original reports abstract

Correlative Biomarker Analysis of Intrinsic Subtypes and Efficacy Across the MONALEESA Phase III Studies

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PURPOSE The prognostic and predictive value of intrinsic subtypes in hormone receptor–positive, human epidermal growth factor receptor 2–negative advanced breast cancer treated with endocrine therapy and ribociclib (RIB) is currently unknown. We evaluated the association of intrinsic subtypes with progression-free survival (PFS) in the MONALEESA trials.

METHODS A retrospective and exploratory PAM50-based analysis of tumor samples from the phase III MONALEESA-2, MONALEESA-3, and MONALEESA-7 trials was undertaken. The prognostic relationship of PAM50-based subtypes with PFS and risk of disease progression by subtype and treatment were evaluated using a multivariable Cox proportional hazards model, adjusting for age, prior chemotherapy, performance status, visceral disease, bone-only metastases, histological grade, number of metastatic sites, prior endocrine therapy, and de novo metastatic disease.

RESULTS Overall, 1,153 tumors from the RIB (n = 667) and placebo (n = 486) cohorts were robustly profiled. Subtype distribution was luminal A (LumA), 46.8%; luminal B (LumB), 24.0%; normal-like, 14.1%; HER2-enriched (HER2E), 12.6%; and basal-like, 2.4% and was generally consistent across treatment arms and trials. The associations between subtypes and PFS were statistically significant in both arms ($P < .001$). The risks of disease progression for LumB, HER2E, and basal-like subtypes were 1.44, 2.32, and 3.87 times higher compared with those for LumA, respectively. All subtypes except basal-like demonstrated significant PFS benefit with RIB. HER2E (hazard ratio [HR], 0.40; $P < .0001$), LumB (HR, 0.52; $P < .0001$), LumA (HR, 0.63; $P = .0007$), and normal-like (HR, 0.47; $P = .0005$) subtypes derived benefit from RIB. Patients with basal-like subtype (n = 28) did not derive benefit from RIB (HR, 1.14; $P = .78$).

CONCLUSION In this retrospective exploratory analysis of hormone receptor–positive and human epidermal growth factor receptor 2–negative advanced breast cancer, each intrinsic subtype exhibited a consistent PFS benefit with RIB, except for basal-like.

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INTRODUCTION

Hormone receptor–positive (HR+) and human epidermal growth factor receptor 2–negative (HER2−) advanced breast cancer (ABC) consists of a clinically heterogeneous group of tumors with different prognoses and responses to endocrine therapy (ET) and chemotherapy.[1,2] Recently, the addition of cyclin-dependent kinase 4 and 6 (CDK4/6) inhibitors to ET has been considered the standard therapy for most patients with HR+ and HER2− ABC because of clinically meaningful increases in both progression-free survival (PFS)[3-5] and overall survival (OS).[6,7] In this context, no prognostic and/or predictive biomarkers have been clinically implemented.

Previously, studies evaluating gene expression patterns in HR+ and HER2− ABC have identified not only the classical luminal A (LumA) and luminal B (LumB) subtypes but also a substantial proportion (5.8%-34.2%) of HER2-enriched (HER2E) and basal-like subtypes.[8-11] In a retrospective correlative analysis of 644 patients with HR+ and HER2– ABC in the EGF3008 trial, a first-line study of letrozole with or without lapatinib in HR+ ABC, intrinsic subtypes were independently associated with PFS and OS.[9] A similar observation was identified in a retrospective correlative analysis of the BOLERO-2 trial.[10] Thus, intrinsic subtypes are prognostic in HR+ and HER2− ABC treated

ASSOCIATED CONTENT

Data Supplement

Author affiliations and support information (if applicable) appear at the end of this article.

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CONTEXT

Key Objective

What is the association of the PAM50-based intrinsic subtypes (ie, luminal A and B, HER2-enriched, and basal-like) with prognosis and treatment benefit among patients with hormone receptor–positive and HER2-negative (HR+ and HER2–) advanced breast cancer enrolled in the MONALEESA (ML) phase III trial program (ML-2, ML-3, and ML-7)?

Knowledge Generated

The intrinsic subtypes are strongly and independently associated with progression-free survival in HR+ and HER2– advanced breast cancer treated with endocrine-based therapy. In addition, a consistent benefit with ribociclib was observed across all subtypes, except for basal-like.

Relevance

HR+ and HER2– breast cancer is a heterogeneous biological disease with different prognosis and treatment benefits. Our results highlight the importance of tissue sample collection in the setting of large randomized trials.

with ET. However, the value of intrinsic subtypes in the context of ET plus CDK4/6 inhibition is unclear.[8,12]

The phase III MONALEESA (ML) trials compared the efficacy of ribociclib (RIB) plus ET with ET alone in patients with HR+ and HER2− ABC. Each ML trial has shown a significant PFS benefit of RIB plus ET compared with ET alone (ML-2: hazard ratio [HR], 0.56 [95% CI, 0.43 to 0.77]; ML-3: HR, 0.59 [95% CI, 0.480 to 0.732]; ML-7: HR, 0.55 [95% CI, 0.44 to 0.69]).[4,5,13]

We evaluated the association of intrinsic subtypes with prognosis and/or treatment benefit in terms of PFS and overall response rate (ORR) in tumor samples from the ML phase III trials.[3,5-7] Pooling samples from the ML trials allowed for increased sample size for this retrospective, exploratory analysis.

