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ORIGINAL ARTICLE

TNBC-DX genomic test in early-stage triple-negative breast cancer treated with neoadjuvant taxane-based therapy☆

M. Martín[1,2,3,4,5†], S. R. Stecklein[6,7,8,9†], O. Gluz[10,11,12†], G. Villacampa[13,14†] M. Monte-Millán[1,2,3], U. Nitz[10,11], S. Cobo[15], M. Christgen[12], F. Brasó-Maristany[15,16], E. L. Álvarez[1,2], I. Echavarría[1,2,3], B. Conte[15], S. Kuemmel[17,18], C. Bueno-Muiño[19], Y. Jerez[1,2,3], R. Kates[10], M. Cebollero[1,2], C. Kolberg-Liedtke[20], O. Bueno[1,2], J. Á. García-Saenz[4,21], F. Moreno[4,21], E.-M. Grischke[22], H. Forstbauer[23], M. Braun[24], M. Warm[25], J. Hackmann[26], C. Uleer[27], B. Aktas[28], C. Schumacher[29], R. Wuerstleins[10,30], M. Graeser[10,11,31], C. zu Eulenburg[10,31], H. H. Kreipe[10], H. Gómez[32], T. Massarrah[1,2,3], B. Herrero[1,2,4], L. Paré[16], U. Bohn[33], S. López-Tarruella[1,2,3,4,5], A. Vivancos[16], E. Sanfeliu[15,34], J. S. Parker[35], C. M. Perou[35], P. Villagrasa[16], A. Prat[15,16,36,37,38*‡], P. Sharma[7‡] & N. Harbeck[10,30‡]

[1]Department of Medical Oncology, Hospital General Universitario Gregorio Marañón, Madrid; [2]Instituto de Investigación Sanitaria Gregorio Marañón, Madrid; [3]Centro de Investigación Biomédica en Red de Cáncer, Madrid; [4]Grupo Español de Investigación en Cáncer de Mama, Madrid; [5]Universidad Complutense de Madrid, Madrid, Spain; [6]Department of Internal Medicine, University of Kansas Medical Center, Westwood; Departments of [7]Radiation Oncology; [8]Pathology & Laboratory Medicine; [9]Cancer Biology, University of Kansas Medical Center, Kansas City, USA; [10]West German Study Group, Monchengladbach; [11]Breast Center Niederrhein, Ev. Hospital Bethesda, Moenchengladbach; [12]University Clinics Cologne, Cologne, Germany; [13]SOLTI Cancer Research Group, Barcelona; [14]Statistics Unit, Vall d'Hebron Institute of Oncology (VHIO), Barcelona; [15]Translational Genomics and Targeted Therapies in Solid Tumors, August Pi I Sunyer Biomedical Research Institute (IDIBAPS), Barcelona; [16]Reveal Genomics, Barcelona, Spain; [17]Medical School Hannover, Institute of Pathology, Hannover; [18]Breast Unit, Clinics Essen Mitte, Essen, Germany; [19]Medical Oncology Department, Hospital Infanta Cristina (Parla), Madrid, Spain; [20]Department of Gynecology and Obstetrics, University Hospital Essen, Essen, Germany; [21]Department of Medical Oncology, Instituto de Investigación Sanitaria Hospital Clinico San Carlos (IdISSC), Madrid, Spain; [22]Department of Gynecology, Women's Clinic, University Clinics Tuebingen, Tuebingen; [23]Practice Network Troisdorf, Troisdorf; [24]Breast Center, Rotkreuz Clinics Munich, Munich; [25]Breast Center, City Hospital Holweide, Cologne; [26]Breast Center, Marien-Hospital, Witten; [27]Practice of Gynecology and Oncology, Hildesheim; [28]Women's Clinic, University Clinics Essen, Essen; [29]Breast Center, St. Elisabeth Hospital, Cologne; [30]Breast Center, Department of Obstetrics and Gynecology and CCC Munich, LMU University Hospital, Munich; [31]University Hospital Hamburg-Eppendorf, Hamburg, Germany; [32]Instituto Nacional de Enfermedades Neoplásicas, Lima, Peru; [33]Hospital Universitario de Gran Canaria Dr. Negrín, Las Palmas; [34]Pathology Department, Hospital Clínic de Barcelona, Barcelona, Spain; [35]Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, USA; [36]Cancer Institute and Blood Disorders, Hospital Clínic de Barcelona, Barcelona; [37]Medicine Department, University of Barcelona, Barcelona; [38]Breast Cancer Unit, IOB-QuirónSalud, Barcelona, Spain

Available online XXX

Background: Identification of biomarkers to optimize treatment strategies for early-stage triple-negative breast cancer (TNBC) is crucial. This study presents the development and validation of TNBC-DX, a novel test aimed at predicting both short- and long-term outcomes in early-stage TNBC. The objective of this study was to evaluate the association between TNBC-DX and efficacy outcomes [pathologic complete response (pCR), distant disease-free survival (DDFS) or event-free survival (EFS), and overall survival (OS)] in the validation cohorts.

Methods: Information from 1259 patients with early-stage TNBC (SCAN-B, CALGB-40603, and BrighTNess) was used to establish the TNBC-DX scores. Independent validation of TNBC-DX was carried out in three studies: (i) WSG-ADAPT-TN; (ii) MMJ-CAR-2014-01; and (iii) NeoPACT, including 527 patients with stage I-III TNBC undergoing neoadjuvant chemotherapy. In WSG-ADAPT-TN, patients were randomized to receive nab-paclitaxel plus gemcitabine or carboplatin. In MMJ-CAR-2014-01, patients received carboplatin plus docetaxel. In NeoPACT, patients received carboplatin plus docetaxel and pembrolizumab.

Results: TNBC-DX test was created incorporating the 10-gene Core Immune Gene module, the 4-gene tumor cell proliferation signature, tumor size, and nodal staging. In the two independent validation cohorts without pembrolizumab, the TNBC-DX pCR score was significantly associated with pCR after adjustment for clinicopathological variables and treatment regimen [odds ratio per 10-unit increment 1.34, 95% confidence interval (CI) 1.20-1.52, $P < 0.001$]. pCR rates for the TNBC-DX pCR-high, pCR-medium, and pCR-low categories were 56.3%, 53.6%, and 22.5% respectively (odds ratio for pCR-high versus pCR-low 3.48, 95% CI 1.72-7.15, $P < 0.001$).

*Correspondence to: Prof. Aleix Prat, Cancer Institute and Blood Disorders, Hospital Clinic of Barcelona, Villarroel 170, Barcelona, 08036, Spain. Tel: +34 93 227 54 00 E-mail: alprat@clinic.cat (A. Prat).

†Equal contribution.

‡Senior authors.

☆Note: This study was presented at the ESMO Congress 2024, 13-17 September 2024, Barcelona, Spain.

In addition, the TNBC-DX risk score was significantly associated with DDFS [hazard ratio (HR) high-risk versus low-risk 0.24, 95% CI 0.15-0.41, $P < 0.001$] and OS (HR 0.19, 95% CI 0.11-0.35, $P < 0.001$). In the validation cohort with pembrolizumab, the TNBC-DX scores were significantly associated with pCR, EFS, and OS.

Conclusions: TNBC-DX predicts pCR to neoadjuvant taxane—carboplatin in stage I-III TNBC and helps to forecast the patient's long-term survival in the absence of neoadjuvant anthracycline—cyclophosphamide, and independent of pembrolizumab use.

Key words: early-stage breast cancer, triple negative, biomarkers, TNBC-DX, genomic test

INTRODUCTION

Triple-negative breast cancer (TNBC) presents a significant treatment challenge due to its aggressive nature and limited targeted therapy options.[1,2] Systemic multiagent chemotherapy improves long-term outcomes and is recommended for stage I-III TNBC disease, with most patients in the current era being treated with neoadjuvant chemotherapy.[3] Anthracyclines and cyclophosphamide (AC) have typically constituted the chemotherapy backbone of multiagent regimens in combination with taxane-based therapy (AC—T), which often includes carboplatin (AC—TCb).[3] More recently, neoadjuvant and adjuvant pembrolizumab have been approved for the treatment of stage II-III TNBC in combination with AC—TCb.[4]

Given the short- and long-term toxicities associated with AC,[4-6] interest in anthracycline-free chemotherapy regimens has been gaining momentum among patients and physicians. Indeed, several studies have evaluated the possibility to eliminate the use of anthracyclines and focus on the use of a taxane—carboplatin regimen. This combination yields pathologic complete response (pCR) rates of 45%-55% in TNBC, and patients achieving a pCR with these regimens demonstrate excellent 3-year outcomes without adjuvant anthracycline.[7-11] In fact, in a randomized phase III trial, six cycles of adjuvant carboplatin plus paclitaxel showed superior disease-free survival compared with an anthracycline plus taxane regimen.[12] It is unclear whether the four-drug AC—TCb chemotherapy backbone is necessary for all patients receiving neoadjuvant pembrolizumab. The SCARLET phase III trial (NCT05929768) is comparing the traditional AC—TCb and pembrolizumab regimen with docetaxel—carboplatin and pembrolizumab to optimize neoadjuvant therapy.

