Transcriptomic Predictors of Survival for Palbociclib + Endocrine Therapy Versus Capecitabine in Aromatase Inhibitor–Resistant Breast Cancer From the GEICAM/2013-02 PEARL Trial

Yash N. Agrawal, MD[1,2]; Aranzazu Fernández-Martínez, MD, PhD[1,3]; Miguel Gil-Gil, MD, PhD[4,5]; Christoph Zielinski, MD[6,7]; Manuel Ruiz-Borrego, MD, MSc[4,8]; Eva María Ciruelos, MD, PhD[4,9,10,11]; Montserrat Muñoz, MD, PhD[4,12]; Mireia Margelí, MD, PhD[4,13]; Begoña Bermejo, MD, PhD[4,14,15,16]; Antonio Antón, MD, PhD[4,14,17]; Zsuzsanna Kahan, MD, PhD, DSc[6,18]; Tibor Csöszi, MD[6,19]; José Luis Alonso-Romero, MD, PhD[4,20]; José Ángel García-Saenz, MD, PhD[4,21]; Pedro Sánchez-Rovira, MD, PhD[4,22]; Elena Álvarez, MD[4,23]; José Ignacio Chacón, MD, PhD[4,24]; Santiago González-Santiago, MD[4,25]; César A. Rodríguez, MD[4,26]; Sonia Servitja, MD, PhD[4,14,27]; Adam D. Pfefferle, PhD[1,3]; Jesús Herranz[4]; Yuan Liu, PhD[28]; Lisa A. Carey, MD, ScM, FASCO[1,2]; Isabel Romero-Camarero, PhD[4]; Rosalía Caballero, PhD[4]; Ángel Guerrero-Zotano, MD, PhD[4,29]; Charles M. Perou, PhD[1,3]; and Miguel Martín, MD, PhD[4,30]

DOI https://doi.org/10.1200/PO-24-00937

ABSTRACT

PURPOSE For hormone receptor–positive/human epidermal growth factor receptor 2–negative (HR+/HER2–) metastatic breast cancer (MBC), first-line cyclin-dependent kinase 4/6 inhibitors (CDK4/6i) + endocrine therapy (ET) is the standard of care. They are also used after progression on first-line aromatase inhibitors (AIs), but some patients may respond better to chemotherapy-based options. We examined tumor features associated with survival from GEICAM/2013-02 PEARL, a phase III trial of palbociclib + ET versus capecitabine in AI-resistant HR+/HER2– MBC.

METHODS For 158 and 155 patients from each arm, 878 previously published gene expression signatures were derived using RNA sequencing on pretreatment tumor specimens, both primary and metastatic. Multivariable Cox models for progression-free survival (PFS) and overall survival (OS) were constructed with 16 preselected signatures related to proliferation, loss of retinoblastoma, and immune infiltration, and via Elastic Net using all signatures.

RESULTS Significant PFS difference by PAM50 intrinsic subtype was observed with palbociclib + ET. Comparing treatment arms, luminal A subtype trended toward longer PFS with palbociclib + ET, and luminal B and nonluminal subtypes had significantly longer PFS with capecitabine. Three B-cell (B-lymphocyte)–associated signatures correlated with shorter OS with palbociclib + ET. The immune-activated Immune1 TCGA breast cancer signature had significant treatment arm interaction for OS. Elastic Net iteratively selected B-cell–associated signatures independently associated with shorter OS with palbociclib + ET.

CONCLUSION PAM50 intrinsic subtype predicted PFS differences between palbociclib + ET and capecitabine. Lower B-cell–associated gene expression predicted longer OS with palbociclib + ET versus capecitabine. These features may help identify HR+/HER2– tumors resistant to further ET-based treatment with CDK4/6i.

ACCOMPANYING CONTENT

- Appendix
- Data Sharing Statement
- Data Supplement

Accepted May 27, 2025
Published July 9, 2025

JCO Precis Oncol 9:e2400937

INTRODUCTION

Hormone receptor–positive/human epidermal growth factor receptor 2–negative (HR+/HER2–) breast cancer comprises 70% of female breast cancer cases.[1] It is driven through pathways downstream of hormone receptor and is treated primarily with a backbone of endocrine therapy (ET), which targets signaling through hormone receptor. Selective estrogen receptor (ER) modulators[2] and degraders[3,4] act directly on the estrogen receptor, and aromatase inhibitors (AIs)[5,6] suppress estrogen production itself.

ET resistance can arise through ER loss of expression[7] or mutation,[8-12] aberrant expression of downstream cell cycle

CONTEXT

Key Objective

For metastatic hormone receptor–positive/human epidermal growth factor receptor 2–negative (HR+/HER2–) breast cancer demonstrating endocrine therapy (ET) resistance in postmenopausal women, can tumor gene expression features differentiate survival outcomes with subsequent treatment using a cyclin-dependent kinase 4/6 inhibitor (CDK4/6i) + ET versus cytotoxic chemotherapy?

Knowledge Generated

In a post hoc transcriptomics analysis of the GEICAM/2013-02 PEARL phase III trial, PAM50 intrinsic subtype predicted progression-free survival differences between palbociclib + ET and capecitabine. Lower expression of the Immune1 TCGA breast cancer signature—an immune-activated B-cell–associated gene expression signature—predicted longer overall survival with palbociclib + ET versus capecitabine.

Relevance

For ET-resistant HR+/HER2– metastatic breast cancer, our analysis supports PAM50 intrinsic subtype and B-cell immune microenvironment activity as predictive markers of response to palbociclib + ET compared with chemotherapy, highlighting these features as promising candidates for validation in larger studies of CDK4/6i + ET after ET resistance.

regulators,[13-18] or alternative proliferation pathways.[19-21] Inhibiting cyclin-dependent kinases 4 and 6 (CDK4/6), which regulate the G1/S transition by complexing with cyclin D, phosphorylating retinoblastoma (Rb), and inducing release of E2F transcription factors,[22] can overcome ET resistance. The CDK4/6 inhibitor (CDK4/6i) palbociclib demonstrated efficacy with ET in the first-line setting for HR+/HER2– metastatic breast cancer (MBC) in PALOMA-1 and PALOMA-2,[23-25] and in the second-line setting in PALOMA-3[26,27] and SONIA.[28] After progression, strategies to continue to subvert ET resistance include switching CDK4/6i or ET, as studied in postMONARCH[29] and MAINTAIN,[30] or replacing the CDK4/6i with another adjunct such as capivasertib.[31] The treatment paradigm for HR+/HER2– MBC favors using ET-based therapy until no further ET-based options remain, upon which the tumor is considered ET-insensitive and chemotherapy-based treatments are used.[32,33]

Not all patients benefit equally from CDK4/6i + ET, but clinical variables have not correlated with response, and molecular biomarkers are lacking.[34-36] Acquired *ESR1* mutation is the only established ET resistance biomarker, but there are no predictive biomarkers for CDK4/6i. In PALOMA-1, cyclin D1 amplification and p16 loss of heterozygosity failed to identify patients who benefitted most from adding palbociclib to ET. Candidate biomarkers include intrinsic subtypes[37-40]; alterations to *RB1*,[41-44] cyclin E,[37,38,43,44] CDK2,[45] and CDK6[46]; and expression of PD-1[39] and of genes corresponding to a T-cell–inflamed tumor microenvironment.[47]

The GEICAM Spanish Breast Cancer Group conducted the phase III PEARL clinical trial comparing palbociclib + ET to single-agent capecitabine in postmenopausal women with AI-resistant HR+/HER2– MBC.[48] The primary objectives were to compare PFS between capecitabine and palbociclib + fulvestrant regardless of *ESR1* status, and between capecitabine and palbociclib + ET (exemestane or fulvestrant) for patients with wild-type *ESR1* on the basis of circulating tumor DNA. Although PEARL failed to meet its primary objectives, it demonstrated superior patient-reported outcomes and fewer serious adverse events with palbociclib + ET.[49,50] This trial is ideal for examining pretreatment transcriptomic markers of resistance to CDK4/6i + ET after AI resistance.

We herein present an analysis of tumor RNA sequencing (RNAseq) features predictive of PFS and OS with palbociclib + ET versus capecitabine from the GEICAM/2013-02 PEARL phase III trial.

METHODS

PEARL Study Design

The GEICAM/2013-02 PEARL phase III trial design (ClinicalTrials.gov identifier: NCT02028507) has been previously described.[37,49] 601 postmenopausal women with AI-resistant HR+/HER2– MBC were randomly assigned to palbociclib + ET (exemestane in cohort 1, fulvestrant in cohort 2) or capecitabine. AI resistance was defined as disease recurrence while on or within 12 months of completing adjuvant AI, or progression while on or within 1 month of completing AI treatment for advanced disease.

Enrollment required measurable disease per RECIST v1.1 or at least one lytic/mixed bone lesion, Eastern Cooperative

Oncology Group performance status 0-1, life expectancy of ≥12 weeks, adequate organ function, and zero to one previous lines of chemotherapy for MBC. Patients were excluded for previous CDK4/6i, mammalian target of rapamycin or phosphoinositide 3-kinase inhibitor, or capecitabine exposure; visceral crisis; or a corrected QT (QTc) interval ≥480 ms, a personal or family history of long or short QT syndrome, Brugada syndrome, torsade de pointes, or known QTc prolongation history.

Primary objectives were to compare PFS between treatment arms for all patients who received palbociclib + fulvestrant versus capecitabine, and for patients with wild-type *ESR1* who received palbociclib + ET (exemestane or fulvestrant) versus capecitabine.

The research protocol was approved by each site's respective institutional review board and each country's regulatory agency, and was conducted in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki. Patients signed written informed consents including permission to submit formalin-fixed paraffin-embedded (FFPE) tumor samples for biomarker research. Samples were collected before entry in PEARL if available, either from a metastatic disease site or from an archival primary sample, and sources were documented.

Gene Expression Analysis

Three hundred sixty-four FFPE tissue samples from 360 patients were sent to the UNC Lineberger Comprehensive Cancer Center Translational Genomics Lab for DNA and RNA isolation using the KingFisher Flex automated extraction instrument (Thermo Fisher Scientific, Waltham, MA, 5400630) and the Applied Biosystems MagMAX FFPE DNA/RNA Ultra Kit (Thermo Fisher Scientific A31881) following manufacturer protocol (Thermo Fisher Scientific MAN0015877), allowing for sequential isolation of DNA and RNA from the same FFPE slides using a magnetic bead–based technology. DNA and RNA quality was analyzed using a TapeStation 4200 (Agilent, Santa Clara, CA, G2991AA) and quantified using a Qubit 3.0 fluorometer (Life Technologies, Waltham, MA, Q33216). Total RNA from 361 samples was converted to RNAseq libraries using the TruSeq Stranded Total RNA Library Prep Kit with Ribo-Zero Gold (Illumina, San Diego, CA), and gene expression profiles from 334 libraries that met sequencing criteria were generated via RNAseq on Illumina NovaSeq 6000 S4 flow cells with 2 × 50 bp paired-end reads, with an average sequencing depth of approximately 115 million clusters per library.

