Oncol 2021;32:1475–95. https://doi.org/10.1016/J.ANNONC.2021.09.019/ATTACHMENT/B5F74122-9168-49E3-8D2B-882C1D11AB4A/MMC1.PDF.
[9] Turner N., Oliveira M., Howell S.J., Dalenc F., Cortés J., et al. **Capivasertib and fulvestrant for patients with aromatase inhibitor-resistant hormone receptor-positive/human epidermal growth factor receptor 2-negative advanced breast cancer: results from the Phase III CAPItello-291 trial . Abstract GS3-04, SABCS 2022**.
[10] Bidard F-C, Hardy-Bessard A-C, Dalenc F, Bachelot T, Pierga J-Y, de la Motte Rouge T, et al. Switch to fulvestrant and palbociclib versus no switch in advanced breast cancer with rising ESR1 mutation during aromatase inhibitor and palbociclib therapy (PADA-1): a randomised, open-label, multicentre, phase 3 trial. Lancet Oncol 2022;23(11):1367–77.

[11] Perou CM, Sørlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, et al. Molecular portraits of human breast tumours. Nature 2000;406(6797):747–52.

[12] Sørlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci USA 2001;98(19):10869–74.

[13] Sørlie T, Tibshirani R, Parker J, Hastie T, Marron JS, Nobel A, et al. Repeated observation of breast tumor subtypes in independent gene expression data sets. Proc Natl Acad Sci USA 2003;100(14):8418–23.

[14] Koboldt DC, Fulton RS, McLellan MD, Schmidt H, Kalicki-Veizer J, McMichael JF, et al. Comprehensive molecular portraits of human breast tumours. Nature 2012 490:7418 2012;490:61–70. https://doi.org/10.1038/nature11412.

[15] Prat A, Parker JS, Karginova O, Fan C, Livasy C, Herschkowitz JI, et al. Phenotypic and molecular characterization of the claudin-low intrinsic subtype of breast cancer. Breast Cancer Res 2010;12(5). https://doi.org/10.1186/BCR2635.

[16] Millikan RC, Newman B, Tse C-K, Moorman PG, Conway K, Smith LV, et al. Epidemiology of basal-like breast cancer. Breast Cancer Res Treat 2008;109(1):123–39.

[17] Anderson WF, Rosenberg PS, Prat A, Perou CM, Sherman ME. How many etiological subtypes of breast cancer: two, three, four, or more? JNCI. J Natl Cancer Inst 2014;106(8).

[18] Prat A, Carey LA, Adamo B, Vidal M, Tabernero J, Cortés J, et al. Molecular features and survival outcomes of the intrinsic subtypes within HER2-positive breast cancer. JNCI J Natl Cancer Inst 2014;106(8). https://doi.org/10.1093/JNCI/DJU152.

[19] Prat A, Bianchini G, Thomas M, Belousov A, Cheang MCU, Koehler A, et al. Research-based PAM50 subtype predictor identifies higher responses and improved survival outcomes in HER2-positive breast cancer in the NOAH study. Clin Cancer Res 2014;20:511–21. https://doi.org/10.1158/1078-0432.CCR-13-0239.

[20] Prat A, Cheang MCU, Martín M, Parker JS, Carrasco E, Caballero R, et al. Prognostic significance of progesterone receptor-positive tumor cells within immunohistochemically defined luminal a breast cancer. J Clin Oncol 2013;31(2):203–9. https://doi.org/10.1200/JCO.2012.43.4134.

[21] Cheang MCU, Martin M, Nielsen TO, Prat A, Voduc D, Rodriguez-Lescure A, et al. Defining breast cancer intrinsic subtypes by quantitative receptor expression. Oncologist 2015;20(5):474–82. https://doi.org/10.1634/theoncologist.2014-0372.

[22] Vidal M, Peg V, Galván P, Tres A, Cortés J, Ramón y Cajal S, et al. Gene expression-based classifications of fibroadenomas and phyllodes tumours of the breast. Mol Oncol 2015;9(6):1081–90. https://doi.org/10.1016/J.MOLONC.2015.01.003.

[23] Parker JS, Mullins M, Cheang MCU, Leung S, Voduc D, Vickery T, et al. Supervised risk predictor of breast cancer based on intrinsic subtypes. J Clin Oncol 2009;27(8):1160–7. https://doi.org/10.1200/JCO.2008.18.1370.

[24] Nielsen TO, Parker JS, Leung S, Voduc D, Ebbert M, Vickery T, et al. A comparison of PAM50 intrinsic subtyping with immunohistochemistry and clinical prognostic factors in tamoxifen-treated estrogen receptor-positive breast cancer. Clin Cancer Res 2010;16:5222–32. https://doi.org/10.1158/1078-0432.CCR-10-1282.

[25] Wallden B, Storhoff J, Nielsen T, Dowidar N, Schaper C, Ferree S, et al. Development and verification of the PAM50-based Prosigna breast cancer gene signature assay. BMC Med Genomics 2015;8(1). https://doi.org/10.1186/S12920-015-0129-6.

