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ORIGINAL ARTICLE

Characterization of HER2-low breast cancer in young women with germline *BRCA1/2* pathogenetic variants: Results of a large international retrospective cohort study

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Abstract

Background: Breast cancer (BC) in women aged ≤40 years carrying germline pathogenetic variants (PVs) in *BRCA1/2* genes is infrequent but often associated with aggressive features. Human epidermal growth factor receptor 2 (HER2)-low-

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expressing BC has recently emerged as a novel therapeutic target but has not been characterized in this rare patient subset.

Methods: Women aged ≤40 years with newly diagnosed early-stage HER2-negative BC (HER2-0 and HER2-low) and germline *BRCA1/2* PVs from 78 health care centers worldwide were retrospectively included. Chi-square test and Student *t*-test were used to describe variable distribution between HER2-0 and HER2-low. Associations with HER2-low status were assessed with logistic regression. Kaplan–Meier method and Cox regression analysis were used to assess disease-free survival (DFS) and overall survival. Statistical significance was considered for $p \leq .05$.

Results: Of 3547 included patients, 32.3% had HER2-low BC, representing 46.3% of hormone receptor–positive and 21.3% of triple-negative (TN) tumors. HER2-low vs. HER2-0 BC were more often of grade 1/2 ($p < .001$), hormone receptor–positive ($p < .001$), and node-positive ($p = .003$). *BRCA2* PVs were more often associated with HER2-low than *BRCA1* PVs ($p < .001$). HER2-low versus HER2-0 showed better DFS (hazard ratio [HR], 0.86; 95% CI, 0.76–0.97) in the overall population and more favorable DFS (HR, 0.78; 95% CI, 0.64–0.95) and overall survival (HR, 0.65; 95% CI, 0.46–0.93) in the TN subgroup. Luminal A–like tumors in HER2-low ($p = .014$) and TN and luminal A-like in HER2-0 ($p = .019$) showed the worst DFS.

Conclusions: In young patients with HER2-negative BC and germline *BRCA1/2* PVs, HER2-low disease was less frequent than expected and more frequently linked to *BRCA2* PVs and associated with luminal-like disease. HER2-low status was associated with a modestly improved prognosis.

KEYWORDS
BRCA, breast cancer, HER2-low, subtypes, young women

INTRODUCTION

In up to 10% of cases, breast cancer (BC) is diagnosed in women aged ≤40 years, featuring more aggressive biological characteristics than tumors in women >40 years.[1,2] Roughly 12% of these tumors are directly related to the presence of germline pathogenic or likely pathogenic variants (PVs) in BC susceptibility genes *BRCA1* and/or *BRCA2*.[3] Approximately 90% of *BRCA*-related tumors do not harbor *ERBB2* overexpression/amplification, being hormone receptor–positive (HR+)/human epidermal growth factor receptor 2 (HER2)-negative or triple negative (TNBC) in most cases.[4,5]

Among HER2-negative BCs, approximately 40% to 55% are classified as HER2-low if presenting with a HER2 immunohistochemical (IHC) score of 1+, or 2+ without *ERBB2* (HER2 gene) amplification by in situ hybridization techniques.[6–8] HER2-low BC as a nosological entity is in the oncology community spotlight since 2018 when impressive response rates to novel anti-HER2 antibody drug conjugates such as trastuzumab-deruxtecan (T-DXd) were demonstrated.[9,10] Its clinicopathological, molecular, and prognostic features have been extensively explored in the past few years.[11–15] Nevertheless, there is currently no data concerning the prevalence of HER2-low status in young women with BC, especially in those harboring germline *BRCA1/2* PVs. Moreover, the potential prognostic implications of this unique patient subset are currently unknown.

We report here an analysis about the clinicopathological features of HER2-low BC and its impact on survival outcomes in germline *BRCA1/2* carriers diagnosed at the age of ≤40 years.

MATERIALS AND METHODS

Study design and procedures

This is an international multicenter retrospective cohort study including women ≤40 years of age at the time of invasive BC diagnosis, who harbored germline PVs in *BRCA1* and/or *BRCA2* genes. Patients were treated in 78 different health care institutions worldwide between January 2000 and December 2020. Inclusion/exclusion criteria were previously reported.[16] For this study, only patients with stage I-III HER2-negative breast tumors with known

HER2 IHC score were considered; HER2-negative cases with missing IHC score were excluded.

Data on country of treatment, year of diagnosis, age at diagnosis, menopausal status, *BRCA1/2* status, primary tumor dimension (T) and axillary lymph-node involvement (N) at diagnosis, treatments received (surgery type, endocrine therapy [ET], chemotherapy [CT]), histology, grade (G), HER2 (detailed IHC score), and hormone receptor status (positive/negative) were retrieved from patients' charts.

HER2, estrogen receptor (ER), and progesterone receptor (PgR) were locally assessed according to the ASCO/CAP guidelines.[7,17] Because detailed PgR and Ki67 levels were not available for this study, IHC surrogates of molecular subtypes were defined according to Partridge et al.,[2] as follow: (a) luminal A–like (HR+, G1-2, and HER2-negative); (b) luminal B–like (HR+, G3, and HER2-negative); (c) TNBC (HR-negative and HER2-negative).

Following local regulations, the study received ethics approval by the local independent ethical review committees/institutional review boards; whenever requested, participants provided written informed consent before inclusion.

Study objectives

The primary objective of this study was to describe the clinicopathological features of early-stage HER2-low versus HER2-0 breast tumors in young women with germline *BRCA1/2* PVs.

Secondary objectives included: assessing the association between HER2 status (low vs. 0) and disease-free survival (DFS) or overall survival (OS) in the whole study cohort, and then according to biological surrogate subtypes, namely luminal A–like, luminal B–like, and TNBC; assessing the association between surrogate subtypes according to HER2 status and patient DFS or OS; and evaluating the association of PVs in *BRCA*1 versus *BRCA2* with HER2-low status and their prognostic significance.

Statistical analyses

Descriptive statistics were used to describe clinicopathological characteristics as well as the pattern of DFS/OS events (Supplementary Material 1). Survival probabilities were computed with the Kaplan–Meier method and compared with the log-rank test. Cox proportional hazards models were used to estimate hazard ratios with 95% CIs, stratifying for selected confounders elected among the clinically meaningful differentially distributed clinicopathological features between the HER2-0 and HER2-low cohorts. Year of diagnosis was the only predefined stratification factor, to mitigate a potential selection bias. The association of *BRCA* status (*BRCA1* vs. *BRCA2*) with HER2-low status was explored with univariate and multivariate logistic regressions to estimate odds ratios (OR) with 95% CI. Homogeneity tests on the hazard ratio were performed using the likelihood ratio test to assess whether there was evidence of an interaction between HER2 status and subgroups of interest.

All statistical analyses were two-sided; $p < .05$ was considered statistically significant. Statistical analyses were performed using SAS 9.4 and Stata 16.1 (StataCorp 2019, Stata Statistical Software: Release 16; College Station, TX: StataCorp LP).

RESULTS

Population characteristics and treatments received

A total of 3547 patients with HER2-negative BC were included in this analysis, of whom 2402 (67.7%) pertained to the HER2-0 cohort, whereas 1145 (32.3%) had HER2-low tumors. The STROBE flowchart outlining patients' selection is reported in Figure 1.

Study population characteristics and treatments received are detailed in Table 1. Median age was 35 years (interquartile range [IQR]: 31–38) and 65.0% of the patients harbored a germline *BRCA1* PV. Most tumors were TNBC (55.9%) and were characterized by ductal histology (86.9%) and G3 (67.9%). Neoadjuvant CT was administered to 92.3% of cases, and 94.2% of patients with HR+ tumors received adjuvant ET.