METHODS

Study Designs and Patients

The ML trials were phase III, randomized, double-blind, placebo (PBO)-controlled, multicenter studies. The ML-2 trial (ClinicalTrials.gov identifier: NCT01958021) included postmenopausal women with locally determined HR+ and HER2− ABC who had not received previous systemic therapy for ABC.[13] In ML-2, 668 postmenopausal women with HR+ and HER2− recurrent or metastatic breast cancer were randomly assigned (1:1) to RIB plus letrozole or PBO plus letrozole.[13]

The ML-3 trial (ClinicalTrials.gov identifier: NCT02422615) evaluated RIB plus fulvestrant versus PBO plus fulvestrant in postmenopausal women and men with locally determined HR+ and HER2− ABC.[5] A total of 726 patients were randomly assigned (2:1) to receive RIB plus fulvestrant (n = 484) or PBO plus fulvestrant (n = 242).

The ML-7 trial (ClinicalTrials.gov identifier: NCT02278120) included women who were premenopausal or perimenopausal at the time of study entry and had locally determined HR+ and HER2− ABC.[4] A total of 672 patients were randomly assigned (1:1) to receive RIB or matching PBO with either tamoxifen or nonsteroidal aromatase inhibitor, all with goserelin.

The primary end point for each ML trial was locally assessed PFS. All patients provided written informed consent. The ML trials were performed in accordance with Good Clinical Practice guidelines and the World Medical Association Declaration of Helsinki. An independent ethics committee or institutional review board at each site approved the study protocols and any modifications. Study conduct was overseen by a steering committee including Novartis representatives and participating international investigators. Safety data were assessed by an independent data monitoring committee.

Procedures

Collection of formalin-fixed paraffin-embedded tumor samples (ie, a tumor block or slides) was mandatory from each ML trial, and a metastatic sample was preferable. A central lab reviewed each tumor sample to assure a minimum of 10% tumor content. Gene expression was assessed using a custom CodeSet gene panel (list available upon request) and the nCounter platform (both from NanoString Technologies, Seattle, WA), including 36 of the 50 Prosigna Breast Cancer Prognostic Gene Signature Assay (formerly known and abbreviated here as PAM50) genes.[14] The testing was performed using input of 100 ng of total RNA extracted from primary (72.0%) or metastatic (28.0%) tumors. Positive control and housekeeping gene normalization were performed on the NanoString raw counts; log2 transformation was performed on the normalized counts. Samples with < 20 counts in $> 80\%$ of genes were discarded.

Gene sets with fewer than 50 PAM50 genes have worse accuracies in subtype calling, particularly for tumors of the

LumB and HER2E subtypes.[15] To overcome this and to robustly identify PAM50 subtypes in the ML tumor samples, 48 independent formalin-fixed paraffin-embedded breast tumors with a known PAM50 or Prosigna (NanoString Technologies, Seattle, WA) subtype (LumA [HR+ and HER2–], 10; LumB [HR+ and HER2–], 10; HER2E [HER2+], 10; basal-like [triple-negative], 9; and true normal, 9) were evaluated using the same protocol used for ML samples. A total of 152 genes were selected based on their ability to identify the PAM50 subtypes in this 48-sample set and the original PAM50 microarray training data set.[14] PAM50 subtyping of the ML tumors was performed as previously described[16] using the 152 PAM50-based genes (Data Supplement, online only). Genomic analyses were performed blinded from clinical data. Samples with fewer than 50% of housekeeping genes above the background noise (defined at 26 counts) were removed from the analysis.

Outcomes

The primary objective of this exploratory analysis was to evaluate the association of intrinsic subtypes with PFS. The secondary objective was to evaluate the association of the intrinsic subtypes with treatment benefit in terms of PFS and ORR.

Statistical Analysis

Multivariable Cox proportional hazard regression analyses were used to investigate the association of the intrinsic subtypes with PFS. Multivariable models were adjusted for known clinical prognostic factors, including age, prior chemotherapy, Eastern Cooperative Oncology Group performance status, presence of visceral disease (liver or lung metastases), presence of bone-only metastases, histological grade, number of metastatic sites, prior ET, and presence of de novo metastatic disease. Kaplan-Meier curves were generated, and median PFS (95% CI) was estimated by subtype and treatment arm. ORRs were expressed as percentage (n) and compared by χ^2 test or Fisher exact test. Statistical analyses were performed using R 3.4.3 software.[17] The *P* values generated are descriptive and were not adjusted for multiplicity or false discovery.

