# Pembrolizumab and Paclitaxel in Patients with HR+/HER2− Breast Cancer with HER2-Enriched or Basal-like Subtypes

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## ABSTRACT

**Purpose:** Hormone receptor–positive (HR+), HER2-negative (HER2−) metastatic breast cancer (mBC) is biologically distinct from early-stage disease, with a higher prevalence of genomically defined nonluminal subtypes, particularly the HER2-enriched (HER2-E) and basal-like subtypes. These tumors are highly proliferative, less dependent on hormone signaling, and associated with poor outcomes and early resistance to endocrine therapy and CDK4/6 inhibition (CDK4/6i). This biological shift highlights the need for biomarker-driven strategies in the CDK4/6i-resistant setting.

**Patients and Methods:** The SOLTI-1716 TATEN trial (NCT04251169) is a phase II, single-arm study evaluating the combination of pembrolizumab and paclitaxel in patients with HR+/HER2− mBC classified as HER2-E or basal-like by PAM50 following progression on CDK4/6i. A total of 126 patients were screened using the PAM50 genomic assay; 20 with HER2-E or basal-like subtypes were enrolled and received pembrolizumab (200 mg every 3 weeks) and paclitaxel (80 mg/m$^2$ weekly).

**Results:** The primary endpoint, overall response rate (ORR), was 61.1% [95% confidence interval (CI), 35.7–82.7], and the clinical benefit rate was 94.4% (95% CI, 72.7–99.9). The median progression-free survival was 8.1 months (95% CI, 5.9–10.4), and the median overall survival was 26 months [95% CI, 18−not reached (NR)]. Three patients achieved durable responses lasting ≥24 months. Gene expression analyses revealed that high expression of proliferation- and immune-related genes predicted improved ORR and survival, whereas luminal-related gene expression was associated with lower clinical benefit.

**Conclusions:** These results suggest that chemo-immunotherapy may be an effective strategy in genomically defined nonluminal subtypes of HR+/HER2− mBC following CDK4/6i resistance and highlight the value of molecular subtyping to guide post–endocrine treatment decisions.

## Introduction

Hormone receptor–positive (HR+), HER2-negative (HER2−) breast cancer accounts for approximately 60% to 70% of all breast tumors and is typically associated with a favorable response to endocrine therapy (ET; ref. 1). In the metastatic setting, the addition of cyclin-dependent kinase 4/6 inhibitors (CDK4/6i) to ET has extended median overall survival (OS) to nearly 5 years for patients with HR+/HER2− disease (2–7). However, the majority of patients eventually develop endocrine resistance, either immediately after ET plus CDK4/6i or following additional ET-based regimens. Once resistance emerges, prognosis remains poor, with median OS ranging from 12 to 24 months (8, 9). Therapeutic options in this setting are limited to antibody–drug conjugates (ADC) and sequential single-agent chemotherapy (CT), both of which are associated with modest benefit and cumulative toxicity (10, 11). In

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## Translational Relevance

This proof-of-concept study demonstrates that chemo-immunotherapy is active in genomically defined nonluminal hormone receptor–positive/HER2-negative metastatic breast cancer after CDK4/6 inhibitors, supporting molecular subtyping to guide treatment in endocrine-resistant disease.

particular, the median progression-free survival (PFS) with first-line CT after CDK4/6i is ~8 months (10).

To improve outcomes, several trials have explored combining immune checkpoint inhibitors (ICI) targeting PD-1/PD-L1 with CT. In the neoadjuvant setting, the combination of multi-agent CT with anti–PD-1 ICIs has shown to increase pathologic complete response (pCR) rates by 8% to 11%, suggesting a potential role of immunotherapy in HR+/HER2− disease (12, 13). However, in the absence of biomarker-driven selection, these combinations have shown limited activity in the metastatic setting, with objective response rates (ORR) of 14% to 41% and median PFS of 4.1 to 5.1 months, offering no clear advantage over CT alone (14–17). HR+/HER2− metastatic breast cancer (mBC), however, is biologically heterogeneous.

Genomic profiling by the PAM50 classifier identifies a nonluminal intrinsic subtype—HER2-enriched (HER2-E) or basal-like—in 15% to 35% of tumors (18–26). These subtypes exhibit greater immune infiltration (25), elevated PD-L1 expression (27), and increased tumor mutational burden (TMB; ref. 26) compared with luminal A and B subtypes, suggesting a greater likelihood of benefit from immunotherapy. HR+/HER2− tumors classified as HER2-E display a gene expression profile very similar to HER2+/HER2-E breast cancer, except for lacking ERBB2 overexpression and dependency (23). Clinically, HER2-E and basal-like tumors are associated with worse outcomes across multiple treatment settings, including et alone and ET plus CDK4/6i (20, 28). Very few trials have attempted to target nonluminal subtypes with specific therapeutic approaches, such as combining ET with anti-HER2 agents, with disappointing results thus far. Hence, there is a need for alternative strategies in this patient population. These biological features provide additional rationale for testing alternative treatment strategies, such as immunotherapy-based combinations.

On this basis, we conducted the SOLTI-1716 TATEN trial, a biomarker-driven phase II study designed to evaluate the activity and safety of the anti–PD-1 antibody pembrolizumab in combination with CT in patients with genomically defined HER2-E or basal-like HR+/HER2− mBC following progression on prior ET and CDK4/6i therapy.

# Patients and Methods

### Study design and procedures

SOLTI-1716 TATEN (NCT04251169) is a nonrandomized, open-label, single-arm, biomarker-driven, phase II study designed to evaluate the antitumor activity of pembrolizumab plus paclitaxel in patients with HR+/HER2− mBC who had progressed on prior CDK4/6i-based therapy and exhibited a nonluminal tumor phenotype by PAM50 gene expression analysis. The study was conducted across seven investigational sites in Spain. Although initially designed to enroll 50 patients, the study was terminated early by the sponsor after successfully completing the first stage of the trial as per the Simon two-stage design because of the decision not to fund the second part of the study.

### Patients and eligibility criteria

Eligible patients were adult females or males with HR+/HER2− mBC who had progressed on prior CDK4/6i therapy. Tumor tissue (formalin fixed, paraffin embedded) obtained after diagnosis of metastatic disease—either before or after CDK4/6i therapy—was submitted after informed consent for central PAM50 subtyping at a central laboratory at Clinic Barcelona Comprehensive Cancer Center (Supplementary Table S1).