[26] Prat A, Pineda E, Adamo B, Galván P, Fernández A, Gaba L, et al. Clinical implications of the intrinsic molecular subtypes of breast cancer. Breast 2015;24:S26–35. https://doi.org/10.1016/J.BREAST.2015.07.008.

[27] Cejalvo JM, Pascual T, Fernández-Martínez A, Brasó-Maristany F, Gomis RR, Perou CM, et al. Clinical implications of the non-luminal intrinsic subtypes in hormone receptor-positive breast cancer. Cancer Treat Rev 2018;67:63–70. https://doi.org/10.1016/J.CTRV.2018.04.015.

[28] Schettini F, Brasó-Maristany F, Kuderer NM, Prat A. A perspective on the development and lack of interchangeability of the breast cancer intrinsic subtypes. npj Breast Cancer 2022;8:85. https://doi.org/10.1038/S41523-022-00451-9.

[29] Lindström LS, Karlsson E, Wilking UM, Johansson U, Hartman J, Lidbrink EK, et al. Clinically used breast cancer markers such as estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 are unstable throughout tumor progression. Https://DoiOrg/101200/JCO2011372482 2012;30:2601–8. https://doi.org/10.1200/JCO.2011.37.2482.

[30] Lüönd F, Tiede S, Christofori G. Breast cancer as an example of tumour heterogeneity and tumour cell plasticity during malignant progression. Br J Cancer 2021;125(2):164–75. https://doi.org/10.1038/s41416-021-01328-7.

[31] Cejalvo JM, de Dueñas EM, Galván P, García-Recio S, Gasión OB, Paré L, et al. Intrinsic subtypes and gene expression profiles in primary and metastatic breast cancer. Cancer Res 2017;77:2213–21. https://doi.org/10.1158/0008-5472.CAN-16-2717.

[32] Aftimos P, Oliveira M, Irrthum A, Fumagalli D, Sotiriou C, Gal-Yam EN, et al. Genomic and transcriptomic analyses of breast cancer primaries and matched metastases in AURORA, the breast international group (BIG) molecular screening initiative. Cancer Discov 2021;11:2796–811. https://doi.org/10.1158/2159-8290.CD-20-1647.

[33] Cheang MCU, Martin M, Nielsen TO, Prat A, Rodriguez-Lescure A, Ruiz A, et al. Quantitative hormone receptors, triple-negative breast cancer (TNBC), and molecular subtypes: A collaborative effort of the BIG-NCI NABCG. Https://DoiOrg/101200/Jco20123015_suppl1008 2012;30:1008–1008. https://doi.org/10.1200/.

[34] Lundberg A, Lindstrom LS, Harrell JC, Falato C, Carlson JW, Wright PK, et al. Gene expression signatures and immunohistochemical subtypes add prognostic value to each other in breast cancer cohorts. Clin Cancer Res 2017;23:7512–20. https://doi.org/10.1158/1078-0432.CCR-17-1535.

[35] Falato C, Tobin NP, Lorent J, Lindström LS, Bergh J, Foukakis T. Intrinsic subtypes and genomic signatures of primary breast cancer and prognosis after systemic relapse. Mol Oncol 2016;10:517. https://doi.org/10.1016/J.MOLONC.2015.11.004.

[36] Llombart-Cussac A, Cortés J, Paré L, Galván P, Bermejo B, Martínez N, et al. HER2-enriched subtype as a predictor of pathological complete response following trastuzumab and lapatinib without chemotherapy in early-stage HER2-positive breast cancer (PAMELA): an open-label, single-group, multicentre, phase 2 trial. Lancet Oncol 2017;18(4):545–54. https://doi.org/10.1016/S1470-2045(17)30021-9.

[37] Prat A, Cheang MCU, Galván P, Nuciforo P, Paré L, Adamo B, et al. Prognostic value of intrinsic subtypes in hormone receptor-positive metastatic breast cancer treated with letrozole with or without lapatinib. JAMA Oncol 2016;2(10):1287.

[38] Brasó-Maristany F, Paré L, Chic N, Martínez-Sáez O, Pascual T, Mallafré-Larrosa M, et al. Gene expression profiles of breast cancer metastasis according to organ site. Mol Oncol 2022;16(1):69–87. https://doi.org/10.1002/1878-0261.13021.

[39] Bastien RRL, Rodríguez-Lescure Á, Ebbert MTW, Prat A, Munárriz B, Rowe L, et al. PAM50 breast cancer subtyping by RT-qPCR and concordance with standard clinical molecular markers. BMC Med Genomics 2012;5(1). https://doi.org/10.1186/1755-8794-5-44.

[40] Prat A, Tsai YH, Pascual T, Pare L, Adamo B, Vidal M, et al. A prognostic model based on PAM50 and clinical variables (PAM50MET) for metastatic hormone receptor–positive HER2-negative breast cancer. Clin Cancer Res 2020;26:6141–8. https://doi.org/10.1158/1078-0432.CCR-20-2793.