When subdividing patients into HER2-low and HER2-0 cohorts, numerically minimal but statistically significant differences in geographical distribution, year of diagnosis, and menopausal status were observed (all $p < .001$; Table 1). HER2-low tumors, compared with HER2-0 cases, showed both statistically and clinically significant differences in G, endocrine receptor status and N, being more frequently G1 or 2 (2.1% and 27.7% vs. 1.5% and 18.4%, $p < .001$) and HR+ (63.0% vs. 34.8%, $p < .001$), and less frequently N0 (50.3% vs. 54.9%, $p = .003$).

We then considered only HR+ BCs with available data to distinguish between luminal A–like and luminal B–like (i.e., 644 HER2-low and 762 HER2-0). A higher proportion of luminal A–like tumors was observed in the HER2-low group (41.3% vs. 34.4%) compared with the HER2-0 group ($p = .008$); in contrast, the HER2-0 group was characterized by a higher proportion of luminal B–like tumors (65.6% vs. 58.7%). Nonetheless, luminal B–like tumors were the most prevalent surrogate subtype in both HR+/HER2-low and HR+/HER2-0.

When tumors were divided according to HER2 IHC scores as 0, 1+, and 2+, a progressive increase in the proportion of HR+ tumors was observed as the HER2 score increased, paralleled by a reduction in the proportion of patients with TNBC ($p < .001$). In addition, the proportion of luminal B–like tumors also significantly increased as the HER2 IHC score increased, whereas the proportion of luminal A–like tumors remained similar between HER2 1+ and 2+ subgroups ($p < .001$) (Figure 2A-B). HR+ tumors showed a higher proportion of HER2-low cases in comparison to TNBC (46.3% vs. 21.3%, $p < .001$) (Figure 2C).

Breast conserving surgery was performed more frequently than mastectomy in patients with HER2-0 versus HER2-low tumors (41.8% vs. 33.4%, $p < .001$). Minimal significant differences were observed in CT use ($p = .005$), type of CT ($p = .006$), and ET use in the HR+ subpopulation ($p = .008$) (Table 1).

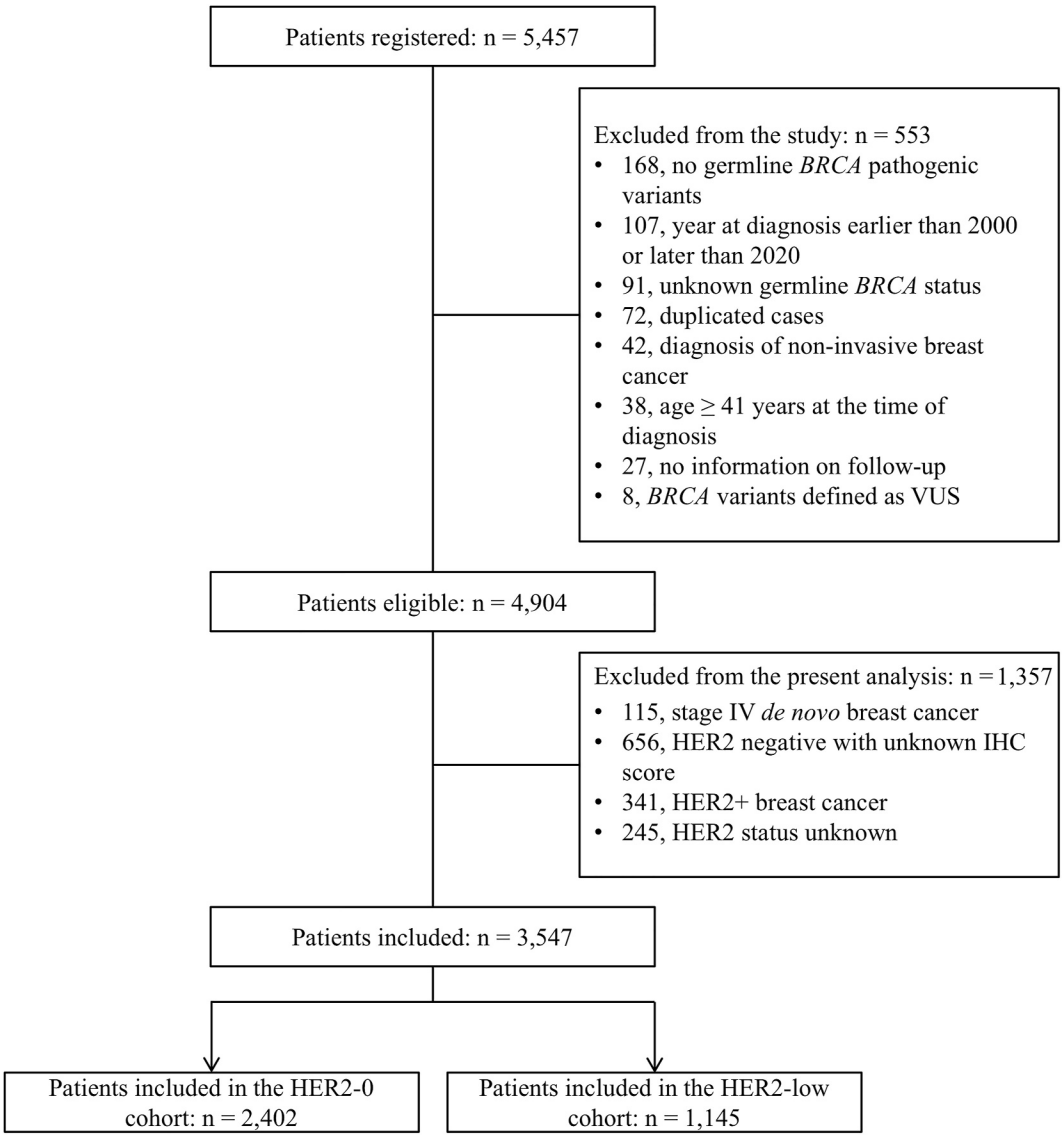

FIGURE 1 STROBE flowchart. + indicates positive; IHC, immunohistochemistry; VUS, variant of uncertain significance.

Association with type of BRCA PVs and HER2 status

When compared with the HER2-0 cohort, the HER2-low cohort was characterized by a significantly higher proportion of PVs in the *BRCA2* (51.0% vs. 26.4%) rather than the *BRCA1* (48.6% vs. 72.7%) gene ($p < .001$). Consistently, the proportion of HER2-low tumors in patients with *BRCA2* PVs was significantly higher than that observed in patients with *BRCA1* PVs (Figure 2C). In univariate analysis, patients with a germline *BRCA2* PV had a significantly higher association with HER2-low status than patients with a germline *BRCA1* PV (OR, 2.89; 95% CI, 2.49–3.35; $p < .001$). The association was confirmed independently of tumor hormone receptor status and histology (adjusted OR, 1.81; 95% CI, 1.51–2.17; $p < .001$). Co-occurrence of germline PVs of *BRCA1* and *BRCA2* was rare (i.e., 0.3% in HER2-low and 0.6% in HER2-0).

Survival analyses

At a median follow-up of 7.3 years (IQR, 4.2–12.2), 878 (36.6%) patients in the HER2-0 and 344 (30.0%) in the HER2-low cohort had experienced a DFS event, including locoregional or distant recurrence, death, and second primary malignancies (Supplementary Table S1). OS events occurred in 320 (13.3%) patients in the HER2-0 and in 115 (10.0%) patients in the HER2-low cohorts; among them, 19 (5.9%) and 3 (2.6%) patients had died without any DFS event, respectively.

