PIK3CA mutations, biomarkers of endocrine resistance, and the effect of palbociclib plus fulvestrant versus placebo plus fulvestrant in women with hormone-receptor–positive, HER2-negative metastatic breast cancer that progressed on prior endocrine therapy (PALOMA-3): a multicentre, double-blind, randomised phase 3 trial

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Keywords: palbociclib; metastatic breast cancer; cyclin-dependent kinases 4 and 6; endocrine therapy

Brief title: PALOMA-3 Updated Efficacy and Safety

Summary

Background Cyclin-dependent kinase (CDK) 4/6 inhibitor palbociclib, when added to fulvestrant in the PALOMA-3 study, significantly improved progression-free survival (PFS) in patients with metastatic breast cancer (MBC). Identification of patients most suitable for the addition of palbociclib to endocrine therapy (ET) after tumour recurrence is critical for treatment optimisation in MBC. We aimed to assess which of the common types of endocrine resistance would be most suitable for treatment with palbociclib in combination with fulvestrant.

Methods In this multicentre, double-blind, randomized phase 3 superiority study, patients with hormone-receptor–positive/HER2-negative MBC, that had progressed on prior ET, were stratified by sensitivity to prior hormonal therapy, menopausal status, and presence of visceral metastasis. Patients were randomly assigned (2:1) to receive oral palbociclib (125 mg daily for 3 weeks followed by 1 week off over 28 day cycles) plus fulvestrant 500 mg (intramuscular injection on days 1 and 15 of cycle 1; then on day 1 of subsequent 28-day cycles) (palbociclib group) or placebo plus fulvestrant (control). The primary endpoint was investigator-assessed PFS in the intention-to-treat population. Study enrolment is closed and overall survival follow-up is in progress. ET resistance by clinical parameters, centrally assessed quantitative hormone receptor expression, and tumour *PIK3CA* mutational status in circulating DNA at baseline were analysed. This study is registered with ClinicalTrials.gov NCT01942135.

Findings Between Oct 7, 2013, and Aug 26, 2014, 521 patients were randomly assigned, 347 to the palbociclib plus fulvestrant and 174 to placebo plus fulvestrant. By

March 2015, 259 PFS events had occurred; median follow-up was 8·9 months (IQR 8·7–9·2). Median PFS was 9·5 months (95% CI 9·2–11·0) for the palbociclib group and 4·6 months (3·5–5·6) for the control group (HR 0·46, [0·36–0·59], p<0·0001). A significantly higher confirmed response rate (intention-to-treat: 19·0% *vs* 8·6%, p=0·002; patients with measurable disease: 24·6% *vs* 10·9%, p<0·001) and clinical benefit rate (intention-to-treat: 66·6% *vs* 39·7%, p<0·0001) was noted. Grade 3/4 adverse events (including asymptomatic neutropenia and leucopenia) occurred in 251/345 patients [72·8%] in the palbociclib group and 38/172 patients [22·1%] in the control group. Palbociclib benefit was consistent for all predefined subsets. *PIK3CA* mutation was detected in the plasma DNA of 129 (33%) of 395 patients. No difference in the magnitude of benefit from palbociclib was noted by *PIK3CA* status (HR=0·45 for *PIK3CA* wild-type, HR=0·48 for *PIK3CA* mutation positive, interaction p=0·83) or hormone receptor expression (Supremum interaction p=0·32 for ER and p=0·54 for PR).

Interpretation Palbociclib plus fulvestrant showed meaningful and consistent improvement in PFS, irrespective of the degree of endocrine resistance, hormone expression levels, and *PIK3CA* mutational status. The combination provides an effective and well-tolerated therapeutic option for patients with recurrent hormone-receptor–positive MBC who have progressed on prior endocrine therapy.

Funding: Pfizer.