At the same time, there is an emerging focus in early-stage TNBC to modulate the intensity of immunotherapy and other therapies—by either escalating or de-escalating—and several phase III trials are underway. The OptimICE-pCR (NCT05812807) trial is investigating if adjuvant pembrolizumab is beneficial for patients who had a pCR following preoperative chemotherapy with pembrolizumab. By contrast, SASCIA (NCT04595565) and OptimICE-RD (NCT05633654) trials are examining the benefits of escalating therapy using the anti-TROP2 antibody—drug conjugate sacituzumab govitecan with or without pembrolizumab for patients with residual disease after neoadjuvant therapy.

Until recently, the percentage of tumor-infiltrating lymphocytes (TILs) has been recognized as a potential biomarker for patients with early-stage TNBC,[13-15] though this has not yet been fully established in clinical guidelines for widespread use. In addition, translational research in the past decade has uncovered robust genomic-based immune biomarkers,[16-18] showing promising potential for clinical application. Among them, the recently developed HER2DX genomic test for early-stage HER2+ breast cancer includes a 14-gene immunoglobulin (IGG) signature.[19-21] In this study, we developed and validated a new genomic test (TNBC-DX) to predict short- and long-term outcomes in patients with early-stage TNBC.

METHODS

TNBC-DX development

The standardized 27-gene HER2DX genomic test for early-stage HER2+ breast cancer[19-24] was used as a reference to develop the TNBC-DX genomic test. The HER2DX assay is based on four different gene signatures comprising 27 genes, including the 14-gene IGG module (i.e. *CD27*, *CD79A*, *HLA-C*, *IGJ*, *IGKC*, *IGL*, *IGLV3-25*, *IL2RG*, *CXCL8*, *LAX1*, *NTN3*, *PIM2*, *POU2AF1* and *TNFRSF17*). The other three gene signatures are a 4-gene tumor cell proliferation signature (*EXO1*, *ASPM*, *NEK2*, and *KIF23*), a 5-gene luminal differentiation signature (*BCL2*, *DNAJC12*, *AGR3*, *AFF3*, and *ESR1*), and the 4-gene HER2 amplicon signature (*ERBB2*, *GRB7*, *STARD3*, and *TCAP*). Two scores are calculated for each patient: (i) the HER2DX pCR score and (ii) the HER2DX risk score (both from 0 to 100). Pre-established cut-offs are used to create the HER2DX pCR groups [low (0-33.3), medium (33.3-66.7), and high (66.7-100)], and to create the HER2DX risk groups [low (0-50) and high (50-100)].

Three *in silico* datasets with information from 1259 patients with early-stage TNBC (i.e. SCAN-B, CALGB-40603, and BrighTNess trials) were used to improve the model in the context of TNBC. Of note, CALGB-40603 and SCAN-B used an estrogen receptor (ER) 10% cut-off, while BrighTNess used an ER 1% cut-off. The signatures defined in the HER2DX assay and individual genes were evaluated across the three studies to assess its association with efficacy outcomes. In addition, a new 10-gene Core Immune Gene (CIG) module (i.e. *CD274*, *CD79A*, *CXCR6*, *IRF4*, *LAX1*, *PDCD1*, *PIM2*, *POU2AF1*, *SLAMF1*, and *TNFRSF17*), which was obtained from a previous analysis,[17] was also evaluated to determine whether incorporating this information could improve the prognostic performance of the model. The development cohorts ($n = 1259$) were solely utilized to

define the TNBC-DX test, without being used for formal validation or for quantifying its association with pCR status and survival outcomes.

TNBC-DX validation studies

After the development of the TNBC-DX, the model was externally validated in 527 patients across the MMJ-CAR-2014-01 ($n = 292$), ADAPT-TN ($n = 126$), and NeoPACT ($n = 109$) studies. TNBC-DX was carried out using RNA in the MMJ-CAR-2014-01 and ADAPT-TN cohorts and using RNA-seq data in the NeoPACT cohort.

The MMJ-CAR-2014-01 (NCT01560663)[11] is an ongoing prospective, multicenter, nonrandomized trial exploring the antitumor activity of neoadjuvant carboplatin and docetaxel in early-stage TNBC, exhibiting <1% expression of ER and progesterone receptor (PR). Eligible patients included females with pathologically confirmed diagnosis of primary invasive breast cancer, stage I-III. The patients were diagnosed at any of the participant academic institutions. From 2013 to 2019, 299 enrolled patients were treated with six cycles of carboplatin [area under the ROC curve (AUC) 6] and docetaxel (75 mg/m^2) administered every 21 days. Patients with non-pCR could receive adjuvant anthracycline-based therapy per investigator's discretion.

The ADAPT-TN study,[8,9] a phase II prospective neoadjuvant trial (WSG-ADAPT TN Trial, NCT01815242), enrolled patients diagnosed with stage I-III TNBC confirmed centrally, exhibiting <1% expression of ER and PR. Participants were randomized in a 1 : 1 ratio, with stratification based on nodal status and study center, to undergo 12 weeks of treatment. Patients were randomized to receive (i) nab-paclitaxel at a dose of 125 mg/m^2 administered on days 1 and 8, combined with either gemcitabine (1000 mg/m^2 on days 1 and 8, referred to as the gem arm) or (ii) carboplatin (AUC 2 on days 1 and 8, referred to as the nab-paclitaxel/carboplatin arm). For patients without a pCR in the breast or axillary nodes during surgery, additional four cycles of anthracycline-based chemotherapy (epirubicin at 90 mg/m^2 and cyclophosphamide at 600 mg/m^2 every 2 or 3 weeks) were mandated. This could also be administered before surgery as additional neoadjuvant chemotherapy upon confirming non-pCR status *via* core biopsy. At the discretion of the investigator, those who had a pCR were allowed to forego further standard chemotherapy. Among patients with pCR, there were 12 instances of invasive disease-free survival (iDFS) events, including 6 distant events, with no significant iDFS risk difference between patients who did and did not receive further chemotherapy.[9]

The NeoPACT (NCT03639948)[25] is an open-label multicenter phase II clinical trial that enrolled 115 female patients with stage I-III TNBC (including tumors with an ER expression up to 10%) who received neoadjuvant carboplatin (AUC 6) and docetaxel (75 mg/m^2) plus pembrolizumab (200 mg) every 21 days for 6 cycles from 2018 to 2022. After surgery, no adjuvant pembrolizumab was indicated. Patients with non-pCR could receive adjuvant anthracycline-based therapy per investigator's discretion.

Further, gene expression and mutation data from 153 TNBC tumors from The Cancer Genome Atlas (TCGA)[26] dataset were obtained from cBioPortal.[27] The TNBC-DX scores were applied on to the RNA-seq data. The TNBC subtype and tumor immune microenvironment (TIME) were obtained from Lehmann et al.[28]

Clinical endpoints

The co-primary endpoints for this analysis were (i) pCR and (ii) distant disease-free survival (DDFS) or event-free survival (EFS). pCR was defined as the absence of residual invasive disease in the breast and axilla with or without ductal carcinoma *in situ* (ypT0/isN0). Pathologic response was determined locally. DDFS was defined as the time from registration, before initiating neoadjuvant therapy, to the time to distant breast cancer recurrence, secondary invasive malignancy, or death, whichever occurs first. EFS was defined as time from diagnosis to first invasive locoregional or distant recurrence, study treatment-related death, or breast cancer-related death. The secondary endpoints were overall survival (OS); iDFS, defined as the time from registration to any invasive cancer event or death; pCR status according to the chemotherapy regimen received during the neoadjuvant treatment; and survival outcomes by pCR status. The TNBC-DX assay was retrospectively evaluated in a blinded manner, with results centrally analyzed and subsequently linked to clinical data.

Statistical analysis

To validate the model, the first objective was to assess the association between the TNBC-DX pCR score (as a continuous variable and group categories) and pCR status in the independent validation cohorts without pembrolizumab. Univariable and multivariable logistic regression models were used to investigate the association for each variable with pCR in terms of odds ratios (ORs) with 95% confidence interval (CI). To evaluate the performance of the TNBC-DX pCR score, the AUC and calibration plots were calculated.[29] The second objective to validate the model was to assess the ability of the TNBC-DX risk score (as a continuous variable and group categories) to predict survival outcome (DDFS and OS) in the independent validation cohorts without pembrolizumab. The Kaplan–Meier method was used to estimate survival outcomes. Stratified univariable and multivariable Cox proportional hazards models were used to obtain hazard ratios (HRs). The cohort type was used as a stratification factor (ADAPT-TN and MMJ-CAR-2014-01), allowing a different baseline hazard function for each study. The proportional hazards assumption was tested and inspected visually by means of Schoenfeld residuals (Supplementary Table S1, available at https://doi.org/10.1016/j.annonc.2024.10.012). All variables evaluated in the univariable analysis were included in the multivariable model. Missing at random values were imputed using the chained equations method.[30] The prevalence of missing data was <5% in all variables (Supplementary Table S2, available at https://doi.org/10.1016/j.annonc.2024.10.012).

Following imputation, a sensitivity analysis was conducted to ensure that these imputations did not alter the obtained results. Details of the adjuvant chemotherapy used in the study MMJ-CAR-2014-01 are provided in Supplementary Table S3, available at https://doi.org/10.1016/j.annonc.2024.10.012. The third objective was to assess the association between the TNBC-DX pCR and risk scores (as a continuous variable and group categories) with pCR status, EFS, and OS in the independent validation cohort with pembrolizumab. The median follow-up was calculated using the reverse Kaplan—Meier method. For all statistical analyses, the significance level was set at two-sided alpha of 0.05 and all analyses were carried out using R statistical software version 4.3.2 (R Foundation, Vienna, Austria).