In the overall HER2-negative population, the 8-year DFS for the HER2-low cohort was 69% (95% CI, 65%–72%), whereas for the HER2-0 cohort this was 64% (95% CI, 62%–66%). The 8-year OS was 90% (95% CI, 87%–92%) for HER2-low cases, compared with the 87% (95% CI, 85%–88%) observed in HER2-0. HER2-low status was associated

TABLE 1 Population demographics.

Patient demographics	Overall population *N* (%) *n* = 3547 (100.0%)	HER2-0 *N* (%) *n* = 2402 (67.7%)	HER2-Low *N* (%) *n* = 1145 (32.3%)	*p*[a]
Country				<.001
North America	237 (6.7)	145 (6.0)	92 (8.0)	
South-Central America	118 (3.3)	115 (4.8)	3 (0.3)	
Asia + Israel	519 (14.6)	323 (13.4)	196 (17.1)	
Oceania	145 (4.1)	129 (5.4)	16 (1.4)	
North Europe	632 (17.8)	376 (15.6)	256 (22.4)	
South Europe	1683 (47.4)	1189 (49.5)	494 (43.1)	
East Europe	213 (6.0)	125 (5.2)	88 (7.7)	
Year of diagnosis				<.001
2000–2004	333 (9.4)	260 (10.8)	73 (6.4)	
2005–2008	550 (15.5)	407 (16.9)	143 (12.5)	
2009–2012	761 (21.4)	521 (21.7)	240 (21.0)	
2013–2016	880 (24.8)	576 (24.0)	304 (26.5)	
2017–2020	1023 (28.8)	638 (26.6)	385 (33.6)	
Age at diagnosis, median (IQR) years	35 (31–38)	35 (31–38)	35 (3–38)	.538
Age at diagnosis				.644
≤30 years	719 (20.3)	497 (20.7)	222 (19.4)	
31–35 years	1297 (36.6)	877 (36.5)	420 (36.7)	
36–40 years	1531 (43.2)	1028 (42.8)	503 (43.9)	
Menopausal status at diagnosis				<.001
Premenopausal	3370 (95.0)	2261 (94.1)	1109 (96.9)	
Perimenopausal	14 (0.4)	10 (0.4)	4 (0.3)	
Postmenopausal	88 (2.5)	76 (3.2)	12 (1.0)	
Missing	75 (2.1)	55 (2.3)	20 (1.7)	
Use of hormonal contraception before cancer diagnosis				.523
No	1074 (30.3)	715 (29.8)	359 (31.3)	
Yes	1499 (42.3)	1017 (42.3)	482 (42.1)	
Missing	974 (27.5)	670 (27.9)	304 (26.5)	
BRCA status				<.001
BRCA1	2304 (65.0)	1747 (72.7)	557 (48.6)	
BRCA2	1218 (34.3)	634 (26.4)	584 (51.0)	
BRCA1 and *BRCA2*	18 (0.5)	15 (0.6)	3 (0.3)	
BRCA mutated (unknown if 1 or 2)	7 (0.2)	6 (0.2)	1 (0.1)	
Histology				.064
Ductal carcinoma	3084 (86.9)	2079 (86.5)	1005 (87.8)	
Lobular carcinoma	108 (3.0)	64 (2.7)	44 (3.8)	
Invasive (histology not specified)	121 (3.4)	91 (3.8)	30 (2.6)	
Others	211 (5.9)	148 (6.2)	63 (5.5)	
Missing	23 (0.6)	20 (0.8)	3 (0.3)	

TABLE 1 (Continued)

Patient demographics	Overall population *N* (%) *n* = 3547 (100.0%)	HER2-0 *N* (%) *n* = 2402 (67.7%)	HER2-Low *N* (%) *n* = 1145 (32.3%)	*p*[a]
Tumor grade				<.001
G1	60 (1.7)	36 (1.5)	24 (2.1)	
G2	758 (21.4)	441 (18.4)	317 (27.7)	
G3	2410 (67.9)	1720 (71.6)	690 (60.3)	
Missing	319 (9.0)	205 (8.5)	114 (10.0)	
Tumor size				.565
T1	1384 (39.0)	912 (38.0)	472 (41.2)	
T2	1563 (44.1)	1058 (44.0)	505 (44.1)	
T3-T4	477 (13.4)	322 (13.4)	155 (13.5)	
Missing	123 (3.5)	110 (4.6)	13 (1.1)	
Nodal status				.003
N0	1894 (53.4)	1318 (54.9)	576 (50.3)	
N1	1161 (32.7)	757 (31.5)	404 (35.3)	
N2-N3	405 (11.4)	252 (10.5)	153 (13.4)	
Missing	87 (2.4)	75 (3.1)	12 (1.0)	
Hormone receptor status				<.001
ER and/or PgR positive	1557 (43.9)	836 (34.8)	721 (63.0)	
ER and PgR negative	1983 (55.9)	1560 (64.9)	423 (36.9)	
Missing	7 (0.2)	6 (0.2)	1 (0.1)	
Breast surgery				<.001
Not done	12 (0.3)	9 (0.4)	3 (0.3)	
Breast-conserving surgery	1387 (39.1)	1004 (41.8)	383 (33.4)	
Mastectomy	2127 (60.0)	1374 (57.2)	753 (65.8)	
Missing	21 (0.6)	15 (0.6)	6 (0.5)	
Breast cancer surrogate subtype[b]				<.001
Luminal A–like	528 (14.9)	262 (10.9)	266 (23.2)	
Luminal B–like	878 (24.7)	500 (20.8)	378 (33.0)	
TNBC	1983 (55.9)	1560 (64.9)	423 (36.9)	
Missing	158 (4.4)	80 (3.3)	78 (6.8)	
Use of chemotherapy				.005
No	255 (7.2)	152 (6.3)	103 (9.0)	
Yes	3274 (92.3)	2236 (93.1)	1038 (90.7)	
Missing	18 (0.5)	14 (0.6)	4 (0.3)	
Type of chemotherapy[c]				.006
Anthracycline- and taxane-based	2453 (74.9)	1648 (73.7)	805 (77.5)	
Anthracycline-based	536 (16.4)	382 (17.1)	154 (14.8)	
Taxane-based	94 (2.9)	54 (2.4)	40 (3.8)	
Others	95 (2.9)	74 (3.3)	21 (2.0)	
Missing	96 (2.9)	78 (3.5)	18 (1.7)	

(Continues)

TABLE 1 (Continued)

Patient demographics	Overall population *N* (%) *n* = 3547 (100.0%)	HER2-0 *N* (%) *n* = 2402 (67.7%)	HER2-Low *N* (%) *n* = 1145 (32.3%)	*p*[a]
Use of endocrine therapy[d]				.008
No	73 (4.7)	50 (6.0)	23 (3.2)	
Yes	1467 (94.2)	771 (92.2)	696 (96.5)	
Missing	17 (1.1)	15 (1.8)	2 (0.3)	
Type of endocrine therapy[e]				.104
Tamoxifen alone	478 (32.6)	273 (35.4)	205 (29.4)	
Tamoxifen + LHRHa	429 (29.2)	212 (27.5)	217 (31.2)	
LHRHa alone	25 (1.7)	16 (2.1)	9 (1.3)	
AI ± LHRHa	273 (18.6)	132 (17.1)	141 (20.3)	
Tamoxifen and AI (± LHRHa)	230 (15.7)	118 (15.3)	112 (16.1)	
Others	18 (1.2)	9 (1.2)	9 (1.3)	
Missing	14 (0.9)	11 (1.4)	3 (0.4)	
Duration of endocrine therapy, median (IQR) months	60 (27–60)	60 (26–60)	58 (27–60)	<.001

Note: T and N refer to baseline clinical staging.