Six samples—four duplicates and two collected after completion of study treatment—were excluded. For the remaining 328 samples, uniquely corresponding to 328 patients, intrinsic subtypes were derived via the PAM50 predictor[51] after HER2/ER subgroup-specific normalization.[52,53] Single-gene expression and gene expression signatures, derived from 108 publications[54-56] and the Molecular Signature Database[57] and representing multiple biologic pathways and cell types, were calculated from tumor RNAseq features. Fifteen normal-like tumors were excluded, and the remaining 313—158 from the palbociclib + ET arm and 155 from the capecitabine arm—were included in the final analysis (Appendix Fig A1).

Statistical Analysis

Median PFS and OS were calculated for PAM50 intrinsic subtypes in each treatment arm using the Kaplan-Meier method.

For gene expression signatures, univariable and multivariable Cox proportional hazards regression models were constructed for PFS and OS in each treatment arm. Multivariable models were adjusted for site of disease, previous sensitivity to ET (defined as disease relapse after 24 months of adjuvant ET, or disease control—complete response, partial response, or stable disease for at least 24 weeks on the most recent ET for advanced disease), previous chemotherapy for MBC, number of involved sites of disease, and—with the exception of models for PAM50 luminal status—were adjusted for PAM50 luminal status as well. Hazard ratios (HRs) and 95% CIs were calculated for each model. Association between gene expression signature and PAM50 luminal status was assessed using analysis of variance. Significance was defined by an adjusted $P < .05$ on the basis of the Bonferroni method.

High-dimensional modeling for PFS and OS for each treatment arm was performed with Elastic Net[58] (R package glmnet). Models were built with 10-fold cross-validation for each survival end point using the training sets over a grid of alpha values (0.1-0.8 by 0.1 increments) with lambda values recommended by glmnet. The most accurate model for each treatment and survival end point was selected using the Harrell C-index.[59]

Study Approval

The research protocol for the GEICAM/2013-02 PEARL clinical trial was approved by each site's respective institutional review board and each country's regulatory agency. This study was determined not to constitute human subjects research as defined under federal regulations (45 CFR 46.102 (e or l) and 21 CFR 56.102(c)(e)(l)) by the University of North Carolina institutional review board (24-2632).

RESULTS

Patient Characteristics

Patient characteristics are displayed in Table 1. Of 601 patients in PEARL, 328 (54.6%) received on-study treatment and had RNAseq derived from pretreatment tumor samples,

TABLE 1. Clinical Characteristics of Patients From the GEICAM/2013-02 PEARL Trial Included in the Biomarker Analysis

Characteristic	ITT (N = 601)	RNAseq Analysis (n = 313)	*P*	Palbociclib + ET (n = 158)	Capecitabine (n = 155)	*P*
Age, years, median (IQR)	—	61 (53-68)		61 (56-69)	61 (52-68)	.35
Site of disease, No. (%)			.57			.73
Nonvisceral	204 (34)	113 (36)		59 (37)	54 (35)	
Visceral	396 (66)	200 (64)		99 (63)	101 (65)	
Number of involved sites, No. (%)			.05			.009
1	170 (28)	111 (35)		69 (44)	42 (27)	
2	229 (38)	117 (37)		52 (33)	65 (42)	
≥3	201 (34)	85 (27)		37 (23)	48 (31)	
Initial M stage, No. (%)			.21			.41
M0	471 (78)	257 (82)		133 (84)	124 (80)	
M1	130 (22)	56 (18)		25 (16)	31 (20)	
Treatment line, No. (%)			.35			.77
First	139 (23)	86 (27)		41 (26)	45 (29)	
Second	266 (44)	135 (43)		71 (45)	64 (41)	
≥Third line	193 (32)	92 (29)		46 (29)	46 (30)	
Previous chemotherapy for MBC, No. (%)			.45			.68
Yes	171 (28)	81 (26)		43 (27)	38 (25)	
No	430 (72)	232 (74)		115 (73)	117 (75)	
Previous sensitivity to ET, No. (%)			.84			.53
Yes	452 (75)	238 (76)		123 (78)	115 (74)	
No	149 (25)	75 (24)		35 (22)	40 (26)	
ER status,[a] No. (%)			.04			1
Positive	589 (98)	312 (100)		157 (99)	155 (100)	
Negative	12 (2.0)	1 (0.3)		1 (0.6)	0 (0)	
Sample type, No. (%)			—			.76
Primary	—	239 (76)		119 (75)	120 (77)	
Metastatic	—	74 (24)		39 (25)	35 (23)	
Intrinsic subtype, No. (%)	AIMS (n = 455)	PAM50 (n = 313)	.55	PAM50	PAM50	.52
Luminal A	232 (51)	172 (55)		86 (54)	86 (55)	
Luminal B	192 (42)	122 (39)		60 (38)	62 (40)	
Nonluminal	31 (6.8)	19 (6.1)		12 (7.6)	7 (4.5)	

Abbreviations: AIMS, Absolute Intrinsic Molecular Subtyping; ER, estrogen receptor; ET, endocrine therapy; ITT, intention-to-treat; M stage, metastasis stage; MBC, metastatic breast cancer; RNAseq, RNA sequencing.
[a]Based on local laboratory determination, ER-positive defined as ≥1% positive cells by immunohistochemistry for ER.

and 313 (52%) were included in the final analysis (Appendix Fig A1). Age, presence of visceral disease, metastatic stage at the time of diagnosis (M0 or M1), previous chemotherapy for MBC, previous ET sensitivity, and ER status were similar to the original patient cohort and between treatment arms. Within our patient population, 18% had de novo metastatic disease. Seventy-six percent had previous ET sensitivity, and 26% had previous chemotherapy for MBC. More patients receiving capecitabine had ≥two disease sites (73%) compared with patients receiving palbociclib + ET (56%). Seventy-six percent of tumor samples were from the primary tumor. PAM50 intrinsic subtype distribution was similar between treatment arms and to the original study, which derived intrinsic subtype with the Absolute Intrinsic Molecular Subtyping classifier.[49]

Cox Proportional Hazards Analysis: Intrinsic Subtype

With palbociclib + ET, median PFS for luminal A, luminal B, and nonluminal tumors was 11.2, 5.6, and 4.3 months, respectively. On multivariable Cox analysis, luminal B (HR, 1.90 [95% CI, 1.28 to 2.82]; $P = .001$) and nonluminal tumors (HR, 3.19 [95% CI, 1.65 to 6.16]; $P < .001$) were associated with significantly worse PFS compared with luminal A tumors (Fig 1A). With capecitabine, median PFS with luminal A, luminal B, and nonluminal tumors was 7.9, 10.6, and 13.0 months, respectively, although these differences were not statistically significant on multivariable Cox analysis (Fig 1B). Intrinsic subtype was not associated with significant differences in OS for either treatment (Figs 1C and 1D). A statistically significant treatment arm interaction for PAM50

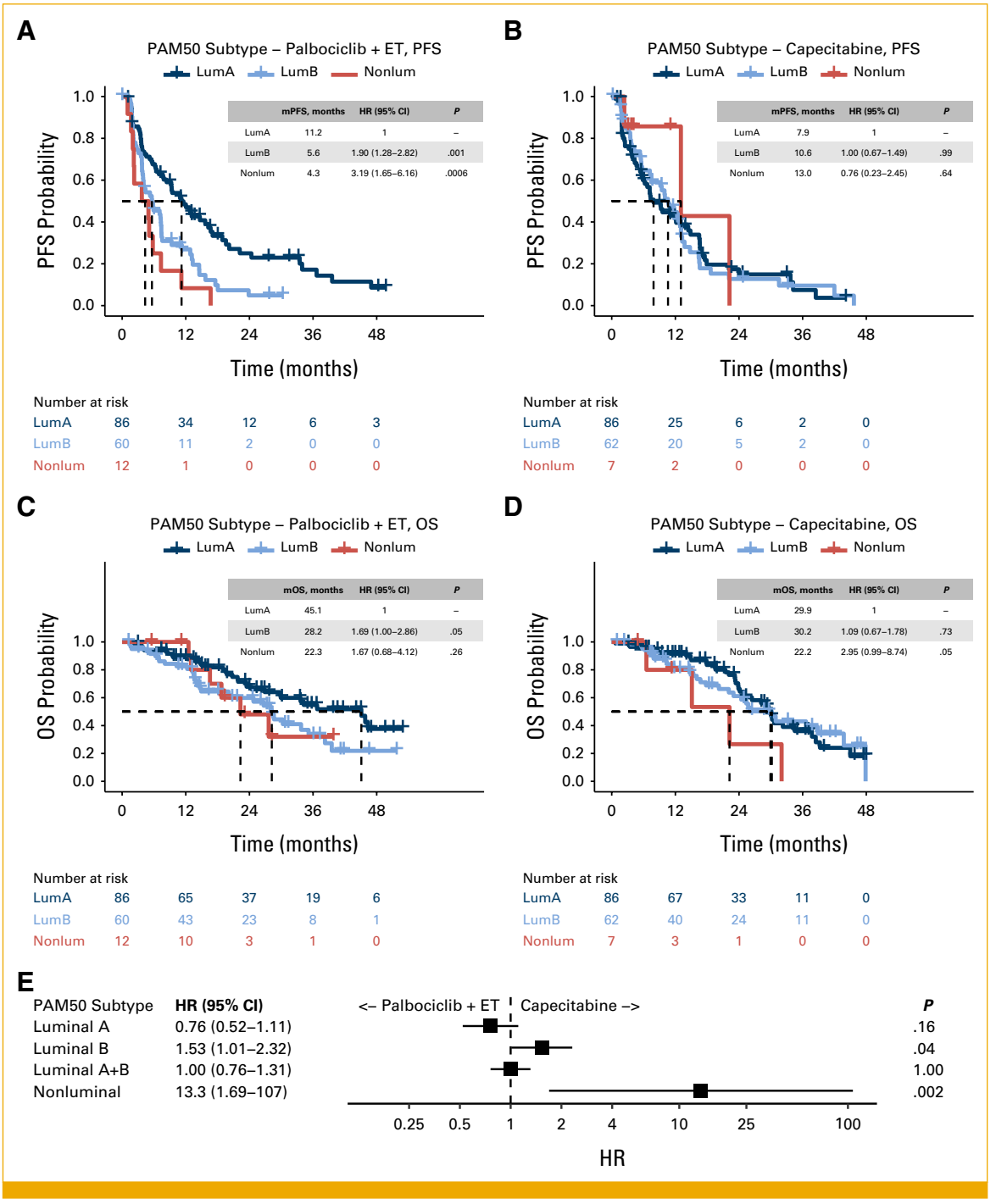

FIG 1. PAM50 intrinsic subtype correlations with PFS and OS for each treatment arm. (A) Kaplan-Meier plot of PFS with palbociclib + ET by PAM50 luminal status. (B) Kaplan-Meier plot of PFS with capecitabine by PAM50 luminal status. (C) Kaplan-Meier plot of OS with palbociclib + ET by PAM50 luminal status. (D) Kaplan-Meier plot of OS with capecitabine by PAM50 luminal status. (E) Forest plot of PAM50 intrinsic subtype correlation with PFS between treatment arms. HRs compare PFS between treatment arms by PAM50 intrinsic subtype. Luminal B and nonluminal subtypes were significantly associated with longer PFS with capecitabine than with palbociclib + ET. ET, endocrine therapy; HR, hazard ratio; LumA, Luminal A; LumB, Luminal B; mOS, median overall survival; mPFS, median progression-free survival; Nonlum, Nonluminal; OS, overall survival; PFS, progression-free survival.

luminal status was observed for PFS (P = .004) but not for OS (P = .33).