[41] Bergamino MA, Morani G, Parker J, Schuster EF, Leal MF, Lopez-Knowles E, et al. Impact of duration of neoadjuvant aromatase inhibitors on molecular expression profiles in estrogen receptor-positive breast cancers. Clin Cancer Res 2022;28:1217–28. https://doi.org/10.1158/1078-0432.CCR-21-2718.

[42] Prat A, Parker JS, Fan C, Cheang MCU, Miller LD, Bergh J, et al. Concordance among gene expression-based predictors for ER-positive breast cancer treated with adjuvant tamoxifen. Ann Oncol 2012;23(11):2866–73. https://doi.org/10.1093/ANNONC/MDS080.

[43] Fujii T, Kogawa T, Dong W, Sahin AA, Moulder S, Litton JK, et al. Revisiting the definition of estrogen receptor positivity in HER2-negative primary breast cancer. Ann Oncol 2017;28(10):2420–8. https://doi.org/10.1093/ANNONC/MDX397.

[44] Prat A, Perou CM. Deconstructing the molecular portraits of breast cancer. Mol Oncol 2011;5:5–23. https://doi.org/10.1016/J.MOLONC.2010.11.003.

[45] Pascual T, Oliveira M, Villagrasa P, Ortega V, Paré L, Bermejo B, et al. Neoadjuvant eribulin in HER2-negative early-stage breast cancer (SOLTI-1007-NeoEribulin): a multicenter, two-cohort, non-randomized phase II trial. npj Breast Cancer 2021;7(1). https://doi.org/10.1038/S41523-021-00351-4.

[46] Prat A, Chaudhury A, Solovieff N, Paré L, Martinez D, Chic N, et al. Correlative biomarker analysis of intrinsic subtypes and efficacy across the MONALEESA phase III studies. J Clin Oncol 2021;39(13):1458–67. https://doi.org/10.1200/JCO.20.02977.

[47] Bergamino MA, López-Knowles E, Morani G, Tovey H, Kilburn L, Schuster EF, et al. HER2-enriched subtype and novel molecular subgroups drive aromatase inhibitor resistance and an increased risk of relapse in early ER+/HER2+ breast cancer. EBioMedicine 2022;83:104205.

[48] Brasó-Maristany F, Griguolo G, Pascual T, Paré L, Nuciforo P, Llombart-Cussac A, et al. Phenotypic changes of HER2-positive breast cancer during and after dual HER2 blockade. Nat Commun 2020 11:1 2020;11:1–11. https://doi.org/10.1038/s41467-019-14111-3.

[49] Kuong KJ, Loeb LA. APOBEC3B mutagenesis in cancer. Nat Genet 2013;45(9):964–5. https://doi.org/10.1038/NG.2736.

[50] Hohmann L, Oliveira D, Sigurjónsdóttir K, Bosch A, Borg Å, Vallon-Christersson J, et al. 17P Clinicopathological and transcriptomic characterization of luminal HER2-enriched breast cancer. Ann Oncol 2022;33:S130–1. https://doi.org/10.1016/j.annonc.2022.03.032.

[51] Prat A, Adamo B, Cheang MCU, Anders CK, Carey LA, Perou CM. Molecular characterization of basal-like and non-basal-like triple-negative breast cancer. Oncologist 2013;18:123. https://doi.org/10.1634/THEONCOLOGIST.2012-0397.

[52] Wolff AC, Hammond MEH, Allison KH, Harvey BE, Mangu PB, Bartlett JMS, et al. Human epidermal growth factor receptor 2 testing in breast cancer: American society of clinical oncology/ college of American pathologists clinical practice guideline focused update. J Clin Oncol 2018;36(20):2105–22. https://doi.org/10.1200/JCO.2018.77.8738.

[53] Schettini F, Chic N, Brasó-Maristany F, Paré L, Pascual T, Conte B, et al. Clinical, pathological, and PAM50 gene expression features of HER2-low breast cancer. Npj Breast Cancer 2021 7:1 2021;7:1–13. https://doi.org/10.1038/s41523-020-00208-2.

[54] Agostinetto E, Rediti M, Fimereli D, Debien V, Piccart M, Aftimos P, et al. HER2-low breast cancer: molecular characteristics and prognosis. Cancers (Basel) 2021;13(11):2824.

[55] Tarantino P, Hamilton E, Tolaney SM, Cortes J, Morganti S, Ferraro E, et al. HER2-low breast cancer: pathological and clinical landscape. J Clin Oncol 2020;38(17):1951–62. https://doi.org/10.1200/JCO.19.02488.

