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Palbociclib and letrozole for hormone receptor-positive HER2-negative breast cancer with residual disease after neoadjuvant chemotherapy

Sonia Pernas[1,2] ✉, Esther Sanfeliu[1,3,4], Guillermo Villacampa[1,5], Javier Salvador[6], Antonia Perelló[7], Xavier González[1,8,9,10], Begoña Jiménez[11], María Merino[12], Patricia Palacios[13], Tomás Pascual[1], Emilio Alba[14], Lorea Villanueva[1], Samyukta Chillara[1], Juan Manuel Ferrero-Cafiero[1], Patricia Galvan[3], Aleix Prat[1,3,15,16] & Eva Ciruelos[1,17] ✉

With the incorporation of cyclin-dependent kinase inhibitors in early breast cancer (BC), a better identification of biomarkers is needed. The PROMETEO II trial aimed to evaluate the antitumor activity of palbociclib plus letrozole and to identify response biomarkers in patients with operable HR+/HER2- BC and residual disease after neoadjuvant chemotherapy (NAC). The primary endpoint was the rate of complete cell cycle arrest (CCCA), centrally determined by Ki67 ≤ 2.7% at surgery. A comprehensive translational analysis was conducted. At surgery, the CCCA rate was 59.1%, with a 44.2% decrease in Ki67 from the end of NAC. Changes in intrinsic subtypes occurred in 48% of patients, with proliferation genes suppressed, and immune genes more upregulated in tumors with CCCA. Overall, 14% of tumors were classified as PD-L1$^+$ after palbociclib. Nine patients experienced grade 3 adverse events (AEs). Palbociclib showed an anti-proliferative effect, with increased immune infiltration in residual tumors with CCCA.

Trial registration: Palbociclib Plus Letrozole in Hormone Receptor Positive Residual Disease After Neoadjuvant Chemotherapy (PROMETEO II) ClinicalTrial.gov number NCT04130152. Study registration; October 17, 2019.

Hormone receptor-positive (HR+) human epidermal growth factor receptor 2-negative (HER2-) breast cancer (BC) subtype accounts for approximately three-quarters of all initial BC diagnoses[1]. Cyclin-dependent kinase 4/6 inhibitors (CDK4/6i) have demonstrated significant efficacy in HR+/HER2- BC. The effectiveness of CDK4/6i is supported by their capacity to disrupt cancer cell proliferation. These inhibitors function by inhibiting the CDK4 and CDK6 enzymes, which play a critical role in cell cycle progression hindering the uncontrolled growth of cancer cells[2,3].

In the advanced disease, combining CDK4/6i (such as palbociclib, ribociclib, abemaciclib and dalpiciclib) along endocrine therapy (ET) has demonstrated an improvement in progression-free survival (PFS) with manageable safety profiles[4–10]. This has facilitated the approval of these drugs and established them as the standard of care in first-line treatment for

[1]SOLTI Cancer Research Group, Barcelona, Spain. [2]Institut Catala d'Oncologia-IDIBELL; L'Hospitalet, Barcelona, Spain. [3]August Pi i Sunyer Biomedical Research Institute (IDIBAPS), Barcelona, Spain. [4]Pathology Department Hospital Clinic de Barcelona, Barcelona, Spain. [5]Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain. [6]Hospital Universitario Virgen del Rocio, Sevilla, Spain. [7]Hospital Son Espases, Palma, Illes Balears, Spain. [8]Institut Oncològic Dr. Rosell, Barcelona, Spain. [9]Hospital General de Catalunya, Sant Cugat del Vallès, Spain. [10]Universitat Internacional de Catalunya, Sant Cugat del Vallès, Spain. [11]Hospitales Regional y Universitario Virgen de la Victoria, Málaga, Spain. [12]Hospital Universitario Infanta Sofía, Madrid, Spain. [13]Hospital Clínico Universitario de Santiago, Santiago de Compostela, Spain. [14]Hospital Clínico Universitario Virgen de la Victoria, Málaga. Instituto de Investigación Biomédica de Málaga, IBIMA, Málaga, Centro de Investigación Biomédica en Red de Oncología, CIBERONC-ISCIII, Madrid, Spain. [15]Hospital Clínic de Barcelona, Barcelona, Spain. [16]Medicine Department, University of Barcelona, Barcelona, Spain. [17]Hospital 12 de Octubre, Madrid, Spain.
✉e-mail: spernas@iconcologia.net; eva.ciruelos@gmail.com

metastatic HR+/HER2- BC. However, in the early setting heterogeneous results have elicited discussion in the oncology community. While the PALLAS and PENELOPE-B trials did not demonstrate an improvement in event-free survival with the addition of adjuvant palbociclib to ET[11,12], the MonarchE and NATALEE trials successfully met their primary endpoints by demonstrating the efficacy of adding abemaciclib and ribociclib, respectively[13,14].