Comparing treatment arms, luminal A tumors had longer PFS with palbociclib + ET (HR, 0.76 [95% CI, 0.52 to 1.11]; P = .16). Luminal B tumors (HR, 1.53 [95% CI, 1.01 to 2.32]; P = .04) and nonluminal tumors (HR, 13.3 [95% CI, 1.69 to 107]; P = .002) had significantly longer PFS with capecitabine (Fig 1E). The analysis notably had a limited number of nonluminal samples.

Luminal A centroid correlation was associated with longer PFS and OS with both treatments, which was significant for OS in the capecitabine arm (HR, 0.47 [95% CI, 0.31 to 0.73]; adjusted P = .01). Basal-like centroid correlation was associated with shorter PFS and OS with both treatments (Figs 2 and 3).

Cox Proportional Hazards Analysis: Gene Expression Features

Sixteen gene expression features were preselected on the basis of existing knowledge: PAM50 proliferation score and correlations to the luminal A, luminal B, basal-like, and HER2-enriched centroids[51]; *CCNE1*[38] and *CD274*[60] expression; and gene expression signatures corresponding to Rb loss of heterozygosity,[61] FGFR4-induced and FGFR4-repressed signatures,[56] a Fos-Jun signature,[54] and immune signatures associated with B-cell and T-cell activity.[54,56,60,62] Multivariable Cox models for PFS (Fig 2) and OS (Fig 3) in each treatment arm were constructed for each signature.

Three B-cell (B-lymphocyte)–associated signatures significantly correlated with shorter OS for palbociclib + ET: the Immune1 TCGA breast cancer signature (TCGA_BRCA_1198_Immune1[56]; HR, 1.52 [95% CI, 1.20 to 1.92]; adjusted P = .009), IgG_Cluster signature[54] (HR, 1.43 [95% CI, 1.13 to 1.80]; adjusted P = .05), and B-cell/T-cell cooperativity signature (Bcell_Tcell_Cooperation[60]; HR, 1.42 [95% CI, 1.14 to 1.78]; adjusted P = .04; Fig 3A). These signatures had a nonsignificant correlation with shorter PFS for palbociclib + ET and did not correlate with PFS or OS for capecitabine. A significant interaction was observed between treatment arm and the immune-activated TCGA_BRCA_1198_Immune1 signature for OS (Appendix Table A1). Low TCGA_BRCA_1198_Immune1 expression was associated with significantly longer OS with palbociclib + ET than capecitabine, whereas high expression was not associated with a significant OS difference (Fig 4). The signature's expression was independent of intrinsic subtype (P = .56).

Exploratory Elastic Net Regression Analysis

To examine the potential of gene expression–based predictive models, we applied 878 gene expression signatures to the RNAseq data set (Data Supplement) and, using a Cox proportional hazards approach with Elastic Net regression[58] (R package glmnet), identified recurrently selected signatures correlating with survival over 20 rounds of iterative modeling with repeated subsampling. This approach was repeated after excluding nonluminal tumors to identify signatures in common.

For palbociclib + ET, the NKI 70-gene signature (Pcorr_NKI70_Good_Correlation) corresponding to MammaPrint[63] was consistently selected in PFS and OS models, as was the IMMUNE_Bindea_Cell_Th17_cells signature,[64] which pertains to Th17-cell activity. Both corresponded with longer survival (Figs 5A and 5B). Frequently selected signatures in OS models including those corresponding to neutrophil (Charoentong_Neutrophil,[65] Neutrophils_MCP[66]), natural killer cell (IMMUNE_Bindea_Cell_NK_cells,[64] NANOSTRING_MODULE_NK_CD56bright_cell), macrophage (NANOSTRING_MODULE_Macrophage_Functions), and Th2 activity (NANOSTRING_MODULE_Th2_cell) correlated with longer OS. Signatures corresponding to T follicular helper cell (IMMUNE_Bindea_TFH_Immunity,[64] TCGA_Tfh_cells_Immunity[67]) and B-cell activity (Bcells_Plasmablast,[68] NANOSTRING_Module_B_cell, TCGA_BRCA_1198_IMMUNE1) correlated with shorter OS (Fig 5B).

For capecitabine, the HS_Green18 signature[54] corresponding to the luminal B subtype, and the IMMUNE_Bindea_Cell_NK_CD56bright_cells signature[64] reflecting CD56^{bright} natural killer cell–related expression were frequently selected in both PFS and OS models and correlated with longer survival (Appendix Fig A2). For PFS models, frequently selected signatures corresponding with metabolism (MM_Green3[54]), p53 status (Duke_Module14_p53[69]) and luminal subtype (TCGA_BRCA_1198_Luminal[56]) were associated with longer PFS. The LumA-Basal score,[56] corresponding with the luminal A subtype, correlated with shorter PFS (Appendix Fig A2A). For OS models, signatures associated with T regulatory cells (T_regulatory_cell_2gene[70]) central memory CD8 T-cells (Charoentong_Central_memory_CD8_T_cell[65]), immunotherapy resistance (Immunoediting_Swarbrick_POSITIVE_MOUSE[71]), and *BCL2* expression correlated with longer OS; and T follicular helper cell–related signatures (IMMUNE_Bindea_TFH_Immunity,[64] TCGA_Tfh_cells_Immunity[67]), a Th17-cell related signature (Charoentong_Type_17_T_helper_cell[65]), an invasiveness signature (Pcorr_IGS_Correlation[72]), *CD3D* expression, *HRAS* expression, and a PTEN pathway signature (GSEA_BIOCARTA_PTEN_PATHWAY) correlated with shorter OS (Appendix Fig A2B).

DISCUSSION

Our analysis sheds light on transcriptomic features associated with survival with palbociclib + ET versus capecitabine in the setting of AI resistance. We demonstrate that PAM50 intrinsic subtype and low B-cell–associated tumor expression may identify patients who would benefit from continuing ET-based therapy with palbociclib.

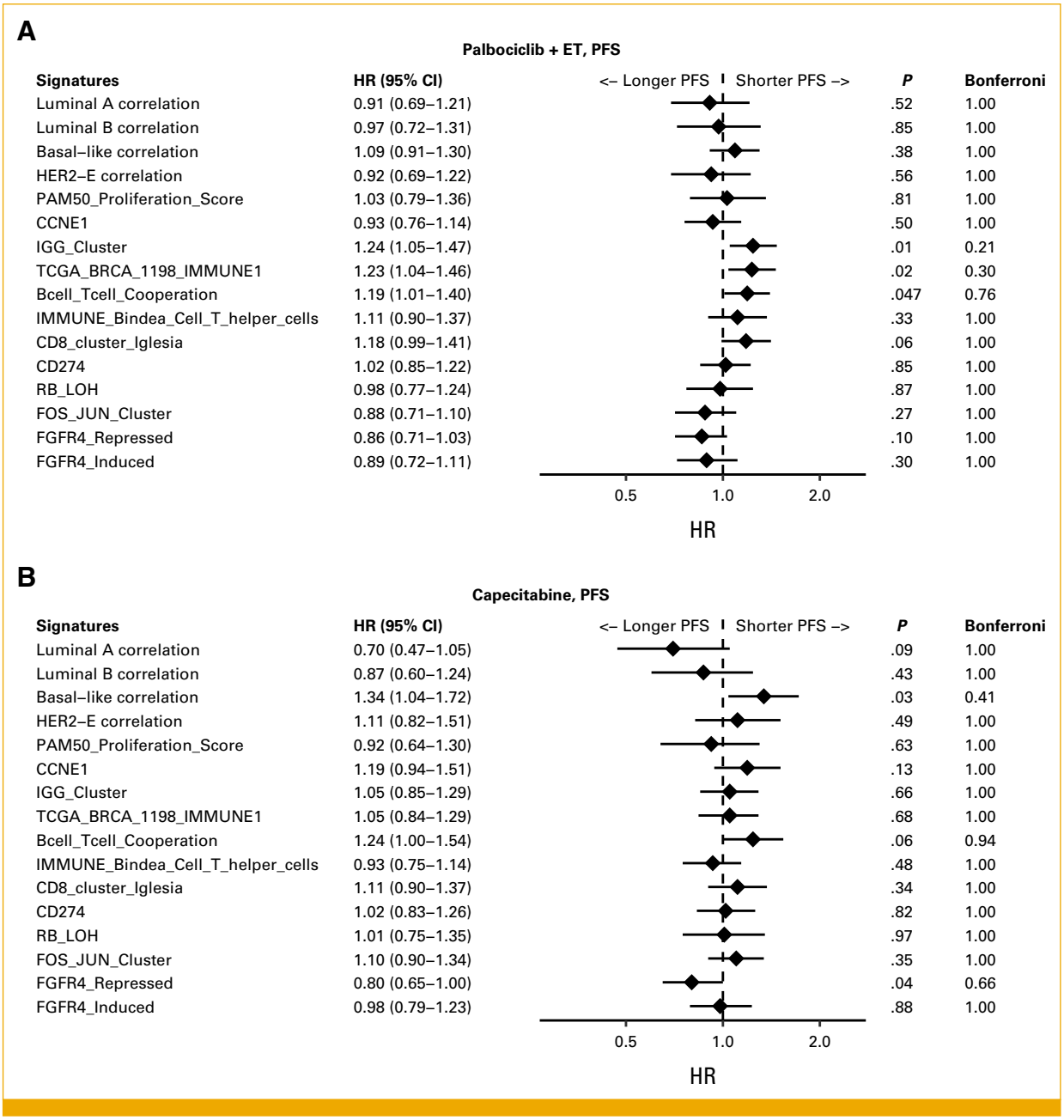

FIG 2. Forest plots for HRs for PFS for each treatment arm for 16 selected biomarker signatures. (A) Adjusted HRs for PFS for patients treated with palbociclib + ET. (B) Adjusted HRs for PFS for patients treated with capecitabine. ET, endocrine therapy; HR, hazard ratio; PFS, progression-free survival; TCGA, The Cancer Genome Atlas.

Although international guidelines recommend switching to chemotherapy after ET resistance, this has not been systematically studied, and usually the timing of this switch is primarily guided by clinical gestalt. The GEICAM/2013-02 PEARL study compared second-line palbociclib + ET versus capecitabine monotherapy in postmenopausal women with AI-resistant HR+/HER2– breast cancer and found no difference in efficacy but did demonstrate lower toxicity with palbociclib + ET. Given the molecular heterogeneity of HR+/HER2– breast cancer,[25,26,37-41,47,73,74] identifying molecular features that could affect response to CDK4/6i + ET versus chemotherapy could help with choosing between these two treatment avenues.