[56] Tarantino P, Jin Q, Tayob N, Jeselsohn RM, Schnitt SJ, Vincuilla J, et al. Prognostic and biologic significance of ERBB2-low expression in early-stage

breast cancer. JAMA Oncol 2022. https://doi.org/10.1001/JAMAONCOL.2022.2286.
[57] Tarantino P, Curigliano G, Tolaney SM. Navigating the HER2-low paradigm in breast oncology: new standards, future horizons. Cancer Discov 2022:OF1–5. https://doi.org/10.1158/2159-8290.CD-22-0703.
[58] Schettini F, Buono G, Cardalesi C, Desideri I, de Placido S, del Mastro L. Hormone receptor/human epidermal growth factor receptor 2-positive breast cancer: Where we are now and where we are going. Cancer Treat Rev 2016;46:20–6. https://doi.org/10.1016/J.CTRV.2016.03.012.
[59] Prat A, Bardia A, Curigliano G, Hammond MEH, Loibl S, Tolaney SM, et al. An overview of clinical development of agents for metastatic or advanced breast cancer without ERBB2 amplification (HER2-low). JAMA Oncol 2022;8(11):1676.
[60] Nielsen DL, Bjerre KD, Jakobsen EH, Cold S, Stenbygaard L, Sørensen PG, et al. Gemcitabine plus docetaxel versus docetaxel in patients with predominantly human epidermal growth factor receptor 2-negative locally advanced or metastatic breast cancer: A randomized, phase III study by the Danish Breast Cancer Cooperative Group. J Clin Oncol 2011;29(36):4748–54. https://doi.org/10.1200/JCO.2010.33.9507.
[61] Jørgensen CLT, Nielsen TO, Bjerre KD, Liu S, Wallden B, Balslev E, et al. PAM50 breast cancer intrinsic subtypes and effect of gemcitabine in advanced breast cancer patients. Acta Oncol 2014;53(6):776–87. https://doi.org/10.3109/0284186X.2013.865076.
[62] Tobin NP, Harrell JC, Lövrot J, Egyhazi Brage S, Frostvik Stolt M, Carlsson L, et al. Molecular subtype and tumor characteristics of breast cancer metastases as assessed by gene expression significantly influence patient post-relapse survival. Ann Oncol 2015;26(1):81–8. https://doi.org/10.1093/ANNONC/MDU498.
[63] Prat A, Brase JC, Cheng Y, Nuciforo P, Paré L, Pascual T, et al. Everolimus plus exemestane for hormone receptor-positive advanced breast cancer: A PAM50 intrinsic subtype analysis of BOLERO-2. Oncologist 2019;24(7):893–900.
[64] Turner NC, Liu Y, Zhu Z, Loi S, Colleoni M, Loibl S, et al. Cyclin E1 expression and palbociclib efficacy in previously treated hormone receptor-positive metastatic breast cancer. J Clin Oncol 2019;37(14):1169–78.
[65] Finn RS, Liu Y, Zhu Z, Martin M, Rugo HS, Diéras V, et al. Biomarker analyses of response to cyclin-dependent kinase 4/6 inhibition and endocrine therapy in women with treatment-naïve metastatic breast cancer. Clin Cancer Res 2020;26:110–21. https://doi.org/10.1158/1078-0432.CCR-19-0751.
[66] Martinez Saez O. et al. CDK4/6 inhibition and endocrine therapy (ET) in the HER2-enriched subtype (HER2-E) in hormone receptor-positive/HER2-negative (HR+/HER2-) advanced breast cancer (ABC): a retrospective analysis of real-world data. Annals of Oncology 2021. https://doi.org/Annals of Oncology (2021) 32 (suppl_2): S21-S36. 10.1016/annonc/annonc503.
[67] Martin M, Zielinski C, Ruiz-Borrego M, Carrasco E, Turner N, Ciruelos EM, et al. Palbociclib in combination with endocrine therapy versus capecitabine in hormonal receptor-positive, human epidermal growth factor 2-negative, aromatase inhibitor-resistant metastatic breast cancer: a phase III randomised controlled trial—PEARL. Ann Oncol 2021;32(4):488–99. https://doi.org/10.1016/J.ANNONC.2020.12.013.
[68] Lee S, Park K, Kim GM, Jung KH, Kang SY, Park IH, et al. Exploratory analysis of biomarkers associated with clinical outcomes from the study of palbociclib plus endocrine therapy in premenopausal women with hormone receptor-positive, HER2-negative metastatic breast cancer. The Breast : Official J Eur Soc Mastology 2022;62:52–60.
[69] Hatschek T, Carlsson L, Einbeigi Z, Lidbrink E, Linderholm B, Lindh B, et al. Individually tailored treatment with epirubicin and paclitaxel with or without capecitabine as first-line chemotherapy in metastatic breast cancer: a randomized multicenter trial. Breast Cancer Res Treat 2012;131(3):939–47. https://doi.org/10.1007/S10549-011-1880-9.