With the incorporation of CDK4/6i into the therapeutical armamentarium for early-stage disease, there is an urgent need to better identify patients who are most likely to benefit from these treatments and to explore whether the distinct features of each CDK4/6 inhibitor may impact their effectiveness across different treatment settings. Although abemaciclib is approved specifically for high-risk, node-positive patients, the NATALEE trial adopted broader inclusion criteria compared to MonarchE trial. However, the widespread introduction of CDK4/6i presents a challenge in terms of cost-effectiveness and may hinder approval and drug reimbursement in some countries. Consequently, a more in-depth understanding of the tumor biology of HR+/HER2- early BC treated with CDK4/6i is necessary for transitioning from a "one-size-fits-all" to a more personalized treatment approach. To date, exploratory biomarker analyses from the PALLAS and MonarchE trials have not contributed to identifying which patients benefit the most from these adjuvant therapies[11,15]. For that purpose, window-of-opportunity trials provide a favorable setting for testing the effectiveness of drugs within a brief timeframe. Additionally, these trials offer an opportunity to delve into the tumor biology of treatment-naïve patients, facilitating the identification of potential biomarkers.

The SOLTI-1710 PROMETEO II trial (NCT04130152) is a window-of-opportunity trial designed to assess the antiproliferative activity of palbociclib when added to letrozole in pre and postmenopausal women with operable primary HR+/HER2- early BC and presence of residual disease following neoadjuvant chemotherapy (NAC). Furthermore, a translational analysis to identify predictive biomarkers and gain comprehensive biological insights was also conducted.

Results

Study population

Between November 2019 and December 2021, 71 patients were screened. Forty-nine patients failed the screening, primarily due to achieving a complete response or not meeting the required Ki67 index for inclusion following NAC. Twenty-two patients were included in the study and all patients received the study treatment and had a post-treatment biopsy (Supplementary Fig. 1). Patients baseline characteristics are summarized in Table 1. Briefly, the median age at inclusion was 56 years, 45% were premenopausal, 50% had ECOG 0, and most patients had clinical T2 tumors (59%), 73% with lymph node involvement. Prior to the initiation of the anthracycline/taxane-based neoadjuvant treatment, the median Ki67 at baseline was 10%, as per local assessment, and 16%, as determined by centralized assessment.

Complete cell cycle arrest and Ki67 variation from baseline

The rate of complete cell cycle arrest (CCCA) at the time of surgery was 59.1% (90% CI 39.5–76.7), while at baseline and after NAC, it was 4.5% (CI 90% 0.2–19.8) and 27.3% (90% CI 12.6–46.8), respectively (Fig. 1). When we evaluated Ki67 index as a continuous variable, patients with high Ki67 index at baseline were associated with less odds to achieve CCCA at surgery (OR per 10-units increment = 0.48, 90%CI 0.20– 0.94). Between baseline and post-NAC evaluation, Ki67 decreased by a geometric mean change of −79.1% (90%CI, −71.5% to −84.7%). Between post-NAC and surgery, Ki67 expression decreased by a geometric mean change of -44.2% (90%CI, −20.2% to −61.0%). Ki67 expression was reduced in all samples between the baseline and post-NAC timepoints, and in most of the patients between the post-NAC and surgery (73.7%, 14/19) (Supplementary Fig. 2).

Table 1 | Patient and tumor characteristics

		Study population ($n = 22$)
Age, years (median and range)		56 (41–69)
Gender, *n* (%)	Female	22 (100.0%)
	Male	0 (0%)
Race, *n* (%)	Caucasian	19 (86.4%)
	Others	3 (13.6%)
Menopausal status, *n* (%)	Postmenopausal	12 (54.5%)
	Premenopausal	10 (45.5%)
ECOG status, *n* (%)	0	11 (50.0%)
	1	11 (50.0%)
Histological grade, *n* (%)	G1	1 (4.5%)
	G2	8 (36.4%)
	G3	5 (22.7%)
	ND	8 (36.4%)
Tumor size, *n* (%)	T1	3 (13.6%)
	T2	13 (59.1%)
	T3- T4	6 (27.3%)
Lymph node status, *n* (%)	N0	6 (27.3%)
	N1	10 (45.5%)
	N2-N3	6 (27.3%)
Clinical tumor stage, *n* (%)	I	1 (4.5%)
	II	11 (50.0%)
	III	10 (45.5%)
Residual disease maximal diameter, mm (median and range)		19 (9–77)
Ki67 expression by local assessment, (median and range)		10% (5–40%)
Ki67 by central assessment, (median and range)		16% (2–60%)

RCB as per local assessment and pCR rate

After treatment with palbociclib and letrozole, conservative surgery was performed in 68.2% of patients and mastectomy in 31.2%. At surgery, the median tumor size was 21 mm (range 2–70). One patient achieved a residual cancer burden (RCB) I (4.5%, CI 90% 0.2–19. 8) and no patients achieved RCB 0 (Supplementary Tables 1 and 2).