In our analysis, luminal A tumors were associated with a significant PFS advantage with palbociclib + ET compared with luminal B tumors. The magnitude of difference is comparable with other studies of palbociclib + ET. In the first-line setting, palbociclib + letrozole was associated with a median PFS of 30.4 versus 19.6 months for luminal A versus

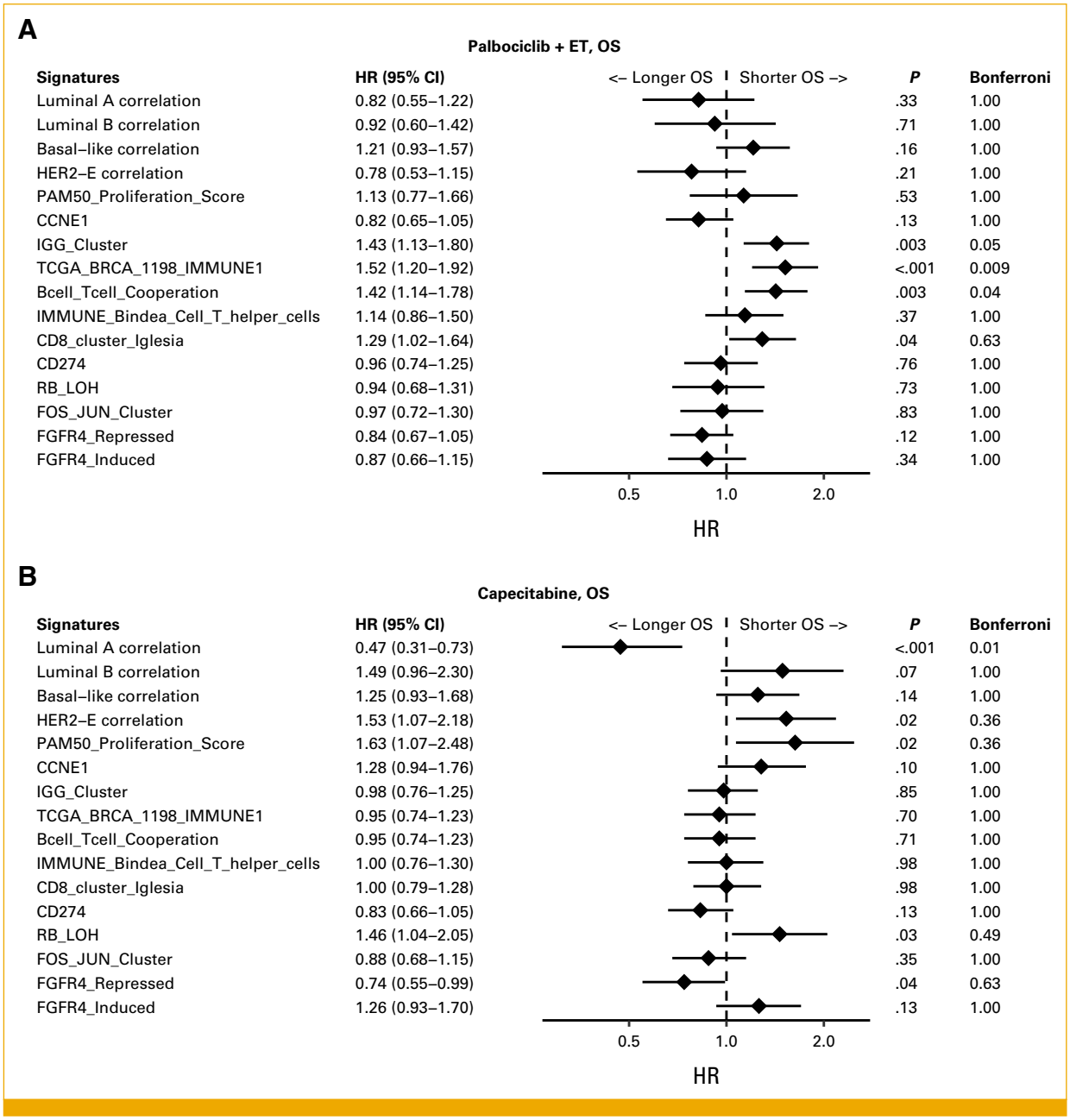

FIG 3. Forest plots for HRs for OS for each treatment arm for 16 selected biomarker signatures. (A) Adjusted HRs for OS for patients treated with palbociclib + ET. (B) Adjusted HRs for OS for patients treated with capecitabine. ET, endocrine therapy; HR, hazard ratio; OS, overall survival; TCGA, The Cancer Genome Atlas.

luminal B tumors in PALOMA-2,[25] and in the second-line in PALOMA-3, luminal A versus luminal B tumors had a median PFS of 16.6 versus 9.2 months.[26]

Although luminal A tumors were associated with longer PFS with palbociclib + ET compared with capecitabine, patients with luminal B and nonluminal tumors had statistically longer PFS with capecitabine as opposed to palbociclib + ET. Although previous trials of palbociclib + ET demonstrate a similar magnitude of survival benefit with palbociclib + ET versus ET for both luminal A and B tumors,[25,26] our findings suggest that PAM50 intrinsic subtype is not only prognostic but may serve a predictive role in this setting by identifying patients who may benefit even more from switching to a chemotherapy-based approach.

Of the intrinsic subtype centroid correlations, only basal-like centroid correlation had a trend toward shorter survival along with the immune-related signatures, suggesting that for palbociclib + ET, specifically having low basal-like features may be an important biological determinant of improved survival. For capecitabine, tumors with greater

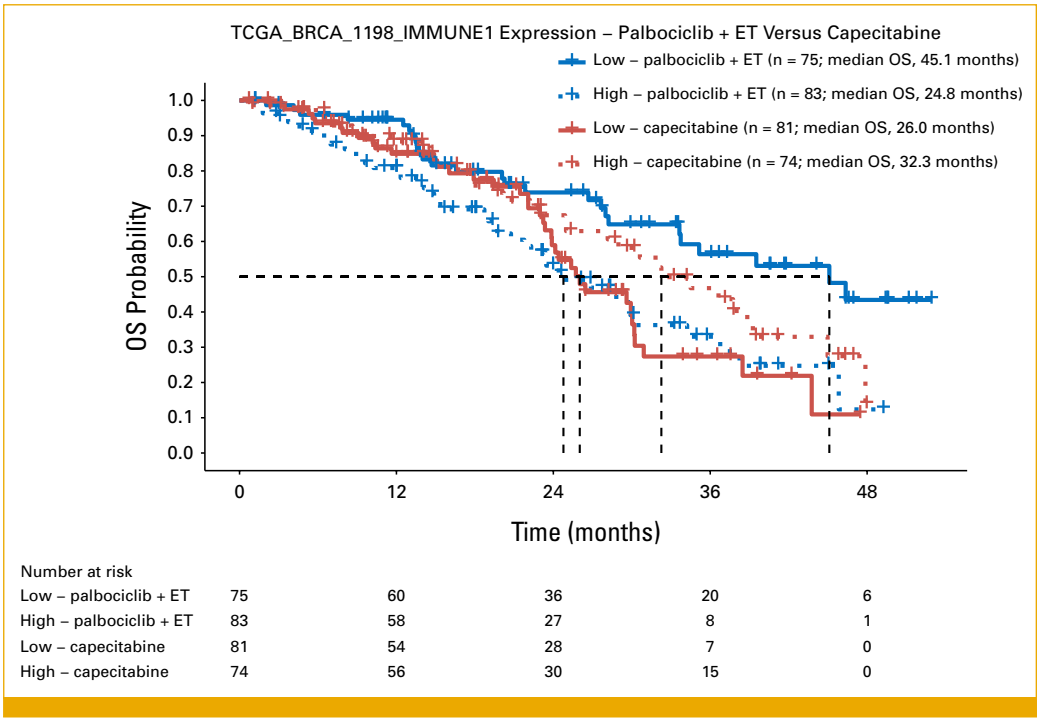

FIG 4. Kaplan-Meier plot of OS for patients treated with palbociclib + ET versus capecitabine by Immune1 TCGA breast cancer (TCGA_BRCA_1198_Immune1) signature expression. Survival curves are separated by low or high tumor expression (with respect to the median; solid or dotted) of the Immune1 TCGA breast cancer signature, and by treatment with palbociclib + ET or capecitabine (blue or red). ET, endocrine therapy; OS, overall survival; TCGA, The Cancer Genome Atlas.

luminal A centroid correlation trended toward longer survival, recapitulating known PAM50 prognostic survival associations[75,76] and suggesting that specifically luminal A alignment, rather than solely low basal-like correlation, is most relevant biologically for survival with cytotoxic therapy in ET-resistant HR+/HER2− breast cancer.

Higher expression of multiple B-cell–associated signatures correlated with worse survival associated with palbociclib + ET and was significant for OS. B-cell–associated signatures were also selected frequently in Cox models constructed via Elastic Net regression for OS with palbociclib + ET, implying a potential immune biological signal that may be associated with a worse prognosis in this setting. Furthermore, independent of intrinsic subtype, low expression of the immune-activated TCGA__BRCA__1198__Immune1 signature had significant treatment arm interaction and was associated with longer OS with palbociclib + ET versus capecitabine. This finding suggests that B-cell–associated gene expression may not just be prognostic but predictive for survival outcomes for palbociclib + ET versus capecitabine in the setting of ET resistance. Analyses of PALOMA-2 and PALOMA-3 found that high expression of PD-1 and a T-cell–inflamed tumor microenvironment signature predicted shorter PFS with palbociclib + ET,[39,47] and a recently presented data from the phase II RIGHT Choice trial demonstrated that lower T-cell expression correlated with longer PFS with ribociclib + ET.[77] Notably, one analysis of B-cell–associated gene expression also showed a correlation with worse survival with ET in ER+ breast cancer.[74] It is possible that adding a CDK4/6i may reverse this association through tumor immune microenvironment remodeling, as shown in in vivo models demonstrating an increase in type III interferons and decreased T regulatory cell proliferation with CDK4/6i.[73] This pattern of lower tumor microenvironment immune activity correlating with improved survival with CDK4/6i + ET in HR+/HER2− breast cancer contrasts with improved survival outcomes seen with higher tumor microenvironment immune activity in triple-negative[78-82] and HER2+[53,83-85] breast cancer.

