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Dynamic Changes in Ki67 Predict Endocrine Responsiveness in Estrogen Receptor–Positive/HER2-Negative Breast Cancer Patients Receiving Preoperative Endocrine Therapy

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Abstract

Although a rapid decline in Ki67 after short-term preoperative endocrine therapy (ET) has been established as a prognostic and predictive marker in controlled studies, its reliability and usefulness in everyday oncology practice are still insufficiently defined. In this real-world analysis, we investigated early on-treatment Ki67 modulation after brief preoperative ET and explored its relationship with tumor biology—including intrinsic molecular subtype and risk of recurrence (ROR)—together with long-term clinical outcomes in patients with early estrogen receptor–positive, human epidermal growth factor receptor 2–negative (ER+/HER2−) breast cancer. This retrospective, registry-based study included 230 consecutive patients with early ER+/HER2− breast cancer treated according to standard clinical protocols at the Breast Unit of the Clinic Barcelona Comprehensive Cancer Center between 2014 and 2023. All patients received neoadjuvant ET, consisting of either tamoxifen or an aromatase inhibitor (AI), administered for 2–12 weeks prior to definitive surgery. Clinicopathological data were collected and patients were stratified according to Ki67 dynamics into "responders" (post-treatment Ki67 between 0% and 10%) and those achieving "complete cell cycle arrest (CCCA)" (Ki67 ≤2.7%). Molecular intrinsic subtypes and ROR scores were derived using the PAM50/Prosigna assay. Event-free survival was evaluated using Kaplan–Meier estimates, and associations with outcomes were examined using Cox proportional hazards models.

The median duration of preoperative ET was 5 weeks (range 2–12 weeks). A reduction in Ki67 consistent with response was observed in 196 patients (85.2%), while 111 patients (48.3%) met criteria for CCCA. Endocrine responsiveness differed significantly by menopausal status, with postmenopausal women showing higher response rates than premenopausal patients (P = 0.004). Treatment allocation reflected this difference, as nearly all postmenopausal women (95.6%) received an AI, whereas tamoxifen was exclusively used in premenopausal patients. Ki67 suppression also varied across intrinsic subtypes, with Luminal A tumors demonstrating the most pronounced response (P = 0.047). Multivariable analyses identified postmenopausal status and higher baseline estrogen receptor expression as independent predictors of both Ki67 response and CCCA, while lower baseline ROR scores were specifically associated with achieving CCCA. After a median follow-up of 47 months, patients who achieved CCCA experienced significantly superior event-free survival [hazard ratio (HR) = 0.19; 95% CI (confidence interval), 0.05–0.72; P value = 0.012]. In a real-world clinical setting, short-duration preoperative endocrine therapy induces marked antiproliferative effects in early ER+/HER2− breast cancer. Early measurement of Ki67 suppression provides a practical surrogate of endocrine sensitivity, and attainment of complete cell cycle arrest delineates a subgroup with particularly favorable prognosis who may be appropriate candidates for treatment de-escalation approaches.

Keywords: Dynamic biomarkers, Ki67, Complete cell cycle arrest, Preoperative endocrine therapy

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Introduction

Breast cancer (BC) remains the leading cancer diagnosis among women globally, with estrogen receptor–positive, human epidermal growth factor receptor 2–negative (ER+/HER2−) disease constituting the predominant biological subtype [1]. Endocrine therapy (ET)

represents the fundamental therapeutic modality for this population, conferring substantial benefits in survival and lowering the risk of recurrence or progression across both early and advanced stages of disease [2–4]. Despite the widespread incorporation of neoadjuvant chemotherapy into routine practice, the use of preoperative ET has been comparatively limited [5–7], even though it is generally well tolerated and offers a unique opportunity to directly evaluate tumor endocrine responsiveness in vivo.

The principal objective of administering ET prior to surgery is not to achieve marked radiological tumor regression, but rather to provoke rapid biological effects, most notably a decrease in tumor cell proliferation. These early biological signals can provide clinically meaningful information to guide subsequent adjuvant treatment decisions. Evidence from prior investigations indicates that a pronounced reduction in proliferative activity during ET is associated with excellent long-term outcomes with endocrine therapy alone and may identify patients in whom chemotherapy can be safely avoided [8, 9]. In this context, the Preoperative Endocrine Prognostic Index—which combines post-treatment tumor size, nodal involvement, hormone receptor expression, and proliferation markers—has been validated as a clinically useful framework to support decisions regarding treatment escalation or de-escalation after surgery [8].