Patients with confirmed nonluminal subtypes (HER2-E or basal-like) were eligible if they had not received prior CT for metastatic disease, had an Eastern Cooperative Oncology Group performance status <2, had measurable disease by RECIST 1.1, had adequate organ function, and no contraindications to immunotherapy. Exclusion criteria included active brain metastases or leptomeningeal disease, prior treatment with ICIs, active autoimmune disease, recent immunosuppressive therapy, or immunodeficiency. Chronic corticosteroid therapy ≤10 mg/day prednisone equivalent was permitted.

### Treatment

All patients received an initial induction dose of pembrolizumab (200 mg i.v.). After 3 weeks, they began combination treatment with pembrolizumab (200 mg i.v. every 3 weeks) and paclitaxel (80 mg/m$^2$ i.v. on days 1, 8, and 15 every 3 weeks) until disease progression or unacceptable toxicity. A minimum of six cycles of paclitaxel were recommended; pembrolizumab could continue as monotherapy for up to 2 years. Concomitant maintenance ET was not permitted.

### Clinical endpoints

The primary endpoint was the overall response rate (ORR), defined as the proportion of patients achieving complete response or partial response (PR) per RECIST 1.1. Secondary endpoints included clinical benefit rate [CBR; complete response/PR or stable disease (SD) ≥24 weeks], PFS, OS, duration of response, time to response (TtR), and safety. ORR and CBR were evaluated in the response-evaluable population (patients who received ≥1 dose of study drug and had ≥1 postbaseline tumor assessment). PFS and OS were analyzed in the intention-to-treat (ITT) population. Safety analyses included all patients who received at least one dose of pembrolizumab.

### Histologic and molecular analyses

Tumor estrogen receptor (ER), progesterone receptor, and HER2 status was determined locally by IHC/FISH per American Society of Clinical Oncology/College of American Pathologists guidelines (29, 30). Tumor-infiltrating lymphocytes (TIL) were assessed on hematoxylin and eosin–stained sections based on International TILs Working Group guidelines (31). PD-L1 expression was evaluated on trial-entry samples using the 22C3 pharmDx assay (Agilent/Dako, RRID: AB_2650491) and reported as Combined Positive Score (CPS).

RNA from prescreening samples was extracted using the High Pure FFPET RNA Isolation Kit (Roche) and analyzed on the nCounter platform using the PAM50 research-use panel (38). Gene expression analysis in enrolled patients was performed using a 192-gene custom panel covering luminal, basal, HER2, immune, and proliferation signatures (32). We also evaluated the standardized B-cell/immunoglobulin G (IGG) signature from the HER2DX genomic test, which comprises 14 immune genes tracking processes of maturation of T- and B-lymphocyte progenitors (*IL2RG*), CD4+

and B-lymphocyte activation (*CD27*, *TNFRSF17*, and *PIM2*), B-lymphocyte differentiation in germinal centers (*POU2AF1*), immunoglobulin production (*CD79a*, *JCHAIN*, *IGKC*, *IGL*, and *IGLV3-25*), chemotaxis (*CXCL8* and *NTN3*), and regulation of B-, T-, and NK-lymphocyte activity (*LAX1* and *HLA-C*).

Tumor DNA was sequenced using the VHIO-300 v4 panel (435 genes; 1.34 Mb capture size), as previously described (25). TMB was calculated as nonsynonymous somatic mutations per Mb. Sequencing was performed on Illumina HiSeq 2500 (2 × 100 bp; average coverage 500×). Bioinformatic processing included BWA alignment to hg19 and variant calling with VarScan2 [reads were aligned to hg19 using BWA (RRID: SCR_010910)].

### Statistical analysis

Descriptive statistics were used for all endpoints. The sample size was based on the Simon optimal two-stage design for the primary endpoint (ORR). Assuming a null ORR of ≤30% and a target ORR of 50%, with $\alpha = 0.05$ and 80% power, 15 patients were planned in stage 1. If >5 responses were observed, up to 31 more patients could be enrolled. The null hypothesis would be rejected if ≥19 responses were observed among 46 patients. Assuming a 20% prevalence of nonluminal subtypes among HR+/HER2− tumors and 10% attrition, ~250 patients were projected for prescreening to recruit 46 evaluable patients.

Associations between baseline variables and ORR were assessed using univariate and multivariate logistic regression. PFS and OS were analyzed using Cox proportional hazards models. Odds ratios (OR), hazard ratios (HR), and 95% confidence intervals (CI) were reported. Wilcoxon rank-sum tests were used to compare gene expression between long and normal responders. Gene Ontology (GO) enrichment analysis was performed using a hypergeometric test with FDR < 0.05. No formal adjustment for multiple testing was applied in the exploratory biomarker analyses. Analyses were conducted in R v4.0.3.

### Ethical statement

The SOLTI-1716 TATEN trial was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice guidelines. The study protocol was approved by the institutional review board and independent ethics committees at each participating centers. All patients provided written informed consent before enrollment.

## Results

### Inclusion and patient demographics

Between April 2020 and March 2024, 162 patients with HR+/HER2− mBC who had progressed on prior ET plus CDK4/6i were prospectively screened across seven sites in Spain. Only those whose tumors exhibited a HER2-E or basal-like phenotype by PAM50 were considered eligible for enrollment (**Fig. 1A**). Of 162 patients assessed for eligibility, 21 (12.9%) were excluded because of inadequate or unavailable tumor tissue for gene expression analysis, 11 because of clinical exclusion criteria, and three because of consent withdrawal. The remaining 126 patients (77.8%) entered molecular prescreening for central PAM50 subtyping (**Fig. 1B**). Biopsy sites included liver ($n = 52$, 41.3%), breast ($n = 30$, 23.8%), and bone ($n = 11$, 8.7%; Supplementary Table S3). Fifty-eight samples (46%) were collected during CDK4/6i progression, 67 (53.2%) prior to CDK4/6i initiation, and one was unspecified. Among the 126 prescreened patients, 105 (83.3%) had luminal A or B tumors, whereas 19 (15.1%) and two (1.6%) had HER2-E and basal-like subtypes, respectively, and were eligible for the trial (**Fig. 1B**).