Introduction

Management of resistance to endocrine therapy is among the most challenging aspects of breast cancer treatment and an active area of research.[1] A number of publications have suggested that responsiveness to endocrine therapy may be associated with oestrogen receptor (ER) expression levels in hormonal-receptor–positive breast cancer, and as such a temporal relationship between them has been reported.[2,3] Studies of resistance to hormonal therapies and ER biology have highlighted the fundamental role of signalling pathway crosstalk with ERs and acquisition of *ESR-1* mutations.[4-6] Preclinical models indicate that an adaptive upregulation of growth factor signalling is associated with acquired and *de novo* resistance to endocrine therapies.[7,8] As an example, the phosphatidylinositol-3-kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) pathway interacts directly and indirectly with ER, and activation of this pathway through mutations of *PIK3CA*/*AKT* confer resistance to selective ER modulators (SERMs) and ER downregulators such as fulvestrant.[9] However, the clinical implications of modulating this pathway by combining PI3K inhibitors with ER modulators or degraders, particularly fulvestrant, are still unclear, with many studies ongoing.

Activation of the cyclin-dependent kinase (CDK) 4/CDK 6/E2F axis is a common feature of luminal ER-positive breast cancer. Hormonal therapies function in part through suppressing CDK4/6 activity, and reactivation of CDK4/6 has been implicated in endocrine resistance.[10] Luminal breast cancer models showed sensitivity to CDK4/6 inhibition as single agent and therapeutic synergy with hormonal blockade in both endocrine therapy–naïve and endocrine resistant preclinical models.[11-13] Combined

CDK4/6 inhibition with fulvestrant produced increased penetrating inhibition of retinoblastoma tumour suppressor protein phosphorylation, leading to durable cell cycle arrest and increased markers of cellular senescence, supporting the clinical investigation of this combination.[11-14]

Palbociclib (PD-0332991) is an orally bioavailable selective inhibitor of CDK4/6 that prevents DNA synthesis by blocking progression of the cell cycle from G1 to S phase[15,16] and that has demonstrated efficacy as first-line treatment of postmenopausal metastatic breast cancer (MBC).[17] A phase 3 trial (PALOMA-3) comparing palbociclib and fulvestrant versus fulvestrant and placebo in women with MBC, irrespective of menopausal status and line of therapy, established an improved hazard ratio (HR) of 0·42 in progression-free survival (PFS) at the first interim analysis.[18] This report, with extended follow-up, now focuses on the analysis of various aspects of endocrine resistance, including degree of tumour ER expression, responsiveness to previous endocrine therapy, and number and type of previous therapies. We analysed the impact of *PIK3CA* mutations in the plasma as the most common potentially targetable mutation in ER-positive breast cancer. The planned analysis of clinical and safety endpoints is included, allowing for a comprehensive conclusion regarding response and clinical benefit data and a complete evaluation of the role of palbociclib in the management of hormone-receptor–positive MBC.

Methods

Study design and participants

PALOMA-3 is a prospective, randomised, double-blind, placebo-controlled phase 3 trial performed in 144 centers in 17 countries (appendix p1) and the study design has been described elsewhere.[18] Briefly, all eligible patients had confirmed hormone-receptor–positive, human epidermal growth factor receptor 2 (HER2)-negative advanced breast cancer. Provision of tumour tissue was required for patient participation. Eligible patients were women aged 18 years or older of any menopausal status and with Eastern Cooperative Oncology Group Performance Status 0–1, who had disease measurable by RECIST (version 1.1) or bone disease only. Disease relapse or progression had to occur after prior endocrine therapy (with an aromatase inhibitor [AI] if patient was postmenopausal or with tamoxifen if premenopausal or perimenopausal) in the advanced setting or during treatment or within 12 months of completion of adjuvant therapy irrespective of menopausal status. One prior line of chemotherapy in the advanced setting was allowed. Patients were excluded from the study if they had previously received any CDK inhibitor, fulvestrant, everolimus, or PI3K/mTOR pathway inhibitor; or had extensive metastatic, symptomatic, visceral spread who were at the risk of life-theartening complications in the short-term, or had uncontrolled central nervous system metastases. All patients provided written, informed consent before enrolment. The study protocol was approved by Independent Institutional Review Boards at all participating centres. The study was conducted in accordance with Good Clinical Practice standards and the Declaration of Helsinki.