Risk stratification can be further refined by integrating dynamic changes in proliferation with genomic assays, such as Oncotype DX, thereby extending prognostic accuracy beyond baseline molecular classification [9, 10]. Notably, even patients with limited nodal involvement (N1 disease) who are categorized as having intermediate genomic risk may be suitable for less intensive therapy if they exhibit marked suppression of proliferation, whereas those showing minimal biological response may warrant more aggressive adjuvant strategies [9, 11].

More recently, neoadjuvant endocrine trials have underscored the importance of achieving complete cell cycle arrest (CCCA), defined by near-total suppression of proliferative activity (Ki67 ≤2.7%), as a particularly robust indicator of endocrine sensitivity and a strong surrogate for favorable long-term outcomes [12, 13].

Nevertheless, the extent to which these concepts can be consistently applied and reproduced in routine clinical settings remains uncertain. Therefore, the present study examines early changes in tumor proliferation induced by short-term preoperative ET in a real-world cohort of patients with early ER+/HER2− BC treated at a single institution, and investigates how these dynamic biomarkers relate to clinicopathological features, genomic profiles, and patient outcomes.

Materials and Methods

Study design and patient population

This retrospective, registry-based cohort study analyzed 230 consecutive patients managed according to standard clinical practice. Eligible individuals had unicentric, unilateral stage I–III ER+/HER2− breast cancer, classified according to the American Joint Committee on Cancer Staging Manual (8th edition), and received a short course of preoperative ET between 2014 and 2023 at Hospital Clínic of Barcelona, Spain. The decision to initiate ET prior to surgery was made at the treating physician's discretion, most commonly in situations where the benefit of adjuvant chemotherapy was uncertain. Patients were treated with ET for 2 to 12 weeks before definitive surgery as a pragmatic, real-world approach to assess endocrine sensitivity and support adjuvant treatment planning. Importantly, the duration of preoperative ET was not protocol driven and was frequently influenced by logistical factors, such as surgical scheduling and operating room availability, rather than by predefined clinical criteria.

Data collection

Baseline clinical and demographic information—including age, menopausal status, body mass index, and treatment-related variables—was extracted from electronic medical records. Imaging data were derived from standard-of-care mammography or breast magnetic resonance imaging performed at diagnosis. Pathological assessment was conducted on baseline core needle biopsies and corresponding surgical specimens using immunohistochemistry (IHC). Estrogen receptor positivity was defined as expression in at least 1% of tumor cells, while HER2 negativity was established in accordance with American Society of Clinical Oncology/College of American Pathologists guidelines (IHC 0–1+, or IHC 2+ with non-amplified in situ hybridization results) [14, 15]. Ki67 evaluation followed local clinical protocols and was performed by the same pathology team on both baseline and post-treatment samples. Tumor sections (3–4 μm) were stained using a rabbit monoclonal anti-Ki67 antibody (clone 30.9,

Roche, Basel, Switzerland) on an automated Ventana Benchmark Ultra platform (Roche, Basel).

IHC-based molecular phenotypes were classified as luminal A–like (ER+/HER2−, progesterone receptor ≥20%, Ki67 <14%) or luminal B–like (ER+/HER2− with Ki67 ≥14%, or ER+/HER2− with Ki67 <14% and progesterone receptor <20%) [16]. Molecular intrinsic subtype and risk of recurrence (ROR) score were determined using the PAM50/Prosigna assay in 138 baseline tumor core biopsies and 44 surgical specimens processed at Hospital Clínic of Barcelona. ROR categories were assigned according to certified PAM50 thresholds [17]: low ROR (≤40 points for node-negative disease or <15 points for tumors with one to three positive nodes), intermediate ROR (41–60 points for node-negative disease or 16–40 points with one to three positive nodes), and high ROR (61–100 points for node-negative disease or 41–100 points with one to three positive nodes).