### Clinical and molecular characteristics of nonluminal subtypes

HER2-E tumors were more prevalent in samples collected at CDK4/6i progression (17.2% vs. 13.4%), and luminal A less frequent (20.7% vs. 29.9%), although the differences were not statistically significant (Supplementary Fig. S1). TIL percentages did not differ significantly by PAM50 subtype ($P = 0.118$) or biopsy timing ($P = 0.128$). The median ER percentage was significantly lower in HER2-E tumors [35% (IQR: 20–78)] compared with luminal A [90% (IQR: 25–100)] and luminal B [95% (IQR: 90–100); $P < 0.0001$] and lower in post-CDK4/6i versus pre-CDK4/6i biopsies [75% (IQR: 50–100) vs. 93% (IQR: 90–100); $P$=0.040; Supplementary Figs. S2–S5].

### Baseline characteristics of the enrolled population

Of 21 eligible patients, 20 were enrolled; one patient was not recruited because of early trial termination. Baseline characteristics of the ITT population are shown in **Table 1**. The median age was 54 years (IQR: 33–70); three patients were premenopausal. Most (75%) had recurrent mBC and received prior (neo)adjuvant CT. The majority (85%) enrolled after progression on first-line CDK4/6i, most frequently ribociclib (55%). Visceral involvement was present in 65%, and 50% had liver metastases. The median TILs were 3% (IQR: 1–5), the median TMB was 8.2 mut/Mb (IQR: 4.5–10), and 55% had a PD-L1 CPS <10%.

### Activity and efficacy

At the data cutoff (October 29, 2024), after a median follow-up of 26 months (IQR: 16.6–NR), 16 progressions and 12 deaths were recorded. Disease progression accounted for 13 of 20 treatment discontinuations (65%). Two patients discontinued early (one because of rapid progression and one because of an unrelated condition) and were excluded from the ORR analysis.

Eight PRs, six SD, and one disease progression were observed among the first 15 enrolled patients, meeting the primary endpoint for the first stage of the trial for which at least five responders were needed. Among the 18 evaluable patients of the whole trial population, 11 achieved a PR, yielding a final ORR of 61.1% (95% CI, 35.7–82.7; **Fig. 2A**). The median TtR was 3.9 months (IQR: 3.9–5.9); the median response duration was 6.3 months (95% CI, 3.7–31.1; **Fig. 2B**). Seventeen patients achieved clinical benefit (PR or SD ≥ 6 months), resulting in a CBR of 94.4% (95% CI, 72.7–99.9). Two patients remained in PR on pembrolizumab monotherapy for 29.4 and 28.1 months, respectively, following an approved exception to extend treatment beyond the 2-year limit. One patient has an ongoing PR (>24 months) after discontinuation of both agents due to an immune-related adverse event (AE; **Fig. 2C**).

In the ITT population ($n = 20$), the median PFS was 8.1 months (95% CI, 5.9–10.4) and the median OS was 26 months (95% CI, 18–NR; Supplementary Figs. S6 and S7). Among responders, the median PFS and OS were 10.2 months (95% CI, 5.9–NR) and 30 months (95% CI, 26–NR), respectively.

### Safety

Nineteen patients (95%) experienced ≥1 treatment-related AE (TRAE). Most common TRAEs for pembrolizumab included fatigue (25%), hypothyroidism (25%), and rash (15%). Paclitaxel-related events included neuropathy (75%), rash (40%), and neutropenia (25%). Grade 3 TRAEs occurred in nine patients (45%), including