Randomisation and masking

Eligible patients were randomly assigned (double-blind) to receive palbociclib plus fulvestrant or placebo plus fulvestrant in a 2:1 ratio by the investigator or their designee

using a centralized interactive web- and voice-based randomisation system to register and randomly assign patients using three stratification factors: sensitivity to prior hormonal therapy (defined as a documented clinical benefit to at least one prior endocrine therapy in the metastatic setting or treatment with at least 24 months of adjuvant therapy before disease recurrence), menopausal status at study entry (postmenopausal vs premenopausal or perimenopausal), and presence of visceral metastases (ie, lung, liver, brain, pleural, and peritoneal involvement). The randomisation system generated the random assignment of the two treatments in a block size of six for each of the stratification levels. This was a double-blind study in which study participants, and investigators or their designee, were masked to treatment group assignment. Sponsor personnel or designees involved in the study design and data analysis were also masked to treatment group assignment until the Independent Data Monitoring Committee (IDMC) recommended stopping the study at the pre-planned interim analysis.

Procedures

Patients received fulvestrant 500 mg intramuscular injection on days 1 and 15 of cycle 1 and then every 4 weeks ±7 days in subsequent cycles. Patients were also administered oral palbociclib 125 mg (palbociclib group) or an identical placebo (control group) once daily for 3 weeks followed by 1 week off in a 28-day cycle. Study treatment continued until disease progression, unacceptable toxicity, withdrawal of consent, or death. All premenopausal or perimenopausal women were required to have commenced treatment with a luteinizing hormone–releasing hormone agonist at least 4 weeks before randomisation. During the treatment period, all premenopausal or perimenopausal

women received goserelin at the time of fulvestrant administration. Every effort was made to keep to the planned schedule and dose. Patients who experienced toxicity related to the investigational product were allowed to have palbociclib/placebo dose interruption, dose reduction, or dose delay per predefined dose modification strategy (appendix p16). Fulvestrant dose reduction was not allowed. Crossover between treatment arms was not allowed.

Tumour assessment was at baseline and every 8 weeks (±7 days) with computed tomography (CT) and/or magnetic resonance imaging (MRI). Patients with bone lesions as the only site of disease at baseline received a follow-up x-ray, CT, or MRI every 8 weeks (±7 days) during active treatment for the first year and, thereafter, every 12 weeks (±7 days) from the date of randomisation and to confirm complete response. Assessment of adverse events included incidence and severity (graded by National Cancer Institute Common Terminology Criteria, version 4.0), timing, seriousness, and relatedness to the study treatment. Haematologic and blood chemistry analyses were performed on day 1 and day 15 for the first 2 cycles and then on day 1 of each subsequent cycle.

Tumour tissue was obtained from a biopsy of recurrent disease in all patients except those with bone-only disease, in which case primary tissue was obtained. Archived formalin-fixed paraffin-embedded specimens were collected. If archival tissue was not available, a *de novo* biopsy was required for patient participation. Plasma samples were also collected on day 1 of cycle 1, day 15 of cycle 1, and at the end of treatment.

Local assessment of ER-positive and/or progesterone receptor [PR]–positive tumour and HER2-negative tumour was via an assay consistent with local standards. Hormone receptor status (ER and/or PR expression) and HER2 status were assessed centrally at a Clinical Laboratory Improvement Amendments–certified laboratory using validated ER (DAKO 1D5 antibody; DAKO, Glosrup, Denmark) and PR (DAKO 1294 antibody) assays and HER2 assay (HercepTest, DAKO, and PathVysion HER2 DNA Probe Kit, Abbott Molecular, Des Plaines, IL, USA). H-score methodology was used for ER and PR expression reporting (scale range: 0–300). For central laboratory analyses, if the H-score was ≥1% then the result was positive. Assessment of HER2-positive status was via immunohistochemistry (IHC) using the HercepTest (range 0–3+). For HER2 results determined using the IHC method: 0 and 1+ were classified as negative, 2+ as equivocal, and 3+ as positive. FISH tests were used to confirm HER2 status when IHC results were equivocal. If the IHC score was 2+, fluorescence in situ hybridisation by PathVysion was required.

