**SUPPLEMENTARY DATA**

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**Supplement to:** Albanell J *et al.* Palbociclib Rechallenge for Hormone Receptor-Positive/Human Epidermal Growth Factor Receptor-Negative Advanced Breast Cancer: Clinical and Biomarker Findings from the Phase II BioPER Trial

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# SUPPLEMENTARY TABLES

## Supplementary Table S1. Selection Criteria

| **Inclusion Criteria** | **Exclusion Criteria** |
| --- | --- |
| Patients are eligible for inclusion only if they meet ALL of the following criteria:1. Female patients over 18 years of age.
2. Pre-menopausal women provided they are being treated with Luteinizing Hormone-Releasing Hormone (LHRH) analogues for at least 28 days (if shorter LHRH treatment period, post-menopausal estrogen levels must be confirmed on laboratory assessments) prior to study entry or post-menopausal women, as defined by any of the following criteria:
	1. Age 60 or over.
	2. Age 45 to 59 years and meets ≥ 1 of the following criteria:
		1. Amenorrhea for ≥ 24 months.
		2. Amenorrhea for < 24 months and follicle-stimulating hormone within the post-menopausal range (including patients with hysterectomy, prior hormone replacement therapy, or chemotherapy-induced amenorrhea).
	3. Patients with bilateral oophorectomy.
3. Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1.
4. Life expectancy greater or equal to 12 weeks.
5. Histologically confirmed recurrent hormone receptor (HR)-positive (estrogen receptor [ER] and/or progesterone receptor [PR]) and human epidermal growth factor receptor 2 (HER2)-negative, locally advanced or metastatic breast cancer.

*Note: According to NCCN and ASCO guidelines, tumors with ≥1% of tumor cells with positive nuclear staining for ER and PR by immunohistochemistry (IHC) should be considered ER-positive and PR-positive, respectively.*1. Confirmed disease progression on immediate previous regimen of palbociclib plus endocrine therapy.
2. Last dose of palbociclib administered no later than eight weeks and not earlier than three weeks from study entry.
3. Patients must have been treated with a stable dose of palbociclib during the last four weeks in the previous palbociclib line, with the exception of patients treated with the lowest dose of palbociclib (75 mg/day) that must have been treated at least during the last eight weeks with the same dose and should not have experienced any grade 3-4 adverse event related to palbociclib.
4. Clinical benefit –in terms of stable disease ≥ 24 weeks, partial response or complete response– on immediate previous regimen of palbociclib plus endocrine therapy.
5. No prior use of at least one of the reasonable endocrine therapy options: tamoxifen, fulvestrant, letrozole/anastrozole, or exemestane.
6. Measurable disease as per Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1.
7. Patients agree to the collection of blood and metastatic tumor sample at the time of inclusion and disease progression (if feasible).
8. No more than two prior lines of endocrine therapy for metastatic disease.
9. No more than one prior line of chemotherapy for adv metastatic disease.
10. Adequate organ function:
	1. Hematological: White blood cell (WBC) count > 3.0 x 109/L, absolute neutrophil count (ANC) > 1.5 x 109/L, platelet count > 75.0 x109/L, and hemoglobin > 10.0 g/dL (>6.2 mmol/L).
	2. Hepatic: Bilirubin < 1.5 times the upper limit of normal (x ULN); alkaline phosphatase (ALP) < 2.5 times ULN; aspartate transaminase (AST) and alanine transaminase (ALT) < 3 times ULN.

*Note: Patients with ALP ≥ 2.5 times ULN are eligible if ALP abnormalities are unequivocally related to bone lesions (radiological assessments performed within 4 weeks prior to inclusion demonstrated bone metastatic disease).** 1. Renal: Serum creatinine < 1.5 x ULN.
1. Exhibit patient compliance and geographic proximity that allow for adequate follow-up.
2. Patients have been informed about the nature of study, and have agreed to participate in the study, and signed the informed consent form prior to participation in any study-related activities.
3. Resolution of all acute toxic effects of prior anti-cancer therapy or surgical procedures to grade ≤ 1 as determined by the National Cancer Institute (NCI) common terminology criteria for adverse events (CTCAE) version 4.0.3 (except alopecia or other toxicities, not considered a safety risk for the patient at investigator's discretion).
 | Patients will be excluded from the study if they meet ANY of the following criteria:1. HR or HER2 unknown disease.
2. HER2-positive disease based on local laboratory results (performed by IHC/fluorescence in situ hybridization [FISH]).
3. Locally advanced breast cancer candidate for a local treatment with a radical intention.
4. Formal contraindication to endocrine therapy.
5. Progressing central nervous system (CNS) disease.
6. Patients with exclusive non-measurable/evaluable disease.
7. Other malignancies within the past five years except adequately treated basal cell or squamous cell skin cancer or carcinoma *in situ* of the cervix.
8. Major surgery (defined as requiring general anesthesia) or significant traumatic injury within four weeks of start of study drug, or patients who have not recovered from the side effects of any major surgery, or patients that may require major surgery during the course of the study.
9. Patients with an active bleeding diathesis, previous history of bleeding diathesis, or anti-coagulation treatment.