Our analysis has some limitations. Sampling bias may be present, as only 54% of samples from the original PEARL study were sequenced. However, most clinical variables were not significantly different between the original study population and our patient subset or between treatment arms, and our analysis adjusted for these variables. A research version of PAM50 used in this study may produce different results than the commercial nCounter-based PAM50 assay. Confounding effects are inherently present in a retrospective exploratory study, although we attempted to limit their impact in our multivariable Cox regression analysis. A smaller sample size affects the power of our analysis, and a larger study would help to detect other expression signature

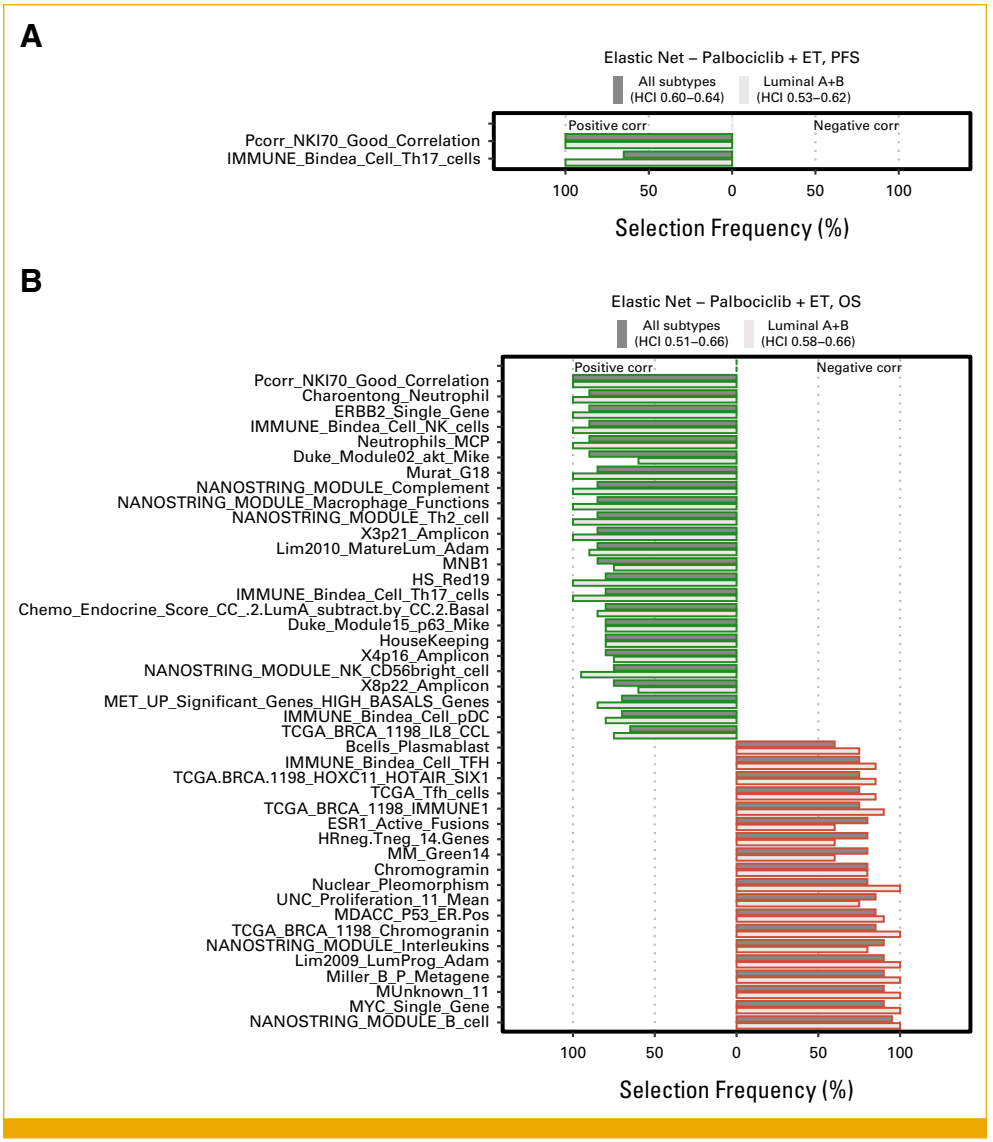

FIG 5. Most frequently selected gene expression signatures in multivariable Cox models for survival with palbociclib + ET. Models based on gene expression signatures were iteratively constructed for all patients treated with palbociclib + ET, as well as patients with luminal A or B tumors only, via high-dimensional modeling using Elastic Net regression. Bar length represents frequency with which each signature was included in a constructed model. Signatures whose values positively correlated with survival are highlighted in green, and those whose values negatively correlated with survival are highlighted in red. (A) Most frequently selected signatures for models for PFS with palbociclib + ET. (B) Most frequently selected signatures in models for OS with palbociclib + ET. ET, endocrine therapy; HCI, Harrell C-index; OS, overall survival; PFS, progression-free survival; TCGA, The Cancer Genome Atlas.

patterns and construct more robust Cox models with Elastic Net regression.

In conclusion, our analysis highlights both PAM50 intrinsic subtype and B-cell–related tumor gene expression signatures as potential predictors of response to CDK4/6i + ET versus chemotherapy for postmenopausal women with AI-resistant HR+/HER2– breast cancer. These gene expression features should be explored further in larger studies of ET with palbociclib and other CDK4/6i, particularly compared with chemotherapy in the setting of ET resistance.

AFFILIATIONS

[1]Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC
[2]Division of Oncology, Department of Medicine, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC
[3]Department of Genetics, University of North Carolina, Chapel Hill, NC
[4]GEICAM Spanish Breast Cancer Group, Madrid, Spain
[5]Institut Català d'Oncologia (ICO) & IDIBELL, L'Hospitalet de Llobregat, Barcelona, Spain
[6]CECOG, Central European Cooperative Oncology Group, Vienna, Austria
[7]Vienna Cancer Center, Medical University of Vienna and Vienna Hospital Association, Vienna, Austria
[8]Hospital Universitario Virgen del Rocío, Sevilla, Spain
[9]Medical Oncology Department, Breast Cancer Unit, University Hospital 12 de Octubre, Madrid, Spain
[10]HM Hospitales, Madrid, Spain
[11]SOLTI Group on Breast Cancer Research, Barcelona, Spain
[12]Hospital Universitari Clinic de Barcelona, Institut del Càncer i malalties de la sang, IDIBAPS, Barcelona, Spain
[13]Badalona Applied Research Group in Oncology (ARGO Group), Institut Catalá D'Oncologia, Hospital Universitari Germans Trias I Pujol, Badalona, Spain
[14]Centro de Investigación Biomédica en Red de Oncología, CIBERONC-ISCIII, Madrid, Spain
[15]Hospital Clínico Universitario de Valencia, Instituto de Investigación Sanitaria-INCLIVA, Valencia, Spain
[16]Facultad de medicina de la Universidad de Valencia, Valencia, Spain
[17]Hospital Universitario Miguel Servet, Instituto de Investigación Sanitaria Aragón-IISA, Zaragoza, Spain
[18]Department of Oncotherapy, University of Szeged, Szeged, Hungary
[19]Semmelweis University, Pankreász Betegségek Intézete, Budapest, Hungary
[20]Hospital Universitario Virgen de La Arrixaca-IMIB, Murcia, Spain
[21]Medical Oncology Department, Hospital Clínico San Carlos, Instituto de Investigación Sanitaria San Carlos (IdISSC), CIBERONC, Madrid, Spain
[22]Oncology Unit, Universitary Hospital Jaén, Jaén, Spain
[23]Hospital Universitario Lucus Augusti, Lugo, Spain
[24]Medical Oncology, Hospital Universitario de Toledo, Toledo, Spain
[25]Hospital Universitario San Pedro de Alcantara, Cáceres, Spain
[26]Hospital Clínico Universitario de Salamanca-IBSAL, Salamanca, Spain
[27]Hospital del Mar, Barcelona, Spain
[28]Former Pfizer employee, La Jolla, CA
[29]Instituto Valenciano de Oncología (IVO), Valencia, Spain
[30]Instituto de Investigación Sanitaria Gregorio Maranon, Madrid, Spain

CORRESPONDING AUTHOR

Charles M. Perou, PhD; e-mail: cperou@med.unc.edu.

DISCLAIMER

Role of the funder: Pfizer, Inc provided palbociclib and exemestane through a collaboration agreement; they did not play a role in the design of this study; or the collection, analysis, and interpretation of the data, although the company reviewed the manuscript. AstraZeneca provided fulvestrant but did not play a role in the design of the study; the collection, analysis, and interpretation of the data; the writing of the manuscript; or the decision to submit the manuscript for publication. The National Cancer Institute, the Breast Cancer Research Foundation, and Susan G. Komen did not play a role in the design of the study; the collection, analysis, and interpretation of the data; the writing of the manuscript; or the decision to submit the manuscript for publication.

PRIOR PRESENTATION

Presented in part at the The San Antonio Breast Cancer Symposium (SABCS), San Antonio, TX, December 5-9, 2023.

SUPPORT

Supported by the GEICAM Spanish Breast Cancer Group by Pfizer Inc; AstraZeneca (which provided fulvestrant); the National Cancer Institute (Breast SPORE program (P50-CA58223) and RO1-CA14876); the Breast Cancer Research Foundation; and Susan G. Komen (SAC-160074).

DATA SHARING STATEMENT

A data sharing statement provided by the authors is available with this article at DOI https://doi.org/10.1200/PO-24-00937. The molecular data in this study is submitted to the European Genome-phenome Archive (EGA)—EGAD00001015613. The data sets used and analyzed, including the clinical database of this study, are available from Dr Miguel Martín(mmartin@geicam.org) upon reasonable request.

AUTHOR CONTRIBUTIONS

Conception and design: Yash N. Agrawal, Christoph Zielinski, Eva María Ciruelos, José Luis Alonso-Romero, Pedro Sánchez-Rovira, Elena Álvarez, Lisa A. Carey, Ángel Guerrero-Zotano, Charles M. Perou
Financial support: Charles M. Perou
Administrative support: Yash N. Agrawal, Isabel Romero-Camarero, Rosalía Caballero, Charles M. Perou
Provision of study materials or patients: Christoph Zielinski, Manuel Ruiz-Borrego, Montserrat Muñoz, Begoña Bermejo, Antonio Antón, Zsuzsanna Kahan, Pedro Sánchez-Rovira, José Ignacio Chacón, Santiago González-Santiago, César A. Rodríguez, Isabel Romero-Camarero, Ángel Guerrero-Zotano, Charles M. Perou, Miguel Martín
Collection and assembly of data: Aranzazu Fernández-Martínez, Miguel Gil-Gil, Christoph Zielinski, Montserrat Muñoz, Mireia Margelí, Begoña Bermejo, Zsuzsanna Kahan, Tibor Csöszi, José Luis Alonso-Romero, Pedro Sánchez-Rovira, José Ignacio Chacón, Santiago González-Santiago, César A. Rodríguez, Sonia Servitja, Adam D. Pfefferle, Isabel Romero-Camarero, Charles M. Perou, Miguel Martín
Data analysis and interpretation: Yash N. Agrawal, Miguel Gil-Gil, Christoph Zielinski, Manuel Ruiz-Borrego, Eva María Ciruelos, Antonio Antón, José Ángel García-Saenz, Pedro Sánchez-Rovira, Jesús Herranz, Yuan Liu, Lisa A. Carey, Isabel Romero-Camarero, Rosalía Caballero, Charles M. Perou, Miguel Martín
Manuscript writing: All authors
Final approval of manuscript: All authors
Accountable for all aspects of the work: All authors

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The following represents disclosure information provided by authors of this manuscript. All relationships are considered compensated unless otherwise noted. Relationships are self-held unless noted. I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the subject matter of this manuscript. For more information about ASCO's conflict of interest policy, please refer to www.asco.org/rwc or ascopubs.org/po/author-center.
Open Payments is a public database containing information reported by companies about payments made to US-licensed physicians (Open Payments).