Circulating free DNA (cfDNA) was isolated from baseline day 1 of cycle 1 plasma samples using QIAamp circulating free nucleic acids purification kit (Qiagen, Venlo, Netherlands) from 396 patients with samples available. *PIK3CA* mutation detection using BEAMing assays were conducted by Sysmex Inostics, Inc. (Baltimore, MD, USA) with assays against Exon 9 1624G>A E542K; Exon 9 1633G>A E545K; Exon 20 3140A>G H1047R; Exon 20 3140A>T H1047L mutations. The detection limit of the BEAMing assay is 0·02% (allele frequency). BEAMing, first described by Dressman et al.,[19] has been shown by Higgins et al. to demonstrate 100% concordance between

PIK3CA mutation detection in tissue samples and circulating tumour DNA when the BEAMing approach was applied to both sample types.[20]

Outcomes

The primary endpoint was investigator-assessed PFS according to Response Evaluation Criteria in Solid Tumors (RECIST version 1.1), calculated as the time from randomisation to radiologic disease progression or death on study. Secondary efficacy endpoints were as follows: confirmed objective response, defined as complete response or partial response according to RECIST version 1.1; clinical benefit response, defined as complete response or progressive disease or stable disease ≥24 weeks; duration of response; tumour tissue biomarkers, including genes (eg, *PIK3CA* mutations), proteins (eg, ER and PR quantitiative expression), RNA expression; safety.

Other prespecified secondary endpoints not reported here include overall survival, survival probabilities at 1, 2, and 3 years, duration of response, patient reported outcomes, and pharmacokinetics.[18]

To correlate the analysis results of the primary endpoint, an audit approach, using a random-sample-based, blinded, independent central review were used. 211 (40%) of 521 patients were selected after enrolment completion and blinded central radiography assessment were performed.

Statistical analysis

The study was designed to test the null hypothesis that the true PFS distributions for both palbociclib plus fulvestrant and placebo plus fulvestrant arms were the same with a

median PFS 6·0 months versus the alternative hypothesis that the true PFS distribution of palbociclib plus fulvestrant arm had a median that was longer than 6·0 months.

The median PFS for the control arm in this study was assumed to be 6·0 months. A total of 238 PFS events were required in the two treatment arms (2:1 randomization) for the study to have a 90% power to detect clinically meaningful improvement in median PFS from 6·0 to 9·38 months (56%; corresponding to a HR=0·64), if tested at a one-sided significance level of alpha=0·025.

The study was planned to have one interim analysis (cutoff date of December 5, 2014) at which time the IDMC recommended stopping the study early because of significant efficacy as the study crossed the prespecified Haybittle-Peto efficacy stopping boundary (α=0.00135). The statistical analyses in this report were conducted from updated data, with a cut-off date of March 16, 2015, after 259 PFS events were reached to support the results of initial interim analysis. It is important to note that at the time of the current analysis (March 16, 2015), IDMC's decision was not known yet, hence all trial procedures followed the original statistical analysis plan. Two-sided p values were used for efficacy analyses comparing treatment arms and interaction tests associated with the subgroup factors. All the statistical analysis results (including the p values) should be considered exploratory.

The enrolment of patients for the PALOMA-3 study was completed before IDMC interim analysis decision was made. Therefore, the manuscript contains all data (up to the data cutoff date of Mar 16, 2015) for patients enrolled in the trial for the intention-to-treat-based analyses. The population assessed for safety include the patients who receive at

least one dose of study medication, with treatment assignments designated according to actual study treatment received. The population used in the biomarker analyses included the patients who had biomarker test results available. No analyses were conducted in the per protocol population for this manuscript; therefore, protocol deviation was not used as a rule for excluding patients in any analyses or data assessment in the manuscript.

The efficacy analyses were performed according to the intention-to-treat principles. PFS data between the treatment groups in the overall population were compared using a log-rank test stratified by the presence or absence of visceral disease and sensitivity to prior endocrine therapy. The HR was estimated from the Cox proportional hazards regression model. The odds ratio estimator and the stratified exact test were used to compare the rates of binary endpoints.

The subpopulation treatment effect pattern plot (STEPP) approach was used to graphically evaluate the heterogeneity of PFS treatment effect across the continuum of H-scores of ER and PR.[21] Each subpopulation was organised into two quantities: *r*1=the largest number of patients in common (or overlapping) among consecutive subpopulations and *r*2=the number of patients in each subpopulation, such that each subpopulation contained approximately 90 (*r*2) patients and approximately 30 (*r*1) overlapping patients. The first subpopulation consisted of the *r*2 patients with the lowest covariate values, whereas the next subpopulation was formed by removing *r*2 minus *r*1 patients with the lowest covariate values from the current subpopulation, and they were replaced with the next *r*2 minus *r*1 patients in the ordered list. This procedure continued until all patients had been included in at least one subpopulation. Patients could