Biomarker analysis

All biomarkers were evaluated at the three study timepoints: i) baseline samples before receiving NAC, ii) after NAC but before starting treatment with palbociclib (post-NAC) and iii) at surgery. Signature immune-based markers, including the Proliferation score, risk of recurrence (ROR) score based on subtype (ROR-S), and proliferation (ROR-P) score, demonstrated a noteworthy decrease in surgery samples in contrast to baseline (Fig. 2). Regarding IGG signature, despite a marked increase within tumor samples collected during surgery (IgG high group 59%) compared to baseline (9%), no significant differences based on CCCA status were observed. However, at surgery, significant higher levels in proliferation score, ROR-S, and ROR-P-based signatures ($p < 0.05$) were observed in non-CCCA samples as opposed to CCCA tumor samples (Fig. 2).

Gene expression analysis was performed to compare the changes i) between post-NAC and baseline, and ii) between surgery and post-NAC evaluations. Between post-NAC and baseline, one gene/module (0.5%) increased and 38 (20.2%) decreased expression. Most downregulated genes were related to cell cycle, including *AURKA*, CCNB1/2 and CC20. In the comparison between post-palbociclib surgical specimens and post-NAC specimens, 35 (18.6%) genes increased expression and 31 (16.5%) decreased

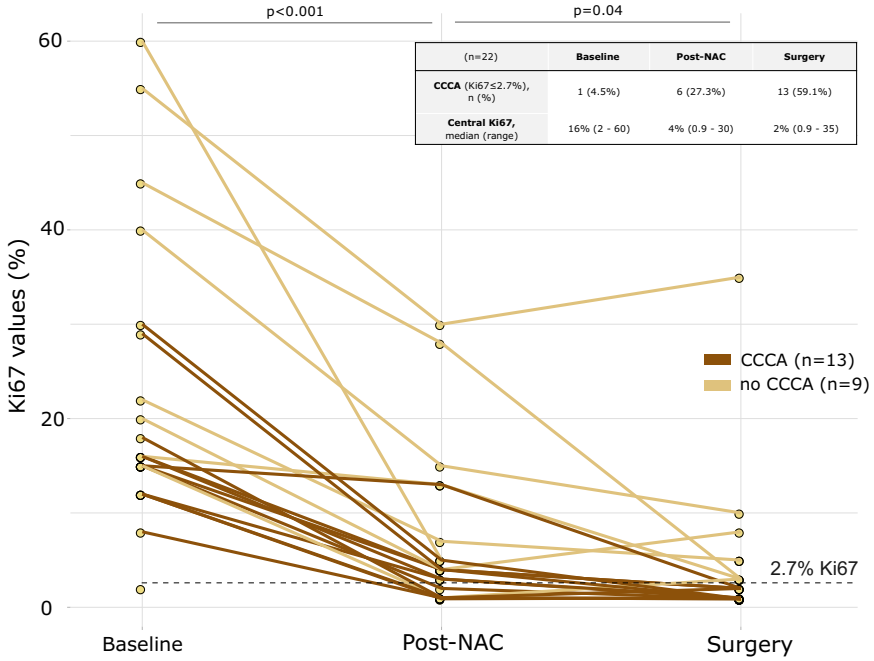

Fig. 1 | Ki67 levels from baseline to surgery. Continuous KI67 levels were represented i) at baseline, ii) after the neoadjuvant treatment but before receiving palbociclib (post-NAC) and iii) at surgery. Colors represent the CCCA status at surgery. The brown lines correspond to samples that showed CCCA at surgery and yellow to non-CCCA.

expression (FDR < 5%) (Fig. 3). In the subset of downregulated genes, in addition to those involved in cell-cycle regulation (e.g., *AURKA, CCND1, CDCA5*), we highlight genes linked to the HER2 amplicon (*ERBB2* and *ERBB3*) and estrogen-signaling genes (e.g., *ESR1, PGR*). Interestingly, genes associated with B-cell signaling (*CD79A, IGKC*), immune-related genes (*CTLA4, CXCL8, CX3CL1*) and cytotoxic cell activation genes (e.g., *KLRB1, GZMB*) were found to be upregulated in samples following palbociclib treatment. The increased expression of some of these genes (e.g.,*CD79A, CX3CL1)* was associated with CCCA at surgery (Supplementary figure 3).

In addition, the hierarchical cluster analysis showed that 129 of 187 genes were significantly downregulated (69 genes) or upregulated (60 genes) across timepoints (Supplementary Fig. 4). Relevant genes involved in BC signatures including *CDCA5, CNE1, ESR1, MKI67* and immune-related genes such as *CD8* or *GZMB* that displayed significant expression levels between timepoints ($p < 0.05$) are shown in Supplementary Fig. 5.