[70] Tobin NP, Lundberg A, Lindström LS, Harrell JC, Foukakis T, Carlsson L, et al. PAM50 provides prognostic information when applied to the lymph node metastases of advanced breast cancer patients. Clin Cancer Res 2017;23:7225. https://doi.org/10.1158/1078-0432.CCR-17-2301.
[71] Kang Y, Siegel PM, Shu W, Drobnjak M, Kakonen SM, Cordón-Cardo C, et al. A multigenic program mediating breast cancer metastasis to bone. Cancer Cell 2003;3(6):537–49. https://doi.org/10.1016/S1535-6108(03)00132-6.
[72] Minn AJ, Gupta GP, Siegel PM, Bos PD, Shu W, Giri DD, et al. Genes that mediate breast cancer metastasis to lung. Nature 2005;436(7050):518–24. https://doi.org/10.1038/NATURE03799.
[73] Johnston S, Pippen J, Pivot X, Lichinitser M, Sadeghi S, Dieras V, et al. Lapatinib combined with letrozole versus letrozole and placebo as first-line therapy for postmenopausal hormone receptor - Positive metastatic breast cancer. J Clin Oncol 2009;27(33):5538–46. https://doi.org/10.1200/JCO.2009.23.3734.
[74] Baselga J, Campone M, Piccart M, Burris HAI, Rugo HS, Sahmoud T, et al. Everolimus in postmenopausal hormone-receptor–positive advanced breast cancer. Http://DxDoiOrg/101056/NEJMoa1109653 2012;366:520–9. https://doi.org/10.1056/NEJMOA1109653.
[75] Finn RS, Martin M, Rugo HS, Jones S, Im S-A, Gelmon K, et al. Palbociclib and letrozole in advanced breast cancer. N Engl J Med 2016;375(20):1925–36.
[76] Cristofanilli M, Turner NC, Bondarenko I, Ro J, Im S-A, Masuda N, et al. Fulvestrant plus palbociclib versus fulvestrant plus placebo for treatment of hormone-receptor-positive, HER2-negative metastatic breast cancer that progressed on previous endocrine therapy (PALOMA-3): final analysis of the multicentre, double-blind, phase 3 randomised controlled trial. Lancet Oncol 2016;17(4):425–39. https://doi.org/10.1016/S1470-2045(15)00613-0.
[77] Hortobagyi GN, Stemmer SM, Burris HA, Yap Y-S, Sonke GS, Paluch-Shimon S, et al. Ribociclib as first-line therapy for HR-positive, advanced breast cancer. N Engl J Med 2016;375(18):1738–48. https://doi.org/10.1056/NEJMOA1609709.
[78] Tripathy D, Im S-A, Colleoni M, Franke F, Bardia A, Harbeck N, et al. Ribociclib plus endocrine therapy for premenopausal women with hormone-receptor-positive, advanced breast cancer (MONALEESA-7): a randomised phase 3 trial. Lancet Oncol 2018;19(7):904–15. https://doi.org/10.1016/S1470-2045(18)30292-4.
[79] Slamon DJ, Neven P, Chia S, Fasching PA, De Laurentiis M, Im S-A, et al. Phase III randomized study of ribociclib and fulvestrant in hormone receptor-positive, human epidermal growth factor receptor 2–negative advanced breast cancer: MONALEESA-3. J Clin Oncol 2018;36(24):2465–72. https://doi.org/10.1200/JCO.2018.78.9909.
[80] Turner NC, Slamon DJ, Ro J, Bondarenko I, Im S-A, Masuda N, et al. Overall survival with palbociclib and fulvestrant in advanced breast cancer. N Engl J Med 2018;379(20):1926–36. https://doi.org/10.1056/NEJMOA1810527.
[81] Im S-A, Lu Y-S, Bardia A, Harbeck N, Colleoni M, Franke F, et al. Overall survival with ribociclib plus endocrine therapy in breast cancer. N Engl J Med 2019;381(4):307–16. https://doi.org/10.1056/NEJMOA1903765.
[82] Sledge GW, Toi M, Neven P, Sohn J, Inoue K, Pivot X, et al. MONARCH 2: Abemaciclib in combination with fulvestrant in women with HR+/HER2- advanced breast cancer who had progressed while receiving endocrine therapy. J Clin Oncol 2017;35(25):2875–84. https://doi.org/10.1200/JCO.2017.73.7585.
[83] Goetz MP, Toi M, Campone M, Sohn J, Paluch-Shimon S, Huober J, et al. MONARCH 3: Abemaciclib as initial therapy for advanced breast cancer. J Clin Oncol 2017;35(32):3638–46. https://doi.org/10.1200/JCO.2017.75.6155.
[84] Slamon DJ, Neven P, Chia S, Fasching PA, de Laurentiis M, Im S-A, et al. Overall survival with ribociclib plus fulvestrant in advanced breast cancer. N Engl J Med 2020;382:514–24. https://doi.org/10.1056/NEJMOA1911149/SUPPL_FILE/NEJMOA1911149_DATA-SHARING.PDF.
