

Distinct Neoadjuvant Chemotherapy Response and 5-Year Outcome in Patients With Estrogen Receptor–Positive, Human Epidermal Growth Factor Receptor 2–Negative Breast Tumors That Reclassify as Basal-Type by the 80-Gene Signature

Pat W. Whitworth, MD^{1,2}; Peter D. Beitsch, MD^{2,3}; James V. Pellicane, MD⁴; Paul L. Baron, MD^{5,6}; Laura A. Lee, MD⁷; Carrie L. Dul, MD⁸; Mary K. Murray, MD^{9,10}; Mark A. Gittleman, MD¹¹; Raye J. Budway, MD¹²; Rakhshanda Layeequr Rahman, MD¹³; Pond R. Kelemen, MD^{14,15}; William C. Dooley, MD^{16,17}; David T. Rock, MD^{18,19}; Kenneth H. Cowan, MD, PhD²⁰; Beth-Ann Lesnikoski, MD^{21,22}; Julie L. Barone, DO^{23,24}; Andrew Y. Ashikari, MD^{15,25}; Beth B. Dupree, MD²⁶; Shiyu Wang, MS²⁷; Andrea R. Menicucci, PhD²⁷; Erin B. Yoder, MS²⁷; Christine Finn, BS²⁷; Kate Corcoran, MPH²⁷; Lisa E. Blumencranz, PhD²⁷; and William Audeh, MD, MS²⁷; NBRST Investigators Group

PURPOSE The 80-gene molecular subtyping signature (80-GS) reclassifies a proportion of immunohistochemistry (IHC)-defined luminal breast cancers (estrogen receptor–positive [ER+], human epidermal growth factor receptor 2–negative [HER2–]) as Basal-Type. We report the association of 80-GS reclassification with neoadjuvant treatment response and 5-year outcome in patients with breast cancer.

METHODS Neoadjuvant Breast Registry Symphony Trial (NBRST; [NCT01479101](#)) is an observational, prospective study that included 1,069 patients with early-stage breast cancer age 18–90 years who received neoadjuvant therapy. Pathologic complete response (pCR) and 5-year distant metastasis-free survival (DMFS) and overall survival (OS) were assessed in 477 patients with IHC-defined ER+, HER2– tumors and in a reference group of 229 patients with IHC-defined triple-negative breast cancer (TNBC).

RESULTS 80-GS reclassified 15% of ER+, HER2– tumors (n = 73) as Basal-Type (ER+/Basal), which had similar pCR compared with TNBC/Basal tumors (34% v 38%; *P* = .52), and significantly higher pCR than ER+/Luminal A (2%; *P* < .001) and ER+/Luminal B (6%; *P* < .001) tumors. The 5-year DMFS (% [95% CI]) was significantly lower for patients with ER+/Basal tumors (66% [52.6 to 77.3]), compared with those with ER+/Luminal A tumors (92.3% [85.2 to 96.1]) and ER+/Luminal B tumors (73.5% [44.5 to 79.3]). Importantly, patients with ER+/Basal or TNBC/Basal tumors that had a pCR exhibited significantly improved DMFS and OS compared with those with residual disease. By contrast, patients with ER+/Luminal B tumors had comparable 5-year DMFS and OS whether or not they achieved pCR.

CONCLUSION Significant differences in chemosensitivity and 5-year outcome suggest patients with ER+/Basal molecular subtype may benefit from neoadjuvant regimens optimized for patients with TNBC/Basal tumors compared with patients with ER+/Luminal subtype. These data highlight the importance of identifying this subset of patients to improve treatment planning and long-term survival.

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INTRODUCTION

Neoadjuvant systemic therapy selection for patients with early-stage breast cancer requires precise classification of breast tumor biology. Currently, breast cancer is classified into surrogate intrinsic subtypes, on the basis of hormone receptors (estrogen receptor [ER] and progesterone receptor [PR]) and human epidermal

growth factor receptor (HER2) via routine immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH). Advances in genomics have enabled the investigation of a robust number of biomarkers simultaneously, thus more accurately characterizing the actual underlying biologic profile and signaling pathways of a tumor. Previous gene expression profiling studies

ASSOCIATED CONTENT

[Data Sharing Statement](#)

[Data Supplement](#)

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

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CONTEXT

Key Objective

Accurately reclassifying breast cancer subtypes with genomics can better predict treatment response and outcomes compared with conventional methods. In this observational, prospective study, the association between the 80-gene molecular subtyping signature (80-GS) and chemosensitivity and 5-year outcome were evaluated in immunohistochemistry-defined luminal breast cancers (estrogen receptor-positive [ER+], human epidermal growth factor receptor 2-negative [HER2-]) that were confirmed genomic Luminal-Type (ER+/Luminal) or reclassified as Basal-Type (ER+/Basal).

Knowledge Generated

Among 477 patients with ER+, HER2- tumors, 80-GS reclassified 15% of tumors as Basal-Type. ER+/Basal tumors exhibited significantly higher pathologic complete response to neoadjuvant therapy and worse 5-year survival compared with ER+/Luminal tumors.

Relevance

Immunohistochemistry-defined ER+, HER2- breast cancers, even when classified as genomically high risk, will exhibit distinct biologic characteristics, treatment response, and outcomes on the basis of their molecular subtype and should not be treated uniformly. Use of the 80-GS may diagnose patients with breast cancer more accurately, resulting in improved treatment planning and long-term survival.

demonstrated that breast cancers consist of intrinsic molecular subtypes with distinct clinical outcomes.¹⁻³

Unlike other intrinsic subtyping assays, the 80-gene molecular classifier, Blueprint, was developed in a supervised training method by evaluating mRNA profiles of samples with concordant ER, PR, and HER2 protein expression by IHC/FISH.^{4,5} Blueprint precisely measures the functionality of these receptors by expression of their downstream target genes and classifies the dominant activated pathway of tumors as Luminal-Type, HER2-Type, or Basal-Type. Together with the 70-gene risk of recurrence signature, MammaPrint, Luminal-Type tumors are further stratified into Luminal A-Type (MammaPrint Low Risk) or Luminal B-Type (MammaPrint High Risk). This is important because genomic profiling predicts prognosis more precisely than traditional phenotypes and is valuable in informing chemotherapy treatment decisions.⁶⁻⁸ Retrospective studies report more accurate prediction of sensitivity to neoadjuvant chemotherapy (NCT) in breast cancer, measured by pathologic complete response (pCR) and distant metastasis-free survival (DMFS), by MammaPrint- and Blueprint-defined molecular subgroups, which were significantly different from clinical phenotypes.^{9,10} Since a substantial number of cancers are more accurately reclassified on the basis of genomics compared with clinical subtyping, there are critical consequences for treatment strategy, expected response, and eventual outcome.

