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ORIGINAL ARTICLE

Tumor Ki67 after a 2-week aromatase inhibitor run-in in patients with ER+ early breast cancer proposed for up-front surgery

Valor do Ki67 no tumor após 2 semanas de inibidor da aromatase em doentes com cancro da mama precoce RH+ propostas para cirurgia direta

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Abstract

Background: *Endocrine therapy (ET) is effective for endocrine receptor-positive (ER+) early breast cancer (EBC). Ki67 is used as a biomarker of response and prognosis. The POETIC trial showed that short pre-operative ET reduces Ki67, and low levels at 2 weeks predict lower recurrence risk.* **Objective:** *This study aims to determine the proportion of patients with Ki67 < 10% in surgical specimens after short-term ET and validate these results in the Portuguese population.* **Methods:** *This was two-center, prospective, and single-arm study. Postmenopausal women with ER+, resectable EBC ≥ 1 cm received aromatase inhibitors (POAI) for ≥ 14 days before surgery. Ki67 was assessed at baseline (Ki67B) and post-surgery (Ki67surg).* **Results***: Twenty-three patients were included, median age 67 (50-84), most ECOG PS 0 (82.6%). All had node-negative (cN0) cT1 - 2 tumors. Median POAI duration: 15 days (14-28). Breast-conserving surgery was performed in 73.9%, sentinel node biopsy in 82.6%, and axillary dissection in 17.4%. Pathologically, 60.9% were pN0 and 39.1% pN1 - 3. Eight patients (34.8%) received adjuvant chemotherapy. At diagnosis, 95.7% had Ki67 ≥ 10%, with median Ki67B of 25% (2-70). After POAI, Ki67surg < 10% was found in 44%. Ki67 dropped in 91.3% of patients, with a median difference of 13%.* **Conclusion:** *Short-course pre-operative ET induced dynamic reductions in Ki67. Ki67 variation may offer a simple, low-cost method to assess treatment response in ER+ EBC.*

Keywords: *Early breast cancer. ER-positive. Pre-operative endocrine therapy. Ki67.*

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Resumo

Introdução: *A hormonoterapia (HT) é eficaz no tratamento do cancro da mama precoce (CM) com recetores hormonais positivos (RH+). O Ki67 é usado como marcador de resposta e prognóstico. O estudo POETIC demonstrou que a HT pré-operatória reduz o Ki67 e que valores baixos após 2 semanas se associam a menor risco de recidiva.* **Objetivo:** *Este estudo visa determinar a proporção de doentes com Ki67 < 10% na peça cirúrgica após HT de curta duração e validar os resultados na população portuguesa.* **Métodos:** *Estudo prospetivo, multicêntrico, de braço único. Mulheres pós-menopáusicas com CM RH+, ressecável, ≥ 1 cm, receberam inibidores da aromatase (HTPO) por ≥ 14 dias antes da cirurgia. O Ki67 foi avaliado na biópsia inicial (Ki67B) e na peça cirúrgica (Ki67Cir).* **Resultados:** *Foram incluídas 23 doentes, mediana de idade 67 anos (50-84), maioria com ECOG 0 (82,6%). Tumores cT1 - 2, todos cN0. Duração mediana da HTPO: 15 dias (14-28). Cirurgia conservadora em 73,9%, biópsia do gânglio sentinela em 82,6%, linfadenectomia em 17,4%. Patologicamente, 60,9% eram pN0 e 39,1% pN1 - 3. Oito doentes (34,8%) fizeram quimioterapia adjuvante. No diagnóstico, 95,7% tinham Ki67 ≥ 10%, mediana de 25% (2-70). Após HTPO, 44% tinham Ki67Cir < 10%. Observou-se queda do Ki67 em 91,3% dos casos, com diferença mediana de 13%.* **Conclusão:** *A HT pré-operatória de curta duração induziu reduções dinâmicas do Ki67. A variação do Ki67 pode ser uma ferramenta simples e de baixo custo para estimar a resposta terapêutica no CM RH+ precoce.*

Palavras-chave: *Câncer de mama precoce. ER-positivo. Terapia endócrina pré-operatória. Ki67.*

