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Association between chemotherapy use and prognosis in young patients with stage I-II ER+ /HER2-negative breast cancer according to Prosigna®: An international real-world analysis with propensity-score matching

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ABSTRACT

Background: Genomic assays (GA) guide chemotherapy (CT) use in stage I-II endocrine receptor-positive (ER+)/HER2-negative (HER2-) breast cancer (BC). In tumors N0/intermediate-risk or N1/low-to-intermediate-risk, randomized trials with OncotypeDX® showed a CT benefit only for women aged≤ 50 years/premenopausal. Comparable data for the Prosigna® GA are lacking.

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Methods: We retrospectively included 567 women aged≤ 50 years with stage I-II ER+ /HER2 − BC tested with Prosigna® across 10 hospitals in Spain/Italy (2014–2023). Patients received endocrine therapy (ET) with/without (neo)adjuvant CT. Event-free survival (EFS) was analyzed using Kaplan-Meier curves, log-rank tests, and Cox regression. Propensity score matching (PSM) was applied. 5-year EFS rates were numerically compared with those of OncotypeDX® trials.
Results: Of 567 patients, 73.7% were N0 and 26.3% N1, 39.7% were Prosigna risk-of-relapse (ROR)-low (RL), 33.0% ROR-intermediate (RI), 27.3% ROR-high (RH) and 48.3% received CT. CT independently improved EFS in N0/RI and N1/RL-RI (5-year EFS 97.9% vs. 86.6%; adjusted hazard ratio=0.07, p = 0.018). In premenopausal women, CT benefit persisted only when adjuvant gonadotropin-releasing hormone analogue was not administered (p = 0.008), especially in N0/RI (p = 0.023). Results were confirmed after PSM. CT-treated N0/RH showed similar EFS to CT-treated N0/RI+N1/RL-RI, while N1/RH showed poor prognosis despite CT use. 5-year EFS rates were generally consistent with OncotypeDX® trials.
Conclusion: Prosigna can help identify young women with stage I-II ER+ /HER2- BC who gain benefit from (neo)adjuvant CT and those in need of further escalated treatments. In premenopausal N0/RI and N1/RL-RI disease, the effect of CT seems to be driven by ovarian function suppression. Prospective validation is required.

1. Introduction

Breast cancer (BC) is the most common malignancy and leading cause of cancer-related death in women worldwide [1]. About 70% of cases are endocrine receptor-positive (ER+)/HER2-negative (HER2-) by immunohistochemistry (IHC). This group is biologically heterogeneous, comprising all four molecular intrinsic subtypes (Luminal A, Luminal B, HER2-enriched, Basal-like), characterized by different gene expression (GE) profiles, oncogenic drivers, and treatment sensitivity[2,3]. Early-stage management includes surgery, radiotherapy, chemotherapy (CT), and endocrine therapy (ET)[4–6], but CT adds short-/long-term toxicities[7,8]. While CT benefit is clear in stage III disease [9,10], its role in stage I-II ER+ /HER2- BC is debated. Clinicopathological features (grade [G], Ki67%, progesterone receptor [PgR]%) guide CT decisions but suffer from variability and fail to adequately capture biological heterogeneity [3,11].

In the last 20 years, GE-based assays (GEA) have improved prognostication with standardized, reproducible methods beyond standard IHC parameters [12–15]. OncotypeDX®, MammaPrint® and Prosigna® are the most widely-adopted GEA. TAILORx, RxPONDER, and MINDACT randomized controlled trials (RCTs) tested for CT benefit prediction OncotypeDX® and MammaPrint®, respectively; none showed formal predictive value [16–18]. However, subgroup analyses revealed meaningful CT benefit in women ≤ 50 years/premenopausal with intermediate OncotypeDX® genomic risk, or even low risk in case of 1–3 positive axillary lymph-nodes (N1) (absolute benefit up to 6.5% in TAILORx and 10% in RxPONDER) [17,18]. MINDACT results with Mammaprint® showed a modest signal for CT benefit in women ≤ 50 years old (yo) and discordant clinical-genomic risk [19], limiting a straightforward clinical applicability compared to OncotypeDX®. No similar prospective or retrospective data exist on Prosigna® risk-of-recurrence (ROR) in stage I-II ER+ /HER2- BC in women ≤ 50 years, except for old trials with outdated CT regimens and mostly involving node positive disease and mixed BC subtypes and pre/-postmenopausal cohorts [20–22]. Moreover, whether CT benefit depends on a direct cytotoxic effect or CT-induced ovarian function suppression (OFS) remains unclear, as < 19% received gonadotropin-releasing hormone analogues (GnRHa) in these studies [23]. Finally, Prosigna® uses the PAM50 algorithm to assign a patient's tumor to an intrinsic subtype (IS). However, the role of IS in the context of ROR score remains unclear.

We designed the PROUDER-BC study to evaluate the performance of the Prosigna® test in detecting (neo)adjuvant CT benefit in real-world ≤ 50yo patients with stage I-II ER+ /HER2- BC, determine the role of OFS in premenopausal women of non-high-risk, and explore the role of IS in this context. In addition, outcomes from the PROUDER-BC cohort were contextualized against those reported in TAILORx and RxPONDER.

2. Methods

2.1. Study population

We retrospectively enrolled ≤ 50yo women at first tumor diagnosis with histologically confirmed stage I-II ER+ /HER2- BC who underwent Prosigna® testing according to national and regional guidelines for test reimbursement, to guide (neo)adjuvant CT decision-making at 10 hospitals in Spain and Italy between 01/2014–06/2023. Completion of (neo)adjuvant CT or a minimum post-surgery follow-up of 6 months for those untreated with CT was required for study inclusion. Prosigna® ROR result availability was mandatory. Patient inclusion is summarized in Figure 1 (detailed inclusion/exclusion criteria in Supplementary methods). Prosigna® ROR integrates IS, a genomic proliferation signature, and primary tumor size (T) to stratify patients into ROR-low (RL), intermediate (RI), and high (RH) groups [14,24,25]. For axillary node negative (N0) cases, the ROR cut-offs we adopted to define RL, RI and RH were 40 and 60, respectively; for N1 cases, cut-offs were set at 15 and 40 [26,27]. Clinicopathological characteristics of interest were collected from medical records at each participating center.

2.2. Study objectives

The primary objective of this study was to assess the effect of CT use on event-free survival (EFS) across subgroups defined by Prosigna® ROR classes (RL, RI, RH) and nodal status (N0/N1), and evaluate the effect of CT on EFS specifically in the non–high-risk patients likely to derive benefit according to TAILORx and RxPONDER criteria (N0/RI and N1/RL-RI). As a contextual analysis, we descriptively compared the 5-year EFS rates observed in our cohort with those reported in the TAILORx and RxPONDER trials, aligning for stage, treatment exposure, and genomic risk categories. Secondary objectives included evaluating whether OFS modified the effect of CT on EFS in the non–high-risk subpopulation of interest, the association of CT exposure with EFS and overall survival (OS) in the entire study population and in subgroups defined according to nodal status, stratified or not by ROR class, as well as the predictive role of PAM50 IS *per se* and stratified according to ROR class and nodal status. All the primary and secondary analyses and their corresponding subcohorts are detailed in Supplementary table 1.