The prospective Neoadjuvant Breast Registry Symphony Trial (NBRST) is a multi-institutional US registry that showed that Blueprint reclassified 22% of tumors from patients with breast cancer undergoing NCT into a different molecular subtype compared with conventional IHC/FISH classification.^{11,12} Two types of breast cancers that were substantially reclassified have a drastic change in risk status designation: (1) IHC/FISH-

defined HER2-positive tumors that were non-HER2-Type by Blueprint,^{11,12} and (2) the most common breast cancer clinical subtype, ER-positive, HER2-negative tumors, 15% of which were reclassified as Blueprint Basal-Type, without evidence of functional hormonal signaling.^{11,12} Blueprint reclassification resulted in better prediction of NCT responses, with tumors reassigned as Blueprint HER2-Type or Basal-Type having higher pCR rates, irrespective of their phenotypic profile, compared with Luminal-Type tumors. This finding supports that accurate identification of molecular subgroups on the basis of gene expression may improve neoadjuvant treatment planning.^{11,12} Furthermore, patients with ER-positive, HER2-negative tumors reclassified as Basal-Type had significantly worse 3-year distant metastasis-free interval, characteristic of aggressive clinically triple-negative breast cancer (TNBC), compared with patients whose ER-positive, HER2-negative tumors were genomically Luminal-Type by Blueprint.¹³ These results reinforce that genomic-based classification more accurately predicts treatment response and is prognostic of outcome. Here, we compared chemosensitivity and 5-year outcomes among patients with ER-positive, HER2-negative tumors classified as Blueprint Basal-Type or Luminal-Type, and with a reference group of patients with triple-negative, Blueprint Basal-Type tumors.

METHODS

Patients

NBRST (NCT01479101) prospectively enrolled 1,091 patients with breast cancer from 67 US institutions between June 2011 and December 2014. This study was conducted in accordance with the ethical standards established in the Declaration of Helsinki. Institutional review boards approved the protocol at all participating institutions. Consent of trial participation, clinical data collection, and use of tissue for

scientific research was obtained for all patients. Of 1,091 patients enrolled, 1,069 patients met eligibility criteria: age 18-90 years, diagnosed with histologically proven breast cancer, and started or were scheduled to start neoadjuvant therapy after MammaPrint and Blueprint testing (Fig 1). Systemic therapy was administered at the discretion of the medical oncologist who was not blinded to MammaPrint and Blueprint results. The current analysis included all patients with ER-positive, HER2-negative tumors (n = 477). As a reference group, 229 patients with TNBCs that were Blueprint Basal-Type were included for a total of 706 patients. Clinical characteristics, treatment, and events were collected via case report forms at 6 weeks after receiving MammaPrint and Blueprint results, 4 weeks after surgery, 2-3 years after surgery, and 5 years after surgery.

Clinical and Molecular Subtyping

ER and PR was locally assessed on pretreated core biopsies (formalin-fixed paraffin-embedded or fresh tissue) by IHC and defined as positive if $\geq 1\%$ of tumor cells had positive

nuclear staining on the basis of ASCO/College of American Pathologists (CAP) guidelines.¹⁴ HER2 was determined locally by IHC and FISH according to 2011-2014 ASCO/CAP guidelines and defined as positive by 3+ IHC staining or FISH positivity.^{15,16} IHC/FISH classified tumors as ER-positive, PR-positive or -negative, and HER2-negative (ER+, HER2-), or TNBC (ER-, PR-, HER2-).

MammaPrint and Blueprint, which are based on microarray gene expression analysis,^{4,8} were successfully performed on RNA isolated from pretreated core biopsies at the Agendia Laboratory (Irvine, CA). All samples were blinded for clinical and pathologic data. MammaPrint categorized tumors as low risk (index > 0.000) or high risk (index ≤ 0.000). Blueprint classified tumors into Luminal-Type, HER2-Type, or Basal-Type. MammaPrint combined with Blueprint stratified Luminal-Type into Luminal A-Type (Low Risk) or Luminal B-Type (High Risk).

Statistical Analysis

NBRST was designed as an observational, exploratory study; sample size calculation was not used because only descriptive statistics were initially planned. The primary end point was pCR, defined as the absence of invasive carcinoma in both breast and axilla at microscopic examination of the surgically resected specimen, regardless of the presence of carcinoma in situ (ypT0/isN0).¹⁷ DMFS and overall survival (OS) were end points for 5-year follow-up. Descriptive statistics were used to summarize age, menopausal status, race/ethnicity, tumor stage, grade, MammaPrint and Blueprint results, and IHC/FISH subtypes. Age differences were evaluated by using one-way analysis of variance. Differences in the frequency of grade 3 ER+/Luminal B tumors and grade 3 ER+/Basal tumors were assessed by using a two-tailed proportional z-test. Differences in other clinical characteristics were determined by using either chi-squared test or Fisher's exact test. Statistical significance was defined by a two-sided $P < .05$ for all tests. pCR rates were calculated for each patient subgroup and compared between two subgroups using a two-tailed z-test for proportions.

The 5-year DMFS and OS survival curves were estimated by using the Kaplan-Meier method; log-rank test determined survival differences. Time to DMFS was calculated from the diagnosis date to date of first distant metastasis, death of any cause if not recurrence, or censored at the last follow-up date. Time to OS was calculated from diagnosis date to death from any cause or censored at the last follow-up date. Statistical analyses were conducted using Stata version 16 (Stata Corp, College Station, TX).