Study endpoints

Patients' response to endocrine treatment was classified using the proliferation marker measured during therapy: "responders" showed post-treatment proliferation between 0% and 10%, with a more stringent subgroup called complete cell cycle arrest (CCCA) responders having Ki67 levels of 2.7% or lower. The key prognostic outcome was event-free survival (EFS), calculated as the time from starting endocrine therapy until disease recurrence or death from any reason.

Statistical methods

Medians and ranges described continuous variables, while frequencies and proportions were used for categorical ones. Associations with treatment response and CCCA were explored via logistic regression, including single-variable analyses and adjusted multi-variable models accounting for major clinical factors. Event-free survival curves were generated with the Kaplan–Meier method and compared by log-rank testing; univariate Cox models provided hazard ratios (HRs) along with their 95% confidence intervals (CIs). Analyses were performed in R software, version 3.6.2.

Ethics

This research complied with EMA/CHMP/ICH/135/1995 guidelines and obtained approval from the Hospital Clinic of Barcelona Ethics Committee (approval number HCB/2024/0328). All participants gave written informed consent through the biobank collection titled “Biospecimens of patients with solid tumors at Hospital Clinic of Barcelona” (reference C.0004038).

Findings

Patient and tumor characteristics are presented in **Table 1**. The cohort consisted of 230 women with a median age of 62 years (ranging from 35 to 95), of whom 181 (78.7%) had reached menopause. Among 227 patients with recorded body mass index, more than half (54.6%) were overweight or obese. Most tumors were invasive ductal carcinomas of no special type (87.0%), histological grade 2 (52.2%), clinically T1 stage (74.8%), and without evident lymph node involvement (96.5%). Pretreatment Ki67 had a median value of 12% (range 1%–80%), with 44.3% of cases starting at 10% or below and 12.2% at 30% or above. Neoadjuvant endocrine therapy lasted a median of 5 weeks (2–12 weeks) and was tailored by menopausal status—tamoxifen was given to all premenopausal women and to eight postmenopausal ones (comprising 24.8% of all regimens)—while ovarian function suppression was not applied in any patient.

Table 1. Characteristics of patients, tumors, and treatments (N = 230)

Feature	Cohort Summary
Age (years)	Median: 62 (Range: 35–95)
Body Mass Index (BMI)	Median: 25.3 (Range: 17.2–48.8)
BMI categories	Underweight/Normal: 103 (45.4%) Overweight: 76 (33.5%) Obese: 48 (21.1%)
Menopause Status	Premenopausal: 49 (21.3%) Postmenopausal: 181 (78.7%)
Tumor Histology	NST: 200 (87.0%) ILC: 21 (9.1%) Other types: 9 (3.9%)
Histologic Grade	Grade 1: 97 (42.1%) Grade 2: 120 (52.5%) Grade 3: 7 (3.0%) Unknown: 6 (2.6%)
Clinical Tumor Stage (cT)	T1: 172 (74.8%) T2: 50 (21.7%) T3: 6 (2.6%) T4: 2 (0.9%)
Clinical Nodal Stage (cN)	N0: 222 (96.5%) N1: 6 (2.6%) Nx: 2 (0.9%)
Baseline Ki67 (%)	Median: 12 (Range: 1–80) ≤10%: 102 (44.3%) 11–29%: 100 (43.5%) ≥30%: 28 (12.2%)

Baseline ER Expression	1–10%: 2 (0.9%) ≥10%: 228 (99.1%)
Baseline PR Expression	<10%: 32 (13.9%) ≥10%: 198 (86.1%)
Length of Endocrine Therapy (weeks)	Median: 5 (Range: 2–12)
Type of Endocrine Therapy	Tamoxifen: 57 (24.8%) Aromatase inhibitor: 173 (75.2%)
Postsurgical Ki67 (%)	Median: 3 (Range: 1–75) ≤10%: 196 (85.2%) 11–29%: 27 (11.7%) ≥30%: 7 (3.0%)
Pathological Tumor Stage (pT)	T1: 180 (78.6%) T2: 43 (18.8%) T3: 5 (2.2%) T4: 1 (0.4%) Unknown: 1
Pathological Nodal Stage (pN)	N0: 160 (73.1%) N1: 52 (23.6%) N2: 6 (2.7%) N3: 1 (0.5%) Unknown: 11
Adjuvant Chemotherapy	Administered: 51 (22.2%) Not administered: 178 (77.4%) Unknown: 1 (0.4%)

Abbreviations: AI= aromatase inhibitor; BMI= body mass index; ER= estrogen receptor; ET= endocrine therapy; ILC= invasive lobular carcinoma; NST= no special type; PR= progesterone receptor; TAM= tamoxifen.