2.3. Statistical analysis

Descriptive statistics and χ^2 tests were used as appropriate. EFS and OS were defined *per* adapted NeoSTEEP criteria to account for the inclusion of a neoadjuvant cohort [28], and estimated using the Kaplan-Meier method, with median follow-up by reverse Kaplan-Meier. 5-year EFS/OS rates were calculated by ROR category and axillary nodal status (N) and descriptively compared with analogous TAILORx and RxPONDER populations [17,18]. Within-cohort survival comparisons

used log-rank tests and uni-/multivariable Cox models to estimate hazard ratios (HR) with 95% confidence interval (CI). Significance was set at $p \leq 0.05$. Propensity score matching (PSM) was performed to validate the main results. Additional and sensitivity analyses are detailed in Supplementary methods. Statistics were conducted in R (versions 4.2.3 for Windows–4.3.3 for MacOSX).

2.4. Study ethics

The study was approved by the Hospital Clinic Barcelona Ethics Committee as the coordinating Institution (IRB n. HCB/2023/0971). Informed consent for retrospective data was requested only if required by local regulations.

3. Results

3.1. Study population characteristics

A total of 567 patients were included in our study, all were ≤ 50yo, 95.9% premenopausal. Most tumors were G2–3 (78.7%), of ductal histology (83.9%), with 19.1% as mean Ki67 (standard deviation 14.2), 71.6% T1 and 73.7% N0. Molecularly, 39.7% tumors were classified as RL, 33.0% RI, and 27.3% RH, with 67.3% of the cases classified by PAM50 as Luminal A, 30.6% Luminal B, 0.4% HER2-enriched, and 1.7% Basal-like. PAM50 IS distribution according to ROR and N is reported in Figure 2A. CT was administered in 8.0%, 60.4% and 91.6% of RL, RI and RH cases, respectively. The vast majority of Luminal B and non-luminal tumors were treated with CT regardless of N and ROR (Figure 2A). Detailed CT administration rates in key subgroups are reported in Figure 2A. Overall, 0.6% rejected any systemic treatment and 0.7% stopped or rejected ET but received CT. ET and CT regimens are reported in detail in **Supplementary results**. Clinicopathological features, locoregional and systemic treatments of the PROUDER-BC cohort are reported in Table 1, which also includes a comparison with the populations of the TAILORx and RxPONDER RCT, for descriptive purposes (more details in **Supplementary results**).

3.2. CT effect in the PROUDER-BC cohort

After a median follow-up of 40.7 months (95%CI: 39.1–44.8), 21 relapses (all invasive, 13 distant/locally unresectable) and 2 deaths were observed, 1 unrelated to BC (Supplementary table 2). We first evaluated the effect of CT on EFS in the overall PROUDER-BC cohort. Without considering ROR category, no significant difference was observed between patients treated with vs. without CT, both in the global population ($p = 0.873$) and when analyzing N0 ($p = 0.444$) and N1 ($p = 0.690$) cases separately. However, when stratifying patients by treatment, N, and ROR class, significant prognostic differences emerged ($p = 0.044$; Table 2). A formal interaction test between continuous ROR and CT use was not significant in the overall population ($p = 0.734$) nor when separating by N (N0, $p = 0.125$; N1, $p = 0.945$), likely due to insufficient statistical power. However, the estimation of 5-year EFS rates for

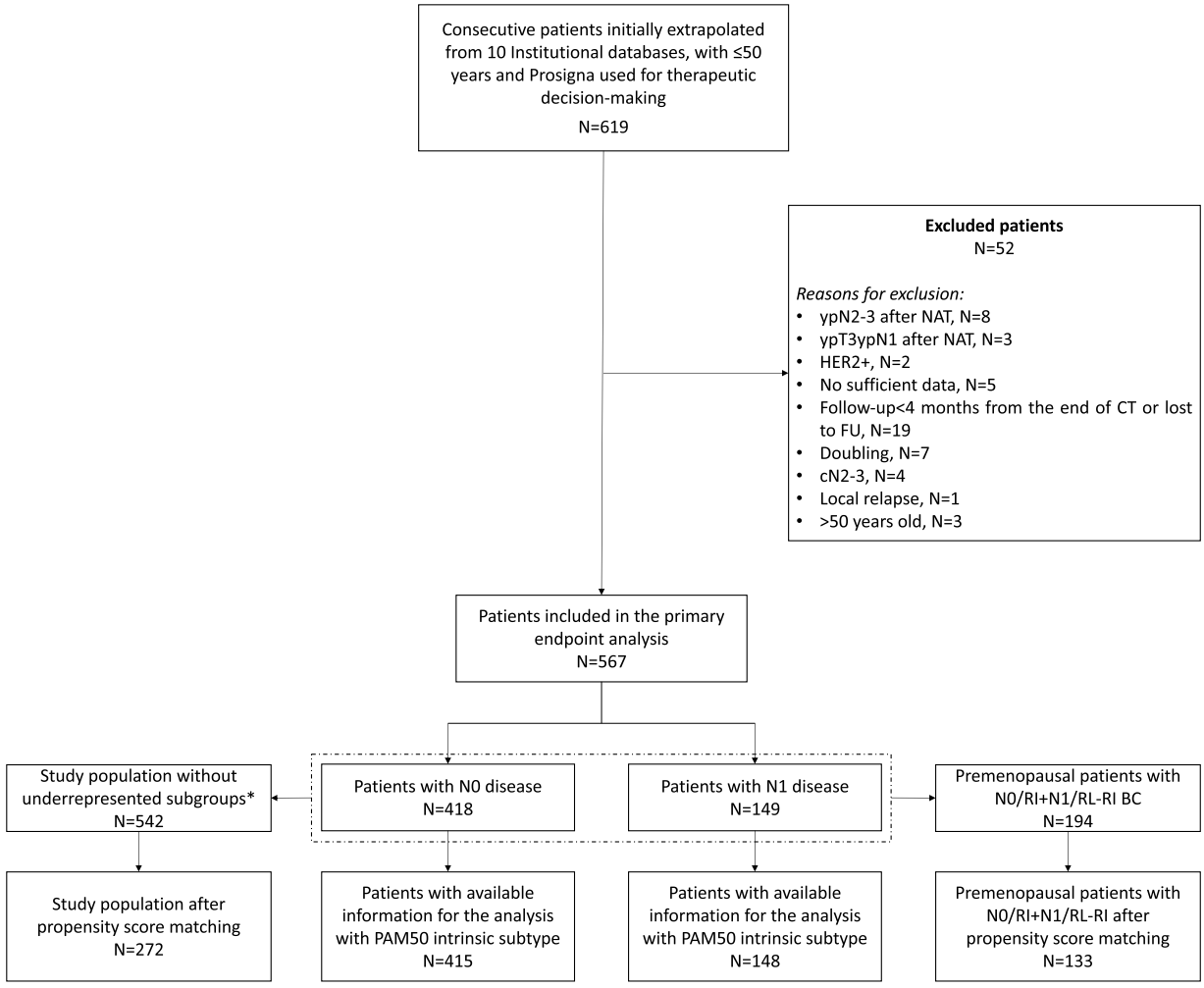

Fig. 1. STROBE flow-chart. **Legend.** FU: follow-up; N: number; NAT: neoadjuvant treatment; N0: axillary lymph-node negative; N1: 1–3 positive axillary lymph-nodes; RL: ROR-low; RI: ROR-intermediate; + : positive. *: N0/RL CT-treated, N0/RH and N1/RH CT-naïve.