contribute to several subpopulations. For the STEPP analyses, the p value of a supremum test statistic was calculated using a permutation approach with the two-sided significance level of 0·05.[22] A p value <0·05 indicates a significant interaction between the treatment effect and hormonal expression levels, that is, a significant treatment-effect heterogeneity across the continuum of H-scores.[22] Hazard ratios for palbociclib plus fulvestrant versus placebo plus fulvestrant with a corresponding 95% point-wise CIs are presented with a HR <1 in favour of palbociclib plus fulvestrant. P values for interaction from a supreme test of the HR are provided based on a permutation distribution approach.

R 3.2.2 was used to perform STEPP analyses. Other statistical analyses were done with the SAS version 9·2 or later (SAS/STAT, Cary, NC, USA). Subset analysis was performed on *PIK3CA*-positive and *PIK3CA*-negative subpopulations using PFS, odds ratio [OR], and clinical benefit response data. A 2x2 contingency table was generated based on the mutation status of *PIK3CA* and ER expression (central laboratory confirmation). The nonparametric Mann-Whitney test was used to compare ER-expression (ie, H-score) between *PIK3CA*-positive and *PIK3CA*-negative patients. In addition, a logistic regression of *PIK3CA* mutation status (positive vs negative) was run on ER H-score and the coefficient estimate with two-sided p value was generated to further assess the correlation between *PIK3CA* mutation status with ER expression.

The study is registered with ClinicalTrials.gov, number NCT01942135.

Role of the funding source

Pfizer Inc (New York, NY, USA) provided funding to the investigators for study design, conduct, treatment administration, and data collection. The study database was held by Pfizer Inc. The study steering committee was involved in all aspects of the study conduct and data analysis. All authors had access to, and had the opportunity to review final study data and are responsible for data interpretation, preparation of the report, and the decision to submit for publication. The sponsor funded medical writing editorial assistance for the purpose of incorporating authors' revisions into the manuscript .The authors attest to study completeness and the accuracy of the data and data analysis. All authors attest to completeness of the data and the accuracy of data analyses.

Results

Patients' characteristics and efficacy

Between Oct 7, 2013, and Aug 26, 2014, 521 patients were enrolled and randomly assigned to palbociclib and fulvestrant (n=347) or placebo and fulvestrant (n=174) (figure 1). There were no major differences in the baseline characteristics of the intention-to-treat population. More than half of the patients had two or more disease sites, and 406 women (78%) had measurable disease. In the palbociclib group, more patients had undergone two prior lines of endocrine therapy compared with the control group (140/347 [40%] *vs* 61/174 [35%] patients). All patients had progressed on prior endocrine therapy consisting of tamoxifen, AIs, or both, and 177 women (34%) had also received chemotherapy in the metastatic setting.

As of March 2015, a total of 259 PFS events occurred (145 events [42%] in the palbociclib group and 114 [66%] in the control group). The median follow-up was 8·9 months (interquartile range [IQR] 8·7–9·2) for the palbociclib and control groups, with 191 patients (55%) in the palbociclib group versus 51 patients (29%) in the control group remaining on treatment. Treatment discontinuation due to disease progression occurred in 128 patients (36%) in the palbociclib group compared with 107 patients (62%) in the control group.

The median PFS was 9·5 months (95% CI 9·2–11·0) in the palbociclib group compared with 4·6 months (95% CI 3·5–5·6) in the control group (HR 0·46, 95% CI 0·36–0·59, two-sided p<0·0001) in the intention-to treat analysis by investigator assessment (figure 2). This was confirmed by a sampling-based independent review in a randomly selected subset of 211 (40%) of 521 study patients (appendix p17). This expanded median follow-up allowed for a comprehensive assessment of confirmed objective response rate (ORR) and clinical benefit rate compared with the previous report. A confirmed ORR was observed in 66 (19·0%, 95% CI 15·0–23·6) patients in the palbociclib group compared with 15 (8·6%, 95% CI 4·9–13·8) in the control group in the intention-to-treat population (OR 2·47, 95% CI 1·36–4·91, two-sided p=0·002) and in 66 (24·6%, 95% CI 19·6%–30·2) vs 15 (10·9%, 95% CI 6·2%–17·3%) patients with measureable disease at baseline (OR 2·69, 95% CI 1·43–5·26, two-sided p=0·001). The median time to response was 112 days (IQR 58–160) for the palbociclib group. Clinical benefit was observed in 231 (66·6%, 95% CI 61·3%–71·5%) vs 69 (39·7%, 95% CI 32·3%–47·3%) patients in the control group (OR 3·05, 95% CI 2·07–4·61, two-sided p<0·001).