Introduction

Breast cancer (BC) is the most frequently diagnosed cancer and the first cause of cancer-related death in women[1]. Most are diagnosed at an early stage, with about 5-15% of patients presenting with metastatic disease up-front[2]. BC is a heterogeneous group of tumors with different histological, prognostic, and clinical aspects[3]. Endocrine-receptor positive (ER+) tumors represent about 66% of BC and are associated with better prognosis[4]. Adjuvant treatment with endocrine therapy (ET) is the cornerstone treatment of early-stage ER+ BC. A subset of high-risk patients will benefit from adjuvant chemotherapy, as determined by clinical, molecular, and genomic assays[5]. However, in clinical practice, baseline clinicopathologic features can not precisely distinguish high-risk patients who could benefit from more intensive treatments, highlighting the need for novel, easily accessible, and dynamic biomarkers[6]. Effort has been made to investigate endocrine sensitivity biomarkers in ER+ BC, and Ki67 is one potential marker, with a well-defined predictive role in a neoadjuvant setting[7]. Among the first prospective trials, IMPACT and the P024 trials were the first studies to demonstrate that pre-operative Ki67 and its change after 2-4 weeks of pre-operative ET were associated with improved long-term outcomes. These trials endorsed that Ki67 was a promising biomarker to be further investigated[8,9]. Furthermore, these trials demonstrated that Ki67 after ET was associated with higher prognostic accuracy than the baseline Ki67 expression in this population. The establishment of Ki67 as an intermediate marker of treatment sensitivity and long-term outcome with ET provided the opportunity for new trial designs with Ki67 as the primary endpoint[10]. The PeriOperative Endocrine Therapy for Individualizing Care (POETIC) trial randomized 4000 ER+ operable BC patients to 2 weeks of pre-operative treatment with a nonsteroidal aromatase inhibitor (POAI) or no presurgical treatment[11]. By analyzing paired Ki67 values (pre-operative and perioperative Ki67), this trial provided a unique opportunity for a detailed study of the determinants of response and resistance to ET as well as for testing the role of pre-operative therapy for improved biomarker-based estimates of prognosis. Patients with low paired Ki67 had a lower risk of recurrent disease but patients with Ki67 > 10% in the surgical specimen had the worst prognosis. Although this study did not show a difference in oncological outcomes with short course ET, the authors concluded that the kinetics of Ki67 after short course POAI may identify prognostic subgroups, and provide an early indication of endocrine sensitivity or resistance and thus assist in guiding adjuvant treatment decisions[11]. Dowsett et al. also showed that a low-priced biomarker assay using Ki67 after short-term pre-operative ET had improved predictive power for BC prognosis[12]. Ki67 levels after pre-operative treatment were significantly lower than the Ki67 baseline levels[13,14]. However, if Ki67 after short-term ET can be predictive of prognosis as precisely as genomic signatures can it still unknown[14].

The short-term presurgical ET strategy is a window-of-opportunity scenario, to gain information on tumor response early in the course of treatment[15,16]. Future studies correlating clinical response to

neoadjuvant therapy with changes in tumor biomarkers and gene expression may ultimately prove useful to tailor therapy for individual patients and to gain a better understanding of the biology of ER+ BC[17].

The present study aimed to determine the proportion of patients with Ki67 < 10% in the surgical specimen after short-term presurgical ET and to validate these results in the Portuguese population.

Materials and methods

Study design

This prospective study was conducted in two Portuguese institutions, Hospital Beatriz Ângelo in Loures and Hospital da Luz in Lisboa. After discussion in the multidisciplinary tumor board, post-menopausal women with ER+ early breast cancer (EBC) proposed for up-front surgery were eligible and included in the study, after providing written informed consent. A minimum breast tumor size of 10 mm was required to minimize the unlikely possibility of compromising intraoperative identification with tumor downsizing after the 2 weeks of POAI.

The primary endpoint was the proportion of patients that had Ki67 value < 10% after a short course of ET (14 days) with aromatase inhibitor (AI) in the pre-operative setting. The Ki67 cutoff value of 10% was considered according to the methodology of the International Ki67 in BC Working Group[12], and to ensure consistency with other trials[11,13].