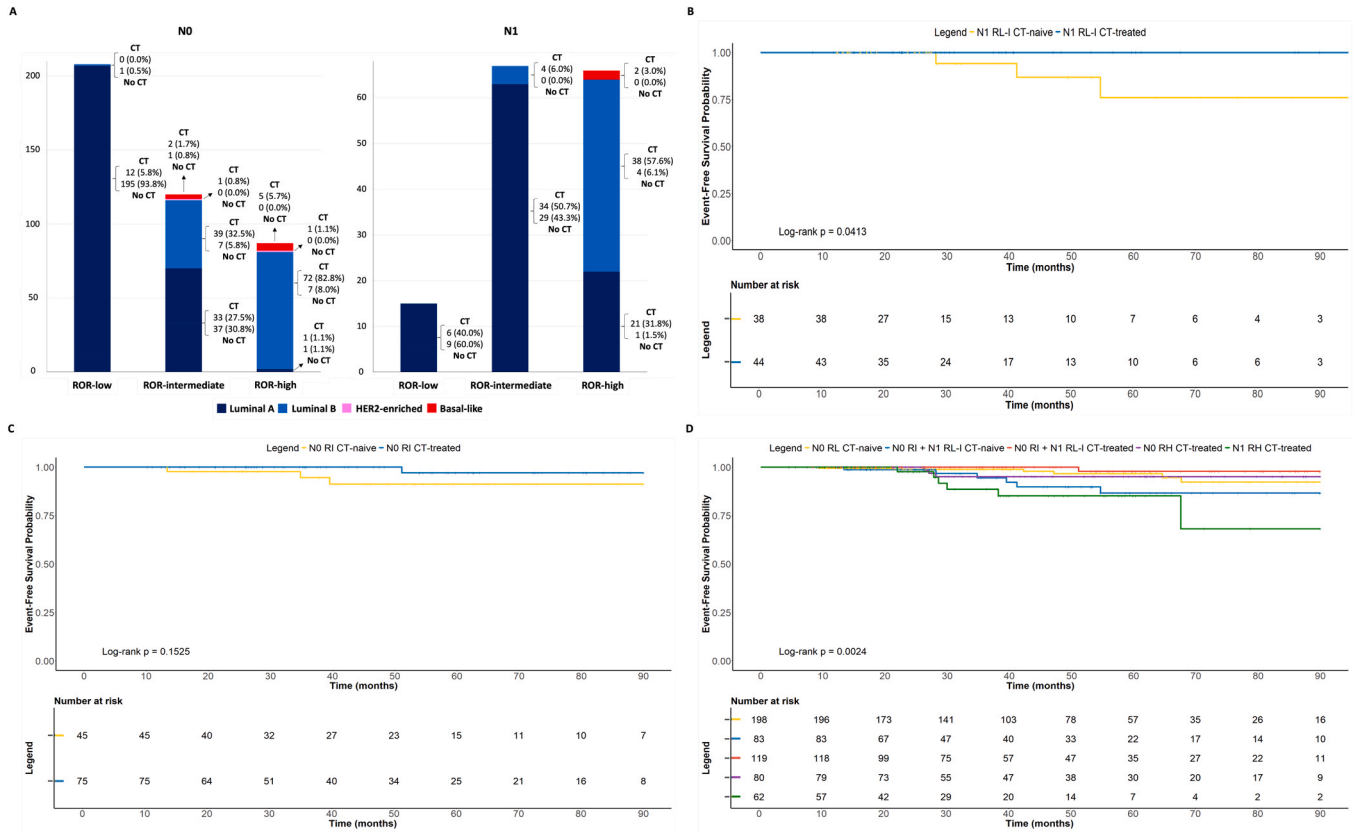

Fig. 2. Molecular and therapeutic description of the PROUDER-BC cohort and KM curves for EFS according to CT use in patients with different risk class and nodal status Legend. A: PAM50 intrinsic subtype distribution according to ROR class and N status, as well as number and proportion of cases receiving or not CT; **B:** EFS in N1/RL-I; **C:** EFS in N0/RI; **D:** EFS according to nodal status, ROR class and CT use without uncommon subpopulations. CT: chemotherapy; EFS: event-free survival; KM: Kaplan-Meier; N0: axillary lymph-node negative; N1: 1–3 positive axillary lymph-nodes; N.: number; RL: ROR-low; RI: ROR-intermediate.

each risk category according to nodal status and treatment allowed to systematically observe higher rates in patients treated with CT and ET vs. ET-only in the non-high-risk groups that would currently receive CT based on OncotypeDX® trials. Namely, 97.1% vs. 91.2% in N0/RI, 100.0% vs. 66.7% in N1/RL and 100.0% vs. 76.4% in N1/RI. Consistently, patients with N1 non-high-risk tumors treated with CT had significantly better EFS than those not receiving CT at univariate analysis ($p = 0.041$, Figure 2B). N0/RI patients showed a similar trend (Figure 2C). Unfortunately, the very low number of patients with N0/RL, N0/RH and N1/RH tumors treated in disconformity with current standard-of-care (SOC) (i.e., CT-treated N0/RL [n = 12, 2.1%], ET-only N0/RH [n = 8, 1.4%] and N1/RH [n = 5, 0.9%]) prevented any meaningful comparison with those treated in line with OncotypeDX® trials. Nonetheless, 5-year EFS rates are fully reported in Table 2 for each subgroup.

We then conducted a multivariable analysis adjusting for age, Ki67%, PgR%, T, and grade (Supplementary table 3). For statistical robustness, N0/RI and N1/RL-RI cases were regrouped (n = 202, 35.6%) as these correspond to subgroups benefiting from CT in TAILORx and RxPONDER. Outcomes of those treated with CT (58.9%) vs. not (41.1%) were compared with other risk groups treated according to SOC; namely, ET-only N0/RL, and CT-treated N0/RH and N1/RH (Figure 2D). Rare subgroups treated against current SOC were excluded (Figure 1). In the adjusted analysis, patients with N0/RI and N1/RL-RI disease receiving CT had a significantly improved EFS (adjusted HR [aHR]: 0.07, 95%CI: 0.01–0.63, $p = 0.018$), with a 5-year EFS of 97.9% vs. 86.6% (Δ11.3%). No significant difference was observed when comparing these patients with N0/RL cases treated with ET ($p = 0.105$). N0/RH patients receiving CT had similar EFS to N0/RI+N1/RL-RI treated with CT ($p = 0.308$), and a borderline benefit compared to N0/RI+N1/RL-RI treated with ET (aHR: 0.24, $p = 0.063$). In contrast, N1/RH patients treated with CT had significantly worse EFS (aHR: 14.71, 95%CI: 1.72–125.53, $p = 0.014$). This benefit was maintained after excluding postmenopausal patients (Supplementary table 3) and confirmed after PSM (aHR: 0.11, $p = 0.048$; Supplementary figure 1).

3.3. *PROUDER-BC results in the context of TAILORx and RxPONDER*

To place these results into context, we assessed the 5-year EFS rates of the PROUDER-BC cohort according to N status, systemic treatment received and risk category according to Prosigna® ROR and numerically compared them to survival rates of the corresponding ≤ 50yo patient categories within the TAILORx and RxPONDER trials [17,18]. We observed that the 5-year EFS rates in ET-only N0 patients appeared similar, while in ET-only N1, RCT patients showed more favorable outcomes (Fig. 3). Among patients who received CT, N0/RI, N0/RH and N1/RI in PROUDER-BC showed numerically higher survival rates than those reported in TAILORx and RxPONDER (Fig. 3). Rare subgroups treated against current SOC in PROUDER-BC had no representation in the two RCT. Furthermore, in RxPONDER patients with N1/high risk according to OncotypeDX® were excluded and not followed-up (differently from N0/high risk in TAILORx). To note, in our cohort, 51.3% of patients received CT plus ET, which is broadly in line with the proportions reported in TAILORx and RxPONDER for the intention-to-treat populations (50.5% and 50.1%, respectively) [17,18].

Due to the very low number of events and short follow-up for the PROUDER-BC cohort, and incomplete availability for TAILORx and RxPONDER, 5-year OS rates are reported for descriptive purposes in Table 2 only for our population.