Molecular subtype distribution

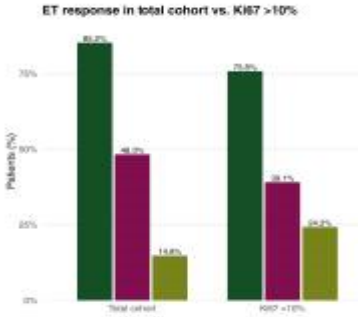
Of the 138 patients, 67.4% had Luminal A tumors, 31.2% had Luminal B, and 1.4 percent had Basal-like. No cases were classified as HER2-enriched. Risk of Relapse (ROR) scores were categorized as low in 45.0 percent, intermediate in 34.8 percent, and high in 20.1 percent of these patients.

Changes in proliferation after preoperative endocrine therapy

After a short course of neoadjuvant endocrine therapy, the median Ki67 level dropped substantially from 12% at baseline to 3% post-treatment, although values after therapy varied widely (1%–75%). In total, 196 patients (85.2percent) achieved a Ki67 ≤10% following treatment, and 111 (48.3%) qualified as complete cell cycle arrest (CCCA). Only 3.0% showed persistently high proliferation (Ki67 ≥30%) after therapy.

In the subgroup with baseline Ki67 >10% (n=128), 75.8% demonstrated a proliferative response (post-treatment Ki67 ≤10%), and 39.1% attained CCCA **(Figure 1a)**. Tumors starting with low baseline Ki67 (≤10 percent) generally remained suppressed, with just 2.9% experiencing a rise above 10% after treatment.

The median duration of endocrine therapy was comparable between responders and non-responders (5 weeks versus 4 weeks; P=0.406), suggesting that small differences in treatment length within this brief preoperative period did not meaningfully affect the degree of Ki67 reduction **(Table 2)**.



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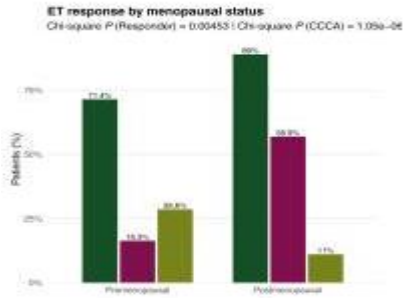

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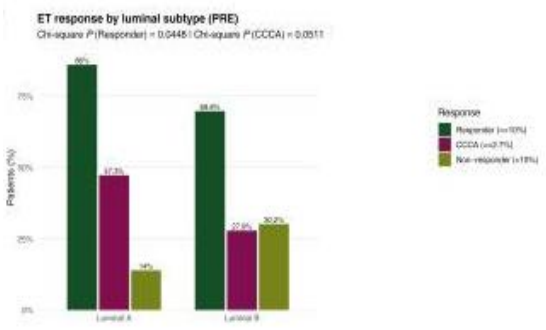

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Figure 1. (a) Proportion of patients achieving a post-treatment Ki67 of ≤10% and those reaching complete cell cycle arrest (CCCA) in the overall population and among individuals with baseline Ki67 above 10%. (b) Post-treatment Ki67 ≤10 percent and CCCA rates stratified by menopausal status. (c) Distribution of Ki67 ≤10 percent and CCCA according to intrinsic tumor subtypes.

Table 2. Clinical and biological differences between responders and non-responders