Yash N. Agrawal
Employment: Pfizer (I)

Miguel Gil-Gil
Honoraria: Pfizer, Daiichi Sankyo, AstraZeneca, Esteve Ph, Menarini Stemline Spain, Lilly
Consulting or Advisory Role: Daiichi Sankyo, AstraZeneca, Gilead Sciences, Seagen
Travel, Accommodations, Expenses: Novartis, Pfizer, AstraZeneca, Lilly

Christoph Zielinski
Honoraria: Pfizer (Inst), MSD Oncology (Inst), Bristol Myers Squibb Foundation (Inst), Servier (Inst), AstraZeneca (Inst), Lilly (Inst)
Consulting or Advisory Role: Roche (Inst), Merck Sharp & Dohme (Inst), AstraZeneca (Inst), Servier (Inst), Lilly (Inst), Bristol Myers Squibb (Inst)
Research Funding: Merck Sharp & Dohme (Inst)
Patents, Royalties, Other Intellectual Property: Patent Property, Imugene

Manuel Ruiz-Borrego
Honoraria: Roche/Genentech, Pfizer, Novartis
Consulting or Advisory Role: Roche, Puma Biotechnology

Eva María Ciruelos
Consulting or Advisory Role: Roche, Pfizer, AstraZeneca, Novartis, Lilly, MSD Oncology, Daiichi Sankyo/AstraZeneca, Gilead Sciences, Seagen, Reveal Genomics, Menarini Group
Speakers' Bureau: Lilly, Roche, Daiichi Sankyo/AstraZeneca, MSD Oncology
Research Funding: Roche/Genentech (Inst), Seagen (Inst), Pfizer
Travel, Accommodations, Expenses: Roche, Pfizer, AstraZeneca

Montserrat Muñoz
Consulting or Advisory Role: Seagen
Speakers' Bureau: Roche, Gilead Sciences, Esteve
Expert Testimony: Novartis, Menarini
Travel, Accommodations, Expenses: Roche, Lilly, Gilead Sciences, Pfizer, Daiichi Sankyo/AstraZeneca

Mireia Margelí
Consulting or Advisory Role: Novartis, Pfizer, Pierre Fabre, Roche, Gylead, Daiichi Sankyo, Lilly, Seagen
Research Funding: Eisai (Inst), AstraZeneca (Inst), Roche (Inst), Gylead (Inst), Seagen (Inst), Pfizer (Inst), Novartis (Inst), Lilly (Inst)

Begoña Bermejo
Consulting or Advisory Role: AstraZeneca/Daiichi Sankyo, Novartis, Lilly, Menarini, Pfizer, Gilead Sciences, Roche/Genentech, MSD Oncology
Speakers' Bureau: Roche, MSD Oncology, Novartis/Pfizer, AstraZeneca/Daiichi Sankyo, Lilly, Gilead Sciences
Travel, Accommodations, Expenses: AstraZeneca, Pfizer, Roche/Genentech

Antonio Antón
Consulting or Advisory Role: Lilly, Gilead Sciences, Daiichi Sankyo/Lilly
Speakers' Bureau: Lilly, Daiichi Sankyo/Lilly, Pfizer, Roche, AstraZeneca Spain, Novartis

Tibor Csöszi
Consulting or Advisory Role: Novartis
Speakers' Bureau: Ipsen, Janssen-Cilag
Travel, Accommodations, Expenses: Sanofi, Pfizer

José Ángel García-Saenz
Consulting or Advisory Role: Novartis, AstraZeneca, Lilly, Daiichi Sankyo, Gilead Sciences, Exact Sciences, Roche, Stemline Therapeutics
Speakers' Bureau: Lilly, Stemline Therapeutics, Exact Sciences, Gilead Sciences
Research Funding: AstraZeneca (Inst)
Travel, Accommodations, Expenses: Roche, AstraZeneca, Novartis, Daiichi Sankyo/AstraZeneca

José Ignacio Chacón
Consulting or Advisory Role: Roche, Novartis, Pfizer, Merck, Eisai, AstraZeneca Spain, Lilly, Daiichi Sankyo/AstraZeneca

Santiago González-Santiago
Consulting or Advisory Role: Pfizer
Speakers' Bureau: Novartis, GlaxoSmithKline, Daiichi Sankyo/AstraZeneca, Lilly
Travel, Accommodations, Expenses: Pfizer

César A. Rodríguez
Consulting or Advisory Role: MSD, Roche, AstraZeneca, Daiichi Sankyo Europe GmbH
Speakers' Bureau: MSD, Roche, Daiichi Sankyo/AstraZeneca, Novartis
Travel, Accommodations, Expenses: Roche, MSD, Lilly, Novartis

Sonia Servitja
Consulting or Advisory Role: Seagen, Daiichi Sankyo/AstraZeneca, Novartis, Gilead Sciences
Speakers' Bureau: Roche, Pfizer, Novartis, Eisai, MSD/AstraZeneca
Travel, Accommodations, Expenses: Daiichi Sankyo/AstraZeneca

Adam D. Pfefferle
Employment: LabCorp (I)
Stock and Other Ownership Interests: LabCorp

Yuan Liu
Employment: Pfizer
Stock and Other Ownership Interests: Pfizer

Lisa A. Carey
Research Funding: NanoString Technologies (Inst), Seagen (Inst), Veracyte (Inst), Gilead Sciences (Inst), Novartis (Inst), Lilly (Inst), Pfizer (Inst), AstraZeneca (Inst)
Uncompensated Relationships: Reveal Genomics
Open Payments Link: https://openpaymentsdata.cms.gov/physician/179671

Ángel Guerrero-Zotano
Consulting or Advisory Role: Pierre Fabre, Exact Sciences, Novartis
Expert Testimony: AstraZeneca, Pierre Fabre, Menarini, Novartis, Lilly, Pfizer
Travel, Accommodations, Expenses: Menarini, Roche, Gilead Sciences, Novartis

Charles M. Perou
Leadership: GeneCentric
Stock and Other Ownership Interests: Bioclassifier, GeneCentric, Reveal Genomics
Consulting or Advisory Role: Bioclassifier, GeneCentric, Veracyte, Reveal Genomics
Patents, Royalties, Other Intellectual Property: Royalties from PAM50 breast cancer gene patent application, and from lung gene signature patent

Miguel Martín
Stock and Other Ownership Interests: Pfizer
Honoraria: Roche/Genentech, Lilly, Pfizer, Novartis, Pierre Fabre, Seagen
Consulting or Advisory Role: Roche/Genentech, Novartis, Pfizer, Lilly, AstraZeneca, Daiichi Sankyo, Stemline-Menarini, Rovi Spain
Speakers' Bureau: Lilly/ImClone, Roche/Genentech, Pierre Fabre
Research Funding: Novartis (Inst), Roche (Inst), Puma Biotechnology (Inst)
Travel, Accommodations, Expenses: Daiichi Sankyo
Other Relationship: Roche, Novartis, AstraZeneca

No other potential conflicts of interest were reported.

REFERENCES

1. SEER*Explorer: An interactive website for SEER cancer statistics. Surveillance Research Program, National Cancer Institute. https://seer.cancer.gov/statistics-network/explorer/
2. Cosman F, Lindsay R: Selective estrogen receptor modulators: Clinical spectrum. Endocr Rev 20:418-434, 1999
3. Bross PF, Baird A, Chen G, et al: Fulvestrant in postmenopausal women with advanced breast cancer. Clin Cancer Res 9:4309-4317, 2003
4. Bidard F-C, Kaklamani VG, Neven P, et al: Elacestrant (oral selective estrogen receptor degrader) versus standard endocrine therapy for estrogen receptor–positive, human epidermal growth factor receptor 2–negative advanced breast cancer: Results from the randomized phase III EMERALD trial. J Clin Oncol 40:3246-3256, 2022