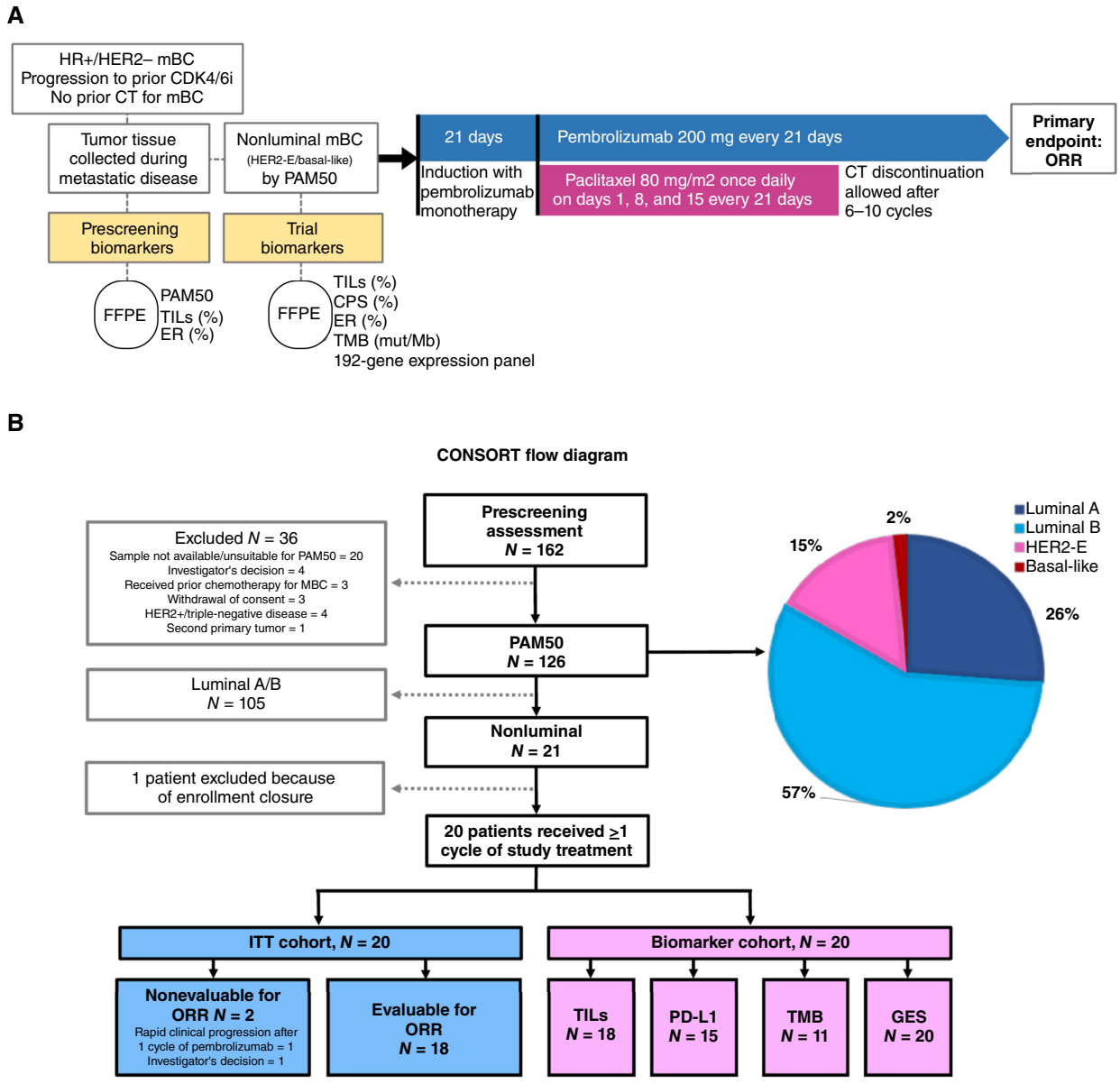


**Figure 1.**

Study design and patient enrollment in the SOLTI-1716 TATEN trial. **A,** Schematic overview of study procedures and planned biomarker analyses. **B,** CONSORT diagram depicting the disposition of 162 patients assessed for eligibility, including a pie chart showing the distribution of PAM50 intrinsic subtypes among prescreened patients. FFPE, formalin-fixed, paraffin-embedded; GES, gene expression signatures; mut, mutation.

six attributed to pembrolizumab, four to paclitaxel, and one to both. No grade 4 or 5 TRAEs occurred. Two patients (10%) discontinued pembrolizumab because of AEs (Supplementary Table S4).

### Biomarkers of response and survival

No significant associations were found between ER, TILs, PD-L1 CPS, TMB, and clinical outcomes (Supplementary Tables S5–S8). The gene expression analysis revealed significant heterogeneity in the expression of luminal, proliferative, and immune-related genes within nonluminal HR+/HER2− mBC (**Fig. 3A**). Higher expressions of luminal genes were associated with poorer outcomes, whereas proliferative and immune-related genes correlated with improved PFS and OS (**Fig. 3B–D**).

Luminal genes (*RRAGA*, *MAPT*, and *DNAJC12*) were negatively associated with ORR. Immune evasion–related genes (*CRYAB*, *SFRP1*, *IL34*, and *IL18R1*) were also linked to lower ORR (**Fig. 3B**). Proliferation genes (*MND1*, *CDCA8*, *CDCA5*, and *CDKN3*) predicted improved PFS (**Fig. 3C**). *PGR* expression was associated with worse OS, whereas *CD4* and *CD68* correlated with improved OS (**Fig. 3D**). The HER2DX immune/IGG signature stratified PFS and OS. HER2DX IGG-high tumors represented three cases, and these showed better PFS and OS than IGG-medium/-low cases (**Fig. 3E–**

**Table 1.** Baseline characteristics of the ITT population.

| ITT population, *n*=20 | No. (%) |
|---|---|
| Age at inclusion, years | |
| Median, IQR | 54 (33–77) |
| Menopausal status | |
| Premenopausal | 3 (15) |
| Postmenopausal | 17 (85) |
| Ethnicity | |
| Caucasian | 16 (80) |
| Arabic | 1 (5) |
| Latino | 1 (5) |
| White | 2 (10) |
| ECOG PS | |
| 0 | 11 (55) |
| 1 | 9 (45) |
| Visceral disease | 13 (65) |
| Liver metastasis | 10 (50) |
| *De novo* breast cancer | 5 (25) |
| Previous (neo)adjuvant CT | 15 (75) |
| Lines of ET for mBC | |
| 1 | 17 (85) |
| 2 | 1 (5) |
| 3 | 2 (10) |
| Most recent treatment line | |
| AI + CDK4/6i | 5 (25) |
| Fulvestrant + CDK46i | 12 (60) |
| Fulvestrant + alpelisib | 2 (10) |
| Exemestane + everolimus | 1 (5) |
| Type CDK4/6i | |
| Abemaciclib | 5 (25) |
| Palbociclib | 4 (20) |
| Ribociclib | 11 (55) |
| TILs, % | |
| Median, IQR | 3 (1–5) |
| PD-L1 CPS, % | |
| <10 | 11 (55) |
| ≥10 | 4 (20) |
| Missing | 5 (25) |
| ER, % | |
| Median, IQR | 35 (20–80) |
| TMB, mut/Mb | |
| Median, IQR | 8.2 (4.5–10) |

Abbreviations: AI, aromatase inhibitors; ECOG PS, Eastern Cooperative Oncology Group performance status.

**G**). Multivariate and bivariate models confirmed independent associations for luminal, proliferative, and immune markers with ORR, PFS, and OS (Supplementary Tables S9–S17).

Transcriptomic analysis of long responders (≥24 months) compared with short responders (<24 months) showed downregulation of luminal genes (*RRAGA* and *ACTR3B*) and upregulation of proliferative and immune genes (*CDKN3*, *CDCA8*, and *IL23A*; **Fig. 3H**). GO analysis showed enrichment in immune and cell cycle pathways (Supplementary Fig. S8). Correlation analysis revealed alignment of beneficial gene profiles with basal-like, immune-enriched features and inverse correlation with luminal A, TILs, PD-L1, PD-1, and proliferation signature (Supplementary Fig. S9)

### Clinical case of sustained response to immunotherapy

One long responder with paired tumor samples (before and after CDK4/6i) exhibited a shift from luminal A subtype at the time of metastatic diagnosis to a HER2-E phenotype following progression on ET plus ribociclib. Prior to initiating pembrolizumab, this patient's tumor showed high IGG expression (based on the pre-established HER2DX cutoff), elevated TILs (20%), and reduced ER expression (30%). The patient experienced a rapid and profound response to treatment, achieving a 75.8% reduction in tumor size within 1.8 months, and maintained a PR for 32.9 months, including 29.4 months on pembrolizumab monotherapy (**Fig. 4A–F**).

## Discussion

In this phase II trial, the combination of pembrolizumab and paclitaxel demonstrated promising activity in patients with HR+/HER2− mBC characterized by HER2-E or basal-like subtypes who had progressed on prior CDK4/6i therapy. Eight objective responses were observed among the first 15 patients enrolled, meeting the prespecified threshold for continuation under the Simon two-stage design. Although the trial was terminated early for nonscientific reasons and did not complete full enrollment, the observed ORR of 61% exceeded the statistical threshold required to reject the null hypothesis in the planned full cohort (≥50%). No new safety signals were observed.