[85] Paquet ER, Hallett MT. Absolute assignment of breast cancer intrinsic molecular subtype. J Natl Cancer Inst 2014:107. https://doi.org/10.1093/JNCI/DJU357.
[86] Carey L, Solovieff N, André F, O'Shaughnessy J, Cameron DA, Janni W, et al. Abstract GS2-00: Correlative analysis of overall survival by intrinsic subtype across the MONALEESA-2, -3, and -7 studies of ribociclib + endocrine therapy in patients with HR+/HER2− advanced breast cancer. Cancer Res 2022;82:GS2-00-GS2-00. https://doi.org/10.1158/1538-7445.SABCS21-GS2-00.
[87] Schettini F, Martínez-Sáez O, Chic N, Brasó-Maristany F, Galván P, Martínez D, et al. Abstract P4-07-08: 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:P4-07-08-P4-07–8. https://doi.org/10.1158/1538-7445.SABCS21-P4-07-08.
[88] Park YH, Kim T-Y, Kim GM, Kang SY, Park IH, Kim JH, et al. Palbociclib plus exemestane with gonadotropin-releasing hormone agonist versus capecitabine in premenopausal women with hormone receptor-positive, HER2-negative metastatic breast cancer (KCSG-BR15-10): a multicentre, open-label, randomised, phase 2 trial. Lancet Oncol 2019;20(12):1750–9. https://doi.org/10.1016/S1470-2045(19)30565-0.
[89] Prat A, Solovieff N, Su F, Bardia A, Neven P, Hortobagyi GN, et al. Abstract PD2-05: Genomic profiling of PAM50-based intrinsic subtypes in HR+/HER2- advanced breast cancer (ABC) across the MONALEESA (ML) studies. Cancer Res 2022;82:PD2-05-PD2-05. https://doi.org/10.1158/1538-7445.SABCS21-PD2-05.
[90] Schettini F, Seguí E, Conte B, Sanfeliu E, Gonzalez-Farre B, Jares P, et al. Intrinsic subtypes and therapeutic decision-making in hormone receptor-positive/HER2-negative metastatic breast cancer with visceral crisis: A case report. Front Oncol 2022;12. https://doi.org/10.3389/fonc.2022.1009352.
[91] Braso-Maristany F et al. Understanding the biologic determinants of ribociclib efficacy in breast cancer. Annals of Oncology 32 (Suppl_2): S21-S36 2021;32 (suppl_2). https://doi.org/10.1016/annonc/annonc503.
[92] Kan Z, Im S-A, Park K, Wen J, Lee K-H, Choi Y-L, et al. Abstract PD2-08: Serial genomic profiling reveals molecular mechanisms of breast cancer resistance to palbociclib. Cancer Res 2022;82:PD2-08-PD2-08. https://doi.org/10.1158/1538-7445.SABCS21-PD2-08.
[93] Zanudo JGT, Barroso-Sousa R, Jain E, Buendia-Buendia J, Li T, Tayob N, et al. Abstract P4-01-06: Genomic and transcriptomic analysis reveals known and novel resistance mechanisms to CDK4/6 inhibitors and sensitivity factors for the response to triplet therapy (palbociclib + everolimus + exemestane) in a phase I/IIb study in hormone-receptor positive (HR+)/HER2- metastatic breast cancer (MBC) after progression on a CDK4/6 inhibitor (CDK4/6i). Cancer Res 2022;82:P4-01-06-P4-01–6. https://doi.org/10.1158/1538-7445.SABCS21-P4-01-06.
[94] Tolosa P, Pascual T, Hernando C, Servitja S, Abad MF, Brasó-Maristany F, et al. Abstract OT2-19-03: Solti-1801. Analysis of the efficacy of CDK4/6 inhibitors in combination with hormonal treatment in luminal breast cancer in relation to the intrinsic subtype and markers of immunity (CDK-predict). Cancer Res 2022;82:OT2-19-03-OT2-19–03. https://doi.org/10.1158/1538-7445.SABCS21-OT2-19-03.
[95] Pascual T., Stover D.G., Thuerigen A. , Perou C.M. , Ciruelos E.M., Casas Fernandez Tejerina A. , et al. 272TiP - HARMONIA SOLTI-2101 / AFT-58: A head-to-head phase III study comparing ribociclib (RIB) and palbociclib (PAL) in patients (pts) with hormone receptor-positive/HER2-negative/HER2-enriched (HR+/HER2-/HER2-E) advanced breast cancer (ABC). Annals of Oncology (2022) 33 (suppl_7): S88-S121. 10.1016/annonc/annonc1040.
[96] Ciruelos E, Saura C, González-Farré X, Bofill JS, Vidal M, Blancas I, et al. Abstract OT2-27-01: Solti-1718 NEREA Trial: Neratinib in hormone receptor (HR)-positive/HER2-negative HER2-enriched (HER2-E) advanced breast cancer (BC). Cancer Res 2022;82:OT2-27-01-OT2-27–01. https://doi.org/10.1158/1538-7445.SABCS21-OT2-27-01.