At the time of analysis, only 57 deaths (29% of planned events needed for an overall survival analysis) had occurred, and overall survival data remain immature with 36 events in the palbociclib group and 21 in the control group.

Endocrine resistance and benefit from palbociclib

The benefit from palbociclib was evaluated in relation to various degrees of clinical endocrine resistance (figure 3). These data demonstrate statistically significant and clinically relevant superiority of palbociclib combined with fulvestrant versus fulvestrant therapy alone in all clinical groups. Among patients with no prior treatment in the advanced setting, those patients who progressed while on or within 12 months of stopping adjuvant endocrine therapy, the PFS was 9·5 with palbociclib plus fulvestrant versus 5·4 months with placebo and fulvestrant (HR 0·55, 95% CI 0·32–0·92, two-sided p=0·02) (figure 2). In patients treated with one line of endocrine therapy, the PFS was 9·5 versus 4·6 months, respectively (HR 0·42, 95% CI 0·29–0·60, two-sided p<0·0001) and in patients treated with two lines of endocrine therapy, the PFS was 9·9 versus 5·1 months, respectively (HR 0·46, 95% CI 0·31–0·69, two-sided p<0·0001). Patients whose disease was responsive to prior endocrine therapy obtained an additional benefit from the combination, achieving a PFS of 10·2 versus 4·2 months (HR 0·42, 95% CI 0·32–0·56, two-sided p<0·0001). Finally, patients in the palbociclib group who were exposed to an AI as their immediate prior treatment had PFS of 9·5 months versus 3·7 months for the control group (HR 0·42, 95% CI 0·31–0·56, two-sided p<0·0001).

Correlative biomarker analysis

We performed central analysis of ER, PR, and HER2 and examined the potential effect of ER and PR expression level on the benefits gained from palbociclib, using STEPP analysis to graphically explore the patterns of treatment effect across the range of ER/PR expression levels (figure 4). There was no significant difference in benefit from palbociclib with decreasing expression of ER or PR, in treatment effect across varying levels of ER or PR H-scores (supremum interaction test two-sided p=0·32 for ER and p=0·54 for PR), or in the probability of having disease progression >6 months from study entry (appendix p18).

Mutations in *PIK3CA*, the alpha catalytic subunit of PI3 kinase, are a common genetic event in ER-positive breast cancer.[23] A baseline cfDNA analysis was performed in all patients with an available baseline plasma sample (396 of 521 patients or 76% of the study cohort). One of the 396 samples (<1%) tested failed the analysis. A *PIK3CA* mutation was detected in 129 analyzed samples (33%). Single mutations were demonstrated in 120 cases (93%), and 9 patients showed multiple mutations (7%). Among the four most common mutations assessed, *PIK3CA* H1047R/L was the most frequent mutation detected (14·4%), followed by E545K (11·6%) and E542K (9·4%). There was no difference in baseline demographic and clinical characteristics by *PIK3CA* status (table 1). Among all patients who had their *PIK3CA* mutation status analyzed, median PFS in patients with detectable mutations was 5·8 months compared with 9·2 months in patients for whom no mutations were detected (HR 1·26, 95% CI 0·94–1·68, one-sided log-rank p=0·94; figure 5). In the patients without *PIK3CA* mutation, the median PFS was 9·9 months in the palbociclib group compared with 4·6 months in the control group (HR 0·45, 95% CI 0·31–0·64, two-sided p<0·0001); in patients with a

PIK3CA mutation, the median PFS was 9·5 months versus 3·6 months, respectively (HR 0·48, 95% CI 0·30–0·78, two-sided p=0·002; figure 5). There was no difference in the magnitude of benefit from palbociclib by *PIK3CA* status (interaction test two-sided p=0·83 [figure 3]) or hormone-receptor status (interaction test two-sided p=0·77). Both groups benefited almost equally. There was no association between ER expression level and presence of *PIK3CA* mutation in cfDNA (p=0·75, Mann-Whitney test, and p=0·67, logistic regression; appendix p19). The Forest-Plot analysis showed that the combination of palbociclib plus fulvestrant is consistently superior to endocrine therapy alone among all subgroups, including prior clinical sensitivity/resistance to endocrine therapy, prior chemotherapy, line of hormonal therapy, hormone receptor expression level, or *PIK3CA* mutational status (figure 3).