Ethical approval

The MMJ-CAR-2014-01, ADAPT-TN, and NeoPACT trials received approval from relevant ethics committees, and institutional review boards, adhering to the Declaration of Helsinki. Participation was contingent upon the provision of written informed consent by all patients. Material transfer agreements were established, and ethical approvals were obtained for the correlative analyses conducted. These approvals cover the use of patient samples and data for the analyses presented in this study.

Role of the funding source

The study was designed and carried out by investigators from the West German Study Group, Gregorio Marañón General Hospital, University of Kansas, and Reveal Genomics. Reveal Genomics funded the study. All authors had full access to all data in the study and had final responsibility for the decision to submit for publication.

RESULTS

TNBC-DX development

The TNBC-DX genomic test was created based on two different gene signatures, the 10-gene CIG module (i.e. *CD274*, *CD79A*, *CXCR6*, *IRF4*, *LAX1*, *PDCD1*, *PIM2*, *POU2AF1*, *SLAMF1*, and *TNFRSF17*) and the 4-gene tumor cell proliferation signature (*EXO1*, *ASPM*, *NEK2*, and *KIF23*), as well as incorporating tumor size and nodal staging.

In the development cohorts (SCAN-B, CALGB-40603, and BrighTNess trials; $n = 1259$), the 10-CIG module and the 4-gene tumor cell proliferation signature were consistently associated with pCR and survival outcomes. The IGG signature was also associated with efficacy outcomes. Of note, 5 of the 10 CIGs (i.e. *CD79A*, *LAX1*, *PIM2*, *POU2AF1*, and *TNFRSF17*) were part of the 14-gene IGG module. As the CIG module presented better results, the IGG signature was not included in the score. Other signatures considered for inclusion, such as the HER2 amplicon signature and luminal differentiation, were not associated with efficacy outcomes and were not incorporated into the model. In addition, the *ERBB2* gene (i.e. the TNBC-DX ERBB2 score) was included to identify clinical HER2 status, but it did not contribute to the calculation of the pCR or risk scores. Thus, the final TNBC-DX test is based on the 10-CIG module, the HER2DX 4-gene tumor cell proliferation signature, tumor size, and nodal staging. Pre-established cut-offs were used to create the TNBC-DX pCR groups [low (0-33.3), medium (33.3-66.7), and high (66.7-100)] and the TNBC-DX risk groups [low (0-58) and high (59-100)]. Further details on the development of TNBC-DX can be found in Supplementary Materials, available at https://doi.org/10.1016/j.annonc.2024.10.012.

Baseline characteristics of the validation cohorts without pembrolizumab

A total of 527 patients with stage I-III TNBC were included in the first two external cohorts to validate the performance of the TNBC-DX test in the absence of pembrolizumab (Table 1). In the combined cohort, the median age was 52 years (range 26-80 years), clinical stage II disease represented 69.7%, and 41.9% had clinically node-positive disease. TILs (i.e. $\geq$10%) were observed in 56.8% of the patients. The TNBC-DX low- and high-risk categories represented 55.4% and 44.6% of the cases, respectively. The TNBC-DX pCR-low, pCR-med, and pCR-high categories represented 33.0%, 33.0%, and 34.0% of the cases, respectively. A significant association was observed between TNBC-DX risk groups and pCR groups, where the pCR-high group was more prevalent in the low-risk group than in the high-risk group (79.9% versus 20.1%). A moderate correlation was observed between both continuous scores ($\rho = -0.56$). Clinicopathologic characteristics according to TNBC-DX pCR and risk groups are provided in Supplementary Table S4, available at https://doi.org/10.1016/j.annonc.2024.10.012.

TNBC-DX association with pCR in the absence of pembrolizumab

The pCR rate was 34.1% (95% CI 26.1% to 43.2%) in the ADAPT-TN cohort, 48.6% (95% CI 42.8% to 54.5%) in the MMJ-CAR-2014-01 cohort, and 44.3% (95% CI 39.5% to 49.2%) in the combined cohort. The pCR rate with taxane—carboplatin and nab-paclitaxel—gemcitabine regimens was 46.4% and 34.7%, respectively. The TNBC-DX pCR score was significantly associated with pCR in the ADAPT-TN cohort (OR per 10-unit increase 1.22, 95% CI 1.06-1.41, $P = 0.006$), in the MMJ-CAR-2014-01 cohort (OR 1.37, 95% CI 1.24-1.52, $P < 0.001$), and in the combined series (OR 1.28, 95% CI 1.18-1.38, $P < 0.001$; Figure 1A). The pCR rate in the TNBC-DX pCR-high group was higher than that in the pCR-low group (56.3% versus 22.5%, OR 4.45, 95% CI 2.67-7.57, $P < 0.001$). Discrimination, calibration plots, and sensitivity analysis are shown in Supplementary Figures S1-S5, available at https://doi.org/10.1016/j.annonc.2024.10.012. In the multivariable model from the combined cohort including clinicopathological factors and treatment, the TNBC-DX pCR score remained significantly associated with pCR (OR 1.34, 95% CI 1.20-1.52, $P < 0.001$) along with clinical nodal stage and the chemotherapy regimen. Of

Table 1. Clinicopathological characteristics of patients in the West German Group ADAPT-TN, the MMJ-CAR-2014-01, and the NeoPACT studies

		Combined (n = 527)	ADAPT-TN (n = 126)	MMJ-CAR-2014-01 (n = 292)	NeoPACT (n = 109[a])
Mean age (range), years		52 (26-80)	52 (26-76)	53 (26-80)	50 (27-70)
Tumor stage, *n* (%)	cT1	110 (20.9)	51 (40.5)	39 (13.4)	20 (18.3)
	cT2	308 (58.4)	66 (52.4)	173 (59.2)	69 (63.3)
	cT3-4	109 (20.7)	9 (7.1)	80 (27.4)	20 (18.3)
Nodal stage, *n* (%)	cN0	306 (58.1)	91 (72.2)	147 (50.3)	68 (62.4)
	cN1	178 (33.8)	31 (24.6)	112 (38.4)	35 (32.1)
	cN2-3	43 (8.1)	4 (3.2)	33 (11.3)	6 (5.5)
Overall stage, *n* (%)	Stage 1	75 (14.2)	40 (31.7)	22 (7.5)	13 (11.9)
	Stage 2	367 (69.7)	79 (62.7)	205 (70.2)	83 (76.1)
	Stage 3	85 (16.1)	7 (5.6)	65 (22.3)	13 (11.9)
TILs mean (range)		26.5 (0-95)	31.3 (0-90)	21.8 (0-90)	33.3 (1-95)
TILs group, *n* (%)	0%-10%	227 (43.2)	34 (27.0)	145 (49.7)	48 (44.4)
	>10%-50%	193 (36.7)	67 (53.2)	103 (35.3)	23 (21.3)
	>50%	106 (20.1)	25 (19.8)	44 (15.1)	37 (34.3)
Histological grade, *n* (%)	1-2	111 (21.1)	9 (7.1)	88 (30.1)	14 (12.8)
	3	416 (79.1)	117 (92.9)	204 (69.9)	95 (87.2)
Neoadjuvant treatment, *n* (%)	Taxane—carboplatin	343 (65.1)	51 (40.5)	292 (100)	0 (0)
	Nab-paclitaxel—gemcitabine	75 (14.2)	75 (59.5)	0 (0)	0 (0)
	Pembrolizumab, carboplatin, and docetaxel	109 (20.7)	0 (0)	0 (0)	109 (100)
Pathological response	pCR	231 (43.8)	43 (34.1)	140 (49.5)	46 (42.2)
	Residual disease	296 (56.2)	83 (65.9)	143 (50.5)	63 (57.8)
TNBC-DX pCR groups	Low	174 (33.0)	35 (27.8)	103 (35.3)	36 (33.0)
	Medium	174 (33.0)	32 (25.4)	106 (36.3)	36 (33.0)
	High	179 (34.0)	59 (46.8)	83 (28.4)	37 (33.9)
TNBC-DX risk groups	Low	292 (55.4)	86 (68.3)	131 (44.9)	75 (68.8)
	High	235 (44.6)	40 (31.7)	161 (55.1)	34 (31.2)

pCR, pathologic complete response; TIL, tumor-infiltrating lymphocyte; TNBC, triple-negative breast cancer.
[a]109/115 (94.8%) of pCR assessable population in Sharma, Stecklein et al., *JAMA Oncology*, 2024. Tumor-infiltrating lymphocytes unavailable for $n = 1$ patient.

note, despite TILs being associated with pCR in univariate analysis, the TILs variable lost its significance in the multivariable analysis when TNBC-DX pCR score was included in the model (OR 1.03, 95% CI 0.94-1.14, $P = 0.48$). Analyses evaluating TNBC-DX and TILs both as a continuous score and as a group category are shown in Supplementary Tables S5, S6 and Figure S6, available at https://doi.org/10.1016/j.annonc.2024.10.012. The association between the TNBC-DX pCR groups and pCR endpoint was consistent across the chemotherapy regimens (Figure 1B).