Previous studies evaluating ICIs in HR+/HER2− mBC—regardless of molecular subtype—have reported modest activity, with ORRs ranging from 14% to 41%, comparable with CT alone. The only randomized trial in this setting (eribulin ± pembrolizumab) showed no improvement in ORR or survival with immunotherapy (14). By contrast, our trial achieved an ORR that was 20% to 47% higher than these studies and 34% higher than that expected with paclitaxel monotherapy in first-line HR+/HER2− mBC (10, 33). This may be partly explained by the inclusion of CT-naïve patients as prior exposure to multiple lines of CT has been shown to reduce ICI efficacy because of progressive immunosuppression of the tumor microenvironment (34). Supporting this notion, the NEWBEAT trial—conducted in a chemo-naïve population—reported an ORR of 74% with nivolumab, paclitaxel, and bevacizumab (35).

An additional key factor in the observed efficacy is biomarker-based patient selection. To our knowledge, the SOLTI-1716 TATEN trial is the first to prospectively select patients with HR+/HER2− mBC for immunotherapy based on PAM50 genomic subtyping. HER2-E and basal-like tumors are known to exhibit higher immune infiltration and expression of immune checkpoints compared with luminal subtypes—even within HR+/HER2− disease (18, 26, 36). These subtypes, although rare in early disease, become more prevalent in metastatic settings, particularly after CDK4/6i treatment (18, 26, 36). In our study, 17% of prescreened tumors were non-luminal, with a numerical increase in HER2-E cases among biopsies obtained after CDK4/6i progression.

In clinical practice, the post-CDK4/6i setting is increasingly crowded with treatment options, including continued ET ± targeted therapies, ADCs, or CT (8–10, 37, 38). Treatment selection often relies on the presence of actionable mutations or clinical indicators of endocrine resistance—such as prior response to CDK4/6i (37, 39). However, both ET-based and CT approaches yield limited benefit, with median PFS ranging from 1.9 to 8 months in patients unselected for PAM50 subtypes (Supplementary Table S18; refs. 8, 37, 38, 40, 41). Patients with non-luminal tumors showed no or limited benefit from ET-based approaches in trials dating back to the pre-CDK4/6is era, raising doubt on the efficacy of ET + targeted agents in a

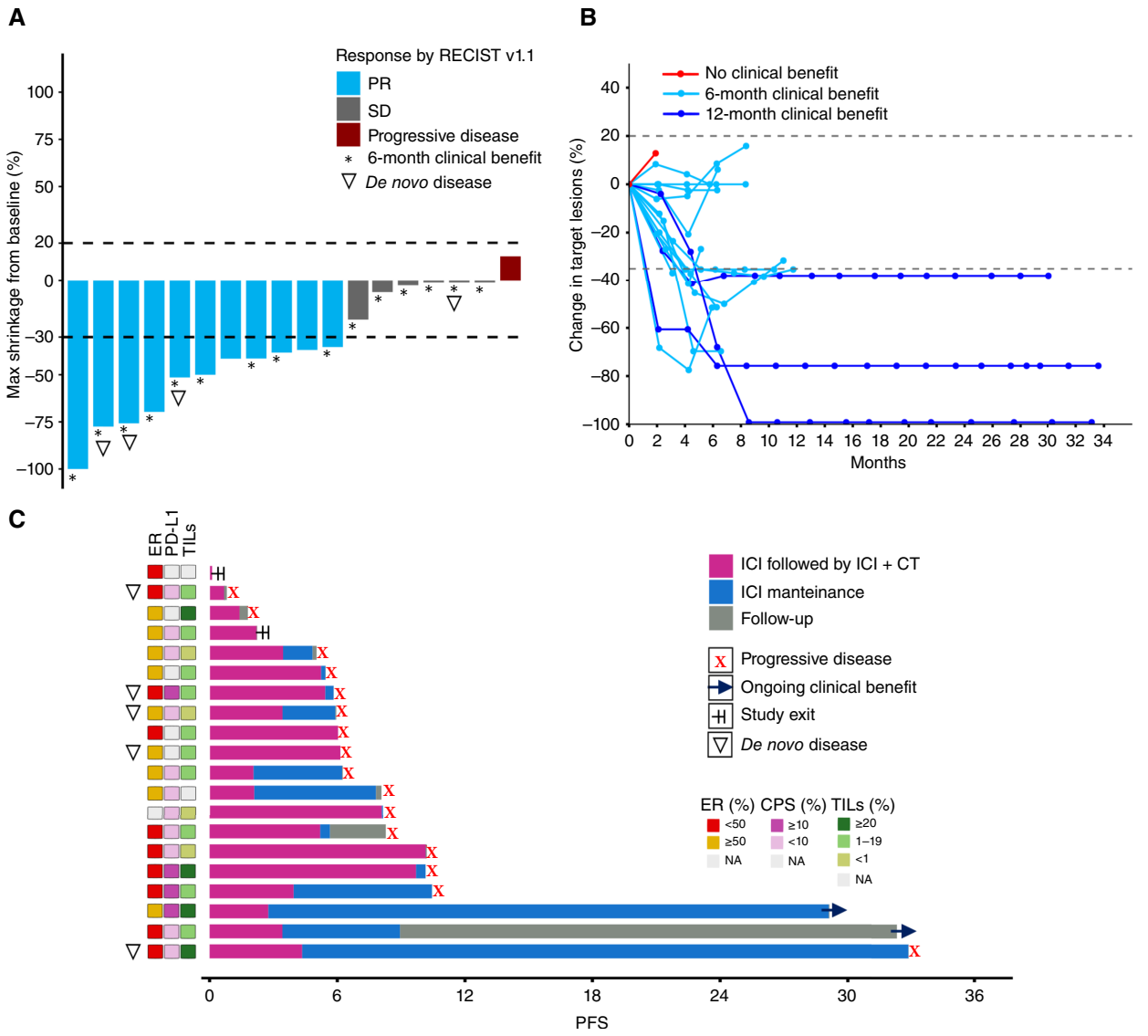


**Figure 2.**

Efficacy outcomes in the SOLTI-1716 TATEN trial. **A,** Waterfall plot showing the maximum percentage change in the sum of target lesion diameters from baseline among the 18 evaluable patients. Each bar represents one patient and is color-coded according to RECIST 1.1 best response (complete response, PR, SD, or progressive disease). **B,** Spider plot illustrating the longitudinal change in target lesion size over time for each evaluable patient. Line colors indicate no clinical benefit (red), clinical benefit at 6 months (light blue), or at 12 months (dark blue). **C,** Swimmer plot showing PFS for all 20 patients in the ITT population. Each horizontal bar represents one patient. Pink segments correspond to combination therapy (pembrolizumab + paclitaxel), blue segments to pembrolizumab maintenance, and gray segments to follow-up without active treatment. Squares on the left denote each patient's ER expression, PD-L1 CPS score, and TIL percentage.