Table 1
Population characteristics.

DEMOGRAPHICS	TAILORx		RxPONDER		PROUDER-BC		P-value (chi-square with Yates correction)
	N	%	N	%	N	%	
	9719	100.0	5018	100.0	567	100.0	
Age (years)*							
≤ 40	448	4.6	147	2.9	94	16.6	< 0.001
41–50	2606	26.8	1077	21.5	473	83.4	
≥ 51	6665	68.6	3794	75.6	0	0.0	
Overall	9719	100.0	5018	100.0	567	100.0	
Menopausal status							
Premenopausal	3300	34.0	1665	33.2	544	95.9	< 0.001
Postmenopausal	6419	66.0	3353	66.8	23	4.1	
Overall	9719	100.0	5018	100.0	567	100.0	
T at diagnosis#							
T1	7271	74.8	2923	58.3	406	71.6	< 0.001
T2	2445	25.2	1843	36.7	153	27.0	
T3	0	0.0	252	5.0	8	1.4	
T4	0	0.0	0	0.0	0	0.0	
Overall	9716	100.0	5018	100.0	567	100.0	
N at diagnosis#							
N0	9719	100.0	0	0.0	418	73.7	< 0.001
N1	0	0.0	5018	100.0	149	26.3	
Overall	9719	100.0	5018	100.0	567	100.0	
G							
1	2512	26.6	1218	24.7	118	21.3	< 0.001
2	5242	55.6	3215	65.0	378	68.4	
3	1676	17.8	507	10.3	57	10.3	
Overall	9430	97.0	4940	98.4	553	97.5	
Histology							
IDC/No special type	NR	-	3673	74.6	469	83.9	< 0.001
ILC and mixed with ILC	NR	-	952	19.3	65	11.6	
Other	NR	-	299	6.1	25	4.5	
Overall	NR	-	4924	98.1	559	98.6	
Estrogen receptor							
Positive	9665	99.4	4987	100.0	563	99.3	< 0.001
Negative	54	0.6	0	0.0	4	0.7	
Overall	9719	100.0	4987	99.4	567	100.0	
Progesterone receptor							
Positive	8564	94.0	4701	94.3	543	95.8	0.181

(*continued on next page*)

Table 1 (*continued*)

DEMOGRAPHICS	TAILORx		RxPONDER		PROUDER-BC		P-value (chi-square with Yates correction)
	N	%	N	%	N	%	
	9719	100.0	5018	100.0	567	100.0	
Negative	551	6.0	286	5.7	24	4.2	
Overall	9115	93.8	4987	99.4	567	100.0	
Molecular risk°							
Low	1619	16.7	2147	42.8	225	39.7	< 0.001
Intermediate	6711	69.0	2871	57.2	187	33.0	
High	1389	14.3	0	0.0	155	27.3	
Overall	9719	100.0	5018	100.0	567	100.0	
PAM50 intrinsic subtype							
Luminal A	-	-	-	-	379	67.3	NA
Luminal B	-	-	-	-	172	30.6	
HER2-enriched	-	-	-	-	2	0.4	
Basal-like	-	-	-	-	10	1.7	
Overall	-	-	-	-	563	99.3	
Axillary management							
ALND	NR	-	3140	62.6	65	11.6	< 0.001
SLNB	NR	-	1878	37.4	473	84.8	
None	NR	-	0	0.0	20	3.6	
Overall	NR	-	5018	100.0	558	98.4	
Systemic therapies type							
Endocrine therapy alone	5522	56.8	2507	50.0	291	51.3	< 0.001
Chemotherapy alone	0	0.0	0	0.0	4	0.7	
Chemoendocrine therapy	4197	43.2	2511	50.0	269	47.4	
None	0	0.0	0	0.0	3	0.6	
Overall	9719	100.0	5018	100.0	567	100.0	
Chemotherapy							
Yes, neoadjuvant	0	0.0	0	0.0	56	9.9	< 0.001
Yes, adjuvant	4197	43.2	2511	50.0	217	38.3	
No	5522	56.8	2507	50.0	294	51.9	
Overall	9719	100.0	5018	100.0	567	100.0	
Endocrine therapy							
Yes, neoadjuvant	0	0.0	0	0.0	59	10.4	< 0.001
Yes, adjuvant	5522	100.0	5018	100.0	501	88.4	
No	0	0.0	0	0.0	7	1.2	
Overall	5522	56.8	5018	100.0	567	100.0	

Legend. ALND: axillary lymph-node dissection; ER: endocrine receptor; G: tumor grade; IDC: invasive ductal carcinoma; IHC: immunohistochemical; ILC: invasive lobular carcinoma; NR: not reported; SLNB: sentinel lymph-node biopsy; TN: triple negative; + : positive; -: negative; *: in the RxPONDER trial the categorization is $\leq$ 40, 41–49, $\geq$ 50 years old; #: in the PROUDER-BC cohort T and N are referred to pathologic staging in patients not treated with neoadjuvant therapies, while in patients treated with neoadjuvant endocrine therapy or chemotherapy T and N are referred to pre-treatment clinical staging; °: in TAILORx and RxPONDER molecular risk is defined by OncotypeDX, while in the PROUDER-BC cohort is defined by Prosigna® ROR.

Table 2
Within-cohort univariate comparison and 5-year EFS and OS rates of the PROUDER-BC cohort according to ROR class, N status and systemic treatment.

Endpoint and treatment group	N. patients	N. events	5-year rate %	Inferior 95%CI %	Superior 95%CI %	P-value (log-rank test)
EFS						
N0 ROR Low endocrine therapy	198	6	96.7	93.4	100.0	0.044
N0 ROR Low chemoendocrine therapy	12	0	100.0	100.0	100.0	
N0 ROR Intermediate endocrine therapy	45	3	91.2	82.0	100.0	
N0 ROR Intermediate chemoendocrine therapy	75	1	97.1	91.5	100.0	
N0 ROR High endocrine therapy	8	0	100.0	100.0	100.0	
N0 ROR High chemoendocrine therapy	80	3	95.1	89.9	100.0	
N1 ROR Low endocrine therapy	9	1	66.7	30.0	100.0	
N1 ROR Low chemoendocrine therapy	6	0	100.0	100.0	100.0	
N1 ROR Intermediate endocrine therapy	29	2	76.4	51.4	100.0	
N1 ROR Intermediate chemoendocrine therapy	38	0	100.0	100.0	100.0	
N1 ROR High endocrine therapy	5	0	100.0	100.0	100.0	
N1 ROR High chemoendocrine therapy	62	6	85.2	74.0	98.2	
OS						
N0 ROR Low endocrine therapy	198	0	100.0	100.0	100.0	NA
N0 ROR Low chemoendocrine therapy	12	0	100.0	100.0	100.0	
N0 ROR Intermediate endocrine therapy	45	0	100.0	100.0	100.0	
N0 ROR Intermediate chemoendocrine therapy	75	1	100.0	100.0	100.0	
N0 ROR High endocrine therapy	8	0	100.0	100.0	100.0	
N0 ROR High chemoendocrine therapy	80	0	100.0	100.0	100.0	
N1 ROR Low endocrine therapy	9	0	100.0	100.0	100.0	
N1 ROR Low chemoendocrine therapy	6	0	100.0	100.0	100.0	
N1 ROR Intermediate endocrine therapy	29	0	100.0	100.0	100.0	
N1 ROR Intermediate chemoendocrine therapy	38	0	100.0	100.0	100.0	
N1 ROR High endocrine therapy	5	0	100.0	100.0	100.0	
N1 ROR High chemoendocrine therapy	62	1	96.9	91.0	100.0	

Legend. CI: confidence interval; EFS: event-free survival; N.: number; N0: axillary lymph-node negative; N1: 1–3 positive axillary lymph-nodes; NA: not assessed; OS: overall survival.