RESULTS

A total of 1,295 of 1,452 (89.2%) tumor samples were profiled for subtype across the ML-2, ML-3, and ML-7 trials; 1,153 samples passed the quality control measures (Data Supplement). The clinical-pathological characteristics of

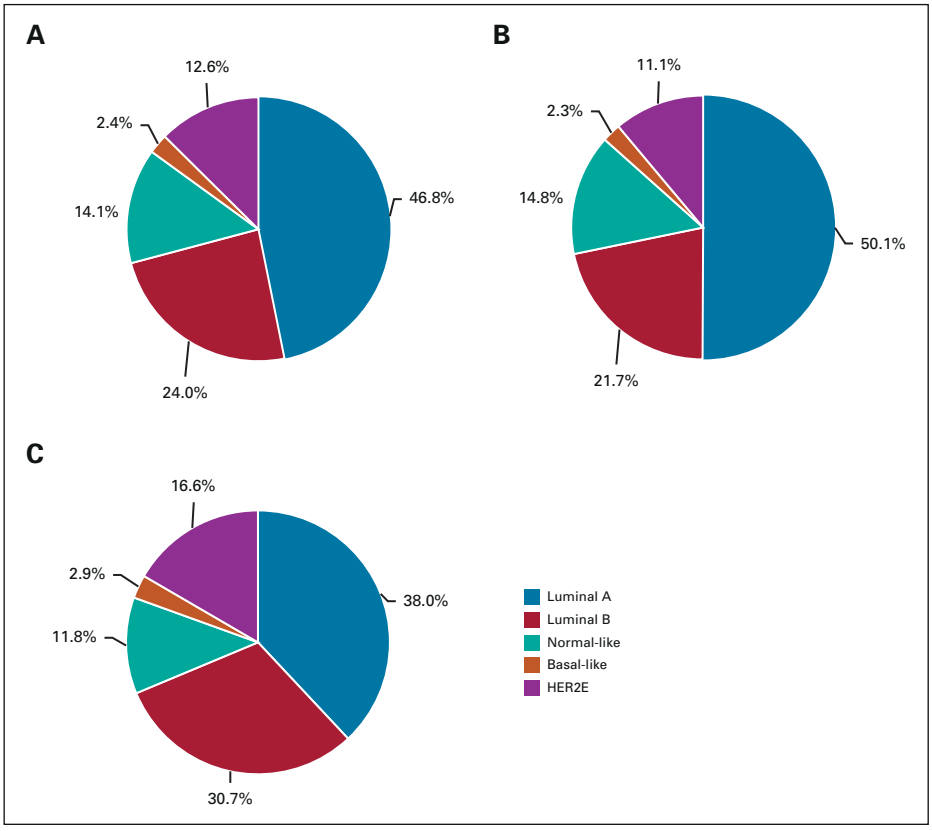

FIG 1. Intrinsic subtype distribution across the ML trials based on PAM50 analysis. (A) All patients pooled from the ML-2, ML-3, and ML-7 trials. (B) Patients with PAM50 performed in primary tumor samples. (C) Patients with PAM50 performed in metastatic tumor samples. HER2E, human epidermal growth factor receptor 2–enriched; ML, MONALEESA.

TABLE 1. Multivariable Cox Analyses of Prognostic Variables in the Combined MONALEESA Data Set

Cohort	Variable	Adjusted HR[a]	95% CI	*P*
RIB arm	LumA	1.00	—	—
	LumB	1.19	0.86 to 1.64	.29
	HER2E	1.79	1.28 to 2.50	.0006
	Basal-like	5.36	2.81 to 10.20	< .0001
	Normal-like	0.92	0.62 to 1.36	.66
PBO arm	LumA	1.00	—	—
	LumB	1.66	1.23 to 2.24	.00099
	HER2E	3.23	2.16 to 4.83	< .0001
	Basal-like	2.92	1.36 to 6.25	.0059
	Normal-like	1.68	1.19 to 2.38	.0035
All patients	LumA	1.00	—	—
	LumB	1.44	1.16 to 1.79	.00089
	HER2E	2.32	1.80 to 2.98	< .0001
	Basal-like	3.87	2.39 to 6.27	< .0001
	Normal-like	1.28	0.99 to 1.65	.058

Abbreviations: HER2E, human epidermal growth factor receptor 2–enriched; HR, hazard ratio; LumA, luminal A; LumB, luminal B; PBO, placebo; RIB, ribociclib.

[a]Obtained from multivariable Cox model including age, race, prior chemotherapy, Eastern Cooperative Oncology Group performance status, presence of visceral disease (liver or lung metastases), presence of bone-only metastases, histological grade, number of metastatic sites, prior endocrine therapy, presence of de novo metastatic disease, and tumor type (primary or metastatic) as covariates.

the patients with subtype data were balanced compared with the patients included in the original studies (Data Supplement), and no systematic bias for low quality of samples was observed based on race, prior chemotherapy, Eastern Cooperative Oncology Group performance status, histological grade, ET sensitivity, or number of metastases. At the same time, low quality of samples did differ by tissue type (metastatic, 30% *v* primary, 15%), site of metastasis (liver or lung *v* bone-only), and de novo disease (15% *v* 22% in patients without de novo disease). Most tumor samples profiled (72.0%) were primary tumors. Subtype distribution was LumA, 46.8% (n = 540); LumB, 24.0% (n = 277); normal-like, 14.1% (n = 163); HER2E, 12.6% (n = 145); and basal-like, 2.4% (n = 28; Fig 1A).