CDK4/6i-resistant setting (**Table 2**; ref. 19). The addition of the anti-HER2 agent lapatinib to ET yielded some benefit in CDK4/6i-naïve patients with HER2-E tumors, but the overall efficacy was modest, with a median PFS of only 6.5 months (22). Preliminary results from the NEREA trial suggest that the efficacy is even smaller after progression to CDK4/6is, with median PFS of only 1.7 months with the association of ET with neratinib (42). In this context, the median PFS of 8.1 months and OS of 26 months observed with pembrolizumab plus paclitaxel in nonluminal tumors compare favorably with current options. Moreover, 15% of patients achieved long-lasting responses (≥24 months), a rate comparable with those seen in malignancies with established roles for immunotherapy, such as non–small cell lung cancer (43).

We observed no significant association between traditional immunotherapy biomarkers—PD-L1 CPS, TILs, or TMB—and treatment outcomes. Although the lack of meaningful associations could be due to the small sample size (only 11 of 20 patients had TMB available) and to different timing of biopsies relative to CDK4/6i treatment, these data are in line with prior studies in HR+/HER2− mBC (14). In contrast, gene expression profiling provided rich prognostic insights, even within a preselected nonluminal population. Luminal-related genes and the luminal A signature—typically

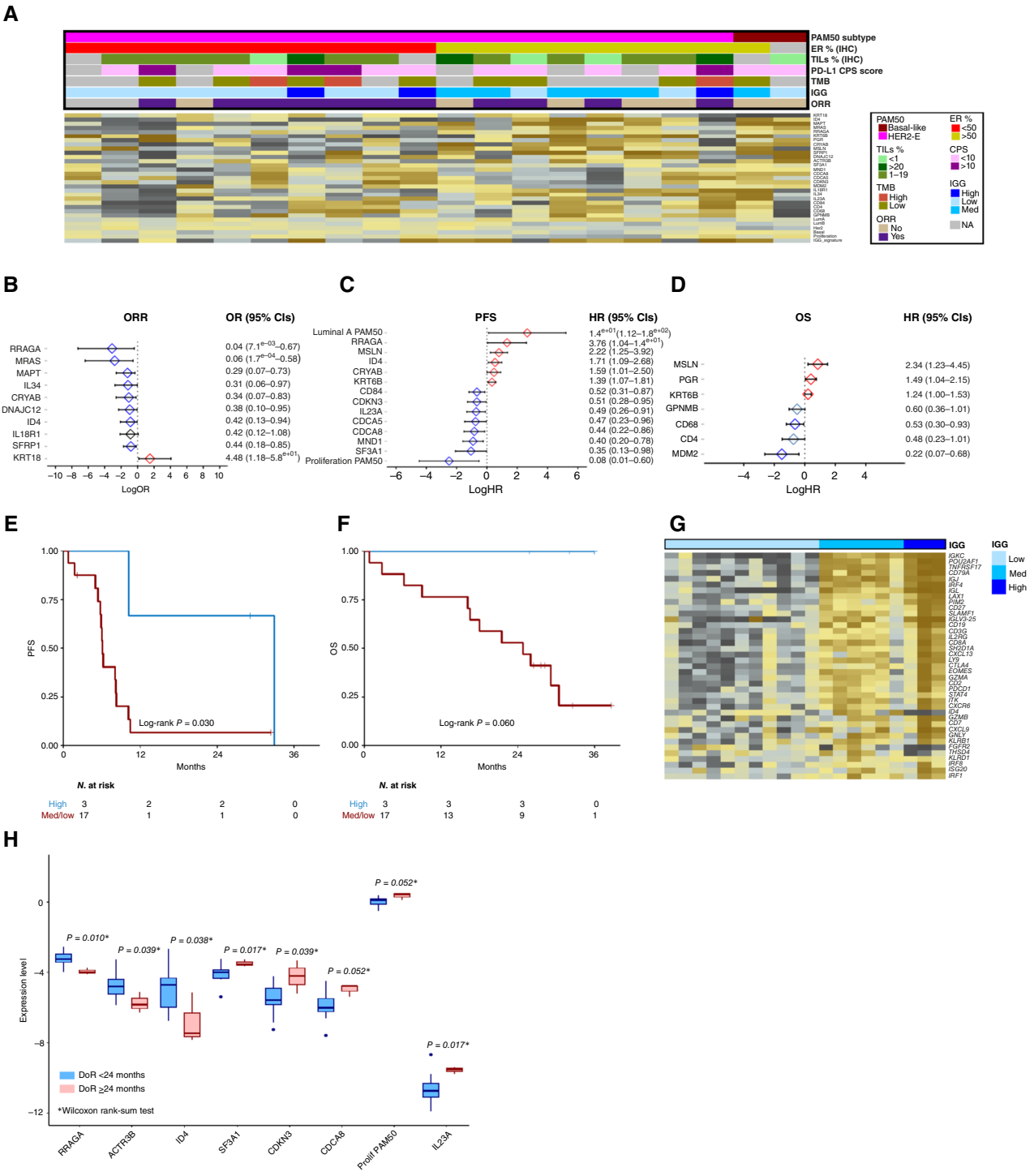


**Figure 3.**

Biomarker analyses on tumor tissue included in the study. **A,** Heatmap showing heterogeneous expression of genes significantly associated with ORR, PFS, and OS within nonluminal HR+/HER2− tumors. Samples are annotated by PAM50 subtype and ER IHC expression. **B,** Forest plot showing results of univariate logistic regression analyses evaluating associations between individual biomarkers and ORR. **C,** Forest plot of univariate Cox proportional hazards regression for PFS. **D,** Forest plot of univariate Cox regression for OS. In **A–C**, blue