RESULTS

Clinical Characteristics

A total of 477 eligible patients had tumors defined by IHC/FISH as ER+, HER2-. Blueprint and MammaPrint classified 29.4% (140/477) as Luminal A-Type (ER+/Luminal A),

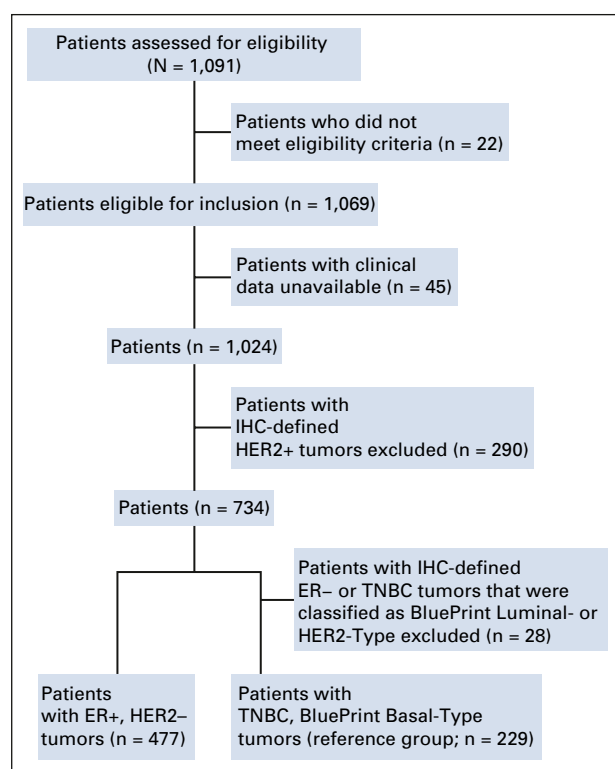


FIG 1. CONSORT diagram. 1,091 patients were assessed for eligibility, of whom 22 were excluded because they did not meet the inclusion criteria, and 45 were excluded because clinical data were unavailable. Patients with IHC-defined HER2+ tumors (n = 290) were excluded. Patients with IHC-defined ER- or TNBC tumors that were classified as Blueprint Luminal- or HER2-Type were excluded. A total of 706 patients were included in the analysis, of whom 477 had ER+, HER2- tumors. The remaining 229 patients had TNBC tumors that were Blueprint Basal-Type and were included as the reference group. ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; TNBC, triple-negative breast cancer.

TABLE 1. Clinical Characteristics for Patients

Characteristics	ER+ HER2-/BP Luminal A-Type	ER+ HER2-/BP Luminal B-Type	ER+ HER2-/BP Basal-Type	TNBC/BP Basal-Type	Total	P ^a
No. of patients	140	262	73	229	704	
Age, mean (SD)	57.7 (12.6)	54.0 (13.0)	49.9 (13.1)	51.2 (11.2)		.088
Menopausal status, No. (%)						
Pre	47 (16.4)	104 (36.2)	40 (13.9)	96 (33.4)	287	.031
Post	91 (22.2)	157 (38.3)	33 (8.0)	129 (31.5)	410	
Unknown	2 (28.6)	1 (14.3)	0	4 (57.1)	7	
Race/ethnicity, No. (%)						
Caucasian	120 (23.2)	187 (36.1)	55 (10.6)	156 (30.1)	518	.001 ^b
African American	8 (7.7)	39 (37.5)	10 (9.6)	47 (45.2)	104	
Asian	3 (23.1)	7 (53.8)	2 (15.4)	1 (7.7)	13	
Hispanic	7 (12.1)	25 (43.1)	4 (6.9)	22 (37.9)	58	
Others	2 (18.2)	4 (36.4)	2 (18.2)	3 (27.3)	11	
cT stage, No. (%)						
T1	17 (16.7)	30 (29.4)	8 (7.8)	47 (46.1)	102	.024
T2	76 (19.1)	143 (36.0)	46 (11.6)	132 (33.2)	397	
T3	42 (25.3)	69 (41.6)	14 (8.4)	41 (24.7)	166	
T4	5 (13.9)	17 (47.2)	5 (13.9)	9 (25.0)	36	
Unknown	0	3 (100.0)	0	0	3	
cN stage, No. (%)						
N0	69 (24.4)	76 (26.9)	30 (10.6)	108 (38.2)	283	< .001 ^c
N1	52 (16.1)	146 (45.2)	29 (9.0)	96 (29.7)	323	
N2	7 (15.6)	20 (44.4)	8 (17.8)	10 (22.2)	45	
N3	3 (18.8)	3 (18.8)	3 (18.8)	7 (43.8)	16	
NX	9 (26.5)	14 (41.2)	3 (8.8)	8 (23.5)	34	
Unknown	0	3 (100.0)	0	0	3	
cN stage, No. (%)						
LN-negative	69 (24.4)	76 (26.9)	30 (10.6)	108 (38.2)	283	< .001
LN-positive	62 (16.1)	169 (44.0)	40 (10.4)	113 (29.4)	384	
Histologic grade, No. (%)						
G1	39 (63.9)	19 (31.1)	1 (1.6)	2 (3.3)	61	
G2	76 (32.1)	119 (50.2)	6 (2.5)	36 (15.2)	237	
G3	17 (4.5)	109 (28.5)	66 (17.3)	190 (49.7)	382	< .001 ^d
GX	8 (33.3)	15 (62.5)	0	1 (4.2)	24	
MammaPrint risk, No. (%)						
Low risk	140	0	0	0	140	< .001
High risk	0	262	73	229	564	

NOTE. For each clinical characteristic, percentages were calculated by row. Differences in age assessed by using one-way ANOVA.

Abbreviations: ANOVA, analysis of variance; BP, Blueprint; cN, clinical nodal stage; cT, clinical T stage; ER, estrogen receptor; FISH, fluorescent in situ hybridization; G, grade; HER2, human epidermal growth factor receptor 2; HR-, hormone receptor-negative; HR+, hormone receptor-positive; IHC, immunohistochemistry; LN, lymph node; SD, standard deviation; TNBC, triple-negative breast cancer.

^aStatistical analysis, using either Fisher's exact or chi-squared test, excluded unknown patients in all comparisons.

^bCompared between African American and Caucasian patients.

^cCompared between N0 and N1.

^dCompared between G3 ER+ HER2-/BP Luminal B-Type and G3 ER+ HER2-/BP Basal-Type.