To simplify the exploratory analysis, and based on the POETIC trial design, Ki67 scores ($Ki67_B$ – baseline Ki67 in the diagnostic biopsy and $Ki67_{surg}$ – Ki67 in the surgical specimen collected 2 weeks after POAI) were dichotomized, and patients divided into four groups as follows: low-low ($Ki67_B$ and $Ki67_{surg}$ < 10%); high-low ($Ki67_B$ ≥ 10% and $Ki67_{surg}$ < 10%); high-high ($Ki67_B$ and $Ki67_{surg}$ > 10%); and low-high ($Ki67_B$ < 10% and $Ki67_{surg}$ ≥ 10%)[11].

Other secondary endpoints were as follows: 1) the proportion of patients who had a Ki67 value reduction between $Ki67_B$ and Ki67surg, regardless of the range; 2) the proportion of patients who had a Ki67 value increase between $Ki67_B$ and Ki67surg, regardless of the range; 3) the comparison between the median values of $Ki67_B$ and $Ki67_{surg}$; and 4) relapse-free survival (RFS) according to the values of $Ki67_B$ and $Ki67_{surg.}$

RFS was defined as the time from the beginning of the short course pre-operative ET until local, regional, or distant tumor recurrence or death from any cause.

Patients

Patients with ER+ EBC who were proposed for up-front surgery were screened between November 2022 and November 2023.

Inclusion criteria were as follows: 1) age ≥ 18 years; 2) post-menopausal woman (≥ 50 years amenorrhoeic for more than 12 months, with bilateral oophorectomy, or who had been on hormone replacement therapy within the previous 12 months, and with follicle-stimulating hormone concentrations in the post-menopausal range if < 55 years age)[11]; 3) histologically documented estrogen receptor-positive or progesterone receptor-positive (≥ 1% of positive cells, by immunohistochemistry [IHC] stain), HER2-positive or HER2-negative (cases with 0 or 1 positive IHC staining for HER2 or with a HER2 gene copy number < 2.0 by fluorescent *in situ* hybridization [FISH] analysis were considered HER2 negative)[18], operable primary BC (ultrasound required size of at least 10 mm); 4) no evidence of metastatic disease according to local guidelines; 5) Eastern Cooperative Oncology Group Performance Status (ECOG PS) of 0-2; and 6) ability to understand and comply with ET.

Men, women who were pregnant or breastfeeding, who were non-candidate for up-front surgery, who had bilateral disease, and/or who were treated with ET or chemotherapy in the last 12 months were excluded from the study. Concurrent use of hormone replacement therapy or any other estrogen-containing medication (within 4 weeks of inclusion), treatment with investigational drug within 4 weeks of inclusion[11], or history of invasive malignancy diagnosed within the previous 5 years were other exclusion criteria.

Procedures

All enrolled patients started POAI treatment. POAI consisted of a non-steroidal AI in standard dosage (oral letrozole 2.5 mg/day or oral anastrozole 1 mg/day); the choice of agent was according to local clinical practice preference. Before enrollment, all patients had surgery scheduled for around 2 weeks (minimum 14 days). POAI should start directly after enrollment allowing the duration of treatment before surgery to be as close as possible to 14 days. If surgery was delayed, POAI duration could be extended for a maximum of 28 days. Treatment was discontinued on the day of the surgery or when completing 28 days (in case of surgery delay). All adjuvant therapy, laboratory investigations, and disease staging were performed according to the standard-of-care local practice (ESMO guidelines[19]) and

after tumor board discussion. All patients had pretreatment mammography and breast ultrasound according to local practice. Nuclear magnetic imaging was optional. There was no specific safety endpoint. No data on adverse event were collected as the safety profiles of the chosen AI are well established. Participants were able to withdraw from the study at any time for any reason.

Formalin-fixed paraffin-embedded tissue samples were required before enrollment (baseline biopsy) and at surgery (surgical specimen)[11]. Baseline samples could be a core-cut diagnostic biopsy or sections from the diagnostic sample. At surgery, samples could be either core biopsies or sections cut from the routine excision.

Tissue samples were processed, stored, and analyzed for Ki67 staining in each center. Ki67 was analyzed with IHC and was estimated as the percentage of cancer cells staining positive. Scoring was according to methodology described by the International Ki67 in BC Working Group[12].

Data collection

Data were collected by the authors from the hospital's electronic medical records and during a patient's enrollment visit.