Characteristics	Responders (*N* = 196)	Non-responders (*N* = 34)	*P* value
Age years – median (range)	62.0 (36-95)	61.5 (35-84)	0.080
BMI – median (range)	25.5 (23.1-29.8)	24.7 (20.89-27.4)	
Underweight/normal	86 (44.6%)	18 (52.9%)	
Overweight	61 (31.6%)	14 (41.2%)	
Obese	46 (23.8%)	2 (5.9%)	**0.030**
Menopausal status			
Premenopausal	35 (17.9%)	14 (41.2%)	**0.004**
Postmenopausal	161 (82.1%)	20 (58.8%)	
Tumor histology			
NST	168 (85.7%)	32 (94.1%)	
ILC	19 (9.7%)	2 (5.9%)	0.168
Other	9 (4.6%)	0 (0.0%)	
Histological grade			
1	90 (45.9%)	7 (20.6%)	
2	97 (49.5%)	23 (67.6%)	**<0.001**
3	3 (1.5%)	4 (11.8%)	
Unknown	6 (3.0%)	0 (0.0%)	
Clinical T stage			0.648
1	148 (75.5%)	24 (70.6)	
2	40 (20.4%)	10 (29.4)	
3	6 (3.1%)	0 (0.0%)	
4	2 (1.0%)	0 (0.9%)	
Clinical N stage			
N0	189 (96.4%)	33 (97.1%)	0.850
N1	5 (2.5%)	1 (2.9%)	
Ki67 baseline – median (range)	10.0 (1.0-55.0)	21.0 (5.0-80.0)	
<10%	99 (50.5%)	3 (8.8%)	
≥10%-29%	83 (42.3%)	17 (50%)	**<0.001**
≥30%	14 (7.1%)	14 (41.2%)	
Baseline ER status			
1-10%	1 (0.5%)	1 (2.9%)	0.683
≥10%	195 (99.5%)	33 (97.1%)	
Baseline PR status			
<10%	33 (16.8%)	3 (8.8%)	0.509
≥10%	163 (83.2%)	31 (91.2%)	
Clinical intrinsic subtype			
Luminal A-like	100 (51%)	5 (14.7%)	
Luminal B-like	96 (49%)	29 (85.3%)	**<0.001**
Baseline PAM50 intrinsic subtype			**0.047**
Luminal A	80 (72.1%)	13 (48.1%)	
Luminal B	30 (27.0%)	13 (48.1%)	

Basal-like	1 (0.9%)	1 (3.7%)	
Baseline ROR			
Low-risk	56 (50.5%)	6 (22.2%)	
Intermediate-risk	36 (32.4%)	12 (44.4%)	**0.023**
High-risk	19 (17.1%)	9 (33.3%)	
ET duration (weeks) – median (range)	5 (2-12)	4 (2-11)	0.406
Endocrine treatment			
TAM	39 (19.9%)	18 (52.9%)	**<0.001**
AI	157 (80.1%)	16 (47.0%)	
Adjuvant chemotherapy			
Yes	31 (15.9%)	20 (58.8%)	**<0.001**
No	164 (84.1%)	14 (41.2%)	

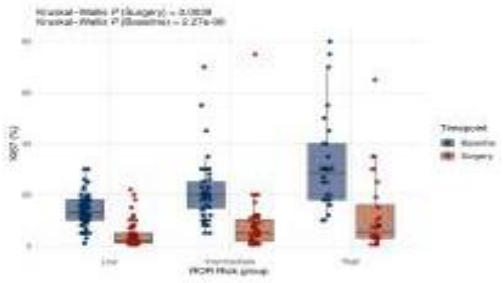
Bold values: statistically significant ($P < 0.05$).

AI= aromatase inhibitor; BMI= body mass index; ER= estrogen receptor; ET,=endocrine therapy; ILC= invasive lobular carcinoma; NST= no special type; PR= progesterone receptor; ROR= risk of recurrence; TAM= tamoxifen.

Endocrine therapy elicited greater responses in postmenopausal patients than in their premenopausal counterparts, with response rates of 88.9 percent versus 71.4 percent (P = 0.004) **(Figure 1b)**; it is notable that all premenopausal women received tamoxifen, whereas only 8 (4.4%) of the postmenopausal group were treated with this agent. Treatment efficacy declined with increasing histological grade, with 92.8% of grade 1 tumors, 80.8% of grade 2, and 42.9% of grade 3 tumors responding (P < 0.001). Overall, 22.3 percent of the cohort received adjuvant chemotherapy, which was disproportionately administered to non-responders compared with responders (58.8% vs. 15.9 percent; P < 0.001) **(Table 2)**.