Abbreviations: AI, aromatase inhibitors; ER, estrogen receptor; G, tumor grade; IQR, interquartile range; LHRHa, luteinizing hormone–releasing hormone agonist; N, nodal status; PgR, progesterone receptor; T, tumor size; TNBC, triple-negative breast cancer.

[a]Calculated after exclusion of missing values.

[b]Luminal A–like defined as hormone receptor–positive/HER2-negative and grade 1-2 at immunohistochemistry; luminal B–like defined as hormone receptor–positive/HER2-negative and grade 3 at immunohistochemistry; TNBC defined as hormone receptor–negative and HER2-negative tumors at immunohistochemistry.[2]

[c]Calculated among patients who received chemotherapy.

[d]Calculated among patients with hormone receptor–positive breast cancer.

[e]Calculated among patients with hormone receptor–positive breast cancer who received endocrine therapy.

with a significantly more favorable DFS (hazard ratio, 0.86; 95% CI, 0.76–0.97) and numerically better OS (hazard ratio, 0.81; 95% CI, 0.66–1.01) in univariate analysis (Figure 3A). The difference in DFS was not significant when stratifying for type of *BRCA* gene, surrogate subtype, CT use, N, and year of diagnosis (stratified hazard ratio, 0.88; 95% CI, 0.77–1.02), whereas a significant difference was observed in OS (stratified hazard ratio, 0.76; 95% CI, 0.60–0.96). Nonetheless, the magnitude of the effect remained similar in all cases. We then evaluated more in detail the prognostic effect of HER2 status in each surrogate subtype group, splitting HR+ tumors in luminal A–like and luminal B–like. Although no significant DFS and OS differences were observed in patients with luminal-like tumors by HER2 status (Figure 3B-C), women with HER2-low BC had more favorable DFS (hazard ratio, 0.78; 95% CI, 0.64–0.95; stratified hazard ratio, 0.79, 95% CI, 0.65–0.97) and OS (hazard ratio, 0.65; 95% CI, 0.46–0.93; stratified hazard ratio, 0.63, 95% CI, 0.43–0.91) when compared with HER2-0 tumors among patients with TNBC (Figure 3D). The 8-year DFS for TNBC/HER2-low was 71% (95% CI, 65%–75%) versus 63% (95% CI, 60%–66%) for TNBC/HER2-0, whereas the 8-year OS was 89% (95% CI, 85%–92%) versus 86% (95% CI, 84%–88%), respectively. The test for interaction between surrogate subtypes and HER2 status was not significant for either DFS ($p = .112$) or OS ($p = .258$).

At the subgroup analysis for DFS, no significant interaction was observed with any of the subgroups of interest, namely type of *BRCA* gene, IHC surrogate molecular subtype, N, G, CT use, and year of diagnosis (Figure 4A). The same was observed at the subgroup analysis for OS (Figure 4B).

To further examine potential biological differences, we explored the prognosis of IHC surrogate subtypes within the HER2-0 and HER2-low cohorts, separately. In the HER2-0 group, a significantly different DFS was observed among luminal A–like, luminal B–like, and TNBC ($p = .019$) (Figure 5A). Luminal B–like tumors exhibited the best prognosis, with an 8-year DFS of 69% (95% CI, 64%–73%) compared with an 8-year DFS of 62% (95% CI, 54%–69%) and 63% (95% CI, 60%–66%) for luminal A–like tumors and TNBC tumors, respectively. When stratifying for age at diagnosis, CT, N, and *BRCA* gene, patients with TNBC showed worse DFS than patients with luminal A–like tumors (stratified hazard ratio, 1.37; 95% CI, 1.03–1.81). In terms of OS, no significant difference was observed according to subtype ($p = .870$) (Figure 5A). In the HER2-low cohort, a significantly different DFS was observed among the three subtypes ($p = .014$) (Figure 5B). Luminal A–like tumors displayed the worst DFS, with an 8-year rate of 61% (95% CI, 52%–68%), followed by an 8-year DFS of 71% (95% CI, 65%–75%) and 73% (95% CI, 67%–78%) in TNBC versus luminal B–like tumors, respectively. Patients with TNBC had numerically better DFS than patients with luminal A–like tumors, although this difference did not reach statistical significance (stratified hazard ratio, 0.78; 95% CI, 0.55–1-10). Despite the absence of significant OS difference ($p = .143$), a numerically worse prognosis was observed for luminal A–like tumors, with an 8-year OS