diamonds indicate ORs or HRs < 1 with statistically significant associations ($P < 0.05$); red diamonds indicate ORs or HRs >1 with statistical significance; and black diamonds indicate nonsignificant associations. **E,** Kaplan–Meier plot for PFS according to IGG expression groups (low/medium vs. high), defined using prespecified HER2DX assay cutoffs. **F,** Kaplan–Meier plot for OS according to IGG expression groups, categorized as in **E**. **G,** Heatmap of immune-related gene expression across tumors stratified by IGG expression groups (low, medium, and high) defined by HER2DX assay cutoffs. Samples are ordered by increasing IGG expression, and colors indicate relative gene expression levels (scaled per gene). **H,** Boxplots comparing the expression of luminal, proliferative, and immune-related genes and signatures between patients with long-lasting responses to pembrolizumab (pink) and those without (light blue). DoR, duration of response.

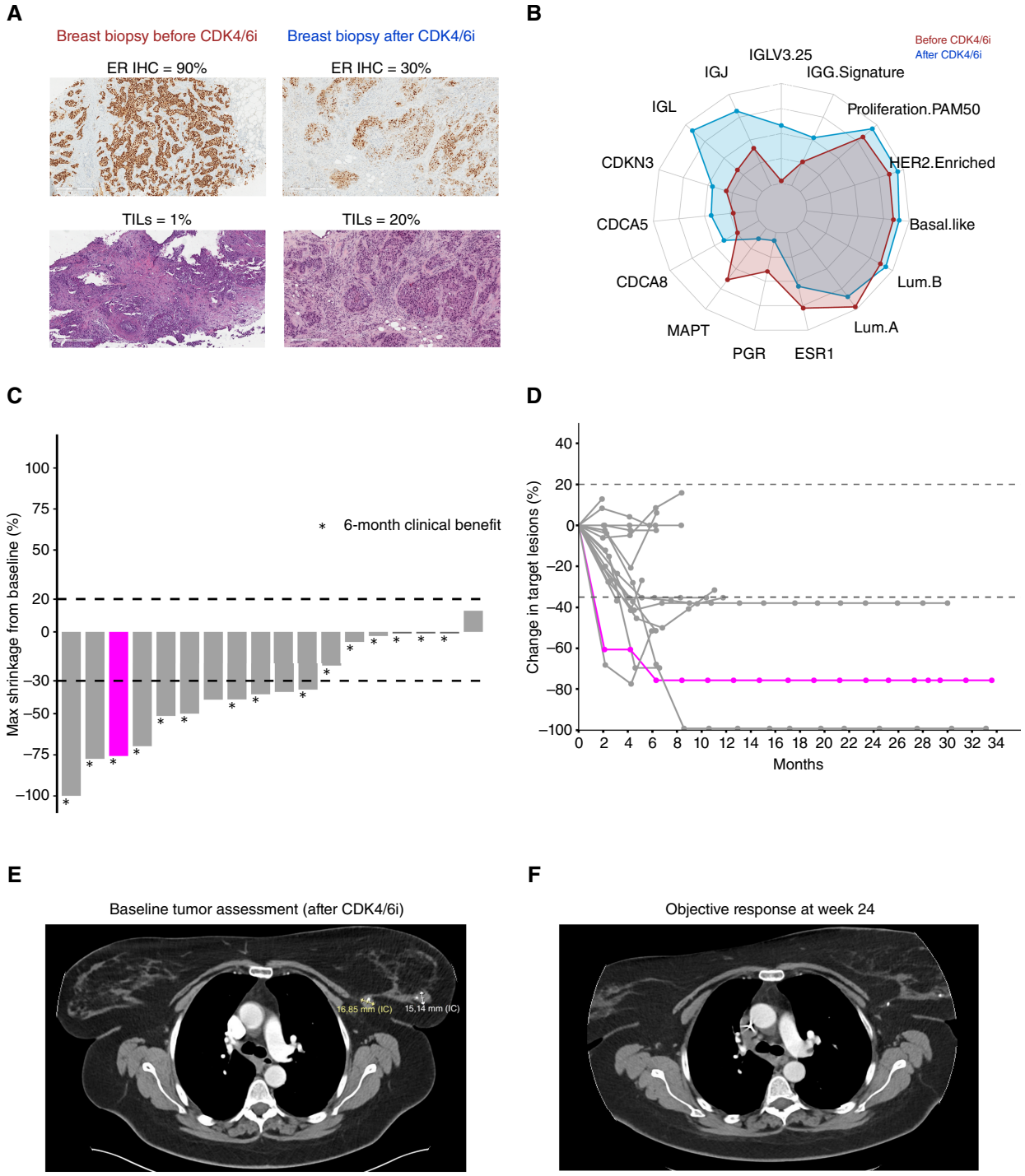


**Figure 4.**

Gene expression dynamics in a patient with long-lasting response to pembrolizumab. **A,** Changes in IHC expression of ER and TILs between the pre-CDK4/6i primary tumor and the metastatic biopsy collected at progression on CDK4/6i therapy. **B,** Wheel plot illustrating global shifts in gene expression between the two tumor samples, highlighting changes in PAM50 subtype signatures, proliferation, immune-related genes, and the IGG signature. **C,** Maximum tumor shrinkage (best overall response) across all evaluable patients ($n$=18), showing percent change in the sum of target lesion diameters from baseline. The patient with long-lasting response to pembrolizumab is highlighted in magenta. **D,** Longitudinal tumor dynamics showing changes in target lesion size over time for each evaluable patient. The patient with long-lasting response is highlighted in magenta. **E,** Baseline tumor burden and **F,** depth of tumor shrinkage, as assessed by computed tomography imaging.