Note: Use of low molecular weight heparin is allowed as soon as it is used as prophylaxis intention.1. Have a serious concomitant systemic disorder (i.e., active infection including HIV, or cardiac disease) incompatible with the study according to the investigator’s criteria.
2. Patients unable to swallow tablets.
3. History of malabsorption syndrome or other condition that would interfere with enteral absorption.
4. Chronic daily treatment with corticosteroids with a dose of ≥ 10 mg/day methylprednisolone equivalent (excluding inhaled steroids).
5. QTc interval > 480 msec on basal assessments, personal history of long or short QT syndrome, Brugada syndrome or known history of QTc prolongation, or Torsade de Pointes (TdP).
6. Uncontrolled electrolyte disorders that can compound the effects of a QTc-prolonging drug (i.e., hypocalcemia, hypokalemia, or hypomagnesemia).
7. Known hypersensitivity to any palbociclib excipients.
8. Participation in the treatment phase of an interventional trial within 30 days prior to study treatment start, with the exception of trials evaluating palbociclib-based treatment.
 |

## Supplementary Table S2. Recruiting Sites, Principal Investigators, and Patient Numbers

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Site ID** | **Recruiting Site** | **Country** | **Principal Investigator** | **Patients per Site** |
| 201 | Hospital Duran i Reynals, ICO l’Hospitalet | Spain | Miguel Gil Gil | 8 |
| 101 | European Institute of Oncology | Italy | Giuseppe Curigliano | 5 |
| 211 | Hospital Universitario Vírgen del Rocío | Spain | Manuel Ruíz Borrego | 4 |
| 202 | Hospital del Mar | Spain | Joan Albanell | 3 |
| 206 | Hospital Universitario La Paz | Spain | Enrique Espinosa | 2 |
| 207 | Hospital Clínicio Universitario de Valencia | Spain | Begoña Bermejo | 2 |
| 210 | Complejo Hospitalario Universitario A Coruña | Spain | Lourdes Calvo | 2 |
| 213 | Hospital Universitario Reina Sofía | Spain | Juan de la Haba | 2 |
| 215 | Vall d’Hebrón Hospital | Spain | Meritxell Bellet | 2 |
| 103 | Azienda Sanitaria Universitaria Integrata di Udine | Italy | Alessandro Minisini | 1 |
| 203 | Hospital Germans Trias i Pujol, ICO Badalona | Spain | Vanessa Quiroga | 1 |
| 208 | Hospital Universitario La Fe | Spain | Ana Santaballa | 1 |
| 102 | Antonio Perrino Hospital | Italy | Saverio Cinieri | 0 |
| 204 | Hospital Universitario 12 de Octubre | Spain | Eva Ciruelos | 0 |
| 205 | Hospital Clínico San Carlos  | Spain | José Ángel García Saenz | 0 |
| 209 | Complejo Hospitalario Universitario de Santiago | Spain | Juan Cueva Bañuelos | 0 |
| 212 | Hospital Universitario de León | Spain | Andrés García Palomo | 0 |
| 214 | Hospital Universitario Miguel Servet | Spain | Antonio Antón | 0 |
| 216 | Hospital Sant Joan de Reus | Spain | Kepa Amilano | 0 |
| 217 | Instituto Valenciano de Oncología | Spain | Joaquín Gávila | 0 |
| 218 | Hospital Provincial de Castellón | Spain | Eduardo Martínez de Dueñas | 0 |
|  |  |  |  |  |
| **Total of Recruiting Sites:** 47 |  | **Total patients included:** | 33 |

## Supplementary Table S3. Study Members

|  |
| --- |
| **Steering Committee Members** |
| Scientific Global Study Coordinator |
| Antonio Llombart-Cussac |
| Clinical Study Coordinator |
| Javier Cortés |
| Exploratory Study CoordinatorJoan Albanell |
| Sponsor Medical Monitor |
| José Manuel Pérez-García |
|  |
| **Pharmacovigilance Responsible** |
| Pharmalex (Actiomed), Zaragoza, Spain |
|  |
| **Sponsor Study Responsible** |
| Leonardo Mina |
|  |
| **Statistician** |
| Miguel Sampayo-Cordero |