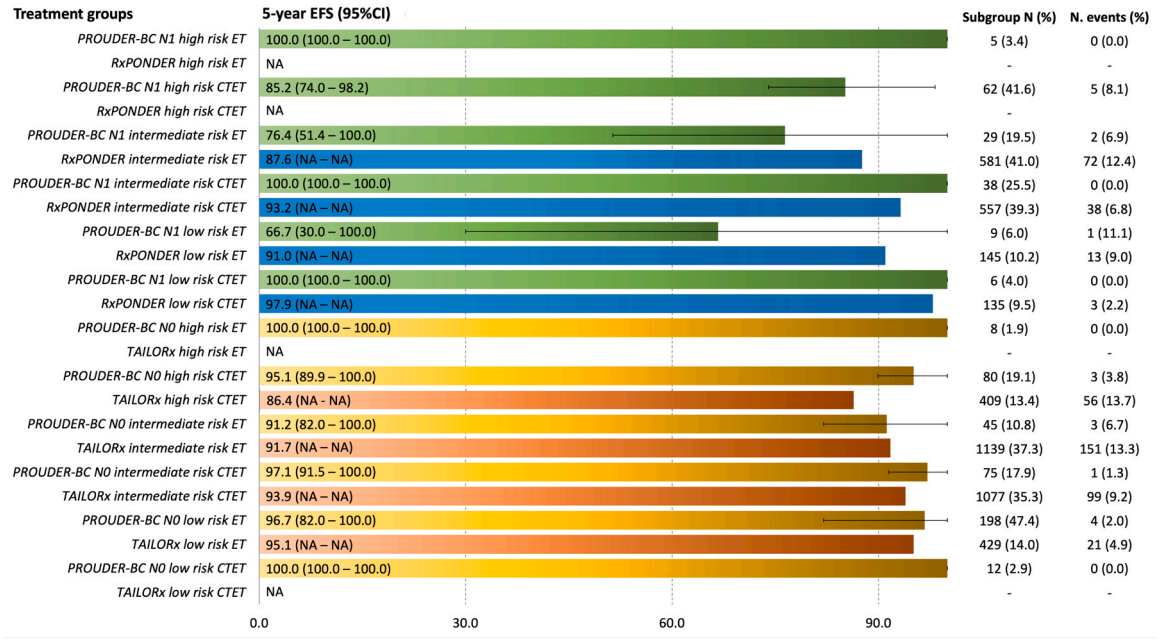

Fig. 3. 5-year EFS rates of the PROUDER-BC cohort, TAILORx and RxPONDER≤ 50 yo populations Legend. 5-year EFS rates according to study, nodal status, systemic (neo)adjuvant treatment, and risk class; CT: chemotherapy; EFS: event-free survival; ET: endocrine therapy; N: number; NA: not available/assessed; N0: negative axillary lymph-nodes; N1: 1–3 positive axillary lymph-nodes; yo: years old. 95%CI for EFS rates were not provided in the randomized trials. The proportions in the N. events column were calculated with respect to the total N of the subpopulation considered, while the proportion for each patient's subgroup was calculated with respect to the total N of the study (TAILORx, RxPONDER, PROUDER-BC N0 and PROUDER-BC N1). OncotypeDX score was considered low risk for values comprised between 0 and 10, while an intermediate risk was considered for values between 11 and 25 and a high risk for values ≥ 26. In the case of Prosigna®, risk category thresholds were reported in the Methods section.

3.4. OFS and CT benefit in subgroups of interest

Among the 221 (40.6%) premenopausal patients of the PROUDER-BC who received a GnRHa in the adjuvant setting, we explored the impact of GnRHa-induced OFS on the benefit of CT in the N0/RI+N1/RL-RI subpopulation (N = 194). CT-naïve patients had a significantly worse EFS than CT-treated patients (p = 0.030) (Figure 4A). When stratified by GnRHa administration, no difference in EFS was observed

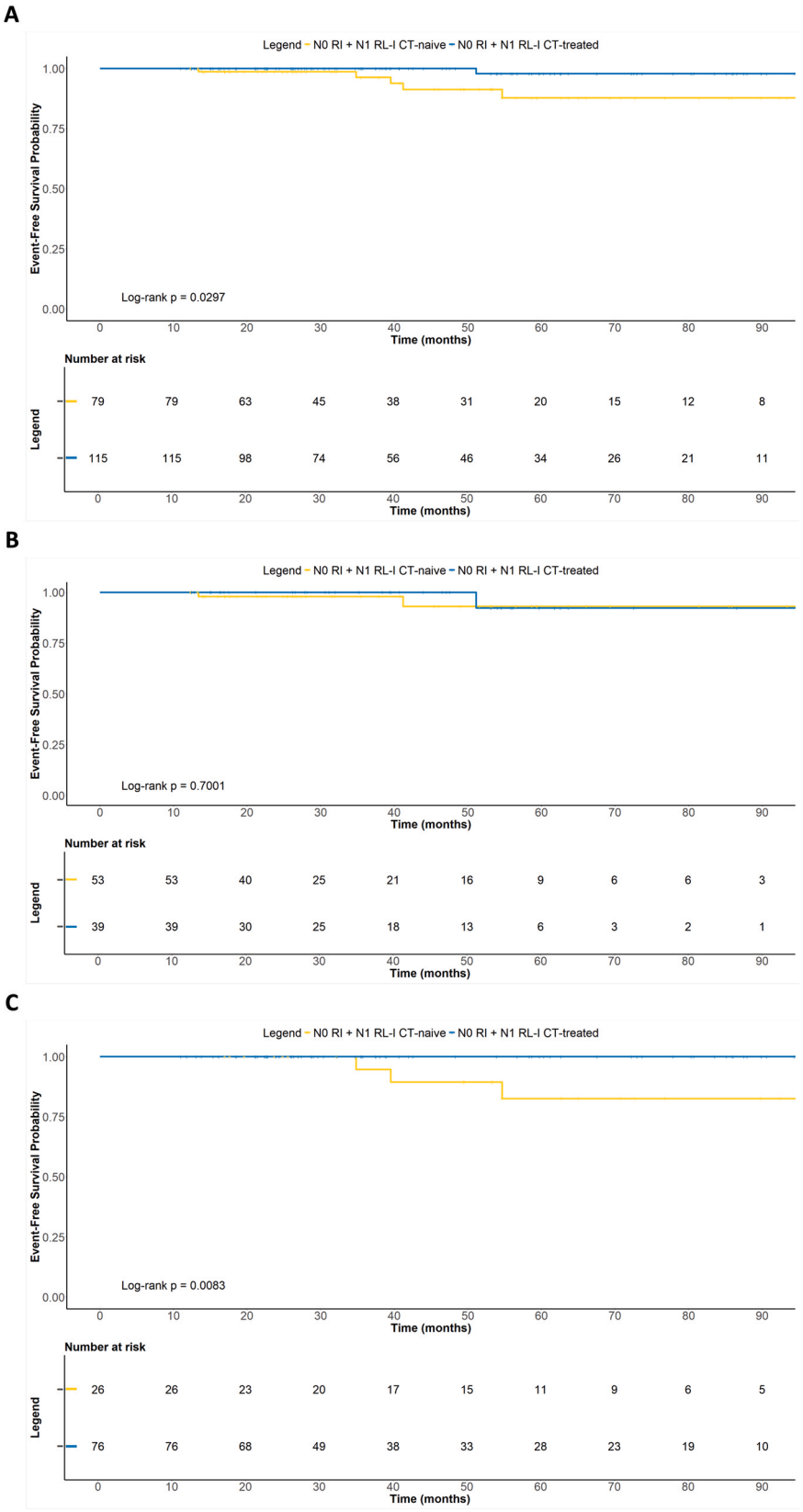

Fig. 4. EFS in premenopausal patients with N0/RI+N1/RL-RI BC according to chemotherapy and adjuvant GnRHa use. Legend. A: EFS according to chemotherapy use in premenopausal patients with N0/RI+N1/RL-RI BC; **B:** EFS according to chemotherapy use in premenopausal patients with N0/RI+N1/RL-RI BC who received GnRHa; **C:** EFS according to chemotherapy use in premenopausal patients with N0/RI+N1/RL-RI BC who did not receive GnRHa; CT: chemotherapy; GnRHa: gonadotropin-releasing hormone analogue; EFS: event-free survival. N.: number; N0: axillary lymph-node negative; N1: 1–3 positive axillary lymph-nodes; RL: ROR-low; RI: ROR-intermediate. The P value is referred to log-rank test.