TNBC-DX association with survival in the absence of pembrolizumab

The median follow-up of the ADAPT-TN and MMJ-CAR-2014-01 cohorts was 60.2 and 50.5 months, respectively. Similar outcomes were observed between both cohorts in DDFS and OS (Supplementary Figures S7 and S8, available at https://doi.org/10.1016/j.annonc.2024.10.012). The 5-year DDFS and OS of the combined cohort was 80.0% (95% 76.1% to 84.2%) and 82.3% (95% 78.4% to 86.4%), respectively. In the DDFS univariable analysis, a statistically significant association between the TNBC-DX risk score and DDFS in each individual study and in the combined cohort (HR per 10-unit increase 1.37, 95% CI 1.25-1.51, $P < 0.001$) was observed (Figure 2A). The 5-year DDFS in the TNBC-DX low-risk group was higher than that in the high-risk group (89.9% versus 69.4%, HR 0.24, 95% CI 0.15-0.41, $P < 0.001$; Figure 2B). In the multivariable model including all the evaluated factors, the TNBC-DX risk score remained significantly associated with DDFS (HR per 10-unit increase 1.33, 95% CI 1.09-1.61, $P = 0.004$). Of note, TILs were not associated with DDFS in the multivariable analysis (HR 1.03, 95% CI 0.92-1.14, $P = 0.61$). Results from analyses evaluating TNBC-DX and TILs, both as continuous scores and as categorical groups, are presented in Supplementary Tables S7 and S8, available at https://doi.org/10.1016/j.annonc.2024.10.012.

Similar results were observed when OS was evaluated (Supplementary Figure S9, available at https://doi.org/10.1016/j.annonc.2024.10.012). TNBC-DX was associated with OS in each validation cohort and in the combined analysis, both as a risk score and as a risk group. The 5-year OS in the combined cohort was 93.8% in the TNBC-DX low-risk group and 70.8% in the high-risk group (HR 0.19, 95% CI 0.11-0.35, $P < 0.001$; Figure 2C). The TNBC-DX risk score remained statistically associated with OS after adjustment by clinical variables, TILs, and treatment regimen (HR per 10-unit increment 1.34, 95% CI 1.09-1.65, $P = 0.006$). However, no association was observed between TILs and OS in the multivariable model (Supplementary Figure S9, available at https://doi.org/10.1016/j.annonc.2024.10.012). Overall, the prognostic value of TNBC-DX, both as a continuous score and as a risk group, in identifying patients with a higher likelihood of achieving a pCR and a lower risk of disease recurrence and death was independent of clinicopathological factors and treatment characteristics (Figure 3). Similar results were observed when using iDFS (Supplementary Figure S10, available at https://doi.org/10.1016/j.annonc.2024.10.012).

A

Variable		N	pCR rate	Univariable analysis OR (95% CI)	*P*-value	Multivariable analysis OR (95% CI)	*P*-value
TNBC-DX pCR score (10-unit increment)		418	-	1.28 (1.18-1.38)	<0.001	1.34 (1.20-1.52)	**<0.001**
TNBC-DX pCR score groups	Low	138	22.5%	Reference		Reference[a]	
	Med	138	53.6%	3.99 (2.39-6.79)	<0.001	3.74 (2.15-6.64)[a]	**<0.001**
	High	142	56.3%	4.45 (2.67-7.57)	<0.001	3.48 (1.72-7.15)[a]	**<0.001**
Age (10-unit increment)		418	-	0.83 (0.71-0.98)	0.024	0.89 (0.75-1.07)	0.221
Clinical tumor stage	T1	90	54.4%	Reference		Reference	
	T2	239	44.8%	0.68 (0.42-1.10)	0.118	1.85 (0.93-3.72)	0.083
	T3−T4	89	32.6%	0.40 (0.22-0.74)	0.003	0.97 (0.44-2.11)	0.934
Clinical nodal stage	N0	238	47.9%	Reference		Reference	
	N1	143	44.8%	0.88 (0.58-1.34)	0.552	1.37 (0.84-2.26)	0.206
	N2+	37	18.9%	0.25 (0.10-0.57)	0.002	0.37 (0.13-0.91)	**0.039**
Histological grade	1−2	97	39.2%	Reference		Reference	
	3	321	45.8%	1.31 (0.83-2.10)	0.251	1.11 (0.65-1.89)	0.696
TILs (%) (10-unit increment)		418	-	1.14 (1.06-1.24)	<0.001	1.03 (0.94-1.14)	0.481
Chemotherapy regimen	Tax+Carbo	343	46.4%	Reference		Reference	
	Tax+Gem	75	34.7%	0.61 (0.36-1.03)	0.066	0.40 (0.22-0.72)	**0.003**

B

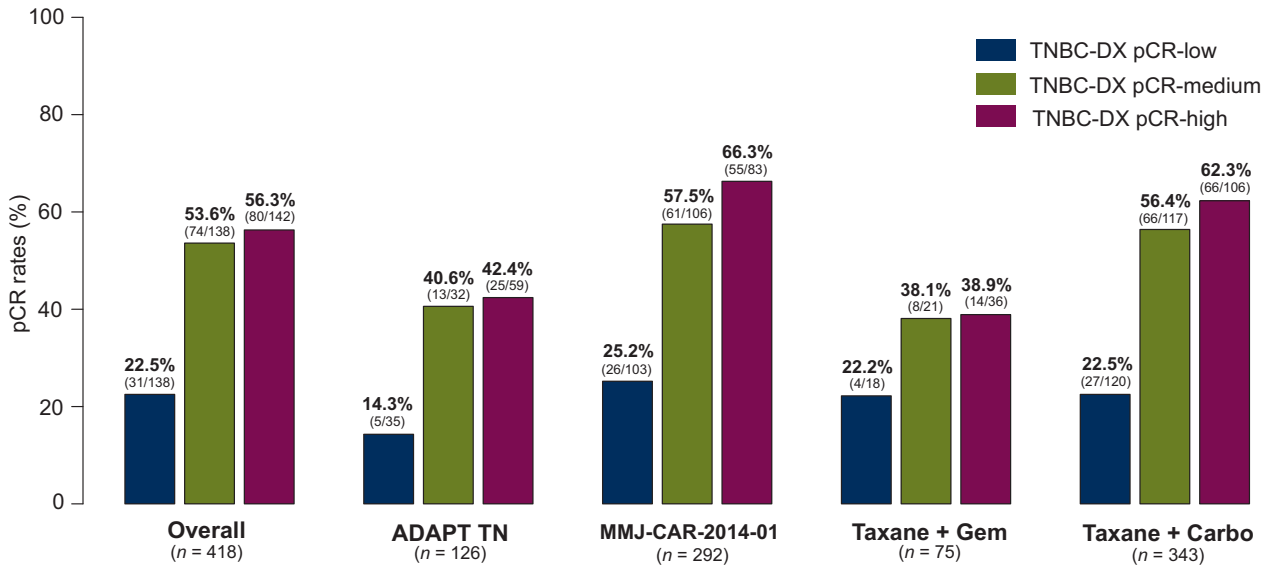


Figure 1. Association of TNBC-DX pCR score with pathological complete response (pCR) endpoint in the combined external validation cohort of 418 patients treated without pembrolizumab. (A) Univariable and multivariable logistic models to predict pCR. A separate multivariable model was estimated using TNBC-DX risk groups instead of TNBC-DX pCR score. To avoid multicollinearity, TNBC-DX pCR groups and TNBC-DX risk score cannot be included in the same model. (B) Bar plots showing the pCR rates across the HER2DX pCR groups based on the study and chemotherapy regimen. The interaction test between the TNBC-DX pCR score and treatment (Tax + Carbo versus Taxane + Gem) resulted in a *P* value of 0.07.

Carbo, carboplatin; CI, confidence interval; Gem, gemcitabine; OR, odds ratio; Tax, taxane; TIL, tumor-infiltrating lymphocyte; TNBC, triple-negative breast cancer.

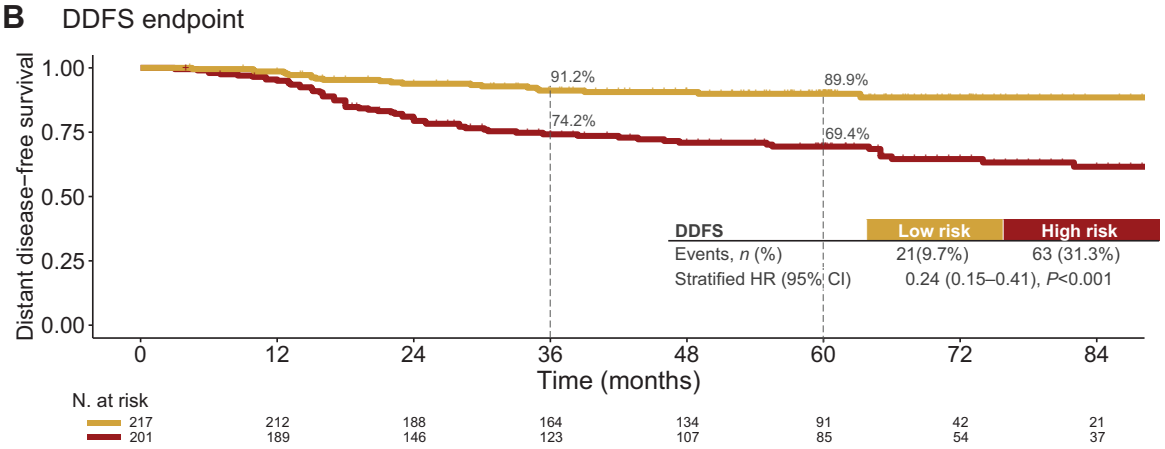
[a]A separate multivariable model has been carried out using TNBC-DX pCR groups instead of TNBC-DX pCR score. To avoid multicollinearity, TNBC-DX pCR groups and TNBC-DX pCR score cannot be included in the same model.