## Supplementary Table S4. List of antibodies used for immunohistochemistry analysis

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Antibody** | **Clone** | **Species** | **Vendor** | **Cat#** | **Assay condition** | **Staining evaluation** |
| CDK4 | D9G3E | Rabbit | Cell Signaling | 12790 | 1:300, 60 minutes, RT | Nuclear |
| CDK6 | EPR4515 | Rabbit | Abcam | AB124821 | 1:800, 60 minutes, RT | Nuclear |
| Cyclin D1 | EP12 | Rabbit | Agilent | M3642 | Ready to use, 20 minutes, RT | Nuclear |
| Cyclin E1 | HE12 | Mouse | Cell Signaling | 4129 | 1:50, 60 minutes, RT | Nuclear |
| pRb | D20B12 | Rabbit | Cell Signaling | 8516S | 1:400, 60 minutes, RT | Nuclear |
| Rb | 4H1 | Mouse | Cell Signaling | 9309 | 1:800, 60 minutes, RT | Nuclear |
| Abbreviations: CDK4, Cyclin-dependent kinase 4; CDK6, Cyclin-dependent kinase 6; pRB, Phosphorylated retinoblastoma; RT, Room temperature; Rb, Retinoblastoma. |

## Supplementary Table S5. Cutoff values for the quantitative assessment of biomarkers by immunohistochemistry

|  |  |
| --- | --- |
| **Biomarker**  | **Selected cutoff****(% of stained cells)** |
| CDK4 | 10 |
| CDK6 | 1 |
| Cyclin D1 | 10 |
| Cyclin E1 | 10 |
| pRb | 1 |
| Rb | 1 |
| Abbreviations: CDK4, Cyclin-dependent kinase 4; CDK6, Cyclin-dependent kinase 6; pRB, Phosphorylated retinoblastoma; Rb, Retinoblastoma. |

## Supplementary Table S6. The 77-gene panel by the AVENIO ctDNA Expanded Kit used for targeted NGS on plasma samples

| ***Gene*** | **All coding regions** | **SNV** | **Indel \*** | **Fusion #** | **CNV #** | ***Gene*** | **All coding regions** | **SNV** | **Indel \*** | **Fusion #** | **CNV #** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| *ABL1* |   | • |   |   |   | *JAK3* |   | • |   |   |   |
| *AKT1* |   | • |   |   |   | *KDR* |   | • |   |   |   |
| *AKT2* |   | • |   |   |   | *KEAP1* | • | • |   |   |   |
| *ALK* |   | • | • | • |   | *KIT* |   | • | • |   |   |
| *APC* |   | • | • |   |   | *KRAS* | • | • |   |   |   |
| *AR* | • | • |   |   |   | *MAP2K1* |   | • |   |   |   |
| *ARAF* |   | • |   |   |   | *MET* | • | • | • |   | • |
| *BRAF* |   | • | • |   |   | *MLH1* | • | • |   |   |   |
| *BRCA1* | • | • |   |   |   | *MSH2* | • | • |   |   |   |
| *BRCA2* | • | • |   |   |   | *MSH6* | • | • |   |   |   |
| *CCND1* | • | • |   |   |   | *MTOR* |   | • |   |   |   |
| *CCND2* | • | • |   |   |   | *NF2* | • | • |   |   |   |
| *CCND3* | • | • |   |   |   | *NFE2L2* |   | • |   |   |   |
| *CD274* | • | • |   |   |   | *NRAS* |   | • |   |   |   |
| *CDK4* | • | • |   |   |   | *NTRK1* |   | • |   | • |   |
| *CDK6* |   | • |   |   |   | *PDCD1LG2* | • | • |   |   |   |
| *CDKN2A* | • | • |   |   |   | *PDGFRA* |   | • |   |   |   |
| *CSF1R* |   | • |   |   |   | *PDGFRB* |   | • |   |   |   |
| *CTNNB1* |   | • | • |   |   | *PIK3CA* |   | • | • |   |   |
| *DDR2* |   | • |   |   |   | *PIK3R1* |   | • |   |   |   |
| *DPYD* |   | • |   |   |   | *PMS2* | • | • |   |   |   |
| *EGFR* | • | • | • |   | • | *PTCH1* |   | • |   |   |   |
| *ERBB2* | • | • | • |   | • | *PTEN* | • | • | • |   |   |
| *ESR1* | • | • |   |   |   | *RAF1* |   | • |   |   |   |
| *EZH2* |   | • |   |   |   | *RB1* | • | • |   |   |   |
| *FBXW7* | • | • |   |   |   | *RET* |   | • |   | • |   |
| *FGFR1* |   | • |   |   |   | *RNF43* |   | • |   |   |   |
| *FGFR2* |   | • |   | • |   | *ROS1* |   | • |   | • |   |
| *FGFR3* |   | • |   | • |   | *SMAD4* | • | • |   |   |   |
| *FLT1* |   | • |   |   |   | *SMO* | • | • |   |   |   |
| *FLT3* |   | • |   |   |   | *STK11* | • | • |   |   |   |
| *FLT4* |   | • |   |   |   | *TERT* † |   | • |   |   |   |
| *GATA3* |   | • |   |   |   | *TP53* | • | • |   |   |   |
| *GNA11* |   | • |   |   |   | *TSC1* |   | • | • |   |   |
| *GNAQ* |   | • |   |   |   | *TSC2* |   | • |   |   |   |
| *IDH1* |   | • |   |   |   | *UGT1A1* |   | • |   |   |   |
| *IDH2* |   | • |   |   |   | *VHL* | • | • |   |   |   |
| *JAK2* |   | • |   |   |   |  |  |  |  |  |  |
| Abbreviations: CNV, Copy number variation; Indel, Insertion/deletion; SNV, Single-nucleotide variants.**\*** Indels are limited to variants in a pre-specified list of positions, referred to as "loci of interest", except for *EGFR* exon 19 long deletions, *EGFR* exon 20 long insertions, and *MET* long insertions, which are not restricted to a pre-defined set of Indels.**#** Detection of Fusions and CNVs are limited to variants in a pre-specified list of positions, referred to as "loci of interest" in the AVENIO analysis software.**†** *TERT* promoter region only. |