Similar median stromal tumor-infiltrating lymphocytes (sTILs) levels were observed at each timepoint: 2.5% at baseline, 2.5% at post-NAC and 4.0% at surgery (Supplementary Fig. 6). Regarding the programmed death ligand 1 (PD-L1) expression, no samples tested positive for PD-L1 at post-NAC evaluation, but three tumors (14%) were converted to PD-L1^{+} at surgery.

Concerning PAM50 subtyping, most tumors displayed a class-switching in PAM50 classification between baseline and post-NAC, from luminal B predominantly to luminal A subtype (Fig. 4). Overall, 17 out of 22 (77.3%) tumors post-NAC exhibited a luminal A subtype. At surgery, 20 out of 22 patients (90.9%) showed subtype shift from baseline, categorized as Luminal A (10 [45.5%]), followed by Normal-like (9 [40.9%]), and Luminal B (2 [9.1%]). At this timepoint, patients classified as Normal-like showed a numerically higher CCCA rate (77.8%) compared to Luminal A (60.0%) and Luminal B (0%).

Safety

Overall, 21 patients (95.5%) experienced treatment-emergent AEs of any grade. The most frequently reported AEs were neutropenia (68%) and asthenia (23%). Nine patients (40.9%) experienced grade 3 AEs, all of them being neutropenia (Supplementary Table 3). No temporary interruptions of palbociclib were reported. One patient (4.5%) halted palbociclib treatment due to grade 3 neutropenia, while another patient (4.5%) experienced grade 1 post-surgery pain related to the surgery.

Discussion

SOLTI-1710 PROMETEO II trial explored the biological effects of short-term palbociclib in combination with letrozole in patients with HR +/HER2- early BC and residual disease following NAC. We observed a complete cell cycle arrest (CCCA, primary endpoint) in 60% of patients. Additionally, there was a significant decrease in Ki67 (≤10%) in most tumors at the time of surgery, with a geometric mean change of 44.2% between receiving NAC and surgery. As expected, no patient achieved a pathological complete response (pCR), and only one (4.5%) had RCB I, indicating a limited pathological response. The safety profile of palbociclib and letrozole in the post-NAC setting was manageable and consistent with previous studies.

Our results are consistent with data reported with palbociclib plus ET regimes in the neoadjuvant setting. PALLET[16], NeoPal[17] and NeoPalAna[18] trials demonstrated a CCCA following treatment with CDK4/6i in most patients, despite the lack of change in the overall response rate. Additionally, in the randomized preoperative POP trial[19], 14 days of palbociclib treatment was associated with significant proliferation inhibition and decreased Rb-pathway activation. These findings highlight the significance of the anti-tumor activity exhibited by CDK4/6i in combination with ET in HR +/HER2- BC, together with the importance of CCCA as a valid endpoint in HR+/HER2- early BC[20,21].

Unlike other studies assessing the effect of short-term CDK4/6i treatment in early BC, SOLTI-1710 PROMETEO II trial explored the impact of palbociclib on the tumor biology within a distinctive patient subset, as all patients had undergone anthracycline-and taxane-based NAC and had a biopsy proven residual and proliferative disease, before surgery.

With the incorporation of CDK4/6i in the therapeutical repertoire of early-stage HR+/HER2- BC, there is an urgent need to better identify patients who are most likely to benefit more from these therapies, avoiding undesired toxicities and saving medical costs[22]. The strategy of treating all patients with CDK4/6i presents a challenge in terms of cost-effectiveness and may hinder approval and drug reimbursement in some countries. The identification of new biomarkers, and the understanding of resistance mechanisms to NAC and to CDK4/6i[23,24], could shed light on this issue[23,25]. In this sense, factors beyond pCR and residual disease burden[26] in the neoadjuvant setting, such as suppression of Ki67[20] and molecular markers[27,28] indicating treatment response, are being increasingly explored as potential prognostic indicators. Moreover, window-of-opportunity trial