Collected demographic and clinical variables were as follows: age at diagnosis; ECOG PS at diagnosis (0,1); date of diagnosis of BC; clinical TNM stage (according to the *American Joint Committee on Cancer* [AJCC] 8th edition); date of start of POAI; date of surgery; date of relapse; site of relapse; date of death; and last follow-up.

Collected pathologic variables were as follows: histological subtype; *World Health Organization* (WHO) histological grade at baseline; estrogen receptor expression and progesterone receptor expression (% - assessed in local pathology laboratories) at diagnosis; Ki67 expression at baseline (%); Ki67 expression in the surgical specimen (after 2-weeks of POAI); and pathological TNM stage (according to the AJCC 8th edition).

Collected treatment variables were as follows: duration of POAI (days), adjuvant chemotherapy and treatment regimen, Oncotype DX® and corresponding recurrence score (RS), adjuvant radiotherapy, and adjuvant treatment with bisphosphonates.

Ethical considerations

This study was approved by the Institutional Review Board/Independent Ethics Committee (IRB/IEC) of both centers and designed according to Good Clinical Practice guidelines and the Declaration of Helsinki. Patients provided written informed consent before enrolment. Clinical data were treated with pseudonymization and kept accessible only to the primary investigators.

Statistical analysis

This is a single-arm and prospective cohort study. The primary endpoint was the proportion of tumors with Ki67 < 10% after a short course of pre-operative AI ET, which we estimated to be around 80%[11]. The inclusion of 100 patients would allow a precision (95% confidence interval) on this estimate of ±8%, according to the formula: $p \pm z\sqrt{\frac{p(1-p)}{n}}$. Based on that estimation, we plan to include 100 patients.

The statistical analysis included descriptive statistics (absolute and relative frequency, median and ranges (OR) mean and standard deviation), and inferential statistics.

Baseline demographic data, tumor characteristics, adjuvant treatment, and Ki67 measurements are presented as descriptive statistics.

To compare the $Ki67_B$ with $Ki67_{surg}$, a non-parametric Wilcoxon Signed-Rank Test was used. An alpha level of 0.05 was used for this analysis.

This manuscript is the preplanned interim analysis after 1 year of inclusion of patients to evaluate the feasibility of the study. RFS, a secondary endpoint will be presented, after complete patient accrual and a MED follow-up of 5 years.

Analyses were performed using SPSS Statistics® for Windows®, Version 29.0.

Results

Patients

From November 2022 to November 2023, a total of 23 patients fulfilled the study eligibility criteria and were included.

Patients median (MED) age was 67 years (range 50-84), and most of them had a PS ECOG 0 (n = 19, 82.6%) (Table 1). All participants had histologically confirmed ER+ HER2 negative BC, the majority no special type (NST) (n = 17, 73.9%) and grade 2 (n = 19, 82.6%). In the clinical staging, 11 tumors had ≤ 2 cm (47.8%) and another 11 > 2-5 cm (47.8%), all clinically node-negative and without evidence of metastatic disease, (cN0M0).

Table 1. Demographics and tumor characteristics at diagnosis

Variable	Overall population (n = 23)
Age-median (range)	67 (50-84)
ECOG PS, n (%)	
0	19 (82.6)
1	4 (17.4)
Hormone receptor status, n (%)	
Estrogen receptor positive	23 (100)
Progesterone receptor positive	20 (86.9)
Histological subtype, n (%)	
Invasive carcinoma of no special type	17 (73.9)
Invasive lobular carcinoma	4 (17.4)
Mucinous carcinoma	1 (4.4)
Invasive micropapillary carcinoma	1 (4.4)
WHO histological grade, n (%)	
G1	2 (8.8)
G2	19 (82.4)
G3	2 (8.8)
Tumor size, cm, n (%)	
≤ 2	11 (47.8)
> 2-5	11 (47.8)
> 5	1 (4.4)

Table 2. Surgery details and tumor characteristics at surgery

Variable	Overall population (n = 23)
Definitive breast surgery, n (%)	
Mastectomy	6 (26.1)
Breast conserving surgery	17 (73.9)
Axillary surgery, n (%)	
Sentinel lymph node biopsy	19 (82.6)
Axillary lymph node dissection	4 (17.4)
Tumor size, cm, n (%)	
≤ 2	12 (52.2)
> 2-5	11 (47.8)
> 5	-
Nodal status	
N0	14 (60.9)
N1-3	9 (39.1)
Multifocal disease, n (%)	
Yes	5 (21.7)
No	18 (78.3)
Oncotype DX®, n (%)	
Yes	9 (39.1)
No	14 (60.9)
Oncotype DX® recurrence score	
0-25	5 (21.7)
26-100	4 (17.4)