## Supplementary Table S7. Representativeness of study participants

|  |  |
| --- | --- |
| Cancer type(s)/subtype(s)/stage(s)/condition | Hormone Receptor-positive/Human Epidermal Growth Factor Receptor 2-negative (HR-/HER2+) unresectable locally advanced not amenable to surgical resection or radiotherapy with curative intent or metastatic breast cancer. |
| Considerations related to: |
| Sex | HR+/HER2-, as all other subtypes of breast cancer, is a predominantly female disease and is rare in men.HR+/HER2- incidence is around 70-80% among all breast cancer subtypes in the overall largely female population.Men account for around 1% of all new diagnoses of this disease and in 15-20% of cases there is a family history that allows a diagnosis of hereditary cancer. |
| Age | This type of tumor usually appears between the ages of 35 and 80, although the age range of 45-65 is the one with the highest incidence, as this is the time when hormonal changes occur in the peri- and post-menopausal periods, an incidence curve that continues to rise as women age. |
| Race/ethnicity | White women are slightly more likely to develop breast cancer than Black, Hispanic, and Asian women. But Black women are more likely to develop more aggressive, more advanced-stage breast cancer that is diagnosed at a young age. |
| Geography | It is estimated that breast cancer accounts for 13.3% of all new cancer cases diagnosed in European Union countries in 2020. This makes it the most frequently occurring cancer. It is estimated that it accounts for 28.7% of all new cancers in women.According to the latest data collected by the European Cancer Information System (ECIS), in 2020 a total of 34,088 new cases of breast cancer were diagnosed in Spain, this type of tumor being the most frequent among women in this country. |
| Overall representativeness of this study | The age distribution of our study is similar to the average age distribution of TNBC in the literature, median age of 59.5.Our study population was limited by the geographic distribution of study sites across 21 study sites in Spain and Italy. Thus, all the included patients were White/Caucasian.Male patients were not allowed to participate to this study. |

## Supplementary Table S8. Association of baseline clinical and pathologic characteristics of trial participants included in the efficacy analysis with clinical benefit.