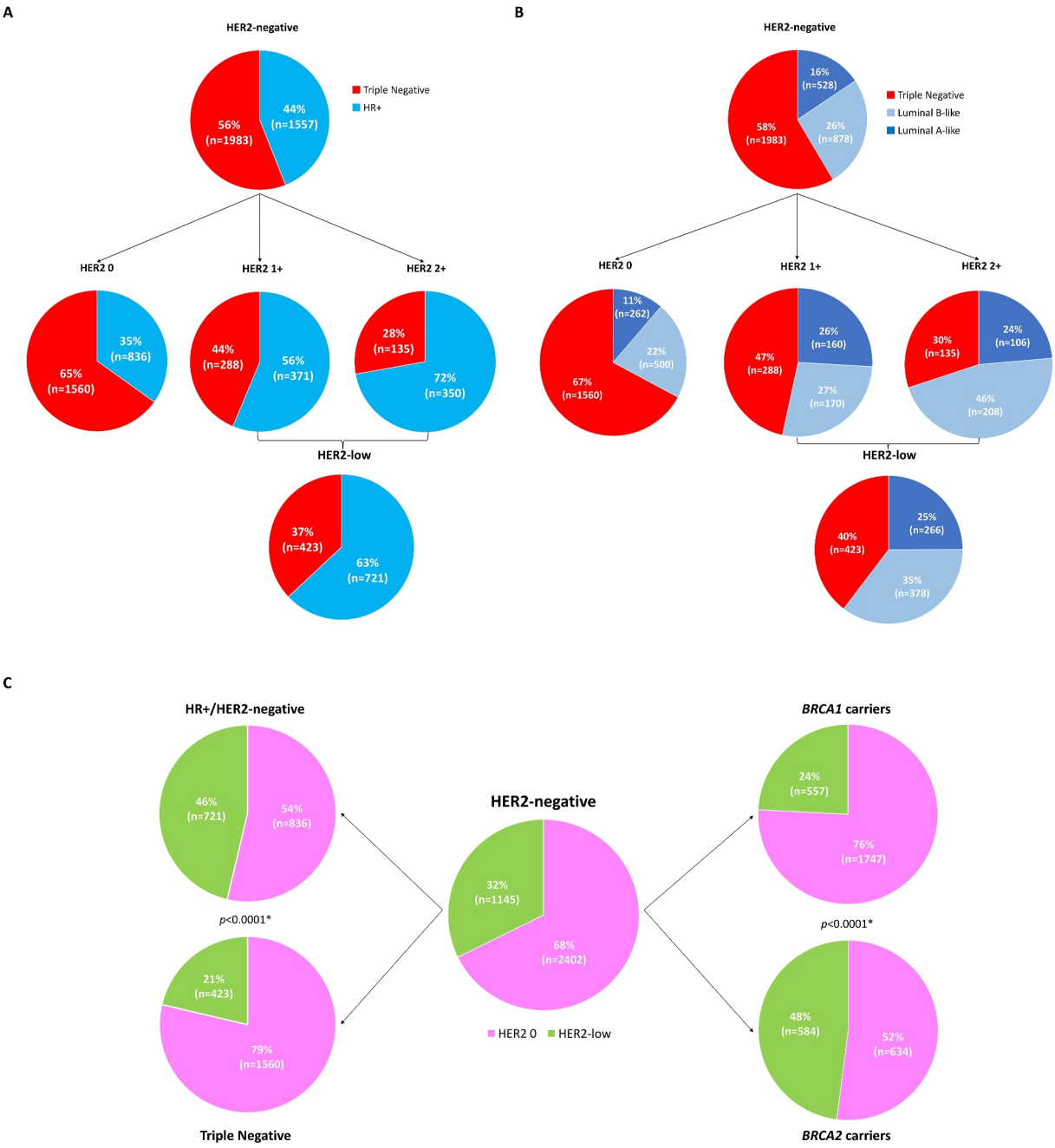

FIGURE 2 Proportion of breast tumors according to hormone receptor status, HER2 IHC score and *BRCA* gene. (A) Distribution of HR+ and TNBC according to HER2 IHC score; (B) distribution of luminal A–like, luminal B–like, and TNBC according to HER2 IHC score; (C) distribution of HER2-0 and HER2-low tumors according to *BRCA* status. Tumors were classified as luminal A–like if ER-positive and/or PgR-positive, HER2-negative, and G1-2. Tumors were classified as luminal B–like if ER-positive and/or PgR-positive, HER2-negative, and G3. Tumors were classified as TNBC if ER-negative, PgR-negative, and HER2-negative, regardless of G. ER indicates estrogen receptor; G, grading; HR+, hormone receptor–positive; PgR, progesterone receptor; TNBC, triple-negative breast cancer. *p values refer to Fisher exact test.

of 86% (95% CI, 79%–91%), compared with an 8-year OS of 89% (95% CI, 85%–92%) and 93% (95% CI, 89%–95%) for TNBC and luminal B–like tumors, respectively (Figure 5B).

Finally, in the HER2-0 cohort, no differences in DFS ($p = .331$) and OS ($p = .951$) were observed between patients harboring germline PVs of *BRCA1* versus *BRCA2*. Similarly, in the HER2-low cohort, no differences in DFS ($p = .799$) and OS ($p = .186$) were observed based on the type of *BRCA* gene (Supplementary Figure S1).

DISCUSSION

To our knowledge, this is the first study specifically assessing the clinical, biological, and prognostic features of HER2-low disease in young women, and more specifically in those harboring germline PVs in the *BRCA* genes. In addition, it reports the first evidence on the relationship between HER2-low status and germline *BRCA* PVs.

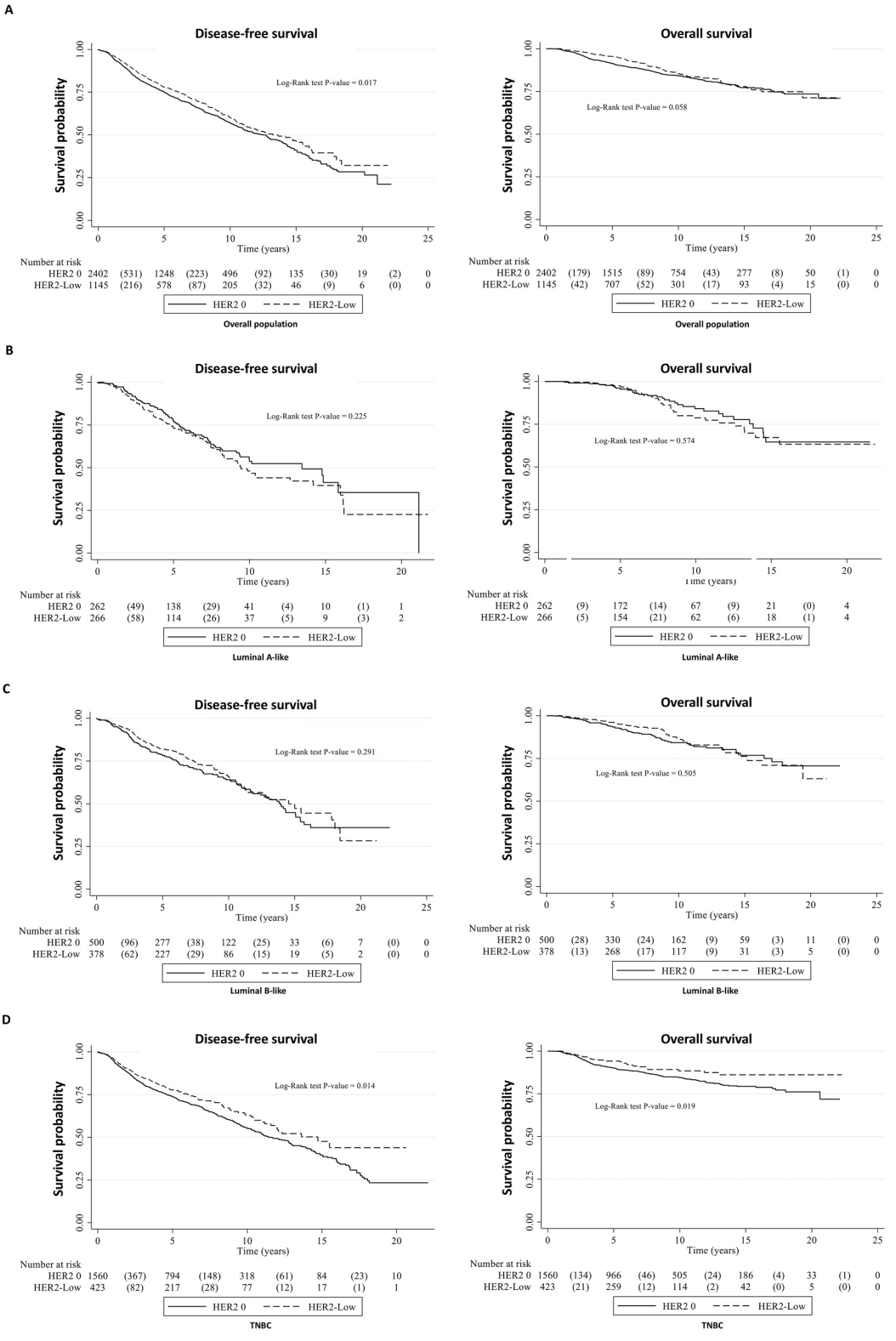

FIGURE 3 Kaplan–Meier curves of DFS and OS according to HER2 status in the overall population and according to surrogate tumor subtypes. (A) DFS and OS for HER2-0 vs. HER2-low in the overall population; (B) DFS and OS for HER2-0 vs. HER2-low in luminal A–like; (C) DFS and OS for HER2-0 vs. HER2-low in luminal B–like. (D) DFS and OS for HER2-0 vs. HER2-low in the TNBC cohort. Tumors were classified according to Partridge et al.[2] as luminal A–like if ER-positive and/or PgR-positive, HER2-negative, and G1-2; luminal B–like if ER-positive and/or PgR-positive, HER2-negative, and G3; as TNBC if HR-negative and HER2-negative. DFS indicates disease-free survival; HR, hormone receptor; OS, overall survival. The number of events at each time point is reported in brackets.