Short course POAI

The MED POAI duration was 15 days (range 14-28). Seventeen patients (73.9%) had breast-conserving surgery, with sentinel lymph node biopsy in 19 (82.6%), and lymph node dissection in 4 patients (17.4%) (Table 2). Regarding pathological nodal status, 14 patients (60.9%) had node-negative disease (pN0), and 9 (39.1%) had node-positive disease. Five patients had multifocal disease. Oncotype DX® was requested in 9 patients (39.1%), 4 with an RS between 26 and 100.

Concerning adjuvant treatments, 8 patients (34.8%) received adjuvant chemotherapy, five received dose-dense doxorubicin and cyclophosphamide (ddAC) for 4 cycles, followed by weekly paclitaxel for 12 cycles; three received docetaxel and cyclophosphamide for 6 cycles; 18 (78%) received adjuvant radiotherapy and 17 (74%) adjuvant bisphosphonates (Table 3). All patients initiated adjuvant AI.

Table 3. Adjuvant treatment

Variable	Overall population (n = 23)
Adjuvant chemotherapy, n (%)	
Yes	8 (34.8)
No	15 (65.2)
Adjuvant radiotherapy, n (%)	
Yes	18 (78.3)
No	5 (21.7)
Adjuvant bisphosphonates, n (%)	
Yes	17 (73.9)
No	6 (26.1)

K67 response variation

Most patients (n = 22; 95.7%) had a Ki67 ≥ 10% on the initial diagnostic biopsy, with a MED $Ki67_B$ of 25% (2-70%). After POAI, tumor expression of Ki67 values reduced in 21 (91%) patients with no change, in the remaining 2 (9%) patients. There were no cases of increase in the $Ki67_{surg}$ value.

A Wilcoxon signed-rank test showed that POAI treatment elicited a statistically significant change between $Ki67_B$ and $Ki67_{surg}$ values, ($Z = -4.030$, $p < 0.001$).

The MED $Ki67_{surg}$ was 10% (1-40%) and the MED Ki67 variation was 13.0% (0-40%). Ten of the 23 (43.5%) tumors had $Ki67_{surg} < 10\%$ after a short course of POAI.

In an exploratory analysis, where Ki67 scores were dichotomized according to the POETIC trial criteria (8),

Table 4. Ki67 variation on POAI

Ki67 variation		n (%)	MED $Ki67_B$ (%)	MED $Ki67_{surg}$ (%)	MED Δ Ki67 (%)
$Ki67_B \geq 10\%$ (High) n = 22 patients	$Ki67_{surg} \geq 10\%$ (High)	13 (56.5%)	30.0% (10;70)	20.0% (10;40)	13.0% (0;40)
	$Ki67_{surg} < 10\%$ (Low)	9 (39.1%)	20.0% (10;25)	5.0% (2;5)	14.0% (5;20)
$Ki67_B < 10\%$ (Low) n = 1 patient	$Ki67_{surg} \geq 10\%$ (High)	0	-	-	-
	$Ki67_{surg} < 10\%$ (Low)	1 (4.3%)	2.0%	1.0%	1.0%
Total		23 (100%)	25.0%	10.0%	13.0%