between patients undergoing or not CT in those receiving the GnRHa ($p = 0.700$) (Figure 4B), whereas CT conferred a significant EFS benefit ($p = 0.008$) in patients not receiving GnRHa (Figure 4C). Clinicopathological characteristics were generally balanced between patients receiving (47.4%) or not receiving (52.6%) GnRHa, except for lower PgR levels ($p = 0.016$), more neoadjuvant CT, and less overall CT use ($p < 0.001$) in the non-GnRHa subgroup (Supplementary table 4). In the propensity score-matched population, 139 patients had N0/RI+N1/RL-RI BC and 133 patients were premenopausal. Again, CT provided better EFS in the subgroup not receiving GnRHa ($p = 0.021$), but not in patients who received GnRHa ($p = 0.987$). Baseline characteristics were similar between patients receiving or not adjuvant GnRHa, whether treated or not with CT, except for lower PgR levels in patients not receiving GnRHa in the CT-treated subgroup (Supplementary table 4–6).

When analyzing N0/RI ($n = 120$), N1/RL ($n = 15$), and N1/RH ($n = 67$) separately, CT improved EFS only in GnRHa-naïve N0/RI patients ($p = 0.023$), with no difference ($p = 0.870$) in those receiving GnRHa (Supplementary figure 2A-**B**). N1 subgroups were too small for separate assessment, but when regrouping N1/RL and N1/RI, a numerical benefit for CT was observed regardless of GnRHa use, though not reaching statistical significance (Supplementary figure 2C-**D**).

3.5. *Exploratory analyses of CT effect according to IS*

PAM50 IS showed significantly different EFS ($p < 0.001$), with HER2-enriched tumors having the worst prognosis compared to Luminal A (HR: 57.6, 95%CI: 6.67–496.26) (Figure 5A). Luminal A and Luminal B tumors, which comprised the majority of the cohort, showed no EFS difference ($p = 0.860$).

When analyzing EFS according to subtype and CT use, significant differences were observed in the overall study cohort ($p < 0.001$) (Figure 5B), and in N0 patients ($p < 0.001$), although statistical significance was not reached in the N1 subgroup ($p = 0.587$) (Figure 5C-D). Formally, CT use did not significantly impact EFS in Luminal A tumors, overall ($p = 0.332$), or within N0 ($p = 0.179$) and N1 ($p = 0.266$) subsets. However, CT-treated Luminal A showed slightly higher 5-year EFS (97.1%, 95%CI: 93.3–100%) than CT-naïve Luminal A (93.7%, 95%CI: 89.9–97.8%) and CT-treated Luminal B (93.7%, 95%CI: 88.9–98.8%) (Supplementary table 7). This numerical benefit of CT in Luminal A disease was retained when separating patients according to N (Supplementary table 7). A similar trend was observed when patients were stratified by ROR, N and subtype (Supplementary table 8 and **Supplementary results**). Notably, a numerical EFS benefit in Luminal A tumors treated with CT was restricted to patients not receiving OFS (Supplementary figure 3A-**B**), regardless of N (Supplementary figure 3C-**F**). Other subtypes, including Luminal B treated only with ET ($n = 19$), HER2-enriched ($n = 2$) and Basal-like ($n = 10$) tumors were too few to calculate meaningful EFS rates or to estimate OS.

4. Discussion

In this international multicenter real-world study, we explored the ability of Prosigna® to estimate the benefit of CT in ≤ 50yo patients with stage I-II ER+ /HER2- BC. Similarly to what observed in OncotypeDX®-based TAILORx and RxPONDER trials, the subgroups appearing to benefit the most from CT were those with N0 tumors with intermediate-to-high genomic risk and N1 tumors, with absolute differences in 5-year EFS rates in favor of CT especially in N0/RI (Δ5.9%), N1/RL (Δ33.3%),