TNBC-DX risk score beyond pCR in the absence of pembrolizumab

The pCR status was significantly associated with survival outcomes (Figure 4A and B). Among patients who had a pCR following neoadjuvant therapy in the combined cohort ($n = 185$), the TNBC-DX low- and high-risk categories represented 41.6% and 58.4% of the cases, respectively. The TNBC-DX risk score as a continuous score was not significantly associated with DDFS (HR per 10-unit increment 1.18, 95% CI 0.95-1.45, $P = 0.14$), but it was associated with OS (HR per 10-unit increment 1.29, 95% CI 1.01-1.65, $P = 0.04$) (Supplementary Table S9, available at https://doi.org/10.1016/j.annonc.2024.10.012). Among patients who did achieve a pCR following neoadjuvant therapy ($n = 233$), the TNBC-DX low- and high-risk categories represented 46.8% and 53.2% of the cases, respectively; TNBC-DX risk score as a continuous score was significantly associated with DDFS (HR per 10-unit increment 1.37, 95% CI 1.22-1.53, $P < 0.001$) and with OS (HR per 10-unit increment 1.36, 95% CI 1.21-1.53, $P < 0.001$) (Supplementary Table S10, available at https://doi.org/10.1016/j.annonc.

A

Variable		N	Hazard ratio	Univariable analysis HR (95% CI)	*P*-value	Multivariable analysis HR (95% CI)	*P*-value
TNBC-DX risk score (10-unit increment)		418		1.37 (1.25-1.51)	**<0.001**	1.33 (1.09-1.61)	**0.004**
TNBC-DX risk groups	High	201		Reference		Reference[a]	
	Low	217		0.24 (0.15-0.41)	**<0.001**	0.52 (0.22-1.23)[a]	0.136
Age (10-unit increment)		418		1.02 (0.85-1.21)	0.86	1.06 (0.89-1.27)	0.521
Clinical tumor stage	T1	90		Reference		Reference	
	T2	239		1.03 (0.55-1.92)	0.93	0.68 (0.34-1.37)	0.279
	T3–T4	89		3.55 (1.82-6.91)	**<0.001**	1.48 (0.68-3.23)	0.321
Clinical nodal stage	N0	238		Reference		Reference	
	N1	143		3.43 (2.06-5.70)	**<0.001**	0.92 (0.37-2.29)	0.863
	N2+	37		6.06 (3.14-11.68)	**<0.001**	1.21 (0.41-3.58)	0.734
Histological grade	1–2	97		Reference		Reference	
	3	321		0.62 (0.38-1.03)	0.06	0.62 (0.37-1.04)	0.069
TILs (%) (10-unit increment)		418		0.93 (0.85-1.03)	0.16	1.03 (0.92-1.14)	0.614
Chemotherapy regimen	Tax+Carbo	343		Reference		Reference	
	Tax+Gem	75		0.41 (0.19-0.88)	**0.02**	0.47 (0.22-1.02)	0.056



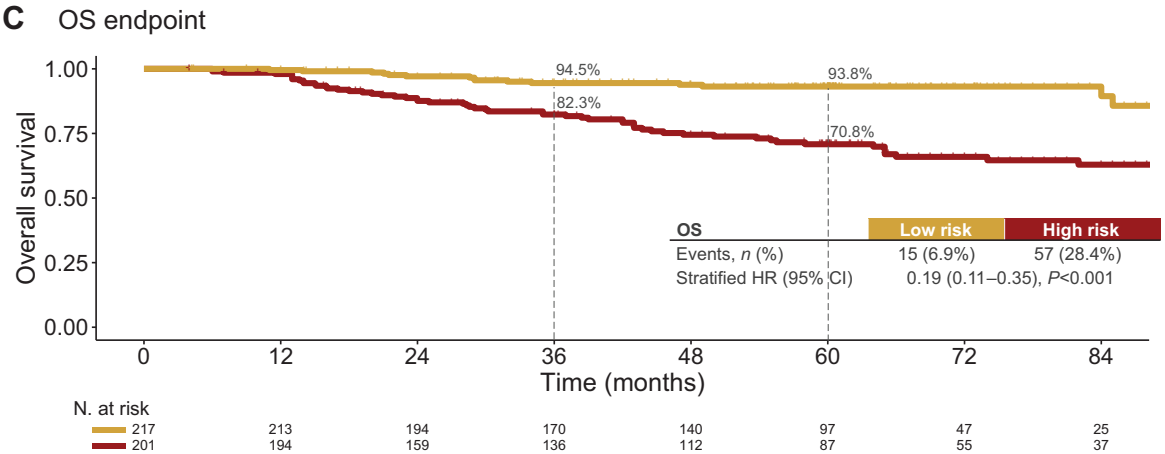


Figure 2. Association of TNBC-DX risk score with survival endpoint in the combined external validation cohort of 418 patients without pembrolizumab. (A) Univariable and multivariable Cox models to predict distant disease-free survival (DDFS). (B) Kaplan—Meier curves by TNBC-DX risk group (low risk versus high risk) in the DDFS endpoint. (C) Kaplan—Meier curves by TNBC-DX risk group (low-risk versus high-risk) in the OS endpoint.
Carbo, carboplatin; CI, confidence interval; Gem, gemcitabine; HR, hazard ratio; OS, overall survival; Tax, taxane; TNBC, triple-negative breast cancer.
[a]A separate multivariable model has been carried out using TNBC-DX risk groups instead of TNBC-DX risk score. To avoid multicollinearity, TNBC-DX risk groups and TNBC-DX risk score cannot be included in the same model.

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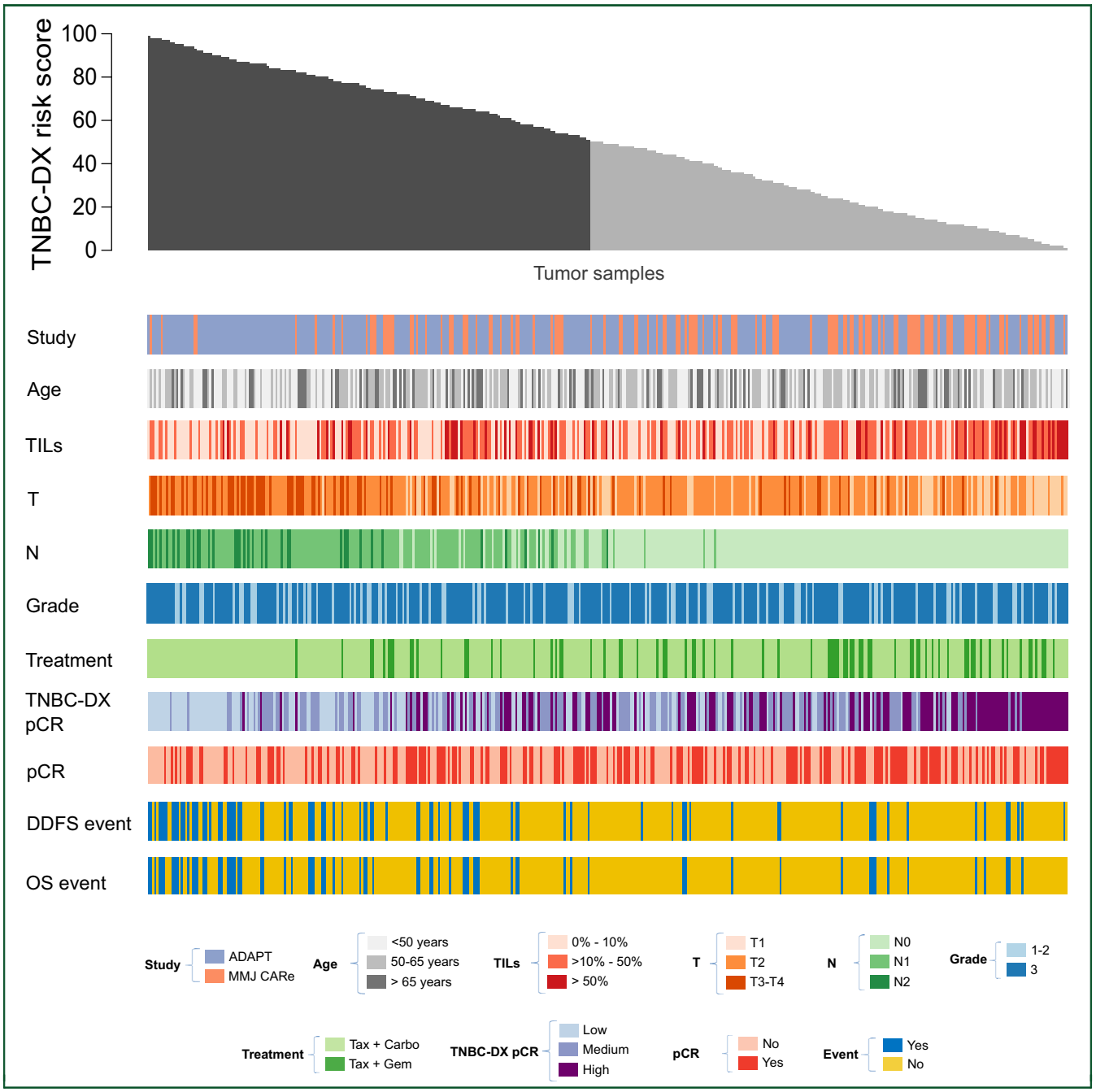

Figure 3. Association of TNBC-DX risk score with clinicopathological variables, pathological complete response (pCR) status, and treatment information in the combined external validation cohort of 418 patients without pembrolizumab. TNBC-DX risk score ranking and association with clinicopathological variables, TNBC-DX pCR groups, pCR endpoint, and type of treatment. Each column represents the information for a patient.
Carbo, carboplatin; DDFS, distant disease-free survival; Gem, gemcitabine; N, clinical nodal stage; OS, overall survival; T, clinical tumor stage; Tax, taxane; TIL, tumor-infiltrating lymphocyte; TNBC, triple-negative breast cancer.