54.9% (262/477) as Luminal B-Type (ER+/Luminal B), 0.4% (2/477) as HER2-Type (ER+/HER2), and 15% (73/477) as Basal-Type (ER+/Basal). Because of the small sample size, ER+, HER2- tumors that reclassified to HER2-Type ($n = 2$) were excluded in downstream analysis. Patients with TNBC tumors that were confirmed Basal-Type (TNBC/Basal) by Blueprint (BP) (n = 229) were included as a reference group. All ER+/Basal and TNBC/Basal tumors were high risk by MammaPrint (Table 1). Within ER+/Luminal tumors, 65.2% (262/402) were MammaPrint High Risk.

The median age was comparable across each subgroup (Table 1). However, premenopausal patients had a higher proportion of ER+/Basal samples than postmenopausal patients ($P = .031$). Compared with Caucasian patients, African American patients had a significantly lower frequency of ER+/Luminal A tumors (7.7% v 23.2%) and a higher percentage of TNBC/Basal tumors (45.2% v 30.1%; Table 1; $P = .001$). For each subgroup, most patients had T2-T3 tumors (76%-84%) and a substantial proportion had lymph node involvement (44%-65%), characteristic of high-risk tumors. Although a substantial percentage (109/262; 41.6%) of ER+/Luminal B tumors were poorly differentiated (grade 3), ER+/Basal tumors comprised significantly more grade 3 tumors (66/73; 90.4%) in comparison (Table 1 and Fig 2A; $P < .001$). ER+/Basal tumors had a broad range of IHC ER-positive staining from 1% to 99%, with 45.2% of tumors showing > 10% ER positivity (Fig 2B).

Among patients with ER+/Luminal A tumors and ER+/Luminal B tumors, a majority (69.3% and 90.8%,

respectively) received NCT (Data Supplement). Most patients (97.3%) with ER+/Basal tumors and all patients with TNBC/Basal tumors received NCT. Among patients who received NCT in each subgroup, most (90.3%-98.6%) received anthracycline- and/or taxane-containing regimens. For patients receiving postoperative adjuvant therapy, treatment was based on IHC/FISH subtype (Data Supplement).

Chemosensitivity and 5-Year Outcome

Following neoadjuvant treatment, patients with ER+/Basal tumors achieved a pCR rate of 34% (25/73), which was comparable with the pCR rate observed in TNBC/Basal tumors (38%; 88/229; $P = .52$) and significantly higher than the pCR rate in ER+/Luminal A (2%; 3/140; $P < .001$) and ER+/Luminal B tumors (6%; 15/262; $P < .001$; Fig 3A). Interestingly, the only patient with an ER+/Basal tumor who received neoadjuvant endocrine therapy (Data Supplement) exhibited progressive disease at surgery, defined as a 25% increase in the longest tumor diameter or detection of new lesions.

We next evaluated 5-year OS and DMFS. The median (range) follow-up was comparable in all subgroups: 5.5 (0.6-7.5) years in the ER+/Luminal A group ($n = 125$), 5.3 (0.3-8.6) years in the ER+/Luminal B group ($n = 230$), 4.9 (0.4-7.1) years in the ER+/Basal group ($n = 61$), and 5.0 (0.4-6.9) years in the TNBC/Basal group ($n = 204$). DMFS was significantly different among the four subgroups of patients ($P < .001$; Fig 3B). ER+/Luminal A tumors exhibited the highest 5-year DMFS

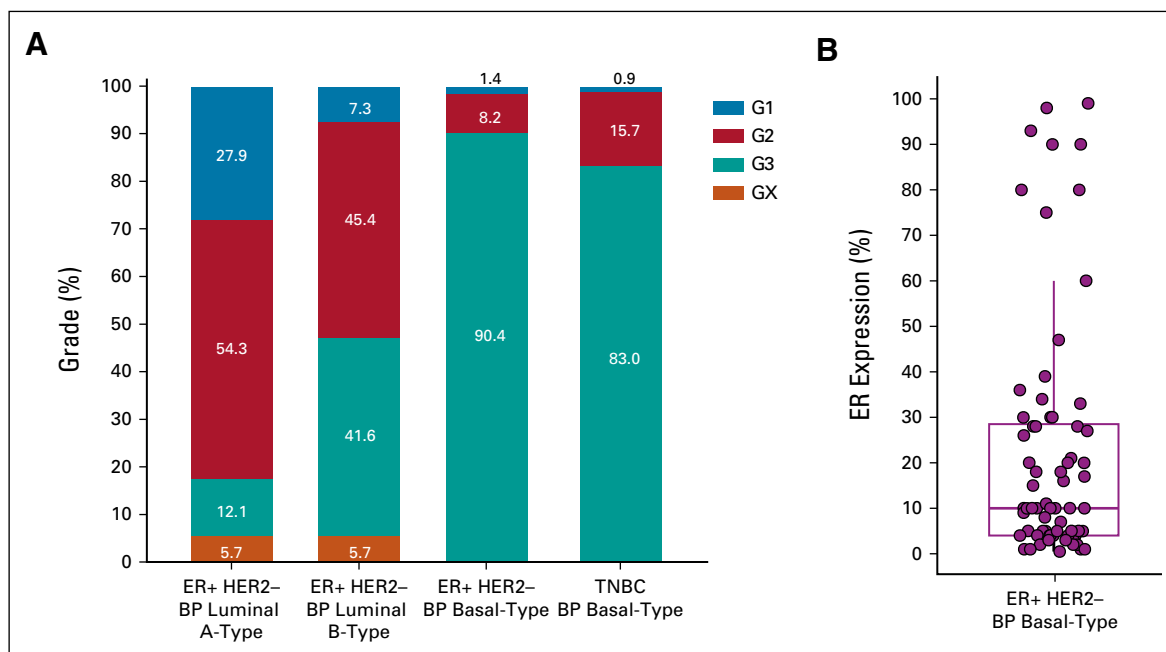


FIG 2. Frequency of grade and distribution of ER% in ER+/Basal tumors. (A) The percentage of each grade (grade 1 = blue, grade 2 = red, grade 3 = teal, grade unknown = orange) is shown for each patient subgroup (ER+/Luminal A, ER+/Luminal B, ER+/Basal, and TNBC/Basal). (B) The distribution of ER expression, shown as percentage, is shown for each patient with an ER+/Basal tumor. BP, Blueprint; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; TNBC, triple-negative breast cancer.