| **Characteristic** | **Efficacy analysis****(*n* = 32)** | **Clinical benefit****(*n* = 11)** | **No clinical benefit****(*n* = 21)** | ***P* value** | **Effect size (95% CI)** |
| --- | --- | --- | --- | --- | --- |
| Age (years), mean (SD) | 60.7 (10.5) | 64.0 (8.9) | 59.0 (11.0) | 0.18 | 4.0 (-2.4–12.4) |
| ECOG performance status |  |  |  |  |  |
| 0 | 14 (43.8) | 8 (57.1) | 6 (42.9) | **0.016** | 44.2 (7.8–67.1) |
| 1 | 18 (56.2) | 3 (16.6) | 15 (83.4) |  |  |
| Estrogen Receptor |   |   |   |   |   |
| 1–10% of stained cells | 1 (3.1) | 0 | 1 (4.8) | 0.35 | 4.7 (-21.4–22.7) |
| >10% of stained cells | 31 (96.9) | 11 (100) | 20 (95.2) |   |   |
| Progesterone Receptor |  |  |  |   |   |
| 1–10% of stained cells | 11 (34.4) | 2 (18.2) | 9 (42.9) | 0.15 | 15.6 (-19.0–42.6) |
| >10% of stained cells | 20 (62.5) | 8 (72.7) | 12 (57.1) |   |   |
| Not evaluated | 1 (3.1) | 1 (9.1) | 0 |   |   |
| Visceral involvement |  |  |  |  |  |
| Yes | 25 (78.1) | 7 (28.0) | 18 (72.0) | 0.16 | -22.1 (-51.8–7.3) |
| No | 7 (21.9) | 4 (57.1) | 3 (42.9) |  |  |
| Number of disease sites |  |  |  |  |  |
| <3 | 13 (40.6) | 5 (38.5) | 8 (61.5) | 0.69 | 7.4 (-24.7–39.1) |
| ≥3 | 19 (59.4) | 6 (31.6) | 13 (68.4) |  |  |
| Previous regimens |  |  |  |  |  |
| (Neo)Adjuvant ET |  |  |  |  |  |
| Yes | 15 (46.9) | 5 (33.3) | 10 (66.7) | 0.91 | 2.6 (-30.6–33.6) |
| No | 17 (53.1) | 6 (35.3) | 11 (64.7) |  |  |
| (Neo)Adjuvant CT |  |  |  |  |  |
| Yes | 15 (46.9) | 4 (26.7) | 11 (73.3) | 0.39 | 16.0 (-18.6–44.7) |
| No | 17 (53.1) | 7 (41.2) | 10 (58.8) |  |  |
| CT for MBC |  |  |  |  |  |
| Yes | 4 (12.5) | 1 (25.0) | 3 (75.0) | 0.67 | 1.7 (0.2–36.2) |
| No | 28 (87.5) | 10 (35.7) | 18 (64.3) |  |  |
| Lines of prior ET for MBC \* |  |  |  |  |  |
| 0 | 27 (84.4) | 9 (33.3) | 18 (66.7) | 0.78 | 3.9 (-20.3–34.8) |
| 1 | 5 (15.6) | 2 (40.0) | 3 (60.0) |  |  |
| ET agent used in prior palbociclib-based regimen |  |  |  |  |  |
| Fulvestrant | 14 (43.8) | 4 (28.6) | 10 (71.4) | 0.47 | Reference |
| Letrozole | 15 (46.9) | 5 (33.3) | 10 (66.7) |  | -2.2 (-33.6–30.6) |
| Exemestane | 3 (9.4) | 2 (66.7) | 1 (33.3) |  | 13.4 (-8.7–43.2) |
| PFS for the prior palbociclib-based regimen (months), mean (SD) | 18.4 (10.7) | 21.2 (14.0) | 16.9 (8.4) | 0.37 | 4.3 (-5.6–14.1) |
| Lines of previous systemic therapy for MBC † |  |  |  |  |  |
| 1 | 24 (75.0) | 8 (33.3) | 16 (66.7) | 0.51 | Reference |
| 2 | 6 (18.8) | 3 (50.0) | 3 (50.0) |  | 12.9 (-13.9–43.7) |
| 3 | 1 (3.1) | 0 | 1 (100) |  | -4.8 (-22.7–21.4) |
| 4 | 1 (3.1) | 0 | 1 (100) |  | -4.8 (-22.7–21.4) |
| ET agent used in the current regimen |  |  |  |  |  |
| Letrozole | 9 (28.1) | 4 (36.4) | 5 (23.8) | 0.19 | Reference |
| Fulvestrant | 18 (56.2) | 7 (63.6) | 11 (52.4) |  | 11.3 (-22.9–40.4) |
| Exemestane | 3 (9.4) | 0 | 3 (14.3) |  | -14.3 (-34.6–13.2) |
| Others | 2 (6.3) | 0 | 2 (9.5) |  | -9.5 (-28.9–17.3) |
|  |  |  |  |  |  |
| Starting dose of palbociclib in the current regimen |  |  |  |  |  |
| 75/100 mg | 9 (28.1) | 1 (9.1) | 8 (38.1) | *0.065* | 29.0 (-4.4–51.3) |
| 125 mg | 23 (71.9) | 10 (90.9) | 13 (61.9) |   |   |
| Abbreviations: CI, Confidence interval; CT, Chemotherapy; ECOG, Eastern Cooperative Oncology Group; ET, Endocrine therapy; MBC, Metastatic breast cancer; PFS, Progression-free survival; SD, Standard deviation.Note: Mean differences have been compared with the T-test. The effect size has been estimated with the likelihood ratio test. The 95% confidence interval for the difference proportions has been estimated with Newcombe method. *P* values in bold show statistical significance at 5% alpha level and those in italics show trend to statistical significance at 1% alpha level.Data are n (%), unless otherwise specified. |