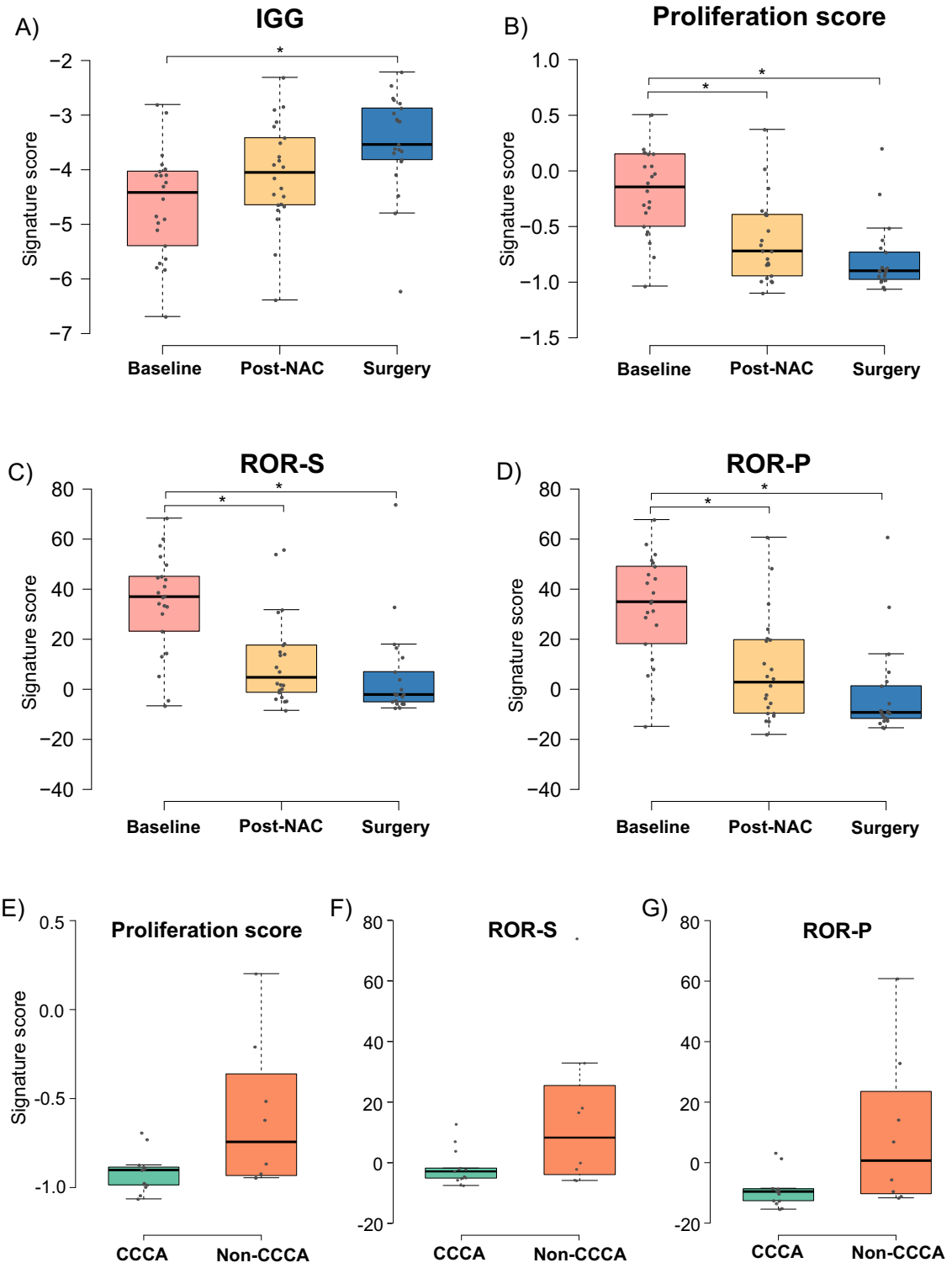

Fig. 2 | Changes in selected immune gene expression-based signatures. A–D Boxplot with the distribution of each score in tumor samples at the three timepoints (baseline, post-NAC, and surgery). **E–G** Boxplot with the distribution of each score stratified by CCCA status. CCCA was defined as a Ki67 ≤ 2.7% at surgery by central laboratory.

design permits a better understanding of tumor biology, identifying potential predictive biomarkers of sensitivity/resistance to the treatment, in contrast to large adjuvant trials.

In our study, gene expression data indicated that the combination treatment induced a profile consistent with high endocrine dependency, upregulating ER-regulated gene signatures (e.g., Luminal A signatures) and downregulating proliferation-related genes. Expression of HER2E and Luminal B signatures decreased post-treatment, suggesting a shift towards a more endocrine-sensitive tumor phenotype. Importantly, the ROR signature, which can differentiate BC patients based on their risk of late distant recurrence beyond conventional clinicopathologic risk factors[29,30], was suppressed. Furthermore, we observed an overexpression of the selective chemokine receptor *CX3CL1*, which together with *CXCL2*, and *SAA1*, are considered a prognostic factor of longer disease-free survival in patients with tamoxifen-resistant BC[31].