Intrinsic tumor subtype, as defined by PAM50, was also a determinant of endocrine response. Luminal A tumors (n = 93) demonstrated superior response rates compared with Luminal B tumors (n = 43) (86.0 percent vs. 69.8 percent; P = 0.047), with a trend toward higher rates of complete cell cycle arrest (CCCA) (47.3% vs. 27.9 percent; P = 0.051) **(Figure 1c)**. Within the Luminal A subgroup, postmenopausal women (n = 68) achieved response and CCCA rates of 94.1% and 60.3%, respectively, which were substantially higher than in premenopausal patients (n = 25; response: 64.0 percent, CCCA: 12.0 percent; P < 0.001 for both). In contrast, Luminal B tumors (n = 43) exhibited comparable outcomes between postmenopausal (n = 36; response: 69.4%, CCCA: 27.8%) and premenopausal patients (n = 7; response: 71.4%, CCCA: 28.6%), with no statistically significant differences (P = 0.899 for response; P = 0.945 for CCCA), reflecting variations in therapy selection based on menopausal status.

Analysis by baseline risk of recurrence (ROR) revealed distinct proliferation dynamics. Both pre- and post-surgical Ki67 levels differed across ROR categories **(Figure 2a)**, with low-risk tumors (n = 62) showing the highest response rate (90.3%), followed by intermediate-risk (n = 48; 75.0 percent) and high-risk tumors (n = 28; 67.9 percent) (P = 0.023). CCCA was similarly correlated with initial ROR, occurring in 54.8% of low-risk, 33.3% of intermediate-risk, and 21.4% of high-risk tumors (P = 0.005) **(Figure 2b)**.



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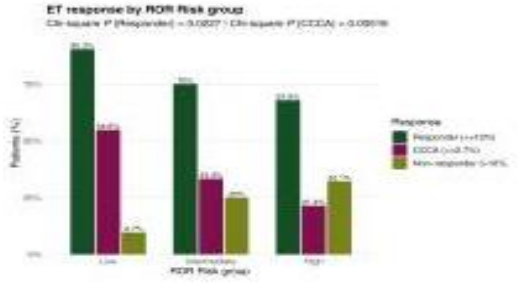

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Figure 2. (a) Ki67 levels at baseline and at the time of surgery, stratified by ROR-score risk groups: high-, intermediate-, and low-risk. (b) Response to endocrine therapy according to baseline ROR-score risk groups.

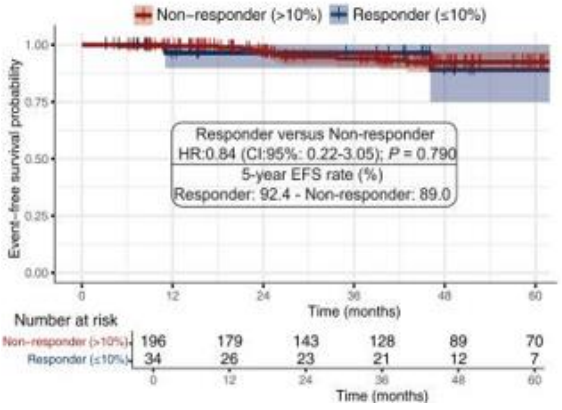
In the subset of patients with baseline proliferation >10 percent and available PAM50 data (n = 108, representing 46.9%), there was a trend for reduced Ki67 response with rising ROR risk categories: 86.0 percent in low-risk (n = 43), 69.2 percent in intermediate-risk (n = 39), and 65.4 percent in high-risk (n = 26), respectively (P = 0.092). By comparison, rates of complete cell cycle arrest (CCCA) showed significant variation across ROR categories: 55.8% in low-risk, 28.2% in intermediate-risk, and 19.2% in high-risk, respectively (P = 0.003).

Multivariable logistic regression analysis revealed postmenopausal status (with 95.6% of these patients receiving aromatase inhibitors) as a robust independent predictor of Ki67 response [odds ratio (OR) 33.39; 95 percent CI 2.78-401.74; P = 0.006], in addition to elevated baseline ER expression (OR 1.12 per 1 percent increase; 95 percent CI 1.02-1.24; P = 0.020) and reduced baseline Ki67 (OR 0.81 per 1 percent increase; 95 percent CI 0.69-0.95; P = 0.008). For CCCA achievement, independent associations persisted with postmenopausal status (OR 9.82; 95 percent CI 2.59-37.22; P < 0.001), baseline ER expression (OR 1.06; 95 percent CI 1.00-1.12; P = 0.048), and elevated ROR-score (OR 0.94; 95% CI 0.90-0.99; P = 0.016). Results from univariate analyses are provided in the Supplementary Material.