Statistically significant differences in subtype distribution were observed across studies ($P < .001$) and based on type of tumor tissue ($P = .005$). The rate of HER2E subtype in ML-2 was lower than that in ML-3 and ML-7 (7.5%, 14.7%, and 15.1%, respectively), and the rate of LumA was lower in ML-7 compared with that in ML-2 and ML-3 (39.9%, 50.0%, and 49.0%, respectively; Data Supplement). The rate of basal-like subtype remained stable at 1.0%-4.5% across the three studies. Although subtype distribution in primary tumors was similar to that observed in the analysis of all tumors, metastatic tumors had higher rates of HER2E and LumB diseases versus primary tumors (HER2E, 16.6% *v* 11.1%; LumB, 30.7% *v* 21.7%) and lower rates of LumA disease (38.0% *v* 50.1%; Figs 1B and 1C). In the ML-3 trial, subtype distribution did not differ by prior ET (Data Supplement). In the ML-7 trial, subtype distribution did not differ by prior chemotherapy for advanced disease (Data Supplement). Finally, time from biopsy and random assignment date was longer for primary biopsies (median, 372 days; Q1, 43 days; Q3, 1,743 days) than metastatic biopsies (median, 42 days; Q1, 28 days; Q3, 106 days).

Prognosis in Each Treatment Arm Based on Intrinsic Subtype

In both treatment arms, intrinsic subtype was independently associated with PFS ($P < .001$), after adjusting for clinical-pathologic variables (Table 1; Data Supplement). Compared with LumA, all other subtypes except for normal-like exhibited a significantly worse PFS. In both treatment arms, median PFS differed across intrinsic subtypes (Fig 2). The small sample size and wide 95% CIs in the basal-like cohort should be noted.

Prognosis in All Patients Based on Intrinsic Subtype

Intrinsic subtype was independently associated with PFS ($P < .0001$), after adjusting for clinical-pathologic variables and treatment arm. Compared with LumA, LumB, HER2E, normal-like, and basal-like subtypes showed 1.4, 2.3, 1.3, and 3.9 times higher risks of disease progression, respectively, after adjusting for other clinical-pathologic variables (Table 1; Data Supplement). Univariate associations of intrinsic markers with outcomes were also evaluated (Data Supplement).

Treatment Benefit Based on Intrinsic Subtype

All subtypes except basal-like exhibited a significant PFS benefit with RIB (Table 2). Patients with HER2E (HR, 0.40; 95% CI, 0.26 to 0.62; $P < .001$), LumB (HR, 0.52; 95% CI, 0.38 to 0.72; $P < .001$), LumA (HR, 0.63; 95% CI, 0.49 to 0.83; $P < .001$), and normal-like (HR, 0.47; 95% CI, 0.30 to 0.72; $P < .001$) subtypes all derived substantial benefit from RIB (Fig 3; Table 2). The absolute median PFS benefit from RIB was 10.9 months in the HER2E, 11.2 months in the normal-like, 9.4 months in the LumB, and 10.1 months in the LumA subtypes. Patients with the basal-like subtype (n = 28) did not derive benefit from RIB (HR, 1.14; 95% CI, 0.46 to 2.83; $P = .78$). The *P* value for the interaction test between PAM50 and treatment arm was .066. Overall, the results regarding biomarkers were consistent across studies for both prognosis and treatment benefit (Data Supplement).

ORR Based on Intrinsic Subtype

The HER2E and LumB subtypes demonstrated a significant increase in ORR with RIB treatment, whereas the other subtypes did not (HER2E: RIB, 40.4%; PBO, 9.8%; $P < .001$; LumB: RIB, 52.3%; PBO, 29.8%; $P < .001$; Data Supplement).