## Supplementary Table S9. Relative dose intensity and drug discontinuation

|  |  |
| --- | --- |
| **Characteristic** | **All patients****(*n* = 33)** |
| Median relative dose intensity (PCT25–PCT75) |  |
| Palbociclib | 100 (96.1–100) |
| Endocrine therapy | 100 (89.1–100) |
| Fulvestrant (*n* = 19) | 100 (89.1–100) |
| Letrozole (*n* = 9) | 99.4 (96.9–100) |
| Exemestane (*n* = 3) | 100 (98.2–100) |
| Anastrozole (*n* = 1) | 97.5 |
| Tamoxifen (*n* = 1) | 100 |
| Treatment discontinuation | 30 (90.9) |
| Disease progression | 29 (87.9) |
| Physician’s decision a | 1 (3.0) |
| Ongoing treatment at data cut-off | 3 (9.1) |
| Dose reduction for palbociclib | 1 (3.0) |
| Dose delayed for the combination | 14 (42.4) |
| Abbreviations: PCT25, percentile 25; PCT75, percentile 75.a The patient excluded from efficacy analysis because did not achieve clinical benefit on prior palbociclib-based regimen was discontinued by disease progression.Data are n (%), unless otherwise specified. |

## Supplementary Table S10. All causality AEs according to NCI-CTCAE version 4.0.3 occurring in at least 5% of the patients

|  |  |  |  |
| --- | --- | --- | --- |
| **Adverse event** | **Any Grade****(*n* = 33)** | **Grade 3** | **Grade 4** |
| **Any** | 30 (90.9) | 15 (45.5) | 2 (6.1) |
| **Hematologic**  | 20 (60.6) | 14 (42.4) | 1 (3.0) |
| Neutropenia | 19 (57.2) | 13 (39.4) | 1 (3.0) |
| Leukopenia | 6 (18.2) | 2 (6.1) | 0 |
| Anemia | 6 (18.2) | 1 (3.0) | 0 |
| Thrombocytopenia | 2 (6.1) | 0 | 0 |
| Lymphopenia | 2 (6.1) | 0 | 0 |
| **Non-Hematologic** | 23 (69.7) | 4 (12.1) | 1 (3.0) |
| Fatigue | 10 (30.3) | 0 | 0 |
| Asthenia | 5 (15.2) | 0 | 0 |
| Diarrhea | 4 (12.1) | 0 | 0 |
| Musculoskeletal pain | 3 (9.1) | 0 | 0 |
| Hot flux | 3 (9.1) | 0 | 0 |
| Arthralgia | 2 (6.1) | 0 | 0 |
| Alanine aminotransferase increased | 2 (6.1) | 0 | 0 |
| Decreased appetite | 2 (6.1) | 0 | 0 |
| Abbreviations: AEs, Adverse events; NCI-CTCAE, National Cancer Institute-Common Terminology Criteria for Adverse Events.Note: No death due to adverse events was reported.Data are n (%), unless otherwise specified. |

## Supplementary Table S11. Adverse events of special interest according to NCI-CTCAE version 4.0.3.

|  |  |  |
| --- | --- | --- |
| **Adverse event** | **Any** | **Grade 3** |
| Pulmonary embolism a  | 1 (3.0) | 1 (3.0) |
| Abbreviations: AEs, Adverse events; NCI-CTCAE, National Cancer Institute-Common Terminology Criteria for Adverse Events.a Adverse event reported during 3-monthly radiological assessment by computed tomography scan. As per investigator criteria, it was not related to progressive disease. Patient was treated with low molecular weight heparin (enoxaparin), that was continuing after the date of final data cut-off.Data are n (%), unless otherwise specified. |

## Supplementary Table S12. Baseline clinical and pathologic characteristics of trial participants included in the retinoblastoma and signature subsets

| **Characteristic** | **Retinoblastoma subset****(*n* = 23)** | **Signature subset****(*n* = 17)** |
| --- | --- | --- |
| Age (years), median (range) | 62.0 (43.0-79.0) | 61.0 (43.0-79.0) |
| ECOG performance status |  |  |
| 0 | 8 (34.8) | 4 (23.5) |
| 1 | 16 (65.2) | 13 (76.5) |
| Visceral involvement |  |  |
| No | 5 (21.7) | 4 (23.5) |
| Yes | 18 (78.3) | 13 (76.5) |
| Number of disease sites |  |  |
| <3 | 10 (43.5) | 6 (35.3) |
| ≥ 3 | 13 (56.5) | 11 (64.7) |
| Previous regimens |  |  |
| (Neo)Adjuvant ET | 8 (34.8) | 7 (41.2) |
| (Neo)Adjuvant CT | 8 (34.8) | 7 (41.2) |
| Lines of prior ET for MBC \* |  |  |
| 0 | 20 (87.0) | 21 (88.2) |
| 1 | 3 (13.0) | 2 (11.8) |
| ET agent used in prior palbociclib-based regimen |  |  |
| Letrozole | 11 (47.8) | 8 (47.1) |
| Fulvestrant | 9 (39.1) | 7 (41.2) |
| Exemestane | 3 (13.0) | 2 (11.8) |
| PFS for the prior palbociclib-based regimen (months), median (range) | 13.9 (7.4-47.1) | 14.5 (9.9-47.1) |
| Lines of previous systemic therapy for MBC, n (range) † |  |  |
| 1 | 19 (82.6) | 14 (82.4) |
| 2 | 4 (17.4) | 3 (17.6) |
| ET agent used in the current regimen |  |  |
| Fulvestrant | 13 (56.5) | 10 (58.8) |
| Letrozole | 7 (30.4) | 5 (29.4) |
| Others | 3 (13.0) | 2 (1.8) |
| Starting dose of palbociclib in the current regimen |  |  |
| 125 mg | 17 (73.9) | 11 (64.7%) |
| 100 mg | 5 (21.7) | 5 (29.4%) |
| 75 mg | 1 (4.3) | 1 (5.9) |
| Abbreviations: CI, Confidence interval; CT, Chemotherapy; ECOG, Eastern Cooperative Oncology Group; ET, Endocrine therapy; MBC, Metastatic breast cancer; PFS, Progression-free survival.\* Excluding the prior palbociclib-based regimen.† Including the prior palbociclib-based regimen.Data are n (%), unless otherwise specified. |