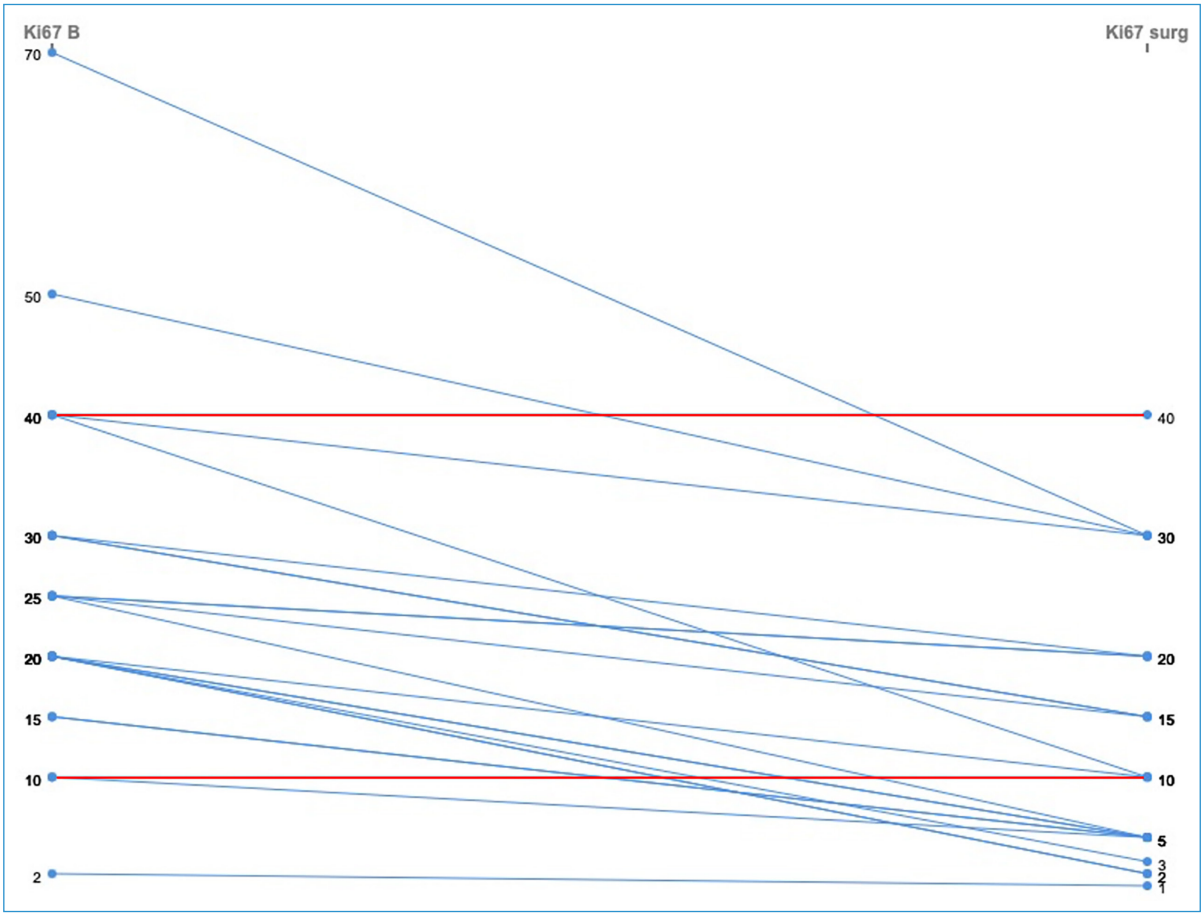

Figure 1. Tumor Ki67 variation dynamics on POAI.

most patients (n = 13, 56.5%) were classified in the high-high category ($Ki67_B$ and $Ki67_{surg} > 10\%$), and 9 in the high-low category ($Ki67_B \geq 10\%$ and $Ki67_{surg} < 10\%$) (Table 4 and Fig. 1). Only one patient fit in the low-low category ($Ki67_B$ and $Ki67_{surg} < 10\%$).

RFS

At the time of the database lock (November 30, 2023), the MED follow-up was only 6 months and no patient experienced disease recurrence or death.

Discussion

The identification of biomarkers with prognostic and/or predictive capacity is a major objective of clinical cancer care. Such biomarkers have the potential to increase treatment in patients who need more treatment and reduce it in those who need less. Published data support the role of tumor Ki67 response to a 14 day-course of POAI as a good prognosis factor. In this study, we evaluated the proportion of patients with Ki67 < 10% after 14 days of POAI, in patients with ER+ EBC eligible for up-front surgery.