2024.10.012). Figure 4C and D shows the association between pCR status, survival outcomes and TNBC-DX risk groups.

Validation of the TNBC-DX scores in the NeoPACT trial

Among the 115 patients originally recruited in the NeoPACT trial[25] (i.e. docetaxel, carboplatin and pembrolizumab), 109 (94.8%) had available TNBC-DX results. In these 109 patients treated, the overall pCR rate was 57.8% (95% CI 48.0% to 67.1%). TNBC-DX pCR score as a continuous score was significantly associated with pCR in the univariable and in the multivariable analysis after adjustment for clinicopathological factors (OR per 10-unit increase 1.28, 95% CI 1.03-1.61, $P = 0.030$) (Supplementary Table S11, available at https://doi.org/10.1016/j.annonc.2024.10.012). The pCR rates in TNBC-DX pCR-high, pCR-medium, and pCR-low groups were 78.4%, 66.1%, and 33.3%, respectively (high versus low: OR 7.25, 95% CI 2.55-20.62, $P < 0.001$; Figure 5A).

With a median follow-up of 31 months, the 3-year EFS and OS was 86.6% (95% CI 78.9% to 95.1%) and 92.4% (95%

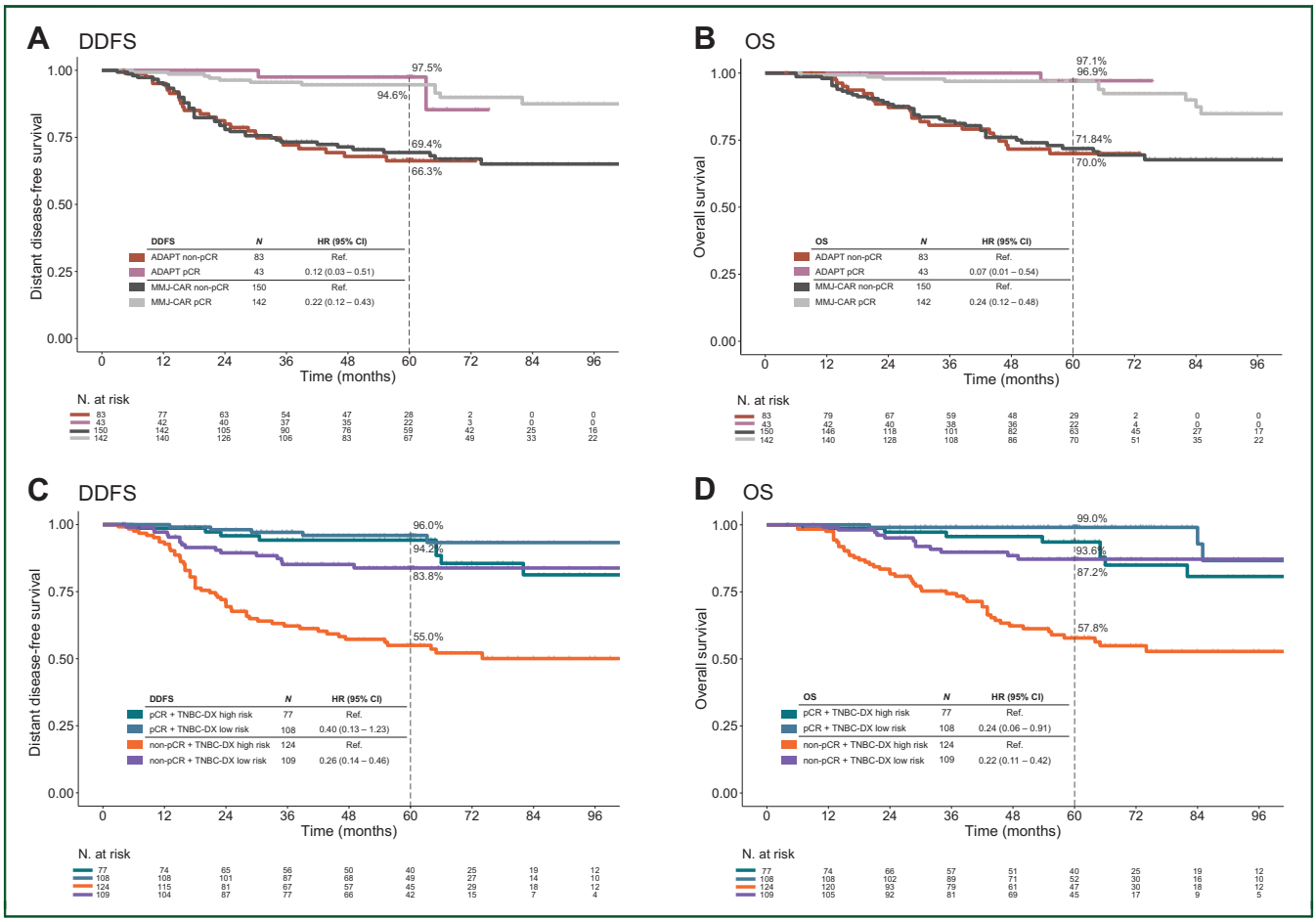

Figure 4. Distant disease-free survival (DDFS) and overall survival (OS) by pathological complete response (pCR) status and TNBC-DX risk group in patients treated without pembrolizumab. (A) DDFS by pCR status across study (ADAPT-TN and MMJ-CAR-2014-01), (B) OS by pCR status across study (ADAPT-TN and MMJ-CAR-2014-01), (C) DDFS by pCR status and by TNBC-DX score, (D) OS by pCR status and by TNBC-DX score.
CI, confidence interval; HR, hazard ratio; pCR, pathological complete response; TNBC, triple-negative breast cancer.

CI 87.0% to 98.0%), respectively. In the EFS univariate analysis, a statistically significant association was observed (HR per 10-unit increase 1.75, 95% CI 1.27-2.42, $P = 0.001$; Supplementary Table S12, available at https://doi.org/10.1016/j.annonc.2024.10.012). The 3-year EFS in the TNBC-DX low-risk group was higher than in the high-risk group (93.6% versus 69.3%, HR 0.08, 95% CI 0.02-0.36, $P = 0.001$; Figure 5B). Of note, TILs were not found to be significantly associated with EFS (HR per 10-unit increase 0.92, 95% CI 0.45-1.14, $P = 0.45$). Similar results were observed when OS was evaluated (Figure 5C and Supplementary Table S13, available at https://doi.org/10.1016/j.annonc.2024.10.012). The association between TNBC-DX risk groups and survival outcomes remained consistent after accounting for pCR status (Figure 5D and E).

Biology associated with TNBC-DX

Finally, to further explore the biology of TNBC-DX scores, we interrogated immune and proliferation gene expression, *TP53* and *PIK3CA* mutations and PAM50, TNBC subtypes, and TIME[28] classification in TNBC tumors from TCGA.[26] TNBC-DX low-risk tumors were enriched for immune gene expression, while the TNBC-DX pCR-high group had higher expression of proliferative genes (Supplementary Figure S11, available at https://doi.org/10.1016/j.annonc.2024.10.012). In addition, the Basal-like 1 TNBC subtype had significantly higher TNBC-DX pCR score ($P = 0.003$). TNBC-DX risk scores were not significantly associated with *TP53* and *PIK3CA* mutations, PAM50, or TIME classification.

DISCUSSION

TNBC-DX is a novel genomic test designed for patients with newly diagnosed stage I-III TNBC. Using a machine learning approach, the assay integrates tumor and nodal staging with immune and proliferation signatures and provides two scores (ranging from 0 to 100): one predicting pCR and another forecasting long-term survival outcomes. In this study, we validated both TNBC-DX scores in 527 patients treated with neoadjuvant taxane-based chemotherapy with or without pembrolizumab across three studies with long-term patient follow-up.