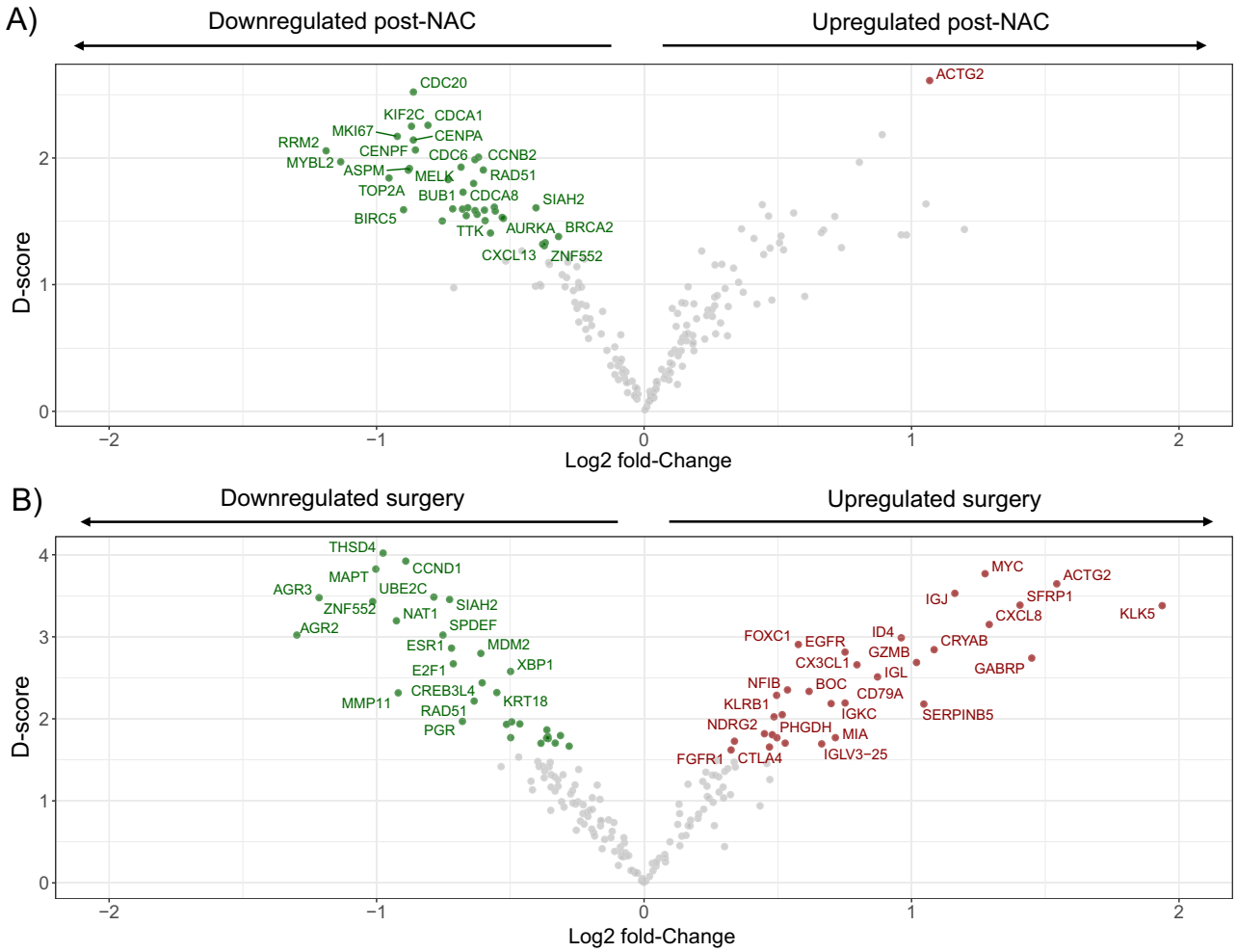

Fig. 3 | Gene expression analysis to identify upregulated and down regulated genes. A Changes in gene expression at post-NAC evaluation compared to baseline. **B** Changes in gene expression at surgery evaluation compared with the post-NAC. Colored dots mark upregulated and downregulated genes with a false discovery rate <5%. Names are reported for the differentially expressed genes. Post-NAC Post neoadjuvant treatment but before starting treatment with palbociclib.

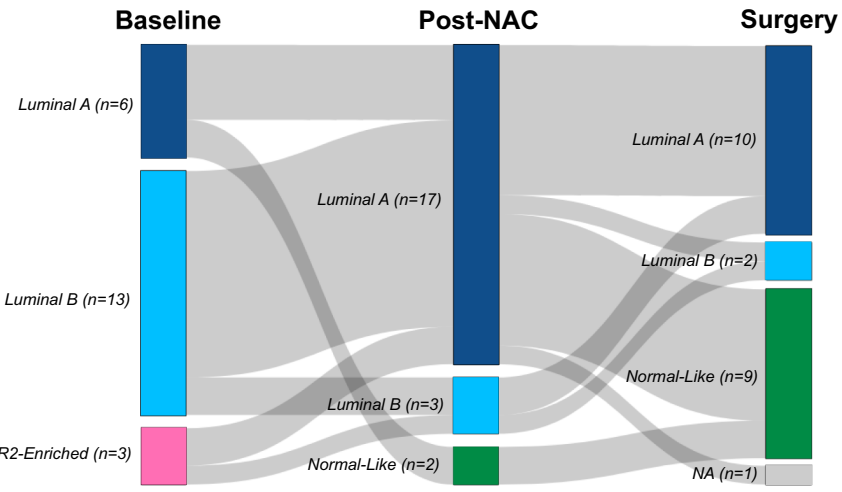

Fig. 4 | PAM50 switching from baseline to surgery. Sankey diagram illustrating the shifts in PAM50 breast cancer intrinsic subtype observed at each timepoint (baseline, post-NAC and surgery). Abbreviations: Lum A/B luminal A/B.