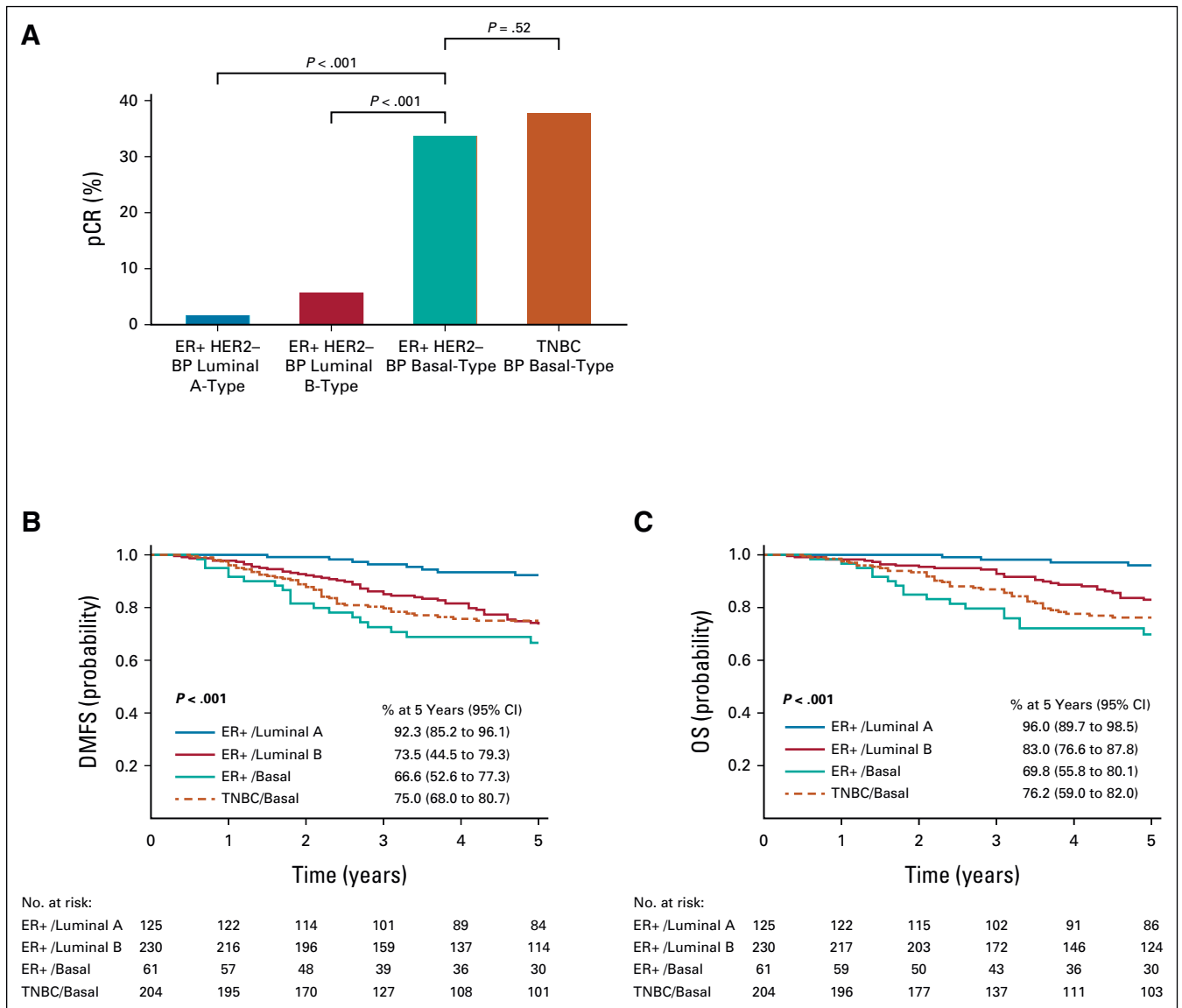


FIG 3. pCR rates and 5-year DMFS and OS. (A) pCR rates in each patient subgroup; significance was assessed by using a two-tailed z-test for proportions. (B) DMFS probability and (C) OS probability for each patient subgroup; significance was assessed by using log-rank test (blue = ER+/Luminal A, red = ER+/Luminal B, teal = ER+/Basal, dashed teal lines = TNBC/Basal). BP, Blueprint; DMFS, distant metastasis-free survival; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; OS, overall survival; pCR, pathologic complete response; TNBC, triple-negative breast cancer.

(92.3%; 95% CI, 85.2 to 96.1), followed by TNBC/Basal tumors (75.0%; 95% CI, 68.0 to 80.7) and ER+/Luminal B tumors (73.5%; 95% CI, 44.5 to 79.3), whereas ER+/Basal tumors had the worst 5-year DMFS probability (66.6%; 95% CI, 52.6 to 77.3; Fig 3B, Data Supplement). Similarly, the 5-year OS for patients with ER+/Luminal A tumors was 96.0% (95% CI, 89.7 to 98.5) compared with 83.0% (95% CI, 76.6 to 87.8) in ER+/Luminal B tumors (Fig 3C, Data Supplement). By contrast, 5-year OS was lower in TNBC/Basal tumors (76.2%; 95% CI, 59.0 to 82.0) and worst in ER+/Basal tumors (69.8%; 95% CI, 55.8 to 80.1) despite similar neoadjuvant treatment regimens (Data Supplement). Within the first 3 years, 70.6% (12/17) of death or distant

recurrence events occurred among ER+/Basal tumors, a temporal pattern more similar to that observed in TNBC/Basal tumors, with 61.0% (25/41) of events occurring within the first 3 years, compared with ER+/Luminal B tumors, with 38.7% (12/31) of events occurring during the same time period.