## Supplementary Table S13. Tumor best response according to RECIST version 1.1 of trial participants included in the retinoblastoma and signature subsets

|  |  |  |
| --- | --- | --- |
| **Characteristic** | **Retinoblastoma subset****(*n* = 23)** | **Signature subset****(*n* = 17)** |
|  | **No. (%)** | **95% CI** | **No. (%)** | **95% CI** |
| CBR | 7 (30.4) | 13.2-52.9 | 4 (23.5) | 6.8-49.9 |
|  |  |  |  |  |
| Best Response |  |  |  |  |
| CR | 0 | - | 0 | - |
| PR | 1 (4.3) | 0-21.9 | 0 | - |
| SD ≥ 24 weeks | 6 (26.1) | 10.2-48.4 | 4 (23.5) | 7-49.9 |
| PD | 15 (65.2) | 42.7-83.6 | 12 (70.6) | 44.0-89.7 |
| NE | 1 (4.3) | 0-21.9 | 1 (5.9) | 0.1-28.7 |
|  |  |  |  |  |
| ORR | 1 (4.3) | 0-21.9 | 0 | - |
|  |  |  |  |  |
| DoR, median (range) | 36.7 (-) |  | - |  |
|  |  |  |  |  |
| PFS, median (months) | 2.8 | 1.8–6.7 | 2.8 | 1.8–5.5 |
|  |  |  |  |  |

Abbreviations: CBR, Clinical benefit rate; CR, Complete response; DoR, Duration of response in patients with response; ORR, Overall response rate; PD, Progressive disease; PFS, Progression-free survival; PR, Partial response; RECIST, Response Evaluation Criteria in Solid Tumors; SD, Stable disease.

Note: Clinical response was evaluated in patients with measurable disease at baseline as per RECIST version 1.1 who received at least one cycle of study treatment. Clinical responses were confirmed at the subsequent tumor assessment as per RECIST version 1.1.

Data are n (%), unless otherwise specified.