[97] Waks AG, Stover DG, Guerriero JL, Dillon D, Barry WT, Gjini E, et al. The immune microenvironment in hormone receptor-positive breast cancer before and after preoperative chemotherapy. Clin Cancer Res 2019;25:4644–55. https://doi.org/10.1158/1078-0432.CCR-19-0173/75093/AM/THE-IMMUNE-MICROENVIRONMENT-IN-HORMONE-RECEPTOR.

[98] Griguolo G, Dieci MV, Paré L, Miglietta F, Generali DG, Frassoldati A, et al. Immune infiltrate composition across intrinsic subtypes in hormone receptor (HR)+/HER2- early breast cancer (BC) enrolled in the prospective LETLOB trial. Ann Oncol 2019;30:v81.

[99] Lefebvre C, Bachelot T, Filleron T, Pedrero M, Campone M, Soria J-C, et al. Mutational profile of metastatic breast cancers: a retrospective analysis. PLoS Med 2016;13(12):e1002201.

[100] O'Leary B, Cutts RJ, Liu Y, Hrebien S, Huang X, Fenwick K, et al. The genetic landscape and clonal evolution of breast cancer resistance to palbociclib plus fulvestrant in the PALOMA-3 trial. Cancer Discov 2018;8:1390–403. https://doi.org/10.1158/2159-8290.CD-18-0264.

[101] Marra A, Gazzo A, Gupta A, Selenica P, Da Silva EM, Pareja F, et al. 210O - Mutational signature analysis reveals patterns of genomic instability linked to resistance to endocrine therapy (ET) +/- CDK 4/6 inhibition (CDK4/6i) in estrogen receptor-positive/HER2-negative (ER+/HER2-) metastatic breast cancer (MBC). Ann Oncol 2022;33:S632.

[102] Brasó-Maristany F, Sansó M, Chic N, Martínez D, González-Farré B, Sanfeliu E, et al. Case report: A case study documenting the activity of atezolizumab in a PD-L1-negative triple-negative breast cancer. Front Oncol 2021;11. https://doi.org/10.3389/FONC.2021.710596.

[103] Asghar US, Kanani R, Roylance R, Mittnacht S. Systematic review of molecular biomarkers predictive of resistance to CDK4/6 inhibition in metastatic breast cancer. JCO Precis Oncol 2022;6:e2100002.

[104] Ciruelos E, Muñoz M, Oliveira M, Chic N, Hernando C, Viruzuela JA, et al. Abstract OT1-17-01: Solti-1716. Targeting with pembrolizumab + paclitaxel non-luminal by PAM50 hormone receptor-positive/HER2-negative advanced/metastatic breast cancer patients who have progressed on or after CDK4/6 inhibitor treatment (TATEN trial). Cancer Res 2022;82:OT1-17-01-OT1-17–01. https://doi.org/10.1158/1538-7445.SABCS21-OT1-17-01.

[105] Collins LC, Cole KS, Marotti JD, Hu R, Schnitt SJ, Tamimi RM. Androgen receptor expression in breast cancer in relation to molecular phenotype: Results from the Nurses' Health Study. Mod Pathol 2011;24(7):924–31.

[106] Peters AA, Buchanan G, Ricciardelli C, Bianco-Miotto T, Centenera MM, Harris JM, et al. Androgen receptor inhibits estrogen receptor-α activity and is prognostic in breast cancer. Cancer Res 2009;69:6131–40. https://doi.org/10.1158/0008-5472.CAN-09-0452.

[107] Vera-Badillo FE, Templeton AJ, de Gouveia P, Diaz-Padilla I, Bedard PL, Al-Mubarak M, et al. Androgen receptor expression and outcomes in early breast cancer: a systematic review and meta-analysis. JNCI: J Natl Cancer Inst 2014;106(1). https://doi.org/10.1093/JNCI/DJT319.

[108] Tsang JYS, Ni Y-B, Chan S-K, Shao M-M, Law BKB, Tan PH, et al. Androgen receptor expression shows distinctive significance in ER positive and negative breast cancers. Ann Surg Oncol 2014;21(7):2218–28. https://doi.org/10.1245/S10434-014-3629-2.

[109] Cochrane DR, Bernales S, Jacobsen BM, Cittelly DM, Howe EN, D'Amato NC, et al. Role of the androgen receptor in breast cancer and preclinical analysis of enzalutamide. Breast Cancer Res 2014;16(1). https://doi.org/10.1186/BCR3599.

[110] Oliveira M, Pernas S, Margelí M, Blanch S, Adamo B, Bofill JS, et al. Abstract OT-09-08: Solti-1502 aRIANNA: Targeting PAM50 HER2-enriched intrinsic subtype with enzalutamide in hormone receptor-positive/HER2-negative metastatic breast cancer. Ongoing Clinical Trials Abstracts, American Association for Cancer Research; 2021, p. OT-09-08-OT-09-08. https://doi.org/10.1158/1538-7445.SABCS20-OT-09-08.