After a median of 16 days of POAI, Ki67 expression decreased significantly, with 91% of patients having a reduction of Ki67 value. In the analyzed population, 10 of 23 patients had Ki67 < 10% on the surgical specimen. This proportion was lower than in the POETIC trial (76%), even though all patients took letrozole up to the day of surgery, even if it meant taking the AI for more than the predicted 14 days from patient inclusion (median days on letrozole 16, range 14-28). Although the clinical characteristics of the population were similar to that of the POAI arm of the POETIC trial, the pathologic features of the diagnostic biopsy suggest that the population included in this cohort might have biologically more aggressive disease, with MED $Ki67_B$ of 25.0% (vs. 15.2% on the POAI arm of the POETIC trial)[11]. Supporting this interpretation, is the fact that 8 patients (35%) were treated with adjuvant chemotherapy, four of them with an Oncotype DX® RS > 26.

While a therapeutic benefit of 14 days of POAI was unexpected, it is reassuring that there were no reported compliance issues, confirming that this is an easily accessible and safe treatment and prognostication approach.

The documentation that Ki67 response to POAI has prognostic value, supports the design of a clinical trial using Ki67 response as a decision tool for treatment tailoring.

A mean overall Ki67 reduction of 13% was observed, similar to that reported by POETIC trial investigators (12% in the POAI arm). The comparison between MED $Ki67_B$ and MED $Ki67_{surg}$ was statistically significant ($p < 0.001$), validating that 2 weeks of POAI may be enough to induce dynamic changes in tumor Ki67, as previously reported[11-13].

Some limitations must be considered in the interpretation of the reported results. First, this is an interim analysis, with a small sample size and short follow-up. We must wait for the final results of patient accrual and survival data to analyze more robust data. Another potential limitation could be the reported variability in interobserver evaluation of Ki67 expression. Furthermore, the tumor samples collected by core needle biopsy at diagnosis and during surgery could vary due to tumor heterogeneity[10]. Nevertheless, our findings are consistent with Ki67 expression changes after short-term ET reported in other studies[11-13].

Recent studies focused on the correlation between Ki67 and prognostic genomic assays with mixed results. Patel et al. failed to document such a correlation, especially in the low-intermediate Ki67 range[14]. Concerning the POAI, a small Japanese study that aimed to test if Ki67 after 2 weeks of ET predicted a similar number of patients with favorable prognosis as genomic markers showed that $Ki67_{surg}$ may have distinct predictive power compared to $Ki67_B$, suggesting that it might be used as a reliable substitute for genomic testing[20].

Conclusion

POAI is an area of research interest. In our study, short-term pre-operative ET induced meaningful dynamic changes in the Ki67 expression. This is an interim analysis with immature data. We will continue to enroll patients up to the planned sample size and follow-up to be able to evaluate the impact of Ki67 variation with POAI as a predictor of RFS. This study endorsed that POAI is a practical and inexpensive intervention with potential to modify clinical practice due to its prognostic value.

Authors' contributions

Conception or design of study: D.N. da Silva, J.A. Albuquerque, P.S. Simões, J.G. Godinho, A.I. Belo, A.C. Catarino, J.L. Passos Coelho, and J.A. Teixeira.

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Conflicts of interest

J.G. Godinho: Pfizer, Janssen, Grunenthal, Novartis (H), Leo Pharma (H; RF); M.C. Casa-Nova: Novartis, Daiichi-Sankyo, Roche, AZ (RF); Roche, Pfizer, Novartis, GSK, PharmaMar, Merk (C/A; SAB); MSD, AZ, GSK, Daiichi Sankyo, Novartis (H); J.L. Passos-Coelho: (C/A) for Novartis and other pharmaceutical companies; The other authors indicated no potential conflicts of interest. (C/A) Consulting/advisory relationship; (RF) Research funding; (E) Employment; (ET) Expert testimony; (H) Honoraria received; (OI) Ownership interests; (IP) Intellectual property rights/inventor/patent holder; (SAB) Scientific advisory board.

Ethical disclosures

This study was approved by the Institutional Review Board/Independent Ethics Committee (IRB/IEC) of both centers and designed according to Good Clinical Practice guidelines and the Declaration of Helsinki. Patients provided written informed consent before enrolment. Clinical data were treated with pseudonymization and kept accessible only to the primary investigators.

The protocol of this study was presented in "1° Congresso de Investigação Luz Saúde" – May, 2022 in Lisbon, Portugal.

The results of this study were presented in "21º Congresso Nacional de Oncologia" – November, 2024 in Coimbra, Portugal.

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