5. Paridaens RJ, Dirix LY, Beex LV, et al: Phase III study comparing exemestane with tamoxifen as first-line hormonal treatment of metastatic breast cancer in postmenopausal women: The European Organisation for Research and Treatment of Cancer Breast Cancer Cooperative Group. J Clin Oncol 26:4883-4890, 2008
6. Mouridsen H, Gershanovich M, Sun Y, et al: Phase III study of letrozole versus tamoxifen as first-line therapy of advanced breast cancer in postmenopausal women: Analysis of survival and update of efficacy from the International Letrozole Breast Cancer Group. J Clin Oncol 21:2101-2109, 2003
7. Clarke R, Skaar TC, Bouker KB, et al: Molecular and pharmacological aspects of antiestrogen resistance. J Steroid Biochem Mol Biol 76:71-84, 2001
8. Robinson DR, Wu YM, Vats P, et al: Activating ESR1 mutations in hormone-resistant metastatic breast cancer. Nat Genet 45:1446-1451, 2013
9. Toy W, Shen Y, Won H, et al: ESR1 ligand-binding domain mutations in hormone-resistant breast cancer. Nat Genet 45:1439-1445, 2013
10. Li S, Shen D, Shao J, et al: Endocrine-therapy-resistant ESR1 variants revealed by genomic characterization of breast-cancer-derived xenografts. Cell Rep 4:1116-1130, 2013
11. Jeselsohn R, Yelensky R, Buchwalter G, et al: Emergence of constitutively active estrogen receptor-α mutations in pretreated advanced estrogen receptor–positive breast cancer. Clin Cancer Res 20:1757-1767, 2014
12. Merenbakh-Lamin K, Ben-Baruch N, Yeheskel A, et al: D538G mutation in estrogen receptor-α: A novel mechanism for acquired endocrine resistance in breast cancer. Cancer Res 73:6856-6864, 2013
13. Caldon CE, Daly RJ, Sutherland RL, et al: Cell cycle control in breast cancer cells. J Cell Biochem 97:261-274, 2006
14. Stendahl M, Kronblad A, Rydén L, et al: Cyclin D1 overexpression is a negative predictive factor for tamoxifen response in postmenopausal breast cancer patients. Br J Cancer 90:1942-1948, 2004
15. Akli S, Zheng PJ, Multani AS, et al: Tumor-specific low molecular weight forms of cyclin E induce genomic instability and resistance to p21, p27, and antiestrogens in breast cancer. Cancer Res 64: 3198-3208, 2004
16. Butt AJ, McNeil CM, Musgrove EA, et al: Downstream targets of growth factor and oestrogen signalling and endocrine resistance: The potential roles of c-myc, cyclin D1 and cyclin E. Endocr Relat Cancer 2005;12(Suppl 1):S47-59
17. Thangavel C, Dean JL, Ertel A, et al: Therapeutically activating RB: Reestablishing cell cycle control in endocrine therapy-resistant breast cancer. Endocr Relat Cancer 18:333-345, 2011
18. Miller TW, Balko JM, Fox EM, et al: ERα-dependent E2F transcription can mediate resistance to estrogen deprivation in human breast cancer. Cancer Discov 1:338-351, 2011
19. Schiff R, Massarweh SA, Shou J, et al: Cross-talk between estrogen receptor and growth factor pathways as a molecular target for overcoming endocrine resistance. Clin Cancer Res 10: 331S-336S, 2004
20. Shou J, Massarweh S, Osborne CK, et al: Mechanisms of tamoxifen resistance: Increased estrogen receptor-HER2/neu cross-talk in ER/HER2-positive breast cancer. J Natl Cancer Inst 96:926-935, 2004
21. Arpino G, Wiechmann L, Osborne CK, et al: Crosstalk between the estrogen receptor and the HER tyrosine kinase receptor family: Molecular mechanism and clinical implications for endocrine therapy resistance. Endocr Rev 29:217-233, 2008
22. Choi YJ, Anders L: Signaling through cyclin D-dependent kinases. Oncogene 33:1890-1903, 2014
23. Finn RS, Crown JP, Lang I, et al: The cyclin-dependent kinase 4/6 inhibitor palbociclib in combination with letrozole versus letrozole alone as first-line treatment of oestrogen receptor-positive, HER2-negative, advanced breast cancer (PALOMA-1/TRIO-18): A randomised phase 2 study. Lancet Oncol 16:25-35, 2015
24. Finn RS, Crown J, Lang I, et al: Overall survival results from the randomized phase II study of palbociclib (P) in combination with letrozole (L) vs letrozole alone for frontline treatment of ER+/ HER2− advanced breast cancer (PALOMA-1; TRIO-18). J Clin Oncol 2017;35(15_suppl):1001
25. Finn RS, Martin M, Rugo HS, et al: Palbociclib and letrozole in advanced breast cancer. N Engl J Med 375:1925-1936, 2016
26. Turner NC, Ro J, André F, et al: Palbociclib in hormone-receptor-positive advanced breast cancer. N Engl J Med 373:209-219, 2015
27. Turner NC, Slamon DJ, Ro J, et al: Overall survival with palbociclib and fulvestrant in advanced breast cancer. N Engl J Med 379:1926-1936, 2018
28. Sonke GS, van Ommen-Nijhof A, Wortelboer N, et al: Primary outcome analysis of the phase 3 SONIA trial (BOOG 2017-03) on selecting the optimal position of cyclin-dependent kinases 4 and 6 (CDK4/6) inhibitors for patients with hormone receptor-positive (HR+), HER2-negative (HER2-) advanced breast cancer (ABC). J Clin Oncol 2023;41(17_suppl):LBA1000
29. Kalinsky K, Bianchini G, Hamilton EP, et al: Abemaciclib plus fulvestrant vs fulvestrant alone for HR+, HER2- advanced breast cancer following progression on a prior CDK4/6 inhibitor plus endocrine therapy: Primary outcome of the phase 3 postMONARCH trial. J Clin Oncol 2024;42(17_suppl):LBA1001
30. Kalinsky K, Accordino MK, Chiuzan C, et al: Randomized phase II trial of endocrine therapy with or without ribociclib after progression on cyclin-dependent kinase 4/6 inhibition in hormone receptor-positive, human epidermal growth factor receptor 2-negative metastatic breast cancer: MAINTAIN trial. J Clin Oncol 41:4004-4013, 2023
31. Turner NC, Oliveira M, Howell SJ, et al: Capivasertib in hormone receptor-positive advanced breast cancer. N Engl J Med 388:2058-2070, 2023
32. Cardoso F, Paluch-Shimon S, Schumacher-Wulf E, et al: 6th and 7th international consensus guidelines for the management of advanced breast cancer (ABC guidelines 6 and 7). Breast 76:103756, 2024
33. Gennari A, André F, Barrios CH, et al: ESMO Clinical Practice Guideline for the diagnosis, staging and treatment of patients with metastatic breast cancer. Ann Oncol 32:1475-1495, 2021
34. Turner NC, Finn RS, Martin M, et al: Clinical considerations of the role of palbociclib in the management of advanced breast cancer patients with and without visceral metastases. Ann Oncol 29: 669-680, 2018
35. Loibl S, Turner NC, Ro J, et al: Palbociclib combined with fulvestrant in premenopausal women with advanced breast cancer and prior progression on endocrine therapy: PALOMA-3 results. Oncologist 22:1028-1038, 2017
36. Iwata H, Im SA, Masuda N, et al. PALOMA-3: Phase III trial of fulvestrant with or without palbociclib in premenopausal and postmenopausal women with hormone receptor-positive, human epidermal growth factor receptor 2-negative metastatic breast cancer that progressed on prior endocrine therapy-safety and efficacy in Asian patients. JCO Glob Oncol 10.1200/jgo.2016.008318
37. Guerrero-Zotano Á, Belli S, Zielinski C, et al: CCNE1 and PLK1 mediate resistance to palbociclib in HR+/HER2- metastatic breast cancer. Clin Cancer Res 29:1557-1568, 2023
38. Turner NC, Liu Y, Zhu Z, et al: Cyclin E1 expression and palbociclib efficacy in previously treated hormone receptor-positive metastatic breast cancer. J Clin Oncol 37:1169-1178, 2019
39. Finn RS, Liu Y, Zhu Z, 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 26:110-121, 2020
40. Prat A, Chaudhury A, Solovieff N, et al: Correlative biomarker analysis of intrinsic subtypes and efficacy across the MONALEESA phase III studies. J Clin Oncol 39:1458-1467, 2021
41. O'Leary B, Cutts RJ, Liu Y, et al: The genetic landscape and clonal evolution of breast cancer resistance to palbociclib plus fulvestrant in the PALOMA-3 trial. Cancer Discov 8:1390-1403, 2018
42. Condorelli R, Spring L, O'Shaughnessy J, et al: Polyclonal RB1 mutations and acquired resistance to CDK 4/6 inhibitors in patients with metastatic breast cancer. Ann Oncol 29:640-645, 2018
43. Wander SA, Han HS, Zangardi ML, et al. Clinical outcomes with abemaciclib after prior CDK4/6 inhibitor progression in breast cancer: A multicenter experience. J Natl Compr Canc Netw. 2021;19: 1–8
44. Wander SA, Cohen O, Gong X, et al: The genomic landscape of intrinsic and acquired resistance to cyclin-dependent kinase 4/6 inhibitors in patients with hormone receptor-positive metastatic breast cancer. Cancer Discov 10:1174-1193, 2020
45. Dean JL, Thangavel C, McClendon AK, et al: Therapeutic CDK4/6 inhibition in breast cancer: Key mechanisms of response and failure. Oncogene 29:4018-4032, 2010
46. Yang C, Li Z, Bhatt T, et al: Acquired CDK6 amplification promotes breast cancer resistance to CDK4/6 inhibitors and loss of ER signaling and dependence. Oncogene 36:2255-2264, 2017
47. Zhu Z, Turner NC, Loi S, et al: Comparative biomarker analysis of PALOMA-2/3 trials for palbociclib. NPJ Precis Oncol 6:56, 2022
48. Turner NC, Swift C, Kilburn L, et al: ESR1 mutations and overall survival on fulvestrant versus exemestane in advanced hormone receptor–positive breast cancer: A combined analysis of the phase III SoFEA and EFECT trials. Clin Cancer Res 26:5172-5177, 2020
49. Martin M, Zielinski C, Ruiz-Borrego M, 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 32:488-499, 2021
50. Martín M, Zielinski C, Ruiz-Borrego M, et al: Overall survival with palbociclib plus endocrine therapy versus capecitabine in postmenopausal patients with hormone receptor-positive, HER2-negative metastatic breast cancer in the PEARL study. Eur J Cancer 168:12-24, 2022
51. Parker JS, Mullins M, Cheang MC, et al: Supervised risk predictor of breast cancer based on intrinsic subtypes. J Clin Oncol 27:1160-1167, 2009
52. Zhao X, Rødland EA, Tibshirani R, et al: Molecular subtyping for clinically defined breast cancer subgroups. Breast Cancer Res 17:29, 2015
53. Fernandez-Martinez A, Krop IE, Hillman DW, et al: Survival, pathologic response, and genomics in CALGB 40601 (Alliance), a neoadjuvant phase III trial of paclitaxel-trastuzumab with or without lapatinib in HER2-positive breast cancer. J Clin Oncol 38:4184-4193, 2020
54. Fan C, Prat A, Parker J, et al: Building prognostic models for breast cancer patients using clinical variables and hundreds of gene expression signatures. BMC Med Genomics 4:3, 2011
55. Gatza ML, Silva GO, Parker JS, et al: An integrated genomics approach identifies drivers of proliferation in luminal-subtype human breast cancer. Nat Genet 46:1051-1059, 2014
56. Garcia-Recio S, Thennavan A, East MP, et al: FGFR4 regulates tumor subtype differentiation in luminal breast cancer and metastatic disease. J Clin Invest 130:4871-4887, 2020
57. Liberzon A, Birger C, Thorvaldsdóttir H, et al: The Molecular Signatures Database (MSigDB) hallmark gene set collection. Cell Syst 1:417-425, 2015
58. Zou H, Hastie T: Regularization and variable selection via the Elastic Net. J R Stat Soc 67:301-320, 2005
59. Harrell FE Jr, Lee KL, Califf RM, et al: Regression modelling strategies for improved prognostic prediction. Stat Med 3:143-152, 1984