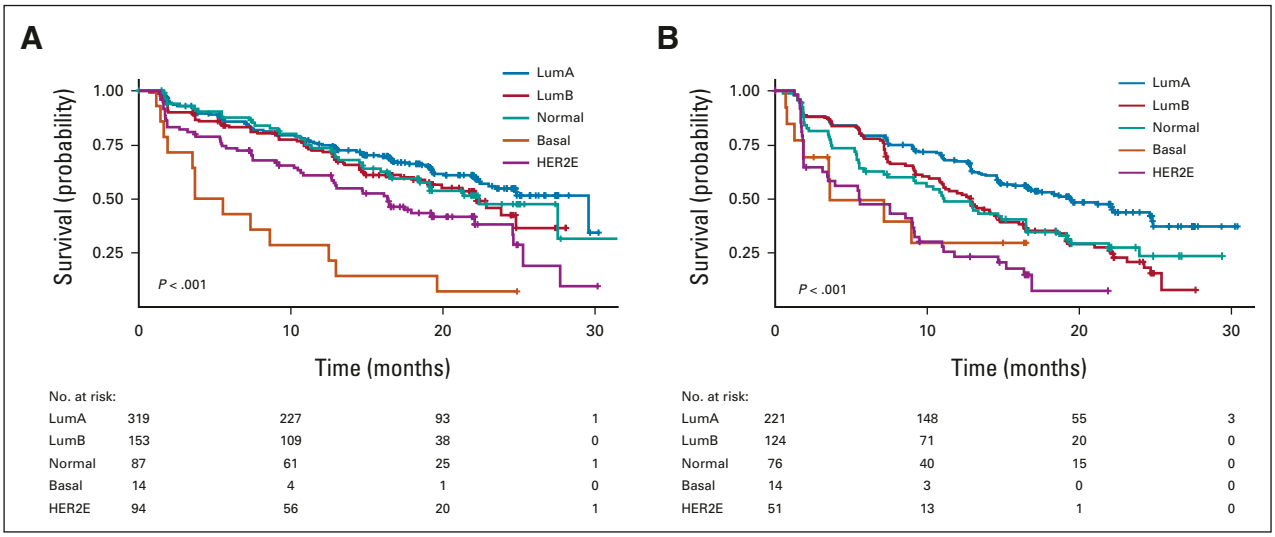

FIG 2. PFS based on intrinsic subtype in the combined MONALEESA data set: (A) in the RIB arm and (B) in the PBO arm. Basal, basal-like; HER2, human epidermal growth factor receptor 2; HER2E, HER2-enriched; LumA, luminal A; LumB, luminal B; NA, not achieved; Normal, normal-like; PBO, placebo; PFS, progression-free survival; RIB, ribociclib.

Biological Features of the Basal-Like Subtype

To better understand the biological features of the basal-like tumors identified in the ML program, we compared expression of the 152 PAM50-based genes found differentially expressed across subtypes (Fig 4A). As expected, the PAM50 subtypes identified in the ML program clustered together with the prototypical PAM50 subtypes. Similar to the triple-negative or basal-like prototypical PAM50 tumors, basal-like tumors in the ML program showed high expression of cyclin E1, epidermal growth factor receptor (*EGFR*), and p16/cyclin-dependent kinase inhibitor 2A, together with low expression of luminal-related genes, such as estrogen receptor 1, progesterone receptor, androgen receptor, and forkhead box 1A transcription factor (Fig 4B).[18]

DISCUSSION

To our knowledge, this is the largest analysis evaluating the correlation of intrinsic subtype with efficacy outcomes in HR+ and HER2− ABC treated with ET and in combination with a CDK4/6 inhibitor. Our results confirm the independent prognostic value of intrinsic subtype in patients treated with ET alone. More importantly, they show that the prognostic value of intrinsic subtype is maintained in the context of ET plus RIB. In addition, all subtypes exhibited a consistent, substantial benefit with RIB treatment except basal-like, which represented 2.4% of all patients. Finally, the HER2E subtype, which represents 12.6% of all patients, exhibited the worst prognosis with ET alone but the greatest relative benefit with RIB and ET.

TABLE 2. Predictive Value of Intrinsic Subtype on PFS by Treatment Arm

Subtype	Treatment Arm	Distribution, n (%)	Events, n (%)	Median PFS Estimate	Median PFS 95% CI	HR Estimate	HR 95% CI	*P*
LumA	PBO	221 (45)	110 (50)	19.48	15.61 to 24.80	0.63	0.49 to 0.83	.00071
	RIB	319 (48)	114 (36)	29.60	23.03 to NA			
LumB	PBO	124 (26)	89 (72)	12.85	10.84 to 14.82	0.52	0.38 to 0.72	< .0001
	RIB	153 (23)	66 (43)	22.21	18.79 to NA			
HER2E	PBO	51 (10)	40 (78)	5.52	3.45 to 9.17	0.40	0.26 to 0.62	< .0001
	RIB	94 (14)	56 (60)	16.39	12.71 to 24.6			
Basal-like	PBO	14 (3)	8 (57)	3.58	1.87 to NA	1.14	0.46 to 2.83	.78
	RIB	14 (2)	13 (93)	4.63	3.55 to 19.6			
Normal-like	PBO	76 (16)	53 (70)	11.10	7.39 to 16.56	0.47	0.30 to 0.72	.0005
	RIB	87 (13)	37 (43)	22.34	16.56 to NA			

Abbreviations: HER2E, human epidermal growth factor receptor 2–enriched; HR, hazard ratio; LumA, luminal A; LumB, luminal B; NA, not applicable; PBO, placebo; PFS, progression-free survival; RIB, ribociclib.