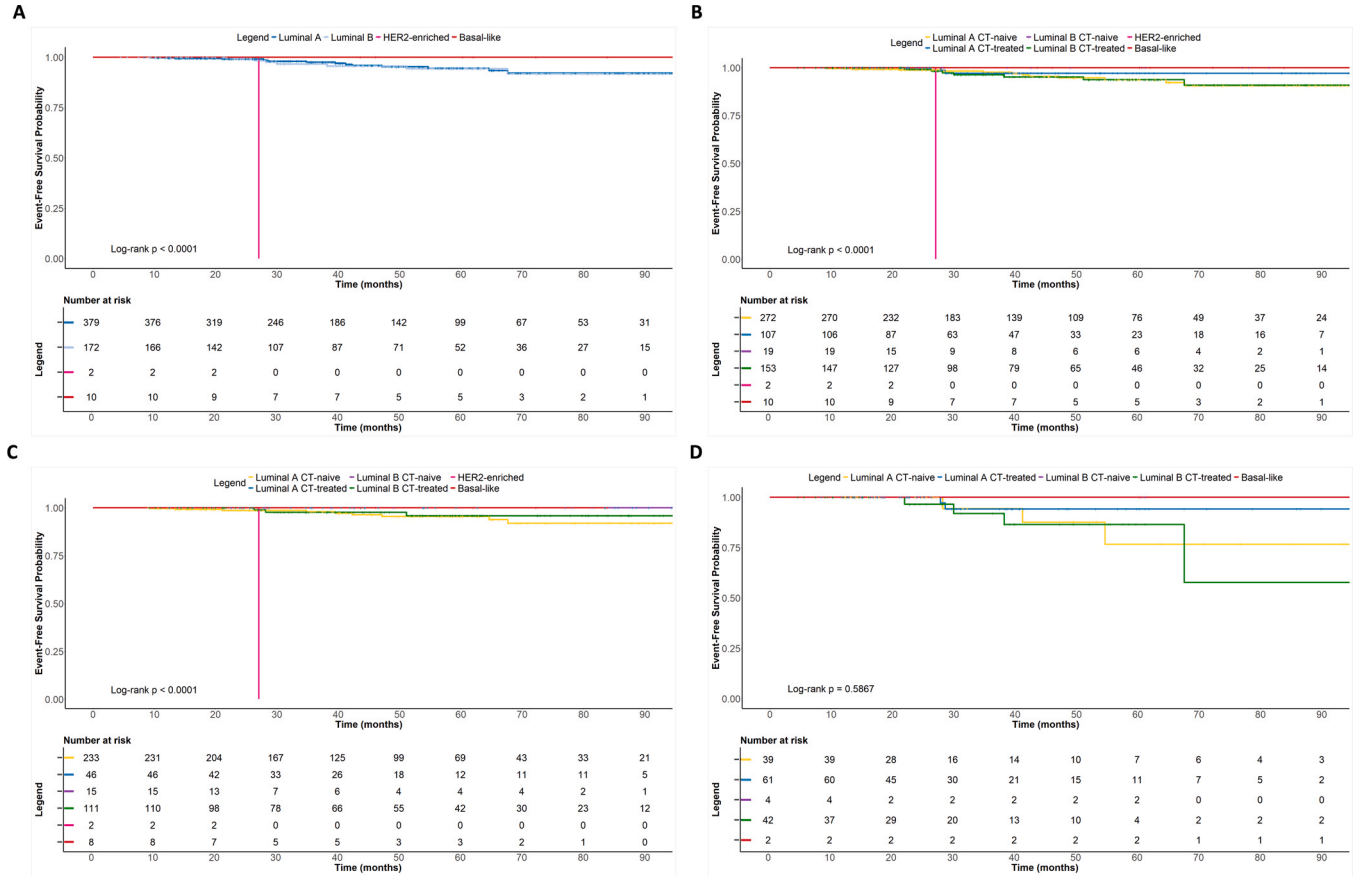

Fig. 5. EFS according to PAM50 intrinsic subtypes in different scenarios. Legend. A: EFS according to PAM50 intrinsic subtypes; **B:** EFS according to PAM50 intrinsic subtypes and treatment; **C:** EFS according to PAM50 intrinsic subtypes and treatment in patients with N0 disease; **D:** EFS according to PAM50 intrinsic subtypes and treatment in patients with N1 disease. CT: chemotherapy; EFS: event-free survival; N.: number.

and N1/RI (Δ23.6%). Notably, in patients with N0/RI+N1/RL-RI BC, EFS after CT was comparable to that of N0/RL cases treated with ET alone. Moreover, the EFS in N0/RH cases treated with CT did not differ from that of N0/RI+N1/RL-RI patients who received CT and was higher than that of N0/RI+N1/RL-RI patients treated only with ET, with 5-year EFS Δ of 8.5%. Altogether, these findings suggest that in these higher-risk subgroups, CT may help mitigate the poorer prognosis associated with intermediate/high genomic risk or nodal involvement, bringing outcomes closer to those of patients with lower genomic risk. Conversely, N1/RH patients, even when treated with CT, still experienced significantly worse EFS than N0/RI+N1/RL-RI patients who received CT, underscoring its limited efficacy and the need for further therapeutic escalation, such as adjuvant CDK4/6-inhibitors [29,30].

To contextualize our findings within the broader literature, we informally compared our results with those of TAILORx and RxPONDER. In our cohort, 5-year EFS landmark analyses stratified by Prosigna® ROR category, nodal status, and systemic treatment were broadly consistent with those reported in OncotypeDX® trials conducted in comparable populations with similar stage, treatment, and genomic risk profiles, supporting the clinical utility of Prosigna® in this setting. A notable exception was observed in the PROUDER-BC N1/RL subgroup treated with endocrine therapy alone, which showed a 5-year EFS of 66.7% compared with 91.0% in RxPONDER. However, this subgroup included only 9 patients, resulting in an unstable estimate with wide confidence intervals. For this reason, the primary analyses grouped the N0/RI, N1/RL, and N1/RI subgroups to improve the statistical robustness of the within-cohort comparisons. Interestingly, 5-year EFS rates for CT-treated PROUDER-BC patients in the N0/RI, N0/RH, N1/RL and N1/RI categories were numerically higher than those observed in the corresponding OncotypeDX® trial populations (Δ of 3.2%, 8.7%, 2.1% and 6.8%, respectively). This difference may reflect the ability of Prosigna® to more accurately identify more aggressive tumors and the subset of patients most likely to derive substantial benefit from CT. In fact, the observed EFS advantage with CT was consistent across all subgroups regardless of genomic risk, whereas EFS rates for patients treated with ET alone were similar between Prosigna® and OncotypeDX® estimates. The biological rationale might be related to the fact that Prosigna® ROR is driven by tumor proliferation, while the estrogen-related gene module drives OncotypeDX® recurrence score [31]. Notably, Prosigna® added significant prognostic information beyond OncotypeDX® in a large validation study with >1000 patients from the ATAC trial [25]. Although the results were obtained in a postmenopausal population, the finding further corroborates our hypothesis.

An open question in the scientific community is whether the observed benefit of CT in premenopausal patients with non-high-risk disease is primarily mediated through OFS rather than direct cytotoxicity. We previously reported that CT downregulated PgR and 19 ER-regulated genes at the mRNA level in premenopausal but not in postmenopausal women, consistent with an OFS effect [32]. The most likely explanation is that CT induces OFS in premenopausal patients, resulting in reduced systemic estradiol levels and decreased expression of ER-regulated genes and hormone receptors in tumor cells. In the PROUDER-BC cohort, CT improved EFS only in OFS-naïve patients, while no benefit was seen when combined with GnRHa, particularly in N0 disease. These findings suggest that CT benefit may be largely mediated by OFS, although CT could retain a role in N1 cases. Ongoing RCTs such as OPTIMA (NCT03416153) and OPTIMA-YOUNG (NCT07106632), where part of our group is directly involved in the study development, will likely provide more definitive evidence.

A previous analysis from the SOFT RCT showed that Prosigna® ROR and PAM50 IS retained their prognostic ability in premenopausal women [33]. In our cohort, the HER2-enriched subtype showed the poorest prognosis, though the small number of cases limited further analysis. Basal-like tumors had unexpectedly favorable outcomes, likely reflecting their known chemosensitivity[34], but also the small sample size and early stage at diagnosis. Interestingly, Luminal A showed outcomes comparable to usually more aggressive Luminal B tumors, in line with previous studies showing worse outcomes than expected for Luminal A disease in young women [33,35–37]. This might be due to the fact that Luminal A may harbor more aggressive biological features than their counterparts in postmenopause, including homologous recombination deficiency, copy number alterations, and mutations in oncogenic drivers [38], as well as higher expression of basal-like- and proliferation-related genes [39]. These features may reduce sensitivity to standard treatments, especially ET, and favor earlier recurrence. Nonetheless, as PSM was not performed for subtype-based analyses, it should be acknowledged that residual confounding related to treatment allocation and disease burden cannot be excluded.

A PAM50-Based Chemoendocrine Score (CES) was proposed to identify ER+ /HER2- BC with sensitivity to CT beyond ROR and PAM50 IS, in RI disease [40]. Besides key limitations derived from the different clinicopathological characteristics of the patients included in the training and validation cohorts (e.g. many postmenopausal, many stage II-III etc.) and the use of research-only ROR-P score instead of Prosigna® ROR, the main issue of CES is that it is unavailable to clinicians, since PAM50 IS correlation scores used to calculate it are not provided in the Prosigna® report [40]. We thus evaluated CT benefit according to PAM50 IS, stratifying the population according to ROR and N. Signals of CT benefit were observed in the RI subgroup regardless of IS and N. In premenopausal patients with Luminal A/RL-RI disease, the impact of OFS seemed to mirror that of the N0/RI and N1/RL-RI subgroups. However, the limited sample size in most subgroups did not allow for meaningful conclusions. Therefore, in $\leq$ 50yo/premenopausal women, treatment decisions should be primarily guided by ROR and N.

It is important to note that in the PROUDER-BC we were unable to evaluate the potential impact of adjuvant CDK4/6-inhibitors (abemaciclib/ribociclib+ET), since these agents were not approved when this population was treated. Therefore, we cannot exclude that the combined use of Prosigna® ROR and IS in young patients may support more biology-driven escalated/de-escalated/alternative approaches, including ET+OFS+CDK4/6-inhibitors without CT, new oral SERDs [41], new target agents, or antibody-drug conjugates.