Today, the conventional approach to treating stage I-III TNBC has predominantly involved multiagent chemotherapy regimens, such as AC and taxane (AC—T or AC—TCb), complemented by pembrolizumab for stage II-III disease.[1,3] While these advancements have improved patient

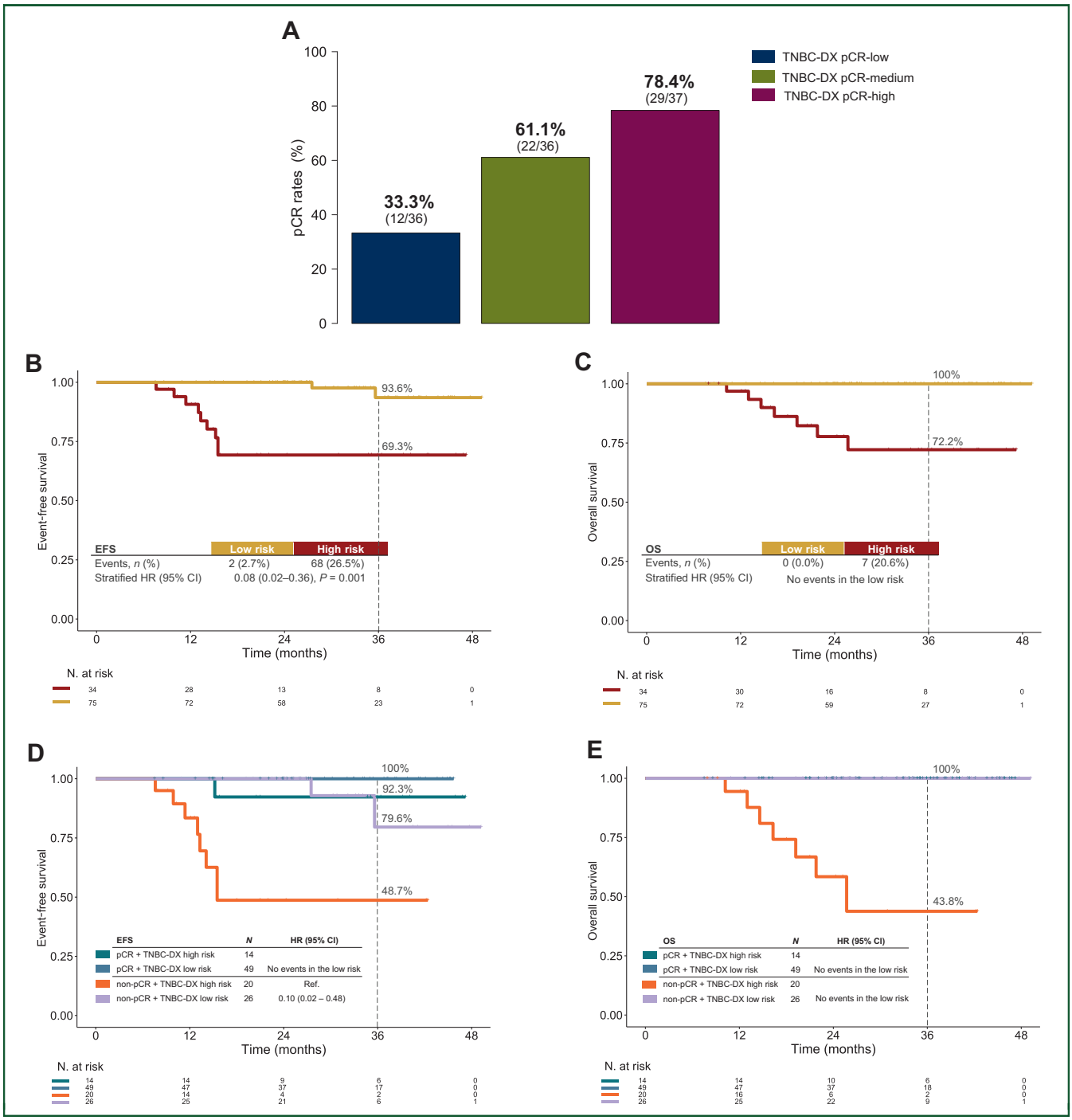

Figure 5. Independent validation of TNBC-DX pathological complete response (pCR) and risk scores in patients treated with neoadjuvant docetaxel, carboplatin, and pembrolizumab in the NeoPACT phase II clinical trial. (A) pCR rates according to the TNBC-DX pCR score groups (i.e. low, medium, and high). (B) Event-free survival (EFS) by TNBC-DX risk score groups. (C) Overall survival (OS) by TNBC-DX risk score groups. (D) EFS in patients with pCR or residual disease at surgery by TNBC-DX risk score groups. (E) OS in patients with pCR or residual disease at surgery by TNBC-DX risk score groups.
TNBC, triple-negative breast cancer.

outcomes, they often lead to overtreatment and associated toxicities, as evidenced by real-world studies evaluating the implementation of neoadjuvant pembrolizumab.[31-33] Consequently, there is a discernible shift toward systemic therapy de-escalation, particularly through omitting anthracyclines in favor of a taxane and carboplatin combination. This strategy, which has shown promise in achieving favorable pCR and 3-year survival rates,[7-11,25] needs further validation.

The potential de-escalation of immunotherapy, especially in the context of the KEYNOTE-522[4] trial findings, has also drawn significant attention.[34] The trial showcased marked improvements in pCR and EFS with the addition of neoadjuvant pembrolizumab plus chemotherapy, followed by adjuvant pembrolizumab for stage II-III TNBC.[4,35] However, no definitive evidence has shown that patients with a pCR following neoadjuvant therapy do not benefit from continued pembrolizumab treatment in the adjuvant

setting. While this raises the possibility that continued pembrolizumab may not substantially improve outcomes after pCR,[36] this hypothesis requires further validation in randomized trials such as OptimICE-pCR. This consideration is supported by findings from the randomized GeparNuevo phase II trial with durvalumab in combination with chemotherapy, where the immune checkpoint inhibitor was only administered during the neoadjuvant phase.[37] Furthermore, while the NeoPACT neoadjuvant phase II trial with docetaxel—carboplatin—pembrolizumab for 18 weeks reported pCR rates (58%) comparable with those observed in the KEYNOTE-522 regimen (64.8%),[4,25] it is important to note that differences in study populations—such as variations in node positivity, inclusion of stage I patients, and ER/PR threshold of 1% versus 10%, limit the direct comparability of these results.

The absence of established biomarkers to calibrate chemotherapy intensity and guide the omission of (neo) adjuvant pembrolizumab underscores the complexity of decision making in patients with stage I-III TNBC. This challenge highlights the potential value of TNBC-DX as a clinical tool. For instance, in patients identified as pCR-high and/or low-risk by TNBC-DX, clinicians could opt for less aggressive treatment regimens. An 18-week course of neoadjuvant docetaxel—carboplatin, with or without pembrolizumab, could be used instead of AC—TCb with pembrolizumab. In addition, stratifying patients in this way would mean that those who achieve a pCR may not require further systemic therapy. This TNBC-DX-tailored approach could reduce unnecessary systemic therapies, their associated side-effects, and positively impact patients' quality of life.

The pCR rates in the TNBC-DX pCR-low group range from 22% to 33%. In clinical stage II-III TNBC, a TNBC-DX pCR-low classification is unlikely to change the standard course of treatment, such as the use of the KEYNOTE-522 regimen. However, combining a pCR-low result with a high-risk score could help identify a subgroup with unmet needs, making them a priority for future trials focused on treatment escalation with novel therapies. In addition, in clinical stage I, where uncertainty exists between opting for primary surgery or neoadjuvant therapy, a TNBC-DX result showing both pCR-low and low-risk disease may favor the decision for primary surgery, potentially avoiding unnecessary neoadjuvant and adjuvant therapy. Further studies are required to better define the clinical utility of TNBC-DX in guiding treatment decisions in these situations.

The moderate correlation observed between the TNBC-DX risk score and pCR score reflects their distinct but complementary roles. While the proliferation component within TNBC-DX is key for predicting pCR, it also helps identify tumors with a high-risk profile for long-term outcomes, demonstrating the dual utility of the TNBC-DX test. In addition, although the pCR rates between the medium and high pCR score groups were similar in our combined dataset without pembrolizumab, the NeoPACT trial showed a numerically higher pCR rate in the pCR-high versus medium group (78.4% versus 61.1%). This high pCR rate approaching 80% in the pCR high group with carboplatin/docetaxel plus pembrolizumab is particularly notable. In addition, the identification of patients with pCR whose tumor is TNBC-DX high risk underscores the complementary nature of the TNBC-DX risk score and pCR. While pCR reflects individual response to therapy, the TNBC-DX risk score captures baseline risk independent of therapy, providing a more comprehensive assessment of prognosis and helping guide further management in patients who achieve pCR but remain at high risk for recurrence.

Several ongoing trials are exploring de-escalation strategies that TNBC-DX could enhance. Notably, the OptimICE-pCR phase III trial (NCT05812807) is comparing the effect of pembrolizumab with observation for the treatment of 1295 patients with early-stage TNBC who achieved a pCR after preoperative chemotherapy in combination with pembrolizumab. The SCARLET phase III trial (NCT05929768) is comparing the effect of pembrolizumab in combination with neoadjuvant docetaxel—carboplatin with pembrolizumab in combination with AC and paclitaxel—carboplatin for the treatment of 2400 patients with stage II-III TNBC. In addition, two phase II trials are exploring other treatment strategies. The ETNA trial (NCT06078384) will evaluate the survival outcomes in 354 patients with surgically resected stage I TNBC following adjuvant treatment with paclitaxel—pembrolizumab or no therapy. Finally, the ADAPT-TN-III neoadjuvant trial (NCT06081244) will evaluate 12 weeks of sacituzumab govitecan with or without pembrolizumab in clinically stage I TNBC.