## Supplementary Table S14. Association of biomarkers and composite signatures with clinical benefit.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Characteristic** | **Efficacy analysis****(*n* = 32)** | **Clinical benefit****(*n* = 11)** | **No clinical benefit****(*n* = 21)** | ***P* value** | **Effect size (95% CI)** |
| CDK4 | *n* = 23 | *n* = 7 | *n* =16 |  |  |
| Mean (SD) | 25.9 (22.9) | 22.9 (12.9) | 27.2 (26.4) | 0.6 | -4.34 (-21.4–12.7) |
| CDK6 | *n* = 25 | *n* = 8 | *n* = 17 |  |  |
| Mean (SD) |  1.1 (3.0) | 2.1 (5.2) | 0.6 (0.9) | 0.46 | 1.5 (-2.9–5.8) |
| Cyclin D1 | *n* = 24 | *n* = 8 | *n* = 16 |  |  |
| Mean (SD) | 72.9 (30.3) | 71.9 (28.0) | 73.4 (32.3) | 0.91 | -1.5 (-28.5–25.6) |
| Cyclin E1 | *n* = 23 | *n* = 7 | *n* = 16 |  |  |
| Mean (SD) | 11.4 (15.1) | 4.1 (1.7) | 14.6 (17.2) | **0.029** | -10.5 (-19.7–1.2) |
| pRb | *n* = 25 | *n* = 8 | *n* = 17 |  |  |
| Mean (SD) | 23.0 (21.8) | 23.8 (24.9) | 22.6 (21.1) | 0.91 | 1.2 (-20.8–23.4) |
| Rb | *n* = 23 | *n* = 7 | *n* = 16 |  |  |
| Mean (SD) | 52.7 (30.5) | 59.7 (22.1) | 49.6 (33.7) | 0.4 | 10.1 (-14.8–35.1) |
| PAM50 intrinsic subtype | *n* = 16 | *n* = 5 | *n* = 11 |  |  |
| Luminal A | 2 (12.5) | 1 (20.0) | 1 (9.1) | 0.57 | Reference |
| Luminal B | 8 (50.0) | 3 (60.0) | 5 (45.5) |  | 14.5 (-30.9–51.7) |
| HER2-enriched | 6 (37.5) | 1 (20.0) | 5 (45.5) |  | -25.4 **(**-56.6–23.4**)** |
| Mutation number on ctDNA | *n* = 21 | *n* = 6 | *n* = 15 |  |  |
| Mean (SD) | 3.1 (2.0) | 2.0 (0.9) | 3.5 (2.2) | **0.033** | 1.5 (-2.9– -0.13) |
| *ESR1* | *n* = 25 | *n* = 7 | *n* = 18 |  |  |
| Wild-type | 12 (48.0) | 6 (85.7) | 6 (33.3) | **0.015** | -52.4 (-73.1– -8.8) |
| Mutated | 13 (52.0) | 1 (14.3) | 12 (66.7) |  |  |
| *TP53* | *n* = 25 | *n* = 7 | *n* = 18 |   |   |
| Wild-type | 21 (81.0) | 5 (71.4) | 16 (88.9) | 0.31 | 17.5 (-12.3–53.9) |
| Mutated | 4 (19.0) | 2 (28.6) | 2 (11.1) |   |   |
| *ERBB2* | *n* = 25 | *n* = 7 | *n* = 18 |   |   |
| Wild-type | 22 (88.0) | 7 (100) | 15 (83.3) | 0.14 | -1.6 **(**-39.2–20.4**)** |
| Mutated | 3 (12.0) | 0 | 3 (16.7) |   |   |
| *MET* | *n* = 25 | *n* = 7 | *n* = 18 |   |   |
| Wild-type | 22 (88.0) | 7 (100) | 15 (83.3) | 0.14 | -1.6 (-39.2–20.4) |
| Mutated | 3 (12.0) | 0 | 3 (16.7) |   |   |
| *PIK3CA*  | *n* = 25 | *n* = 7 | *n* = 18 |  |  |
| Wild-type | 22 (88.0) | 6 (85.7) | 16 (88.9) | 0.83 | 3.1 (-21.4–41.1) |
| Mutated | 3 (12.0) | 1 (14.3) | 2 (11.1) |  |  |
| High cyclin E1 score/Low Rb score | *n* = 17 | *n* = 4 | *n* = 13 |  |  |
| No | 9 (52.9) | 4 (100) | 5 (38.5) | **0.01** | -61.5 (-82.3– -6.1) |
| Yes | 8 (47.1) | 0 | 8 (61.5) |   |  |
| High cyclin E1 score/Low Rb score/*ESR1* mutated | *n* = 17 | *n* = 4 | *n* = 13 |  |  |
| No | 4 (23.5) | 3 (75.0) | 1 (7.7) | **0.008** | -67.3 (-88.7– -15.5) |
| Yes | 13 (76.4) | 1 (25.0) | 12 (92.3) |  |  |
| High cyclin E1 score/Low Rb score/HER2-enriched | *n* = 17 | *n* = 4 | *n* = 13 |  |  |
| No | 7 (41.2) | 3 (75.0) | 4 (30.8) | 0.11 | -44.2 (-71.5–8.1) |
| Yes | 10 (58.8) | 1 (25.0) | 9 (69.2) |  |  |
| High cyclin E1 score/Low Rb score/*ESR1* mutated/HER2-enriched | *n* = 17 | *n* = 4 | *n* = 13 |  |  |
| No | 3 (17.6) | 2 (50.0) | 1 (7.7) | *0.07* | -42.3 (-77.9–1.1) |
| Yes | 14 (82.4) | 2 (50.0) | 12 (92.3) |  |  |

Abbreviations: CI, Confidence interval; CT, Chemotherapy; ECOG, Eastern Cooperative Oncology Group; ET, Endocrine therapy; IQR: Interquartile range; MBC, Metastatic breast cancer; PFS, Progression-free survival.

Note: Mean differences have been compared with the T-test. The effect size has been estimated with the likelihood ratio test. The 95% confidence interval for the difference proportions has been estimated with Newcombe method. *P* values in bold show statistical significance at 5% alpha level and those in italics show trend to statistical significance at 1% alpha level.

Data are n (%), unless otherwise specified.

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