Among the 44 patients with PAM50 data available after treatment, 81.8% were classified as Luminal A and 13.6 percent as Luminal B, with corresponding median post-treatment proliferation (Ki67) values of 5 percent and 18 percent, respectively. In the 24 patients with paired baseline and post-treatment subtype information, 50 percent (n = 12) remained Luminal A at both assessments. Of note, 88.9 percent (n = 8) of patients initially Luminal B converted to Luminal A following endocrine therapy. Basal-like subtypes showed no change (n = 2) (available at https://doi.org/10.1016/j.esmoop.2025.105845).

Furthermore, patients attaining CCCA exhibited markedly lower post-treatment ROR scores (median 22 compared to 32; P = 0.045).

With a median follow-up duration of 47 months, 16 patients (6.9 percent) developed recurrence or died. The 5-year event-free survival (EFS) rate for the overall cohort was 92.1 percent (95 percent CI 88.0 percent-96.4 percent. No notable EFS differences emerged based on post-treatment proliferation response (≤10% vs. >10%; 92.4% vs. 89.0 percent, P = 0.790) **(Figure 3a)**, or when considering endocrine response categories using baseline or post-treatment thresholds (log-rank P = 0.893).



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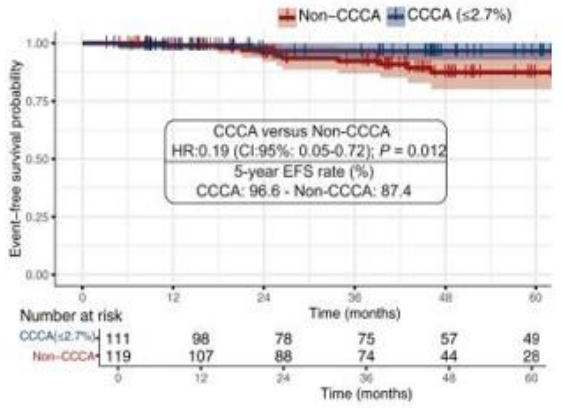

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Figure 3. (a) Event-free survival stratified by post-treatment Ki67 response. (b) Event-free survival stratified by achievement of post-treatment complete cell cycle arrest (CCCA).

In comparison, patients who attained CCCA demonstrated markedly superior 5-year EFS (96.6 percent versus 87.4 percent; P = 0.012), corresponding to an unadjusted hazard ratio (HR) of 0.19 (95 percent CI 0.05-0.72; P = 0.012) **(Figure 3b)**. Neither intrinsic subtype nor baseline ROR-score was predictive of EFS in this clinically low-risk population.

In an exploratory multivariable Cox regression analysis incorporating tumor size, nodal status, baseline proliferation, and receipt of adjuvant chemotherapy, CCCA retained independent prognostic significance

(adjusted HR 0.25; 95 percent CI 0.07-0.92; P = 0.037), alongside tumor size (HR 1.04 per mm; 95 percent CI 1.00-1.09; P = 0.029).

This study reinforces the clinical value of brief preoperative endocrine therapy (ET) as a biologically insightful approach in early ER-positive/HER2-negative breast cancer (BC). A considerable number of tumors exhibited substantial antiproliferative effects, especially in postmenopausal patients—most of whom received aromatase inhibitors (AI)—relative to premenopausal patients treated with tamoxifen (TAM). Furthermore, biological features including elevated baseline ER expression, Luminal A subtype, and low ROR-score category emerged as robust predictors of response, consistent with their association with highly endocrine-dependent disease. The strength of this investigation also stems from its real-world context, mirroring standard clinical practice in cases where chemotherapy benefit was unclear. This method provided a straightforward, inexpensive way to evaluate endocrine sensitivity in vivo prior to surgery. Notably, preoperative ET duration was frequently dictated by surgical scheduling rather than rigid protocols; however, this variability did not affect Ki67 response, underscoring the practicality of incorporating short-course ET into routine care. Consequently, preoperative ET serves as an accessible prognostic and predictive tool across various healthcare systems, particularly appealing in resource-limited settings.