[111] Nielsen T, Wallden B, Schaper C, Ferree S, Liu S, Gao D, et al. Analytical validation of the PAM50-based Prosigna Breast Cancer Prognostic Gene Signature Assay and nCounter Analysis System using formalin-fixed paraffin-embedded breast tumor specimens. BMC Cancer 2014;14(1). https://doi.org/10.1186/1471-2407-14-177.

[112] Wuerstlein R, Sotlar K, Gluz O, Otremba B, von Schumann R, Witzel I, et al. The West German Study Group Breast Cancer Intrinsic Subtype study: a prospective multicenter decision impact study utilizing the Prosigna assay for adjuvant treatment decision-making in estrogen-receptor-positive, HER2-negative early-stage breast cancer. Curr Med Res Opin 2016;32(7):1217–24.

[113] Martín M, González-Rivera M, Morales S, de la Haba-Rodriguez J, González-Cortijo L, Manso L, et al. Prospective study of the impact of the Prosigna assay on adjuvant clinical decision-making in unselected patients with estrogen receptor positive, human epidermal growth factor receptor negative, node negative early-stage breast cancer. Curr Med Res Opin 2015;31(6):1129–37.

[114] Hequet D, Callens C, Gentien D, Albaud B, Mouret-Reynier M-A, Dubot C, et al. Prospective, multicenter French study evaluating the clinical impact of the Breast Cancer Intrinsic Subtype-Prosigna® Test in the management of early-stage breast cancers. PLoS One 2017;12(10):e0185753.

[115] Prat A, Saura C, Pascual T, Hernando C, Muñoz M, Paré L, et al. Ribociclib plus letrozole versus chemotherapy for postmenopausal women with hormone receptor-positive, HER2-negative, luminal B breast cancer (CORALLEEN): an open-label, multicentre, randomised, phase 2 trial. Lancet Oncol 2020;21(1):33–43. https://doi.org/10.1016/S1470-2045(19)30786-7.

[116] Pascual T, Chic N, Martinez Saez O, Sanfeliu Torres E, Adamo B, Cebrecos I, et al. 132TiP HCB-ONC001 ELPIS TRIAL: Omission of surgery and sentinel lymph node dissection in clinically low-risk HER2-positive breast cancer with high HER2 addiction and a complete response following standard anti-HER2-based neoadjuvant therapy. Ann Oncol 2022;33:S182–3. https://doi.org/10.1016/j.annonc.2022.03.149.

[117] Cottu P, D'Hondt V, Dureau S, Lerebours F, Desmoulins I, Heudel P-E, et al. Letrozole and palbociclib versus chemotherapy as neoadjuvant therapy of high-risk luminal breast cancer. Ann Oncol 2018;29(12):2334–40. https://doi.org/10.1093/annonc/mdy448.

[118] Adamo B, Perez Fidalgo JA, Ciruelos E, Vidal M, Blanch S, Lopez A, et al. VENTANA (SOLTI-1501): Antiproliferative effect of the addition of oral metronomic vinorelbine to endocrine therapy in luminal/HER2-negative early breast cancer: A window of opportunity trial. Ann Oncol 2017;28:v66.

[119] Bellet M, Morales S, Gasol A, Amillano K, Chic N, González-Farré X, et al. Abstract P1-07-02: Primary results of ONAWA (SOLTI-1802) trial: A window of opportunity trial of onapristone in postmenopausal women with progesterone receptor-positive/HER2-negative early breast cancer (EBC). Cancer Res 2022;82:P1-07-02-P1-07–02. https://doi.org/10.1158/1538-7445.SABCS21-P1-07-02.

[120] Vidal M, Muñoz M, Margeli M, González X, Amillano K, Sánchez-Bayona R, et al. Abstract OT2-11-07: Solti-1905. Elacestrant in preoperative setting, a window of opportunity study (ELIPSE trial). Cancer Res 2022;82:OT2-11-07-OT2-11–07. https://doi.org/10.1158/1538-7445.SABCS21-OT2-11-07.

[121] Liu MC, Pitcher BN, Mardis ER, Davies SR, Friedman PN, Snider JE, et al. PAM50 gene signatures and breast cancer prognosis with adjuvant anthracycline- and taxane-based chemotherapy: correlative analysis of C9741 (Alliance). npj Breast Cancer 2016;2(1). https://doi.org/10.1038/npjbcancer.2015.23.

[122] Martín M, Prat A, Rodríguez-Lescure Á, Caballero R, Ebbert MTW, Munárriz B, et al. PAM50 proliferation score as a predictor of weekly paclitaxel benefit in breast cancer. Breast Cancer Res Treat 2013;138(2):457–66. https://doi.org/10.1007/s10549-013-2416-2.

[123] Loibl S, Marmé F, Martin M, Untch M, Bonnefoi H, Kim S-B, et al. Palbociclib for residual high-risk invasive HR-positive and HER2-negative early breast cancer—the penelope-B trial. J Clin Oncol 2021;39(14):1518–30. https://doi.org/10.1200/JCO.20.03639.