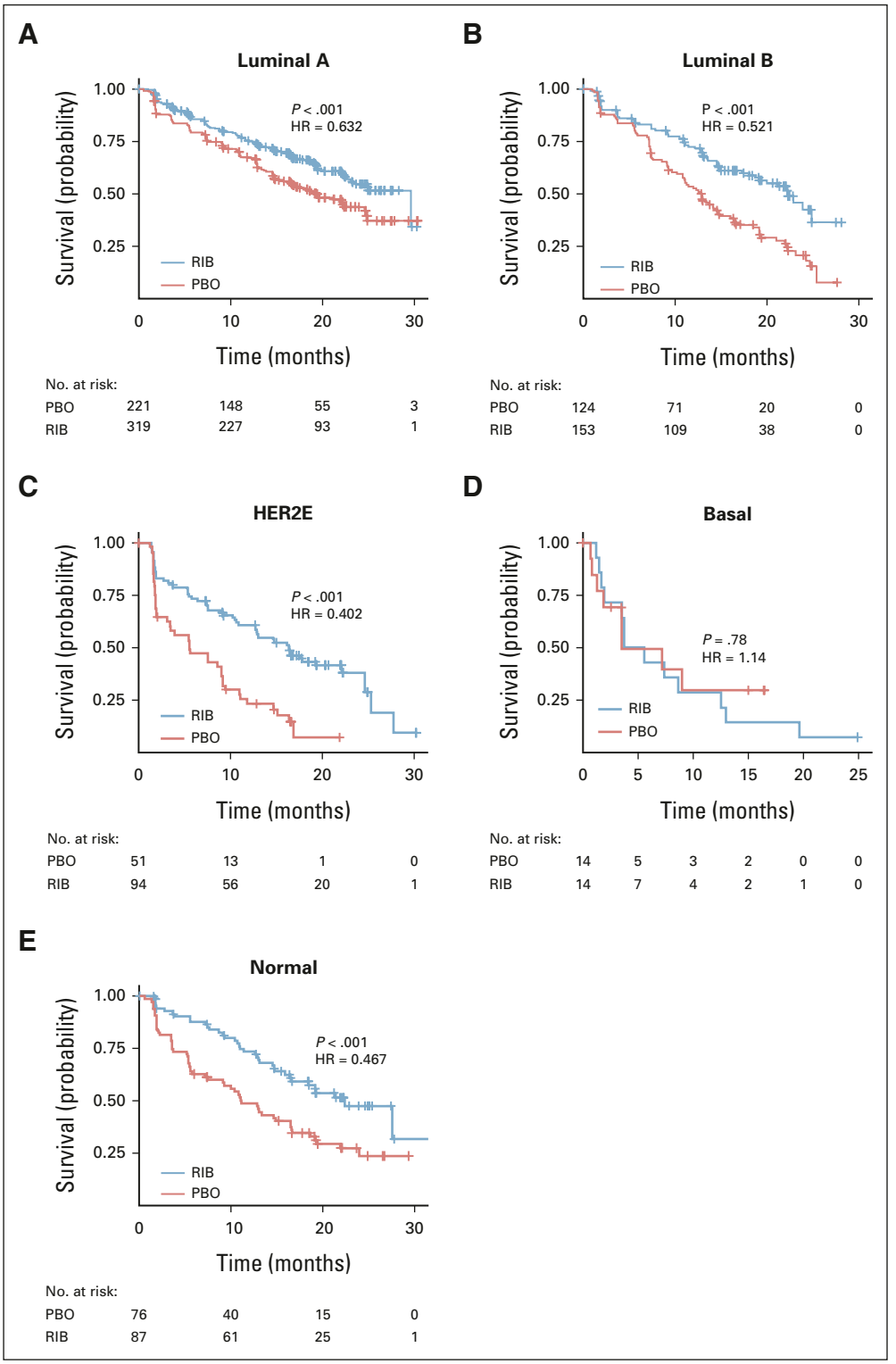

FIG 3. PFS based on treatment within each intrinsic subtype in the combined MONALEESA data set. (A) Within luminal A, (B) within luminal B, (C) within HER2E, (D) within basal-like, and (E) within normal-like. HER2E, human epidermal growth factor receptor 2–enriched; HR, hazard ratio; PBO, placebo; PFS, progression-free survival; RIB, ribociclib.