Interestingly, CDK 4/6i treatment led to an upregulation of immune-related genes, despite HR+/HER2−BC typically being considered non-immunogenic, as described in previous studies[32,33]. In line with prior investigations, only 13.6% of baseline tumor samples and 22.7% of post-NAC samples showed ≥10% stromal sTILs. Moreover, there was an increase in immune-related genes and signatures after four weeks of treatment. This finding suggests that evaluating the immune status in residual tumors post-preoperative therapy could be crucial in assessing drug sensitivity and could

open new opportunities for designing biomarker-driven trials with immunotherapeutic agents in the early setting.

This study has several limitations. Given that the primary goal of the PROMETEO II study was exploratory, the sample size of 22 patients was selected to minimize exposure to palbociclib and letrozole while acquiring valuable translational insights. Consequently, the small sample size limited our capacity to conduct analyses with sufficient statistical power, particularly for subset analysis. Additionally, we acknowledge that comparing pre- and post-treatment samples might be influenced by unavoidable sampling bias and changes in tumor cellularity due to treatment. Reduced tumor cellularity in post-treatment specimens, particularly following surgical resection, is primarily attributed to the cytotoxic effects of therapeutic interventions. Furthermore, the body's reparative response often results in fibrosis and scar tissue formation, replacing areas previously occupied by the tumor. It's important to note that baseline and screening tissues were obtained via core needle biopsies, that selectively target regions of higher cellularity to ensure sufficient diagnostic material. In contrast, post-treatment samples were surgically resected, encompassing a broader tissue area, which may include zones of tumor regression or areas replaced by fibrosis, typically resulting in lower tumor cellularity, even in cases with residual disease.

In conclusion, the PROMETEO II study showed that a single cycle of palbociblib plus letrozole following NAC induces significant changes in tumor biology, while providing to be safe and suggesting the potential of immune infiltration as a marker for treatment efficacy in the neoadjuvant setting. To validate these findings, further studies with larger cohorts are necessary, along with exploration of potential combination therapies. An adaptive study design to adjust treatment regimens based on response and immune infiltration profiles could be pursued to enhance patient selection and refine individualized treatment strategies.

Methods

Study design and patient population

The SOLTI-1710 PROMETEO II trial (ClinicalTrial.gov number NCT04130152; Study registration date: October 17, 2019) is an open-label, multi-center, single-arm, window-of-opportunity study conducted in eight Spanish sites to evaluate the antitumor activity of palbociclib in combination with letrozole in women with operable early HR+/HER2- BC and residual disease after NAC (Supplementary Fig. 7).

The trial was conducted in compliance with the Declaration of Helsinki, Good Clinical Practice, International Conference on Harmonisation guidelines, and applicable local laws and regulations. The trial protocol and relevant study documentation were approved by the Ethics Committee for Clinical Research with Medicinal Products (CEIm) Hospital Universitario 12 de Octubre and the Spanish Agency for Medicines and Health Products. All patients provided written informed consent. Eligible patients were pre- or post-menopausal women aged ≥18 years with operable and histologically confirmed non-metastatic HR-positive/HER2- BC as defined by the current ASCO/CAP criteria. Patients could be enrolled after completing ≥80% of total dose of anthracycline/taxane-based NAC. Residual disease showing a breast tumor diameter ≥10 mm had to be confirmed with an ultrasound and a core-biopsy detecting the presence of invasive tumor with Ki67% ≥5% by local IHC (immunohistochemistry). Patients with only residual disease in the axilla and no residual disease in the breast were not eligible. Additional eligibility criteria included Eastern Cooperative Oncology Group performance status of 0 or 1, no prior therapy with any CDK inhibitor, and any previous treatment using aromatase inhibitors (AI) or Selective Estrogen Receptor Modulators (SERMs) in the past 5 years.

Treatment and procedures

Patients received palbociclib 125 mg, orally, once daily for 21 days followed by 7 days off treatment and letrozole 2.5 mg, continuously during 28-day cycle, until surgery. For pre-menopausal patients, ovarian suppression with luteinizing hormone-releasing hormone (LHRH) analogs (i.e., triptorelin 3.75 mg intramuscular or Goserelin 3,6 mg subcutaneous) had to be initiated at least 2 weeks before palbociclib plus letrozole administration. Breast and axillary surgery were performed according to local practice procedures one week (±3 days) after the last dose of treatment with palbociclib and ER/PgR, HER2 and Ki67 status was centrally assessed in tumor samples. Following surgery, patients were treated as per local standards of care at the investigator's discretion and had to return for an end-of-treatment visit 4 weeks (±7 days) after surgery to monitor the patient's safety.