60. Hollern DP, Xu N, Thennavan A, et al: B cells and T follicular helper cells mediate response to checkpoint inhibitors in high mutation burden mouse models of breast cancer. Cell 179: 1191-1206.e21, 2019
61. Herschkowitz JI, He X, Fan C, et al: The functional loss of the retinoblastoma tumour suppressor is a common event in basal-like and luminal B breast carcinomas. Breast Cancer Res 10:R75, 2008
62. Iglesia MD, Vincent BG, Parker JS, et al: Prognostic B-cell signatures using mRNA-seq in patients with subtype-specific breast and ovarian cancer. Clin Cancer Res 20:3818-3829, 2014
63. van de Vijver MJ, He YD, van't Veer LJ, et al: A gene-expression signature as a predictor of survival in breast cancer. N Engl J Med 347:1999-2009, 2002
64. Bindea G, Mlecnik B, Tosolini M, et al: Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer. Immunity 39:782-795, 2013
65. Charoentong P, Finotello F, Angelova M, et al: Pan-cancer immunogenomic analyses reveal genotype-immunophenotype relationships and predictors of response to checkpoint blockade. Cell Rep 18:248-262, 2017
66. Helmink BA, Reddy SM, Gao J, et al: B cells and tertiary lymphoid structures promote immunotherapy response. Nature 577:549-555, 2020
67. Thorsson V, Gibbs DL, Brown SD, et al: The immune landscape of cancer. Immunity 48:812-830.e14, 2018
68. Dybkær K, Bøgsted M, Falgreen S, et al: Diffuse large B-cell lymphoma classification system that associates normal B-cell subset phenotypes with prognosis. J Clin Oncol 33:1379-1388, 2015
69. Gatza ML, Lucas JE, Barry WT, et al: A pathway-based classification of human breast cancer. Proc Natl Acad Sci USA 107:6994-6999, 2010
70. Petitprez F, de Reyniès A, Keung EZ, et al: B cells are associated with survival and immunotherapy response in sarcoma. Nature 577:556-560, 2020
71. Baldwin LA, Bartonicek N, Yang J, et al: DNA barcoding reveals ongoing immunoediting of clonal cancer populations during metastatic progression and immunotherapy response. Nat Commun 13:6539, 2022
72. Liu R, Wang X, Chen GY, et al: The prognostic role of a gene signature from tumorigenic breast-cancer cells. N Engl J Med 356:217-226, 2007
73. Goel S, DeCristo MJ, Watt AC, et al: CDK4/6 inhibition triggers anti-tumour immunity. Nature 548:471-475, 2017
74. Lee S, Kang BH, Lee HB, et al: B-cell-mediated immunity predicts survival of patients with estrogen receptor-positive breast cancer. JCO Precis Oncol 10.1200/po.23.00263
75. Prat A, Pineda E, Adamo B, et al: Clinical implications of the intrinsic molecular subtypes of breast cancer. Breast 2015;24(Suppl 2):S26–S35
76. Wallden B, Storhoff J, Nielsen T, et al: Development and verification of the PAM50-based Prosigna breast cancer gene signature assay. BMC Med Genomics 8:54, 2015
77. Lu Y-S, Hsu C-L, Bin Mohd Mahidin EI, et al: First-line (1L) ribociclib (RIB) + endocrine therapy (ET) vs combination chemotherapy (combo CT) in clinically aggressive HR+/HER2- advanced breast cancer (ABC): A subgroup analysis of RIGHT Choice by intrinsic subtype & gene & signature expression. Clin Cancer Res 2025;31(12_Suppl):PS2–06
78. Quintana Á, Arenas EJ, Bernadó C, et al: Evaluation of triple negative breast cancer with heterogeneous immune infiltration. Front Immunol 14:1149747, 2023
79. Tomioka N, Azuma M, Ikarashi M, et al: The therapeutic candidate for immune checkpoint inhibitors elucidated by the status of tumor-infiltrating lymphocytes (TILs) and programmed death ligand 1 (PD-L1) expression in triple negative breast cancer (TNBC). Breast Cancer 25:34-42, 2018
80. Denkert C, von Minckwitz G, Brase JC, et al: Tumor-infiltrating lymphocytes and response to neoadjuvant chemotherapy with or without carboplatin in human epidermal growth factor receptor 2-positive and triple-negative primary breast cancers. J Clin Oncol 33:983-991, 2015
81. Denkert C, von Minckwitz G, Darb-Esfahani S, et al: Tumour-infiltrating lymphocytes and prognosis in different subtypes of breast cancer: A pooled analysis of 3771 patients treated with neoadjuvant therapy. Lancet Oncol 19:40-50, 2018
82. Cortes J, Rugo HS, Cescon DW, et al: Pembrolizumab plus chemotherapy in advanced triple-negative breast cancer. N Engl J Med 387:217-226, 2022
83. Prat A, Guarneri V, Pascual T, et al: Development and validation of the new HER2DX assay for predicting pathological response and survival outcome in early-stage HER2-positive breast cancer. EBioMedicine 75:103801, 2022
84. Fernandez-Martinez A, Pascual T, Singh B, et al: Prognostic and predictive value of immune-related gene expression signatures vs tumor-infiltrating lymphocytes in early-stage ERBB2/HER2-positive breast cancer: A correlative analysis of the CALGB 40601 and PAMELA trials. JAMA Oncol 9:490-499, 2023
85. Rediti M, Fernandez-Martinez A, Venet D, et al: Immunological and clinicopathological features predict HER2-positive breast cancer prognosis in the neoadjuvant NeoALTTO and CALGB 40601 randomized trials. Nat Commun 14:7053, 2023

APPENDIX

TABLE A1. Biomarker Signature Interaction With Treatment

	PFS		OS	
Signature (2-tile)	*P*	Bonferroni	*P*	Bonferroni
Basal-like correlation	.68	1.00	.94	1.00
HER2-E correlation	.10	1.00	.85	1.00
Luminal A correlation	.13	1.00	.95	1.00
Luminal B correlation	.13	1.00	.40	1.00
PAM50_Proliferation_Score	.03	0.48	.37	1.00
CCNE1	.76	1.00	.17	1.00
IGG_Cluster	.07	1.00	.006	0.10
TCGA_BRCA_1198_IMMUNE1	.04	0.63	<.001	0.01
Bcell_Tcell_Cooperation	.68	1.00	.40	1.00
IMMUNE_Bindea_ Cell_T_helper_cells	.13	1.00	.59	1.00
CD8_cluster_Iglesia	.87	1.00	.56	1.00
CD274	.72	1.00	.65	1.00
RB_LOH	.07	1.00	.36	1.00
FOS_JUN	.02	0.38	.26	1.00
FGFR4_Repressed	.85	1.00	.87	1.00
FGFR4_Induced	.32	1.00	1.00	1.00

NOTE. *P* values (unadjusted and adjusted via Bonferroni method) for treatment interaction are reported for the 16 selected biomarker signatures with respect to PFS and OS end points. Signatures are treated as binary variables with respect to median expression.
Abbreviations: OS, overall survival; PFS, progression-free survival; TCGA, The Cancer Genome Atlas.

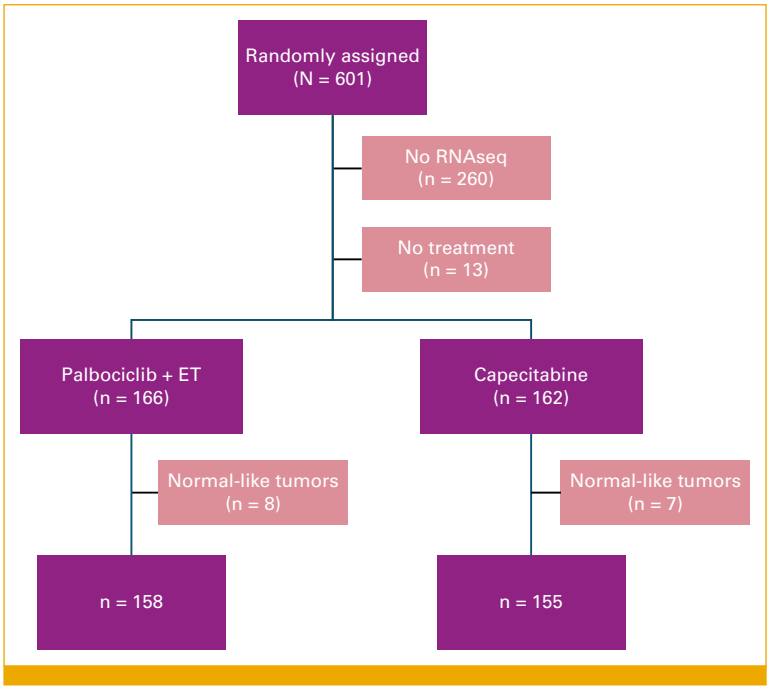

FIG A1. CONSORT diagram of the study. ET, endocrine therapy.

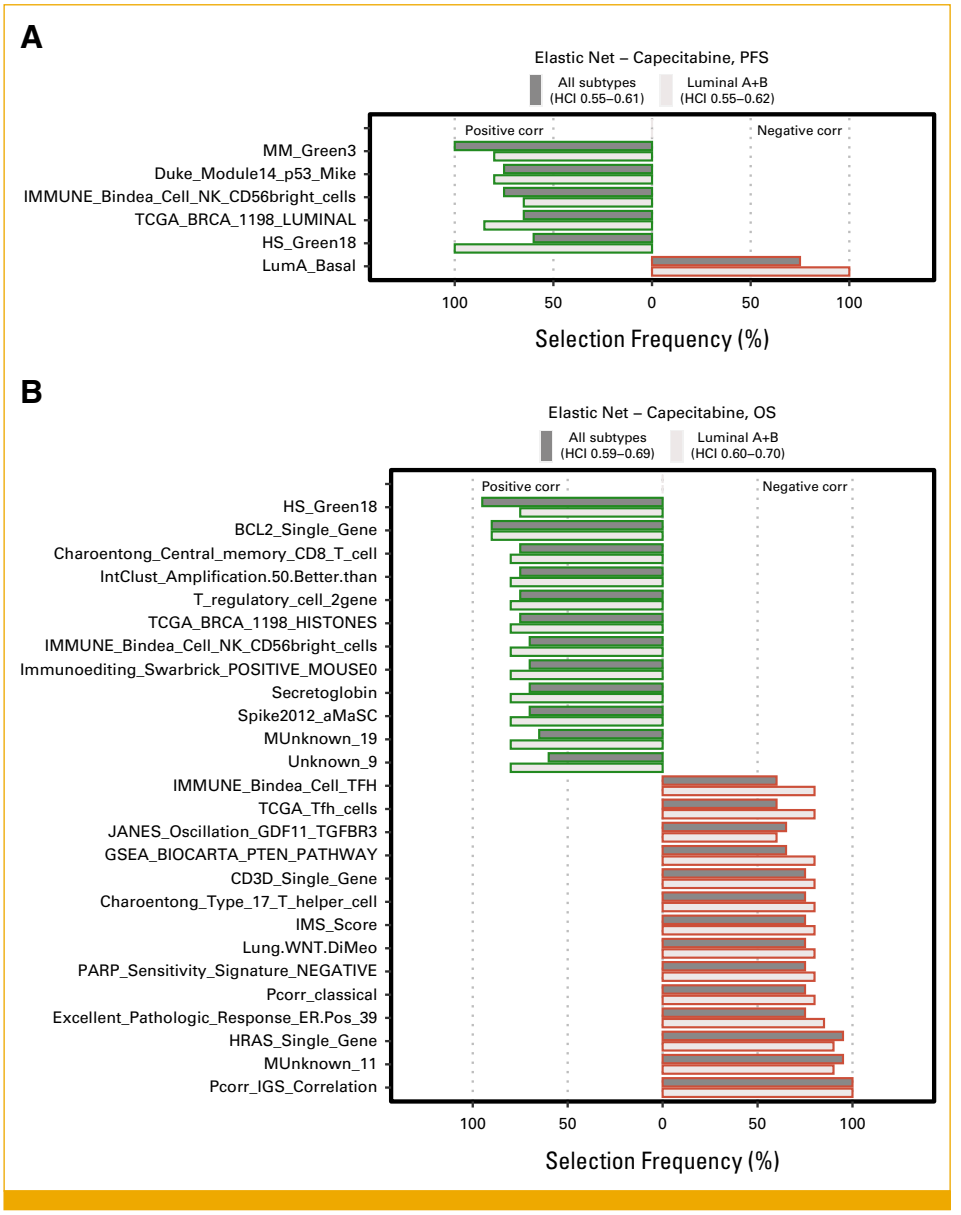

FIG A2. Most frequently selected gene expression signatures in multivariable Cox models for survival with capecitabine. Models based on gene expression signatures were iteratively constructed for all patients treated with capecitabine, as well as patients with luminal A or B tumors only, via high-dimensional modeling using Elastic Net regression. Bar length represents frequency with which each signature was included in a constructed model. Signatures whose values positively correlated with survival are highlighted in green, and those whose values negatively correlated with survival are highlighted in red. (A) Most frequently selected signatures for models for PFS with capecitabine. (B) Most frequently selected signatures in models for OS with capecitabine. corr, correlation; HCI, Harrell C-index; OS, overall survival; PFS, progression-free survival; TCGA, The Cancer Genome Atlas.