Patients with ER+/Luminal B tumors who did not achieve pCR had disease-related events earlier than those who achieved pCR. However, at 5 years, DMFS was similar between ER+/Luminal B tumors that achieved pCR versus those with residual disease (75% v 73.5%, respectively; $P = .672$; Fig 4A, Data Supplement). By contrast, patients with ER+/Basal tumors who had a pCR exhibited a clinically significant better 5-year DMFS

(82.6%; 95% CI, 55.3 to 94.1) compared with those with residual disease (59.0%; 95% CI, 41.7 to 72.8), with a 23.6% benefit albeit not statistically significant ($P = .075$; Fig 4A). OS evaluation demonstrated similar results (Fig 4B, Data Supplement). Additionally, 5-year DMFS and OS evaluation revealed that, regardless of ER status, patients with Blueprint Basal-Type tumors who achieved pCR exhibited significantly improved 5-year probability of DMFS and OS compared with those with residual disease (Figs 4C and 4D, Data Supplement). The recurrence profile was similar between ER+/Basal and TNBC/Basal tumors that did not achieve a pCR. Interestingly, despite achieving pCR, 5-year DMFS and OS was significantly lower in ER+/Basal tumors (82.6%

and 82.6%, respectively) compared with TNBC/Basal tumors (98.6% and 98.4%, respectively; $P = .006$ for DMFS and $P = .005$ for OS; Figs 4C and 4D).

DISCUSSION

We evaluated the association of Blueprint and MammaPrint reclassification with pCR and 5-year outcome among patients with ER+, HER2- breast cancer. Blueprint reclassified 15% of ER+, HER2- tumors as Basal-Type on the basis of underlying gene expression patterns. Similarly, analysis of 5,836 ER+, HER2- early-stage breast cancer specimens from the The Cancer Genome Atlas database revealed that the 80-gene signature reclassified 16% as Basal-Type.¹⁸ In the I-SPY2

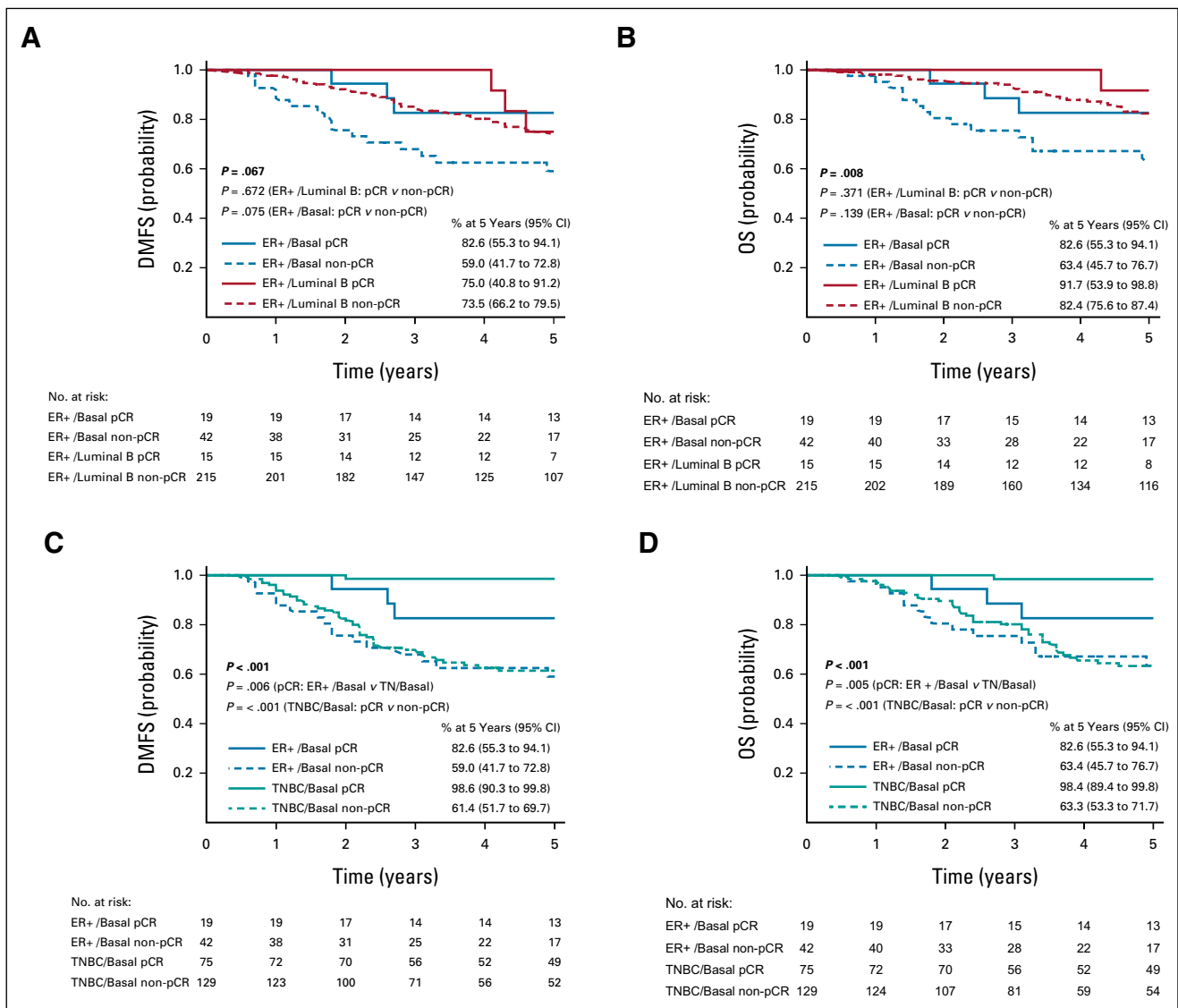


FIG 4. Association between pCR and 5-year DMFS and OS. (A) DMFS and (B) OS in patients with ER+/Luminal B tumors (red) and ER+/Basal tumors (blue) that achieved pCR (solid line) or had residual disease (dashed line). (C) DMFS and (D) OS in patients with TNBC/Basal tumors (teal) and ER+/Basal tumors (blue) that achieved pCR (solid line) or had residual disease (dashed line). Significance was assessed by using log-rank test. DMFS, distant metastasis-free survival; ER, estrogen receptor; OS, overall survival; pCR, pathologic complete response; TNBC, triple-negative breast cancer.

trial, 29% of HR+, HER2– tumors from 375 patients were reclassified as Basal-Type.¹⁹ One explanation for the observed IHC and Blueprint discordant rates is low ER protein expression. However, we report that Blueprint can detect ER+/Basal tumors with ER positivity up to 99%, a large proportion (45.2%) of which had ER positive staining in > 10% of the tumor specimen. This indicates that a significant contributor to basal molecular reclassification is the failure of the ER, which is present but nonfunctional, to elicit downstream transcriptional responses. Compared with ER+ Blueprint Luminal-Type tumors, ER+/Basal samples displayed a significantly higher frequency of the dominant-negative ER Δ 7 splice variant,^{13,20} which has been shown to inhibit estrogen-dependent transcriptional activation by wild-type ER.²¹ Despite differences in functional response, both ER Δ 7 variant and wild-type ER are detected by IHC. These data suggest that Blueprint can precisely identify a subgroup of patients with breast cancer that present with non-functional ER and downstream transcriptional activity more characteristic of Basal-Type tumors, potentially affecting treatment decisions.