A central observation in this cohort was that CCCA achievement was independently linked to improved EFS, even after adjustment for standard clinicopathological variables. These results are consistent with prior clinical trials indicating that dynamic proliferation changes following short ET are superior predictors of long-term outcomes compared to baseline markers alone [12]. Although a 10% proliferation cutoff (as employed in the ADAPT trial) has been established as an indicator of endocrine sensitivity, it lacked prognostic value here—possibly due to the modest follow-up period, few events, and prevalence of early-stage, low-volume disease [11].

The results also illustrate the interconnected roles of menopausal status, ET agent, and regimen intensity in determining endocrine response. Response rates were notably lower in premenopausal women, all of whom received TAM alone in this series. This uniformity precludes distinguishing whether reduced responses stemmed from menopausal status itself or from the ET type. This differs from observations in the ADAPT cycle trial, where premenopausal patients—including those under 40—achieved comparable responses to postmenopausal women when given AI plus ovarian function suppression [9]. Such evidence emphasizes the need for more intensive endocrine suppression in younger patients and implies that the treatment regimen, rather than menopausal status alone, may primarily drive response. Current trials, such as EMPRESS (NCT05659563) and PREMIERE (NCT05982093), are investigating whether oral selective estrogen receptor degraders (SERDs) can elicit robust responses in premenopausal patients without ovarian suppression.

Beyond fixed biomarkers, these data underscore the promise of dynamic markers—like CCCA and molecular subtype conversions—for optimizing therapeutic decisions. In this series, many Luminal B tumors showed profound proliferation inhibition, with a substantial proportion shifting to Luminal A post short-course ET, mirroring patterns seen with extended neoadjuvant ET [18, 19]. This aligns with the idea of molecular downstaging as an indicator of endocrine sensitivity, as demonstrated in trials like CORALLEEN [20], and now under scrutiny in the RIBOLARIS trial (NCT05296746), which is assessing whether patients reaching ROR-low status after neoadjuvant ribociclib plus ET can omit chemotherapy safely [21]. Interestingly, patients exhibiting Ki67 response in our cohort were significantly less often treated with chemotherapy, suggesting that observed biological responses already guided physicians' decisions.

However, the temporary character of these alterations prompts caution. Earlier reports indicate that proliferation and intrinsic subtypes may rebound upon ET cessation, implying that short-term effects represent reversible inhibition rather than permanent alteration [13, 22, 23]. This affects the interpretation of dynamic markers, particularly proliferation-driven ones like ROR-score. Emerging data also implicate genomic and epigenetic elements—such as TP53 mutations and chromatin modifications—as influencers of enduring endocrine response [24]. Additionally, immunological changes during ET are gaining recognition as contributors to response variability and rebound potential [20].

This study carries certain limitations. Its retrospective nature and limited follow-up hinder full evaluation of long-term survival effects in hormone receptor-positive BC. The cohort was largely low-risk with few events, reducing the strength of prognostic inferences. Adjuvant

chemotherapy administration lacked standardization, potentially confounding survival outcomes. Paired pre- and post-treatment molecular data were available only for a subgroup, restricting analysis of transcriptional shifts; correlations with commercial assays like Oncotype DX or MammaPrint were not possible due to absent registry data. Preoperative Endocrine Prognostic Index scores could not be reliably computed given the variable short ET durations. Compliance and tolerability were not formally recorded, though deemed non-issue given the brief exposure. Lastly, differing ET regimens by menopausal status prevent isolating whether response disparities arose from biology or treatment type.

Overall, these results contribute to accumulating evidence favoring dynamic biomarkers in clinical decision-making. CCCA stands out as a strong indicator of endocrine sensitivity, linked to favorable tumor biology and improved long-term outcomes.

Conclusion

Brief preoperative ET provides a feasible, real-time means to evaluate endocrine sensitivity in early ER-positive/HER2-negative BC. Although proliferation suppression was frequent, CCCA appears the most dependable prognostic marker. Molecular downstaging and subtype conversions further indicate clinically relevant responses. Given its ease, affordability, and low resource demands, this approach is suitable for broad adoption across varied healthcare environments, including those without access to advanced genomic testing.

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