Study endpoints

The primary endpoint was CCCA rate, determined by Ki67 ≤ 2.7% at surgery by a central laboratory. Secondary endpoints included (i) changes in Ki67 between tumor samples collected at three timepoints (baseline samples before receiving NAC [baseline], after NAC [post-NAC] and at surgery), (ii) RCB 0-I index according to the MD Anderson Cancer Center procedures, (iii) pathological complete response (pCR) in the breast and axilla at surgery and (iv) incidence and severity of adverse events (AEs) assessed using the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 5.0. Exploratory endpoints included (i) changes in gene expression between the three timepoints and its association with biological response, (ii) changes of the PAM50 intrinsic subtypes between the three timepoints and its association with biological response and (iii) changes in the stromal tumor-infiltrating lymphocytes (sTILs) and PD-L1 expression by IHC in lesions before and after treatment.

Gene expression analysis

All biomarker assessments, including Ki67, gene expression, sTILs and PD-L1 IHC assessment were centrally performed in the Translational Genomics and Targeted Therapeutics in Solid Tumors Laboratory at IDIBAPS (Barcelona, Spain) blinded to clinical data. Ki67, gene expression and sTILs were determined at three timepoints: i) baseline, before receiving NAC, ii) post-NAC, and iii) at surgery. PD-L1 IHC was performed on samples collected before and after CDK4/6i treatment. Briefly, formalin-fixed, paraffin-embedded (FFPE) tumor samples were obtained. Ki67 was evaluated in three to four-micrometer tumor sections using rabbit anti-ki67 monoclonal antibody (clone 30.9, Roche, Basel, Switzerland) in an automated Ventana Benchmark Ultra stainer (Roche, Basel, Switzerland). sTILs and tumor cellularity proportions were centrally determined from FFPE hematoxylin and eosin (H&E) staining of tumor tissues[34]. PD-L1 expression was evaluated using the SP142 assay, with samples considered positive if PD-L1 expression was detected in ≥1% of tumor or immune cells. RNA was used to measure the expression of 192 genes from a customized panel, which includes, among others, the PAM50 genes, 5 housekeeping genes (*ACTB, MRPL19, PSMC4, RPLP0*, and *SF3A1*), and 47 immune genes using the nCounter platform (Nanostring Technologies). Gene counts were log base 2 transformed and normalized. Using the PAM50 subtype predictor, tumors were assigned to one of the four intrinsic subtypes (Luminal A, Luminal B, HER2-enriched, Basal-like) or the Normal-like group, as previously reported[35–37]. The PAM50-based risk of recurrence (ROR) scores based on subtype (ROR-S) and on subtype and proliferation (ROR-P) were computed using weighted coefficients for the four subtypes, and the proliferation score was derived using 11 proliferation genes among the PAM50 genes[38].

Statistical analysis

The study was designed with a sample size of 22 patients to perform a cost-efficient proof-of-concept study, which allowed obtaining reasonable estimation for the true CCCA rate. No formal hypothesis testing was predefined. The efficacy analysis was performed in the intention-to-treat (ITT) population, which included all evaluable patients enrolled in the study. The percentage of CCCA (Ki67 ≤ 2.7%) was reported along with the 90% confidence interval (CI) according to Clopper-Pearson method. To evaluate the association between baseline continuous Ki67 levels and CCCA, a logistic model was estimated to obtain odds-ratio (ORs) with 90%CI. The Ki67 geometric mean change and corresponding 90%CI were calculated using the *Gmean* function of the *DescTools* package for R statistical software. A

paired two-class significance analysis of microarrays (SAM) was conducted to identify genes that were significantly upregulated or downregulated after i) undergoing NAC comparing with the baseline biopsy and ii) at surgery (day 28) vs. post-NAC. A false discovery rate of 5% was used in both SAM models to control for multiple comparisons. The threshold for statistical significance was defined as 0.05 (two-sided). No data imputation were performed. The analysis was undertaken using R statistical software version 4.2.1.

Data availability

The datasets generated and analyzed during this study are available from the corresponding authors upon reasonable request.

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Author contributions

Conceptualization: E.C., S.P., T.P., A.P.; Formal analysis: G.V., T.P., L.V., S.C.; Writing-original draft: S.P., E.C., T.P., G.V., L.V.; Investigation: E.S.F., P.G., A.P.; Writing-review and editing: S.P., E.C., G.V., E.S.F.; J.S., A.P., X.G.F., B.J., M.M.S., P.P., T.P., E.A., L.V., S.C., J.M.F.C., P.G., A.P.

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Additional information

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Correspondence and requests for materials should be addressed to Sonia Pernas or Eva Ciruelos.