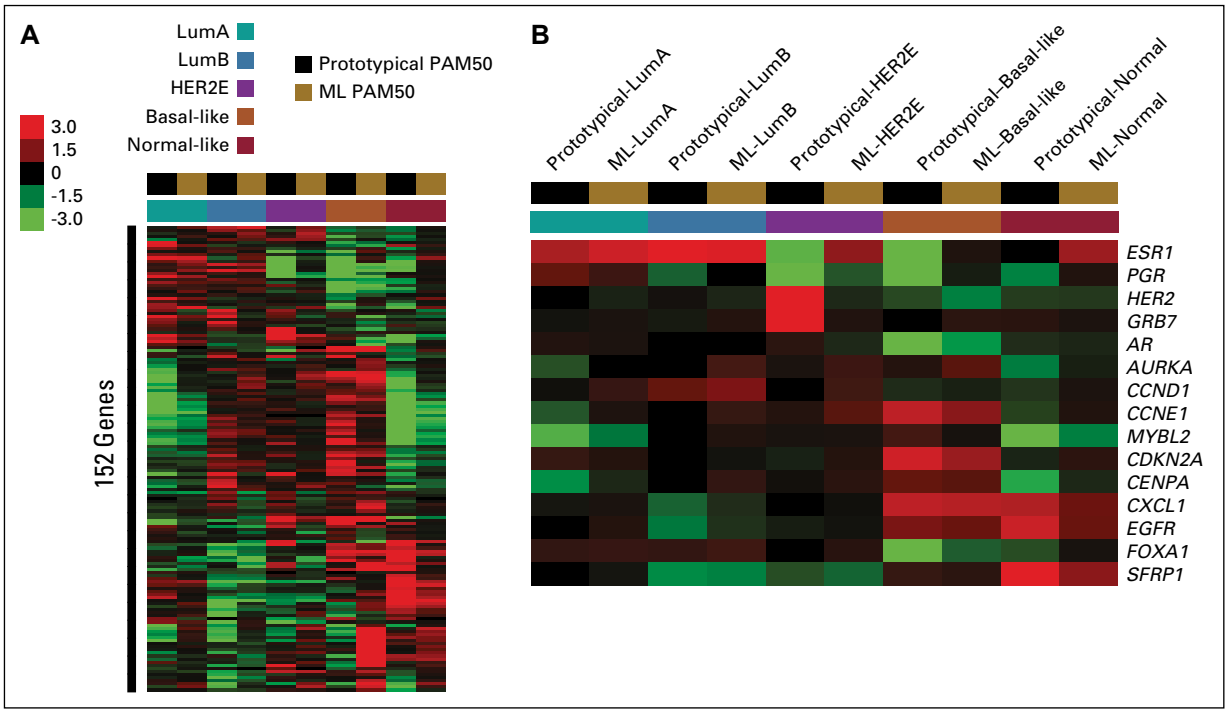

FIG 4. Gene expression differences across the intrinsic subtypes of breast cancer. (A) Unsupervised clustering using 152 genes across the prototypical PAM50 subtypes and the subtypes identified in the ML program. Sample and gene expression data from tumor samples of the same subtype have been combined into a single category. For each gene in a class, we calculated the standardized mean difference between the gene's expression in that class and its overall mean expression in the data set using a five-class Significance Analysis of Microarrays on the ML data set. Red represents relatively high gene expression, green represents relatively low gene expression, and black represents median gene expression. (B) Expression of selected breast cancer–related genes, obtained from the 152-gene list, across the prototypical PAM50 subtypes and the subtypes identified in the ML program. *AR*, androgen receptor; *CCNE1*, cyclin E1; *CDKN2A*, p16/cyclin-dependent kinase inhibitor 2A; *EGFR*, epidermal growth factor receptor; *ESR1*, estrogen receptor 1; *FOXA1*, forkhead box A1; HER2, human epidermal growth factor receptor 2; HER2E, HER2-enriched; LumA, luminal A; LumB, luminal B; ML, MONALEESA; PGR, progesterone receptor.

Two studies have evaluated the prognostic value of the PAM50 subtypes in HR+ and HER2− ABC treated with ET without CDK4/6 inhibition. In the first study, 644 HR+ and HER2− tumors from the EGF3008 phase III clinical trial were gene expression profiled.[9] In this trial, patients with advanced or metastatic disease were treated with first-line letrozole with or without lapatinib. Compared with the LumA subtype, the other subtypes demonstrated significantly decreased PFS and OS independent of other clinical-pathological variables.

In the second study, PAM50 was performed retrospectively in 261 tumor samples of 724 patients from the BOLERO-2 phase III trial.[10] This study randomly assigned (2:1) postmenopausal women with HR+ and HER2− advanced or metastatic breast cancer, previously treated with a nonsteroidal aromatase inhibitor, to exemestane with or without everolimus. The nonluminal subtypes were independently associated with worse PFS and OS compared with the remaining subtypes.

Similar to our study, the majority (≈86%) of PAM50 tumor samples from the EGF3008 and BOLERO-2 studies were from primary tumors and not metastatic samples.[9,10] In 2017, Cejalvo et al[11] performed a PAM50 paired analysis of primary versus metastatic tumors in 123 patients with ABC. High subtype concordance was observed in basal-like (100%), HER2E (76.9%), and LumB (70.0%) tumors. Among LumA primary tumors, 44.7% remained LumA in the metastasis, whereas 40.4% of cases switched to LumB and 14.9% of cases switched to HER2E. Thus, it is plausible that the results in EGF3008, BOLERO-2, and ML studies might have been improved if PAM50 was performed in a metastatic sample. This hypothesis needs confirmation.