4.1. *Limitations and strengths*

Our study is not exempt from limitations. First, it is a retrospective analysis, inherently prone to selection bias and unmeasured confounding. Second, some subgroups were too small to draw any meaningful conclusion. Third, for at least one-fifth of cases Prosigna® was requested irrespective of IHC parameters, potentially explaining the very low proportion of non-luminal subtypes and the unexpectedly high proportion of Luminal A/RI-RH tumors. This lack of clinical pre-selection (e.g. based on Ki67, G or ER) may have influenced outcomes, although it also reflects real-world clinical practice. Fourth, survival outcomes in our analyses should be interpreted with caution given the relatively short median follow-up (40.7 months) and the limited number of events (21 relapses and 2 deaths), particularly in the context of the natural history of HR+ /HER2- disease, which is characterized by a persistent risk of late recurrence [42]. The small number of events, together with multiple subgroup analyses, also increases the risk of chance findings, especially in underrepresented cohorts. Moreover, the current follow-up was insufficient to robustly estimate OS or capture relapses beyond 5 years, which will require long-term observation in a future analysis. Fifth, there were methodological differences in endpoint definition compared with OncotypeDX® RCTs. We used EFS as the primary endpoint, whereas TAILORx and RxPONDER, used invasive disease-free survival (iDFS) in patients not exposed to neoadjuvant treatment. However, only 20.3% of patients received neoadjuvant therapy (9.9% NACT, 10.4% NET) and, by definition [28], EFS does not include *in situ* relapses, closely resembling iDFS. Furthermore, the PSM mitigated the lack of randomization and prospective recruitment, with results supporting all main analyses. At the same time, this approach cannot fully eliminate

residual confounding inherent to retrospective observational designs, such as the decision to request Prosigna® testing and to administer chemotherapy across different centers, where the decision may have been influenced by unmeasured institutional practices, physician preferences, or patient-related factors that were not captured in the database. Finally, direct comparisons to TAILORx and RxPONDER should be interpreted with caution, given the different designs, eligibility criteria, different features of the GEA and populations of the three studies. Similarly, in light of the retrospective and exploratory design, analyses were not adjusted for multiple testing and should be interpreted accordingly.

To our knowledge, this is the first to assess the predictive relevance of Prosigna® in ≤ 50yo/premenopausal women with stage I-II ER+ / HER2- BC and put it in the context of available literature with OncotypeDX®. Although no formal interaction between ROR score and CT was observed, similarly to TAILORx and RxPONDER, we detected clinically meaningful differences in survival rates across subgroups stratified according to genomic risk and treatment received. Importantly, prospective data from RCTs specially addressing this population are not expected soon. Moreover, previous evidence on the predictive role of Prosigna® ROR or its research-based versions in premenopausal women was limited to trials with outdated regimens (cyclophosphamide or CMF), no HER2 testing, mixed pre/postmenopausal cohorts and/or higher tumor burden (N2–3, T3N+, T4 tumors) [20–22,43]. Importantly, our study adhered carefully to STROBE guidelines and used the validated (FDA-cleared and CE-Marked) Prosigna® assay following standard operating procedures specified by the manufacturer at each participating site.

5. Conclusions

In this international real-world analysis, Prosigna® ROR could identify among ≤ 50 yo/premenopausal women with stage I-II ER+ / HER2- BC, those who might benefit from (neo)adjuvant CT, consistent with what observed with other GEA. Besides the genomic high-risk population, CT significantly improved long-term outcomes in patients with N0/RI and N1/RL-RI BC, independently of main clinicopathological features. However, the prognostic advantage of CT seemed to be related to OFS, especially in N0 disease. These findings require prospective confirmation in larger cohorts, but further corroborate the hypothesis that, in certain premenopausal patients, optimal endocrine manipulation might replace the need for CT. Integrating genomic risk assessment with IS classification could further refine treatment selection.

CRediT authorship contribution statement

Rodrigo Sánchez Bayona: Writing – review & editing, Investigation, Data curation. **Michelino De Laurentiis:** Writing – review & editing, Conceptualization. **Francisco Javier Calleja-Holgado:** Writing – review & editing, Investigation, Data curation. **César A. Rodríguez:** Writing – review & editing, Investigation. **David Pelegrina Sánchez:** Writing – review & editing, Investigation, Data curation. **Benjamin Wallbaum:** Writing – review & editing, Investigation, Data curation. **Tomás Pascual:** Writing – review & editing, Investigation. **Grazia Arpino:** Writing – review & editing, Investigation. **Assumpció López Paradís:** Writing – review & editing, Investigation, Data curation. **Gimena Barroso:** Writing – review & editing, Investigation, Data curation. **Ona Cano Cano:** Writing – review & editing, Investigation, Data curation. **Pablo Rivera Vargas:** Writing – review & editing, Investigation, Data curation. **Vidal Losada María J:** Writing – review & editing, Supervision, Project administration, Methodology, Conceptualization. **Francesco Schettini:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Michela Palleschi:** Writing – review & editing, Investigation, Data curation. **Antonino Musolino:** Writing – review & editing, Investigation. **Gastón Zatta Cobos:** Writing – review & editing, Investigation, Data curation. **Elsa Dalmau Portulas:** Writing – review & editing, Investigation, Data curation. **Luis Figuero-Pérez:** Writing – review & editing, Investigation, Data curation. **Aldo Caltavituro:** Writing – review & editing, Investigation, Data curation. **Giuseppe Buono:** Writing – review & editing, Investigation, Data curation. **Yolanda Jerez Gillaranz:** Writing – review & editing, Investigation, Data curation. **Giuseppe Di Grazia:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Vincenzo Di Lauro:** Writing – review & editing, Investigation, Data curation. **Daniel Morchón-Araujo:** Writing – review & editing, Investigation, Data curation. **Sabrina Nucera:** Writing – review & editing, Data curation. **Barbara Adamo:** Writing – review & editing, Investigation. **Montserrat Muñoz Mateu:** Writing – review & editing, Investigation. **Isabel Echavarría:** Writing – review & editing, Investigation, Data curation. **Sara López-Tarruella:** Writing – review & editing, Investigation, Data curation. **Milana Bergamino Sirvén:** Writing – review & editing, Investigation, Data curation. **Olga Martínez-Sáez:** Writing – review & editing, Investigation.

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None.

Data sharing statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: **F. Schettini** reports honoraria from Novartis, Gilead, Veracyte, AstraZeneca and Daiichy-Sankyo for educational events/materials, advisory fees from Pfizer, Astra-Zeneca, Daiichy-Sankyo and Veracyte, and travel expenses from Novartis, Astra-Zeneca, Gilead and Daiichy-Sankyo. **D. Morchón-Araujo** reports honoraria for lectures and educational activities from Astellas Pharma, PharmaMar, Roche, Janssen, AstraZeneca and travel expenses from Novartis, Lilly, Bristol-Myers Squibb, Merck and Gilead. **Luis Figuero-Pérez** declares speaker fees from AstraZeneca, Novartis, Gilead, Sanofi, MSD and travel support from Roche, MSD, Novartis, Lilly and Gilead. **C.A.Rodríguez** reports honoraria for lectures and educational activities from Novartis, Pfizer, Lilly, AstraZeneca, Daiichi Sankyo, MSD, Veracyte, Roche, Eisai, Gilead and consultant/advisory roles from Novartis, Lilly, AstraZeneca, Daiichi Sankyo, MSD, Pierre Fabre and Gilead.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ejca.2026.116818.

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