**Table 2.** Clinical outcomes of HER2-E and basal-like subtypes across trials in HR+/HER2− and HER2-low mBC[a].

| Trial | Population | Sample size (genomic) | Prognostic value | Predictive value | Key notes |
|---|---|---|---|---|---|
| MONALEESA-02/03/07 (20, 28) | HR+/HER2-1L–2L<br>ET ± Ribo | 933 | Yes (PFS and OS) | No | HER2-E:<br>*mPFS*:<br>PBO = 5.5 (3.5–9.2),<br>RIB = 13.9 (12.7–24.6)<br>HR = 0.40 (0.26–0.62)<br>*mOS*:<br>PBO = 29.4 (23.9–42.0)<br>RIB = 40.3 (33.4–49.0)<br>HR = 0.60 (0.40–0.92)<br>Basal-like:<br>*mPFS*:<br>PBO = 3.6 (1.9–NA)<br>RIB = 4.6 (3.6–19.6)<br>HR = 1.14 (0.46–2.83)<br>*mOS*:<br>PBO = 21.2 (12.8–NA)<br>RIB = 19.0 (10.7–33.2)<br>HR = 1.92 (0.81–4.53) |
| BOLERO-2 (19) | HR+/HER2-2L<br>Exe ± Eve | 261 | Yes (PFS) | No | HER2-E: mPFS = 5.2 months (3.9–6.7) |
| NEREA (SOLTI-1718) (42) | HR+/HER2-HER2-E<br>≤1 prior CT neratinib + ET | 18 (12 treated) | — | — | HER2-E:<br>mPFS = 1.7 months (95% CI, 1.6–1.9)<br>PFS6 8.3% (one of 12). |
| EGF30008 (22) | HR+/HER2-1L<br>Let ± Lap | 644 | Yes (PFS and OS) | Yes | HER2-E:<br>mPFS = 4.7 months (95% CI, 2.7–10.8)<br>mOS = 16 months (95% CI, 10–NA)<br>Basal-like:<br>mPFS = 4.1 months (95% CI, 2.5–13.8)<br>mOS = 23 months (95% CI, 12–NA). |
| DESTINY-BREAST-04 (41) | HR+/HER2-low prior ET ± CT<br>T-DXd vs. TPC | 219 | Yes (PFS) | No | HER2-E:<br>*mPFS*:<br>T-DXd = 11 (6.6–NA)<br>TPC = 2.7 (1.4–5.9)<br>HR = 0.15 (0.05–0.40) |

Abbreviations: Eve, everolimus; Exe, exemestane; Lap, lapatinib; Let, letrozole; mOS, median OS; mPFS, median PFS; NA, not applicable; Nera, neratinib; PBO, placebo; Ribo, ribociclib; T-DXd, trastuzumab deruxtecan; TPC, treatment of physician's choice.

[a]Prognostic value refers to whether intrinsic subtypes were significantly associated with clinical outcomes (PFS and/or OS). Predictive value refers to whether subtypes were associated with differential benefit from the treatment evaluated. The median PFS and median OS are both expressed in months. HER2-E, luminal A, luminal B, and basal-like correspond to PAM50 intrinsic molecular subtypes.

favorable predictors in patients receiving ET and CDK4/6is—were associated with reduced response and worse survival outcomes in the context of chemo-immunotherapy (20, 28, 44–46). These luminal tumors exhibited lower immune infiltration and PD-L1/PD-1 expression, features consistent with immune-cold phenotypes.

Conversely, genes associated with proliferation and immune activation emerged as favorable prognostic indicators. High expression of proliferative genes and the PAM50 Proliferation signature were linked to improved PFS, aligning with prior observations that ICIs are more effective in highly proliferative cancers. Interestingly, these same genes are associated with resistance to CDK4/6i (46, 47), underscoring a therapeutic divergence. Similarly, immune-related genes, including those captured by the IGG signature, were associated with improved OS. Patients with IGG-high tumors experienced a median PFS of 32.9 months. This B cell–related signature has also been shown to predict pCR to chemo-immunotherapy in early TNBC (48), suggesting cross-context and cross-subtype relevance of certain immune biomarkers. Notably, luminal, proliferation, and immune features showed reversed prognostic value in CDK4/6i-treated populations (46), emphasizing the dynamic biology of HR+/HER2− mBC and the importance of context-specific biomarker interpretation.

This biological complexity highlights the potential of integrating molecular subtyping with genomic profiling to optimize treatment decisions. One patient in our study exemplified this approach: after long-term ET + CDK4/6i, the tumor evolved into a highly proliferative, TIL- high, IGG-high, HER2-E phenotype—predictive of ICI sensitivity—and responded with a durable PR lasting over 32 months. This case aligns with prior clinical evidence suggesting that treatment with CDK4/6is may increase TILs and sensitivity to immunotherapy. Preliminary results from previous trials suggest potential meaningful activity of the combination of ET with ICIs and CDK4/6is, supporting the hypothesis that the exposure to CDK4/6is may reshape tumor's phenotype and immunogenicity (Supplementary Table S18; refs. 49, 50). Taken together, these findings illustrate how a biomarker-guided strategy can improve therapeutic alignment beyond clinical indicators alone.

This study has several limitations. First, premature study termination prevented accrual of the planned 50 patients and the formal assessment

of the primary endpoint. Hence, the activity of chemo-immunotherapy in nonluminal, CDK4/6i-resistant HR+/HER2− mBC cannot formally be claimed, and the presented results are to be considered exploratory and hypothesis generating. Second, trial's nonrandomized design limits comparative conclusions and restricts the strength of biomarker validation. Third, a proportion of prescreening samples were obtained before CDK4/6i progression, potentially limiting their relevance to the phenotype at enrollment. Fourth, gene expression analysis on metastatic tumor biopsies does not account for potential tumor heterogeneity, which may have had an impact on response to immunotherapy. Lastly, PD-L1, TMB, and TIL data were only available in a subset, reducing statistical power for these exploratory analyses.

In conclusion, these exploratory results indicate potential meaningful clinical activity of the combination of pembrolizumab and paclitaxel in a genomically selected subgroup of HR+/HER2− mBC with nonluminal subtypes following CDK4/6i progression. Gene expression features related to luminal differentiation, proliferation, and immunity provided additional prognostic information. These findings support a biomarker-driven strategy in the post-endocrine setting and highlight the value of molecular subtyping for guiding immunotherapy use in HR+/HER2− breast cancer.

## Data Availability

The protocol of the SOLTI-1716 TATEN study is available as Supplementary Note 1. The nCounter raw gene expression data generated in this study, together with the corresponding best objective response for each patient, are provided in Supplementary Table S2. These data are sufficient to reproduce the primary transcriptomic analyses reported in this article. Additional molecular and clinical data used to generate explorative results will be made available upon reasonable request to the corresponding author.

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## Authors' Contributions

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