Two studies have previously evaluated the prognostic value of PAM50 subtypes in HR+ and HER2− ABC treated with ET and CDK4/6 inhibition with palbociclib.[8,12,19] Both studies used earlier iterations of the PAM50 assay.[20] In the first study, Turner et al[8] evaluated retrospectively the PAM50-based subtypes in 302 tumor samples from the PALOMA-3 trial, which randomly assigned (2:1) endocrine-pretreated patients with HR+ and HER2− ABC to fulvestrant with or without palbociclib. In the 302 (53% primary and 47% metastatic) analyzed tumor samples, subtype distribution was LumA, 44.0%; LumB, 30.8%;

HER2E, 20.9%; normal-like, 2.6%; and basal-like, 1.7%. LumA had improved PFS compared with LumB. Regarding treatment benefit, LumA, LumB, and nonluminal (as one group) subtypes benefited from palbociclib; however, the identification of five patients with basal-like disease precluded any subanalysis in nonluminal disease. In the second study, Finn and colleagues[12,19] evaluated retrospectively the PAM50-based subtypes in 455 tumor samples from the PALOMA-2 trial, which randomly assigned (2:1) patients with HR+ and HER2− ABC to letrozole with or without palbociclib. Subtype distribution was LumA, 50.3%; LumB, 29.7%; HER2E, 18.7%; normal-like, 0.9%; and basal-like, 0.5%. Although LumA and LumB subtypes benefited substantially from palbociclib, similar median PFS was observed in both treatment arms in the HER2E (11.0 *v* 13.8 months) and basal-like (6.4 *v* 5.6 months) subtypes.[21] Contrasting the results for palbociclib, a consistent benefit of RIB was observed for all intrinsic subtypes, including HER2E but excluding basal-like.

The basal-like subtype in the ML program represented 2.4% of all HR+ and HER2− tumors and was associated with poor outcome and lack of benefit from RIB. From a clinical and biological perspective, these tumors seem more similar to triple-negative breast cancer than HR+ and HER2− breast cancer.[22] Future studies should explore if treatment strategies that are effective in triple-negative breast cancer, such as chemotherapy, chemotherapy plus immunotherapy,[23] or novel antibody-drug conjugates,[24] could be effective in this patient population.

The HER2E subtype represented 7.5% to 15.1% of all HR+ and HER2− tumors in the ML program (Data Supplement). Biologically, the HER2E subtype tumors in the ML program resemble classical HER2+ and HER2E tumors but without overexpressing the HER2 amplicon genes such as *ERBB2* and *GRB7* (Fig 4B).[25] Compared with LumA tumors, the HER2E subtype was associated with worse outcome but exhibited the highest relative and absolute benefit from RIB, in contrast to findings with palbociclib, in which HER2E had similar median PFS in both treatment arms.[21] Previous studies have observed that HR+ and HER2− tumors with an HER2E profile are less endocrine sensitive and have a worse outcome than LumA and B tumors.[26-28] A hypothesis behind the high efficacy of RIB in HER2E tumors could be that, beyond cell cycle inhibition, it restores endocrine sensitivity and has a synergistic effect with ET through potential immunomodulation activities. However, these findings are hypothesis generating, and future studies are needed to elucidate this mechanism and the underlying biology of HER2E tumors in HR+ and HER2− ABC. A previous retrospective study suggested that HER2E tumors in HR+ and HER2– ABC might be sensitive to lapatinib, an EGFR or HER2 tyrosine kinase inhibitor.[9] Thus, whether HER2E tumors within HR+ and HER2– ABC should be treated with ET and either lapatinib or RIB needs further studies. For example, the SOLTI-1718 NEREA trial (ClinicalTrials.gov identifier: NCT04460430) is currently testing the value of neratinib, a pan-EGFR tyrosine kinase inhibitor, in HER2E tumors within HR+ and HER2– ABC with or without prior CDK4/6 inhibitor treatment.

Our study has several limitations. First, this is an ad hoc exploratory analysis combining three phase III randomized clinical trials. Thus, the lack of statistical significance in some of this analysis could be due to a lack of statistical power. Additionally, statistically significant findings were not adjusted for multiplicity or false discovery. Second, we were unable to address whether the type of tumor tissue affects prognosis or treatment benefit. Third, we did not evaluate OS, because this end point is still immature in ML-2. Fourth, a substantial proportion (14.1%) of tumor samples were identified as normal-like, which is a group of tumors highly contaminated by normal breast tissue. Additionally, we did not use the standardized 50-gene PAM50 test, and only 36 of the 50 PAM50 genes were available. To overcome this, we derived a 152-gene set from prototypic PAM50 samples to correctly and robustly identify the PAM50 intrinsic subtypes using our custom-made CodeSet. Nonetheless, the generalizability of our observations to the clinic is contingent on additional testing in independent data sets using the same version of the PAM50 test.

Overall, patients in the ML trials exhibited a consistent substantial PFS benefit from RIB across all subtypes except basal-like. Following our results, the question remains whether intrinsic subtyping should guide the use of CDK4/6 inhibitors in ABC. Our opinion is that we are not ready to embrace intrinsic subtype as a biomarker until validation studies and clinical guidelines establish its clinical utility. A critical aspect is that the same version of the PAM50 assay must be used across studies to allow for comparability. Nonetheless, our results suggest that RIB should be explored in studies with an adequate sample size of patients with LumA, LumB, and HER2E subtypes independent of hormone receptor or HER2 status. Finally, our study highlights the importance of tumor tissue sample collection in the setting of large randomized trials.

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Correlative Biomarker Analysis of Intrinsic Subtypes and Efficacy Across ML Phase III Studies

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