Other phase III trials are underway to explore escalation strategies. The SASCIA phase III trial (NCT04595565) is comparing the effect of sacituzumab govitecan with capecitabine or platinum therapy for the treatment of 1332 patients with early-stage HER2-negative disease, including TNBC, who did not achieve a pCR after preoperative chemotherapy. The ASCENT-05/OptimICE-RD phase III trial (NCT05633654) will evaluate the efficacy and safety of sacituzumab govitecan in combination with pembrolizumab versus pembrolizumab (with or without capecitabine, per treating physician discretion) in 1500 patients with TNBC without a pCR following neoadjuvant chemotherapy. Finally, the MK-2870-012 phase III trial (NCT06393374) will evaluate the efficacy and safety of sacituzumab tirumotecan in combination with pembrolizumab versus treatment of physician's choice in 1530 patients with TNBC without a pCR following neoadjuvant chemotherapy. These trials highlight TNBC-DX's potential to guide personalized treatments and optimize outcomes.

Beyond TNBC-DX, pretreatment baseline TILs have been extensively investigated in early-stage TNBC. A high proportion of TILs has been shown to predict pCR to neoadjuvant chemotherapy and better survival outcomes, even in the absence of (neo)adjuvant chemotherapy.[13-15,38,39] In our study, we found that while the immune signature of TNBC-DX moderately correlated with TILs levels, TNBC-DX scores demonstrated superior predictive power for pCR and survival outcomes compared with TILs alone. However, it is important to acknowledge that the most significant

potential clinical use of TILs, particularly in small, lymphocyte-rich TNBC, is the possibility of completely forgoing adjuvant treatment—an area where TNBC-DX data are not currently available. Another added value of TNBC-DX scores over TILs is their potential for standardization, offering more consistent and reproducible measurements across different laboratories. The superiority of gene expression over TILs for predicting patient outcomes has also been observed in early-stage HER2+ breast cancer.[40]

The use of other biomarkers in early-stage TNBC, such as dynamics of circulating tumor DNA (ctDNA) during neoadjuvant therapy, is also being actively investigated.[41,42] TNBC-DX differs from ctDNA in several critical ways, primarily in its timing and application. Thus ctDNA could eventually complement TNBC-DX by offering additional, real-time information that can guide adjustments in therapy midcourse or after surgery, ensuring that treatment remains aligned with the patient's evolving response.

Our study has limitations. For example, the retrospective design and its reliance on nonrandomized cohorts could introduce selection bias, limiting our capacity to precisely gauge the predictive power of TNBC-DX scores for specific therapeutic interventions. In addition, in the ADAPT-TN trial, detailed information on adjuvant chemotherapy was not captured, which limits our ability to adjust for its impact on patient outcomes. Moreover, the routine administration of anthracycline-based therapy in the adjuvant setting for most patients with residual disease, the median follow-up period of ∼5-6 years, and the absence of long-term outcome data beyond this timeframe necessitates further inquiry. However, it is important to note that TNBC recurrences are most common within the first 3-5 years after treatment.[43] Furthermore, TNBC-DX low-risk classification in patients without a pCR may currently lack clinical utility in guiding adjuvant systemic therapy, although it could aid in future patient selection for adjuvant trials. We also acknowledge that the ability of the TNBC-DX pCR score to predict pCR is lower compared with the HER2DX pCR score in HER2-positive breast cancer. This difference may be partially explained by the higher heterogeneity of HER2-positive disease,[44] which involves more distinct molecular subtypes and therapeutic targets compared with the relatively homogeneous nature of TNBC. Finally, we have not explored the value of TNBC-DX in the 5%-15% of patients with TNBC who have germline BRCA1/2 mutations and are candidates for receiving 1 year of adjuvant olaparib.[45]

To conclude, the TNBC-DX genomic test offers a valuable tool for predicting pCR and survival outcomes in early-stage TNBC. This advancement supports the shift toward more personalized and potentially less intensive treatment options, helping to better align therapeutic strategies with the unique profiles and needs of patients with TNBC.

ACKNOWLEDGEMENTS

We thank the patients who participated in these trials, their families, and caregivers.

FUNDING

This work was supported by Reveal Genomics; the European Union's Horizon 2020 Research and Innovation Programme under the Marie Skłodowska—Curie grant [grant number 955951 to BC]; Fundación CRIS contra el cáncer PR_EX_2021-14, to AP Agència de Gestó d'Ajuts Universitaris i de Recerca 2021 SGR 01156 to AP, Fundación Fero BECA ONCOXXI21, "PI22/01017", funded by Instituto de Salud Carlos III (ISCIII) and co-funded by the European Union to AP, Asociación Cáncer de Mama Metastásico IV Premios M. Chiara Giorgetti to AP, Breast Cancer Research Foundation BCRF-22-198, Associació Beca Marta Santamaria to AP, and RESCUER to AP, funded by the European Union's Horizon 2020 Research and Innovation Programme under [grant number 847912 to AP]; Fundación científica AECC Ayudas Investigador AECC 2021 [grant INVES21943-BRAS to FBM] and Fundación Contigo (HIMALAIA). The NeoPACT trial was supported by The University of Kansas Cancer Center (KUCC) to FBM, the Cancer Center Support Grant to KUCC [grant P30 CA168524] (Biospecimen Repository Core Facility), the KUCC ACS-IRG Pilot award, University of Kansas Medical Center Research Institute (Lied Pilot award), National Institutes of Health KL2 award [grant KL2TR002367-05], Team Michelle, and by Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, NJ, USA.

DISCLOSURE

LP is an employee of Reveal Genomics and has the following patents filed: PCT/EP2021/070788, EP23382703, and EP23383369. GV has received a speaker's fee from MSD, Pfizer, GSK, and Pierre Fabre; has held an advisory role with AstraZeneca and received consultant fees from Reveal Genomics. CMP reports stockholder and consulting fees from Reveal Genomics. AP reports advisory and consulting fees from AstraZeneca, Roche, Pfizer, Novartis, Daiichi Sankyo, and Peptomyc; lecture fees from AstraZeneca, Roche, Novartis, and Daiichi Sankyo; institutional financial interests from AstraZeneca, Novartis, Roche, and Daiichi Sankyo; stockholder and employee of Reveal Genomics; patents filed PCT/EP2016/080056, PCT/EP2022/086493, PCT/EP2023/060810, EP23382703, and EP23383369. FBM reports part-time employment from Reveal Genomics, and has the following patents filed: PCT/EP2022/086493, PCT/EP2023/060810, EP23382703, and EP23383369. MM has received research grants from Roche, PUMA, and Novartis; consulting/advisory fees from AstraZeneca, Amgen, Taiho Oncology, Roche/Genentech, Novartis, PharmaMar, Eli Lilly, PUMA, Taiho Oncology, Daiichi Sankyo, Menarini/Stemline, and Pfizer; and speakers' honoraria from AstraZeneca, Lilly, Amgen, Roche/Genentech, Novartis, and Pfizer. OG reports personal fees from Celgene, Roche, AstraZeneca, Amgen, MSD, Novartis, Pfizer, Lilly, Gilead, Genomic Health/Exact Sciences, Molecular Health, NanoString Technologies, Pierre Fabre, and Seagen; nonfinancial support from Daiichi

Sankyo, all outside the submitted work; and serving as a co-director of the West German Study Group. BC reports speaker fees from Veracyte and payment for educational events from Medsite and Novartis. FM reports performing consultant/advisory role for Astra Zeneca, Roche, Pfizer, Pierre-Fabre, Seagen, Daiichi-Sankyo, and MSD; research funding (paid to the institution) from Pfizer; serves on the speakers bureau for Pfizer and Daiichi-Sankyo. TM reports consultant or advisory boards fees from AstraZeneca, Novartis, Roche, GSK, and Menarini; travel grants from Novartis and Astra Zeneca. SLT reports financial interests in Astra Zeneca; serves on the advisory board (personal capacity) of Daiichi Sankyo, Gebro Pharma, Gilead, GSK, Lilly, Menarini—Stemline, Personal MSD, Novartis, Pfizer, Pierre Fabre, Roche, Seagen, and Veracyte; and was an invited speaker for Lilly (personal capacity). AV has the DNADX patent filed (EP22382387.3). ES has received fees as consultant, participated in advisory boards from AstraZeneca. JSP is an equity stockholder and consultant for Reveal Genomics and is also listed as an inventor on patent applications for the Breast PAM50 assay. PV is a stockholder of Reveal Genomics; reports personal fees from NanoString Technologies. PS reports research funding from Merck, Novartis, and Bristol Myers Squibb; royalties from UpToDate; and personal fees from Novartis, Merck, AstraZeneca, Pfizer, Gilead, GlaxoSmithKline, and Sanofi. NH reports honoraria for lectures and/or consulting from Amgen, AstraZeneca, Daiichi-Sankyo, EPG Communication, Gilead, Eli Lilly and Company, MEDSCAPE, MSD, Novartis, Pierre-Fabre, Pfizer, Roche, Sandoz, Sanofi, Seagen, Springer, Viatris, and Zuellig Pharma; ownership interest (minority) as a co-director of the West German Study Group (WSG), outside of the submitted work. All other authors have declared no conflicts of interest.

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