Comprehensive whole transcriptome analysis in 1,500 patients with ER+ breast cancer tumors further support ER+/Basal tumors as a biologically distinct subtype. Clustering analysis showed high similarity in the transcriptional profile between ER+/Basal and ER–/Basal tumors, translating into limited gene expression differences. By contrast, the highest variance was observed between ER+/Basal and ER+/Luminal B tumors, corresponding to substantial gene expression differences associated with increased immune responses and cell proliferation and downregulation of estrogen response in ER+/Basal tumors relative to ER+/Luminal B tumors.²²

The diverse molecular biology between these two ER+ subtypes may contribute to the significantly higher chemosensitivity rates observed in ER+/Basal tumors compared with ER+/Luminal tumors, consistent with contrasting pCR rates observed between clinically defined TNBC and luminal breast cancers.²³ Previous studies demonstrated similar pCR rates in patients with ER+/Basal tumors, ranging from 29%–41%.¹⁹ Furthermore, all ER+/Basal tumors in this study were High Risk by MammaPrint and therefore predicted to benefit from chemotherapy.⁸ By contrast, MammaPrint Low Risk, ER+/Luminal A tumors had a low response to NCT, yet exhibited superior 5-year outcomes. These patients may omit chemotherapy, as demonstrated by excellent 9-year DMFS in MammaPrint Low Risk patients treated with endocrine therapy alone in the MINDACT trial.²⁴

Patients with either ER+/Basal or TNBC/Basal tumors that achieved pCR had significantly improved survival compared with those with residual disease, in line with other evidence demonstrating a strong correlation of pCR with long-term outcomes in TNBC.²⁵ However, patients with

ER+/Luminal B tumors had comparable 5-year DMFS and OS, whether or not they achieved pCR. Notably, among patients with ER+ breast cancer with residual disease, Blueprint further distinguished 5-year outcomes on the basis of molecular subtype. Specifically, Basal-Type tumors that did not achieve pCR exhibited poor outcomes, regardless of ER status, which was significantly worse than that observed for ER+/Luminal B tumors with residual disease. This striking finding highlights the importance of using genomic profiling to distinguish ER+ breast cancers that are intrinsically Basal-Type since they cannot be identified solely by clinicopathologic features, such as ER expression or grade. Additionally, patients with ER+/Basal tumors with residual disease may be good candidates to receive additional adjuvant chemotherapy such as capecitabine, which demonstrated significantly improved disease-free survival and OS in clinically HER2-negative and TNBC patients with residual disease in the CREATE-X trial.²⁶

Overall, our results strongly indicate that ER+/Basal and ER+/Luminal subtypes should not be treated uniformly and that ER+/Basal tumors may benefit from chemotherapy options emerging for TNBC. The biologic similarities of ER+/Basal to TNBC/Basal subtype suggest these tumors may display improved response to a doxorubicin and cyclophosphamide with taxane regimen compared with docetaxel and cyclophosphamide, as was demonstrated in the ABC trials for patients with TNBC and extensive nodal involvement.²⁷ In silico gene expression analysis of a meta-data set predicted ER+/Basal tumors to more likely benefit from PARP inhibitors, platinum salts, and immune therapy compared with ER+/Luminal tumors.¹⁸ Furthermore, we report that the ER+/Basal tumor in the one patient who received neoadjuvant endocrine therapy progressed. Because ER+/Basal tumors are more likely to harbor a functionally dominant negative variant of ER, these patients may not benefit from hormonal therapy or may display endocrine resistance. Bertucci et al¹⁸ reported that patients with ER+, genomic Luminal tumors significantly benefitted from adjuvant hormone therapy (8% improvement in 5-year distant recurrence-free interval), whereas patients with ER+, genomic Basal tumors did not. Finally, despite achieving pCR, patients with ER+/Basal tumors had significantly worse DMFS and OS compared with those with TNBC/Basal tumors in the current analysis. The only noteworthy difference between these groups was, in contrast to patients with TNBC, ER+ patients received adjuvant endocrine therapy. Further studies are required to investigate the effect of adjuvant hormone therapy on the clinical outcome of patients with genomic Basal-Type tumors.

A limitation of this study is the relatively small number of ER+/Basal patients because of the observational registry trial design. Furthermore, the study design introduced variability in the neoadjuvant and adjuvant treatment strategies for each tumor subtype, and treating physicians

were not blinded to MammaPrint and Blueprint results. Therefore, we could not determine the impact of treatment differences on chemosensitivity or outcome among patients with ER+/Basal or ER+/Luminal tumors. However, future studies will assess the effectiveness of a docetaxel and carboplatin regimen compared with standard-of-care chemotherapy in patients with ER+/Basal tumors, as measured by using pCR and 5-year outcome, which demonstrated superior pCR rates in patients with TNBC.^{28,29} Patients for this study are currently being enrolled through the prospective FLEX trial (NCT03053193),

which captures full genomic profiles and comprehensive clinical data from all non-stage 4 breast cancer patients who receive MammaPrint with or without Blueprint as standard of care.³⁰

In conclusion, Blueprint identified a subgroup of ER+ patients with distinct treatment response and 5-year outcomes, which could not otherwise be detected by using traditional clinical or pathologic methods. These results reinforce the importance of using genomic assays such as the 80-gene signature to diagnose patients with breast cancer more accurately and optimize treatment strategies.