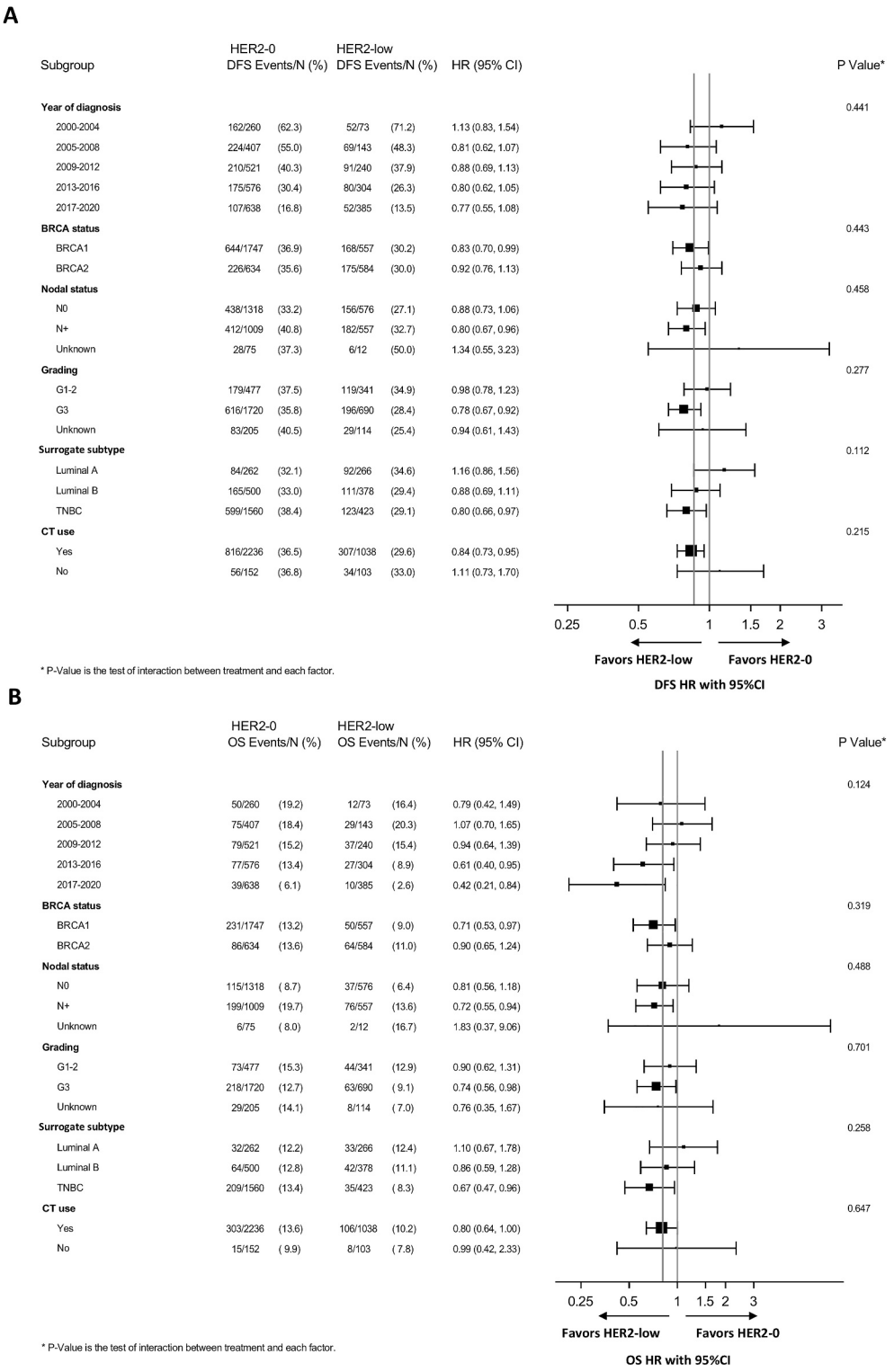

FIGURE 4 Subgroup analysis of DFS and OS for HER2-low vs. HER2-0. (A) Subgroup analysis of DFS; (B) Subgroup analysis of OS. Tumors were classified according to Partridge et al. as luminal A–like if ER-positive and/or PgR-positive, HER2-negative, and G1-2; luminal B–like if ER-positive and/or PgR-positive, HER2-negative, and G3; as TNBC if HR-negative and HER2-negative. DFS indicates disease-free survival; HR, hazard ratio; IHC-defined, immunohistochemically defined; OS, overall survival; TNBC, triple-negative breast cancer. *p values concerning the test for interaction between each variable and HER2 status.

Several studies convincingly showed that HER2-low should not be considered as a new, independent BC subtype.[11,13,15,18,19] In fact, all molecular intrinsic BC subtypes can be found among patients with HER2-low disease,[11,20] with the majority of TNBC/HER2-low being usually basal-like, and the majority of HR+/HER2-low tumors being luminal A or B.[11,12,19,20] This underlying biology is consistent with the

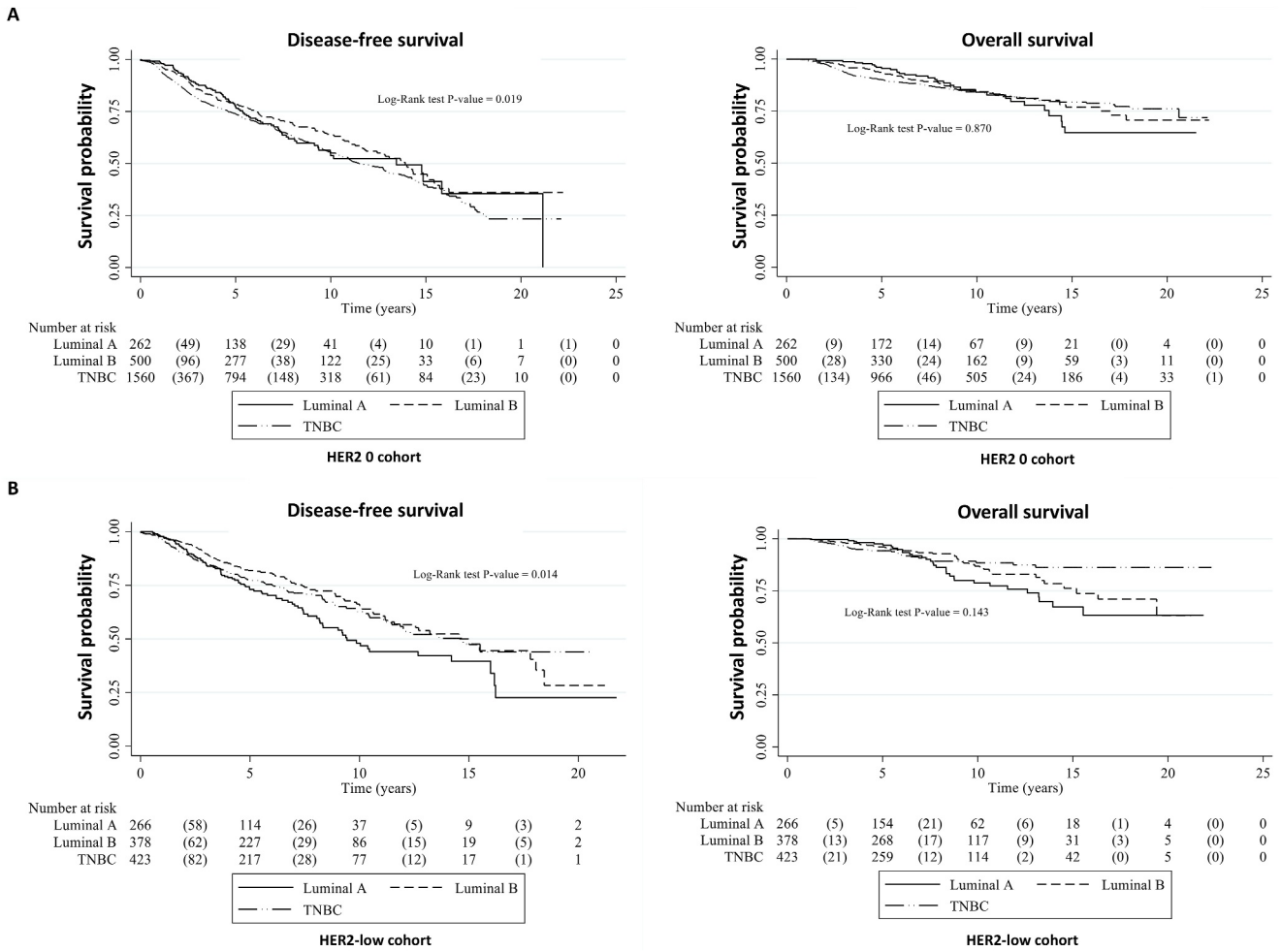

FIGURE 5 Kaplan–Meier curves of DFS and OS according to IHC surrogate molecular subtypes in the HER2-0 and HER2-low cohorts. (A) DFS and OS according to surrogate IHC subtypes in the HER2-0 cohort; (B) DFS and OS according to surrogate IHC subtypes in the HER2-low cohort. Surrogate subtypes were defined according to Partridge et al.[2] DFS indicates disease-free survival; IHC, immunohistochemistry; OS, overall survival; TNBC, triple-negative breast cancer. The number of events at each time point is reported in brackets.

fact that HER2-low disease is significantly more frequent in HR+ BC than TNBC across almost all published studies.[14] We observed that also in young women who were carriers of germline *BRCA1/2* PVs, HER2-low tumors were more commonly HR+, and an increasing HER2 score (2+ vs. 1+ vs. 0) was associated with a progressively lower proportion of TNBCs. Nevertheless, in this study, the proportion of HER2-low tumors, compared with HER2-0, was lower than what is typically observed in the published literature, with observed peaks of more than 65%.[8] On the contrary, in our study population, HER2-0 cases were significantly more frequent, representing ~68% of our entire HER2-negative cohort. The proportions that have been reported in the literature are mostly or exclusively based on postmenopausal, older, patient cohorts.[8] Therefore, a potential explanation for the lower proportion of HER2-low tumors observed in our study might reside in the fact that in young patients, and especially if they are germline *BRCA* carriers, there is a significant enrichment in TNBC.[1,21] Notably, in our cohort, <45% tumors were HR+. In addition, distinguishing between HER2-0 and HER2-low according to ASCO/CAP guidelines has acquired a critical importance only recently because of its therapeutic implications. However, significant discordance has been observed elsewhere among pathologists,[11,22,23] with an increased proportion of HER2-low cases diagnosed in the past decade compared with previous years.[22] As a result, by including patients diagnosed between 2000 and 2020, we cannot exclude that this issues could have partially influenced our results with a lower frequency of HER2-low cases diagnosed in earlier years.

A recent meta-analysis showed that HER2-low status is associated with a slightly better patients prognosis when compared with HER2-0 status, especially in patients with HR+ tumors.[14] In our study, the favorable prognostic effect observed in the HER2-low cohort was overall modest, in line with published literature, but seemed to be driven by the TNBC population instead of HR+. In the TNBC/HER2-low cohort, a 21% relative reduction in the risk of relapse and a 37% relative reduction in the risk of death were observed when compared with HER2-0 TNBC, with a delta in 8-year DFS and OS rates of 8% and 3%, respectively. The test for interaction between hormone receptors and HER2 status was not significant for both DFS and OS, suggesting that this specific result should be

considered with caution. The risk reduction was observed independently of type of *BRCA* gene, subtype, CT use, N, and year of diagnosis, which were the most relevant features differing significantly between the HER2-0 and HER2-low cohorts.

Interestingly, in our patient cohort, *BRCA2* PVs were more frequently associated with HER2-low status than *BRCA1*, independently of tumor hormone receptor status and histology. Notably, a higher prevalence of HER2-positive disease in germline *BRCA2* versus *BRCA1* carriers was previously observed.[4,24] Here, we report for the first time a higher prevalence of HER2-low tumors in patients with *BRCA2* PVs. A recent study showed that *ERBB2* is a key pathway regulator in germline *BRCA*-associated tumors, both in HER2-positive and HER2-negative cases.[25] Whether this was related to unidentified epigenetic mechanisms and/or a potential influence of HER2-low tumors remains unclear. These data suggest that *BRCA2* may be involved in the regulation of *ERBB2* expression, or, alternatively, that DNA alterations resulting from deficient BRCA2 protein may indirectly affect *ERBB2* gene transcription. Further preclinical studies are needed to fully understand the underlying biology and envision potential therapeutic implications of this association (e.g., higher efficacy of PARP inhibitors in *BRCA2*-associated HER2-positive tumors that might lead to more personalized therapeutic approaches). Besides biological considerations, HER2-low breast tumors have recently become a relevant therapeutic target because T-DXd was approved in the metastatic setting, following results from the DESTINY-Breast04 phase 3 trial.[26] Notably, HER2-low status might vary after treatment (including non-HER2–directed CT and ET),[27,28] from primary to metastatic disease,[29] or within the same patient at different metastatic sites or within the same organ.[30,31] Nevertheless, a subanalysis of the DESTINY-Breast04 trial proved that the benefit of T-DXd was consistent with the main study result regardless of the characteristics of the tumor sample used for HER2-low status determination (i.e., biopsy of the primary or metastasis, archived or newly obtained).[32] Therefore, taking into account that just one HER2-low diagnosis along the patients' oncological history is deemed sufficient to grant access to T-DXd,[33] our findings might suggest that, in clinical practice, when the available archival tumor tissue is HER2-0 in at least two samples (e.g., early-stage diagnostic biopsy/surgical pathology and biopsy at metastatic relapse), rebiopsy could be particularly encouraged especially in *BRCA2*-associated/HER2-negative breast malignancies.

When subdividing patients' tumors within the HER2-0 and HER2-low cohorts according to surrogate IHC subtypes,[2] the worst outcomes in the HER2-0 group were observed for TNBC and luminal A–like tumors, which displayed similar unfavorable prognostic effects. Within the HER2-low cohort, the worst outcomes were clearly observed for luminal A–like tumors. These findings are apparently discrepant with common knowledge on BC intrinsic subtypes.[34–36] However, Partridge et al. had previously observed that young age was associated with worse outcomes, especially in luminal A/B–like and mostly in the luminal A–like subgroup.[2] Several peculiar genomic patterns might explain, at least in part, this finding.[37] HR+/HER2-negative tumors in patients aged <40 years versus tumors in older women seemed to be more frequently characterized by poor prognostic alterations, namely genomic features associated with homologous recombination deficiency, higher frequency of gene copy number alterations, higher frequency of mutations in the oncogenic BC driver *GATA3* (especially in the luminal A subtype), and high-risk *PIK3CA* alterations.[37] The poorer prognosis observed for luminal A-like tumors in HER2-0 and HER2-low cases could be driven by such alterations, even more when considering that the presence of *BRCA* PV per se is considered a homologous recombination deficiency feature. Furthermore, previous studies showed a reverse association of the prognostic value of hormone receptor status in young women with BC and known germline *BRCA* PVs,[38] which was particularly pronounced in *BRCA2* carriers.[39–41] In addition, Goodwin et al. observed a significantly increased risk of death with adjuvant ET in patients with *BRCA2* PVs and HR+/HER2-negative BC versus *BRCA2* wild-type disease. This was not seen when adjuvant ET was not administered.[42] This evidence supports a potential interaction between PVs in the *BRCA2* gene and estrogen signaling, which might translate into lower efficacy of endocrine treatments, which are instead considered the most effective therapeutic options for patients with luminal A-like disease, thus contributing to explaining the poorer outcomes observed in our cohort.[35,43] Furthermore, now, the most effective adjuvant ET strategy for premenopausal patients at higher risk of relapse is the combination of ovarian function suppression with an aromatase inhibitor.[44] Approximately 92% of our patients received CT, including most luminal-like cases, suggesting the presence of high-risk features. Hence, the low rate (~20%) of patients with luminal-like tumors receiving ovarian function suppression + aromatase inhibitor could be another factor contributing to the poorer-than-expected long-term outcomes.