AFFILIATIONS

- ¹Nashville Breast Center, Nashville, TN
- ²Targeted Medical Education, Cupertino, CA
- ³Dallas Surgical Group, Dallas, TX
- ⁴Bon Secours Cancer Institute, Richmond, VA
- ⁵Breast and Melanoma Specialist of Charleston, Charleston, SC
- ⁶Lenox Hill Hospital/Northwell Health, New York, NY
- ⁷Comprehensive Cancer Center, Palm Springs, CA
- ⁸Ascension St John Hospital Great Lakes Cancer Management Specialists, Grosse Pointe Woods, MI
- ⁹Akron General Medical Center, Akron, OH
- ¹⁰Cleveland Clinic Akron General, Akron, OH
- ¹¹Breast Care Specialists, Allentown, PA
- ¹²St Clair Hospital, Pittsburgh, PA
- ¹³Texas Tech University, Lubbock, TX
- ¹⁴Ashikari Breast Center, Sleepy Hollow, NY
- ¹⁵Zucker School of Medicine, Hofstra University, Hempstead, NY
- ¹⁶Breast Institute, University of Oklahoma Health Sciences, Oklahoma City, OK
- ¹⁷Stephenson Cancer Center, Oklahoma City, OK
- ¹⁸Regional Breast Care, Fort Myers, FL
- ¹⁹Genesis Care, Fort Myers, FL
- ²⁰Fred and Pamela Buffet Cancer Center and Eppley Institute for Research in Cancer at University of Nebraska Medical Center, Omaha, NE
- ²¹The Breast Institute at JFK Medical Center, Atlantis, FL
- ²²Baptist MD Anderson Cancer Center, Jacksonville, FL
- ²³Exempla Saint Joseph Hospital, Denver, CO
- ²⁴Vail Health, Vail, CO
- ²⁵Northwell Health Physician Partners, Mount Kisco, NY
- ²⁶St Mary Medical Alliance Cancer Specialists, Langhorne, PA
- ²⁷Agendia Inc, Irvine, CA

CORRESPONDING AUTHOR

William Audeh, MD, MS, Medical Affairs, Agendia Inc, 22 Morgan, Irvine, CA 92618; e-mail: william.audeh@agendia.com.

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AUTHOR CONTRIBUTIONS

Conception and design: Pat W. Whitworth, Laura A. Lee, Andrea R. Menicucci, Erin B. Yoder, William Audeh

Provision of study materials or patients: Pat W. Whitworth, Peter D. Beitsch, Laura A. Lee, Carrie L. Dul, Mary K. Murray, Rakhshanda

Layeequr Rahman, Pond R. Kelemen, William C. Dooley, Kenneth H. Cowan, Beth-Ann Lesnikoski, Beth B. Dupree, Lisa E. Blumencranz
Collection and assembly of data: Pat W. Whitworth, Peter D. Beitsch, James V. Pellicane, Carrie L. Dul, Mary K. Murray, Mark A. Gittleman, Raye J. Budway, Pond R. Kelemen, William C. Dooley, David T. Rock, Kenneth H. Cowan, Andrew Y. Ashikari, Beth B. Dupree, Erin B. Yoder, Christine Finn, Kate Corcoran, Lisa E. Blumencranz
Data analysis and interpretation: Pat W. Whitworth, Peter D. Beitsch, Paul L. Baron, Laura A. Lee, Rakhshanda Layeequr Rahman, William C. Dooley, David T. Rock, Beth-Ann Lesnikoski, Julie L. Barone, Beth B. Dupree, Shiyu Wang, Andrea R. Menicucci, Erin B. Yoder, William Audeh
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

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Open Payments is a public database containing information reported by companies about payments made to US-licensed physicians ([Open Payments](http://OpenPayments)).

Pat W. Whitworth

Employment: Integra LifeSciences

Leadership: Integra LifeSciences

Stock and Other Ownership Interests: Targeted Medical Education, Inc, Cerebrotech Medical Systems, Medneon, Integra LifeSciences

Honoraria: Puma Biotechnology

Consulting or Advisory Role: ImpediMed, Prelude Therapeutics, Becton Dickinson

Research Funding: Prelude Therapeutics, Agendia, Medneon

Travel, Accommodations, Expenses: Targeted Medical Education, Inc

Peter D. Beitsch

Employment: InVita

Leadership: Targeted Medical Education, Inc

Stock and Other Ownership Interests: Targeted Medical Education, Inc, InVita

Research Funding: InVita

Expert Testimony: Dune Medical Devices, ImpediMed

Uncompensated Relationships: Medneon

James V. Pellicane

Stock and Other Ownership Interests: PreludeDx

Honoraria: Agendia, PreludeDx

Speakers' Bureau: Agendia, PreludeDx

Paul L. Baron

Honoraria: Myriad Genetics

Consulting or Advisory Role: Myriad Genetics

Speakers' Bureau: Myriad Genetics

Travel, Accommodations, Expenses: Myriad Genetics

William C. Dooley

Leadership: Shaga Medical, LLC

Stock and Other Ownership Interests: Shaga Medical

Research Funding: Agendia, Xofig

Patents, Royalties, Other Intellectual Property: Patent pending: microendoscopy system

Kenneth H. Cowan

Stock and Other Ownership Interests: United Health Group

Consulting or Advisory Role: Merck

Research Funding: Merck

Beth-Ann Lesnikoski

Employment: HCA Healthcare

Stock and Other Ownership Interests: HCA Healthcare

Research Funding: Agendia (Inst), Seattle Genetics (Inst)

Open Payments Link: <https://openpaymentsdata.cms.gov/physician/246359>

Beth B. Dupree

Honoraria: Medtronic

Shiyu Wang

Employment: Agendia

Stock and Other Ownership Interests: Agendia

Andrea R. Menicucci

Employment: Agendia

Erin B. Yoder

Employment: Agendia

Stock and Other Ownership Interests: Agendia

Travel, Accommodations, Expenses: Agendia

Kate Corcoran

Employment: Agendia

Lisa E. Blumencranz

Employment: Agendia

William Audeh

Employment: Agendia

Leadership: Agendia

Stock and Other Ownership Interests: Agendia

Consulting or Advisory Role: Celanese, Private Health

Research Funding: Agendia

Travel, Accommodations, Expenses: Agendia

No other potential conflicts of interest were reported.

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