Unfortunately, the absence of molecular characterization prevents us from clearly elucidating the cause for such prognostic diversity. Nonetheless, our analysis seems to support the concept that luminal A-like tumors in young patients, especially in case of germline *BRCA* PVs, present with peculiar aggressive biological features, regardless of HER2 status. Different ER-related gene expression changes induced by CT based on menopausal status,[45] and greater beneficial prognostic effect in premenopausal women for the addition of CT to the adjuvant systemic strategy in HR+/HER2-negative disease[46–48] further support the need to find more appropriate therapeutic strategies to tackle this biological diversity.

This study is not exempt from limitations. The first is represented by its retrospective nature. Moreover, during the 20 years considered for patient inclusion in this study, several therapeutic options, particularly for HR+ disease, have been included in the management of early breast cancer. Considering that 78 centers worldwide from 26 countries in four continents contributed in the study, differences in health care systems and availability of therapeutic innovations, including access to genomic testing, may have influenced the results. Additionally, patients diagnosed closer to the end of the time period allowed for study inclusion had less

observation time to evaluate survival outcomes. Thus, a confounding effect of all these variables on long-term outcomes should be considered in the interpretation of the results. Another important limitation is the lack of centralized IHC assessment, which might result in some misclassification,[49] especially when it comes to the identification of HER2-low cases, because of reproducibility issues.[11,23] Furthermore, it has been demonstrated that all IHC surrogate subtypes can be found in molecular intrinsic subtypes and vice versa, with a discordance rate of approximately 30% on average.[50] Unfortunately, we could not perform genomic analyses, limiting the possibility to provide more specific insights into tumor biology and adding some degree of approximation regarding the true biological nature of molecular subtypes in this study. Finally, information on the treatment of metastatic disease was not collected; therefore, it is not possible to investigate the potential impact on survival outcomes of newer treatment options, including T-DXd. However, based on the inclusion criteria of diagnosis up to December 2020, it is likely that T-DXd was not available yet for the majority of patients with distant recurrences. However, these limitations are counterbalanced by the fact that, to our knowledge, this is the largest data set worldwide to include the rare population of young *BRCA* carriers with BC. Finally, before this study, there was no published data specifically concerning HER2-low disease in young premenopausal patients with *BRCA*-associated BC.

In conclusion, in a large international cohort of young women with BC harboring germline *BRCA1/2* PVs, we provided the first evidence that HER2-low disease is overall less common than in older patients, though still more commonly associated with luminal-like tumors. We also reported on an association between HER2-low status and more favorable patient prognosis when compared with HER2-0, although this difference in long-term outcomes might be more evident in TNBC. Germline *BRCA2* versus *BRCA1* PVs were more frequently observed in HER2-low tumors than in HER2-0, suggesting a potential relationship with *ERBB2* transcription or in the mutational processes leading to its amplification and HER2 overexpression. Finally, we found that women with luminal A–like tumors had worse prognosis regardless of HER2 status, thus highlighting the need for a better understanding of the biology underlying this tumor subgroup in young patients and tackle it with more appropriate therapeutic strategies.

AUTHOR CONTRIBUTIONS

Francesco Schettini: Conceptualization, methodology, supervision, visualization, and writing – original draft. **Eva Blondeaux**: Formal analysis, methodology, visualization, and writing – original draft. **Luca Carmisciano**: Formal analysis and methodology. **Marco Bruzzone**: Formal analysis, visualization, and writing – original draft. **Matteo Lambertini**: Conceptualization, funding acquisition, methodology, project administration, supervision, and writing – original draft. **All authors**: Data curation, investigation, validation, and writing – review & editing: all authors.

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CONFLICT OF INTEREST STATEMENT

Francesco Schettini reports honoraria and travel expenses from Novartis, Gilead, and Daiichy Sankyo, and an advisory role for Pfizer. Eva Blondeaux reports research support (to the Institution) from Gilead outside the submitted work. Chiara Molinelli reports fees from Novartis and Lilly, travel grants from Gilead. Katarzyna Pogoda reports honoraria for consultations/lectures/training/clinical trials and payment of conferences fees from AstraZeneca, Gilead, Roche, Novartis, Eli Lilly, Pfizer, MSD, Egis, and Vipharm, all outside the submitted work. Angela Toss reports advisory role for Lilly, Novartis, AstraZeneca, Pfizer, Seagen, Gilead, and MSD; speaker honoraria from Lilly, Novartis, Pfizer; Travel Grants from Gilead, Daiichi Sankyo and Novartis. Fabio Puglisi reports honoraria for advisory boards, activities as a speaker, travel grants and research grants from Amgen, AstraZeneca, Daiichi Sankyo, Celgene, Eisai, Eli Lilly, Exact Sciences, Gilead, Ipsen, Menarini, MSD, Novartis, Pierre Fabre, Pfizer, Roche, Seagen, Takeda, and Viatris; research funding from AstraZeneca, Eisai and Roche. Maria Vittoria Dieci received personal fees for consultancy/advisory role from Eli Lilly, Pfizer, Novartis, Seagen, Gilead, MSD, Exact Sciences, AstraZeneca, Roche, Daiichi Sankyo, and Roche. Christine Rousset-Jablonski reports advisory role for Bristol-Myers Squibb, Roche (paid to institution), and Theramex (paid to institution); speaker honoraria from Novartis (paid to institution), Organon (paid to institution), and Theramex (paid to institution); research funding (paid to institution) from Bayer HealthCare. Marion Acheritogaray reports speaker honoraria from MSD. Claudio Vernieri reports an advisory role for Lilly, Novartis, Pfizer, Menarini, Daiichi Sankyo and speaker honoraria from Lilly, Novartis, Pfizer, Istituto Gentili, Accademia di Medicina, and research support (to the Institution) from Roche. Sabine C. Linn reports institutional research grants from AstraZeneca, Eurocept Plaza, Roche, Genentech, Immunomedics (now Gilead Sciences), Tesaro (now GSK), Novartis, and Agendia outside the submitted work; consulting fees (to the institution) from AstraZeneca; payment or honoraria (to the institution) from Daiichi Sankyo; other financial support for attending meetings

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DATA AVAILABILITY STATEMENT

Clinicopathological data are available upon reasonable request to Prof. Matteo Lambertini (matteo.lambertini@unige.it), after proper revision of the data transfer agreement of each participating center and if ultimately allowed by local Ethic Committees.

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