Safety data

Treatment with palbociclib plus fulvestrant versus placebo plus fulvestrant was associated with more frequent neutropenia (all grades, 279/345 [81%] *vs* 6/172 [3%] patients) and leucopenia (171/345 [50%] *vs* 7/172 [4%] patients) (table 3). Grade 3/4 neutropenia was reported in 223 patients (65%) receiving palbociclib plus fulvestrant compared with one patient (1%) receiving placebo plus fulvestrant; grade 3/4 leucopenia occurred in 95 patients (28%) compared with two patients (1%), respectively. Importantly, the rates of febrile neutropenia were low, occurring in three patients (1%) in the palbociclib group and in one patient (1%) in the control group. Other adverse events of any cause found to be higher in the palbociclib group compared with the control group when assessed based on an incidence of ≥10% in the palbociclib group included infections, fatigue, nausea, anaemia, thrombocytopenia, alopecia, rash,

and stomatitis. Serious adverse events (all causalities) occurred in 44 (12·8%) of 345 patients in the palbociclib group and in 30 (17·4%) of 172 patients in the control group. Among the safety population, 187(54%) of 345 patients in the palbociclib group and 10(6%) of 172 patients in the control group had a dose interruption due an adverse event, 123 (36%) and 3 (2%), respectively, had a cycle delay due to an adverse event, and 117 (34%) and 3 (2%), respectively, had at least one dose reduction during the study. Discontinuation due to adverse events occurred in 14 (4%) of 345 patients in the palbociclib group and in 3 (2%) of 172 patients in the control group. No deaths (defined as grade 5 adverse events) occurred in either treatment group as a result of study drug treatment-related toxicity during the trial or during the follow-up period occurring 28 days after the last dose of study medication.

Discussion

The double-blind, phase 3, placebo-controlled PALOMA-3 study met its primary endpoint. A significant improvement in PFS was demonstrated in patients who received palbociclib plus fulvestrant over placebo plus fulvestrant to treat hormone-receptor–positive, HER2-negative MBC after prior endocrine therapy resistance. The performance of fulvestrant in combination with palbociclib is reported by study predefined clinical and biomarker segments and demonstrates that the benefit from palbociclib extends across all subsets analysed. This report confirms the important observation that single-agent endocrine therapy has limited efficacy in patients with disease progression after previous exposure to endocrine therapy, irrespective of

clinical or molecularly defined endocrine sensitivity, suggesting a need for the routine use of more effective combination regimens.[18,24,25]

The current report demonstrates that the combination of palbociclib plus fulvestrant is associated with significant improvement in all parameters of clinical activity compared with placebo plus fulvestrant, including objective response, clinical benefit, and PFS. The improvement in efficacy was observed regardless of the degree of endocrine resistance as defined by number of previous therapies, reported sensitivity to previously received endocrine therapy, and biomarker parameters known to affect sensitivity to endocrine therapy such as the degree of ER and PR expression. The combination treatment was very effective, even after failure of two lines of prior endocrine therapy. This population is generally considered endocrine-refractory and is traditionally treated with single-agent cytotoxic chemotherapy (eg, taxane, capecitabine, or eribulin), often with limited benefit, rarely with meaningful objective response, and frequently with increased toxicity and reduced quality of life.[26] The combination of palbociclib plus fulvestrant resulted in a confirmed ORR of 24·6% in patients with measurable disease, which compares favourably with cytotoxic chemotherapy. Of note, we learned from the longer follow-up in this study that responses to palbociclib were relatively slow to manifest, with a median time to response of almost 4 months. Palbociclib in combination with fulvestrant may offer not only increased efficacy, but also a more favourable safety profile than chemotherapy, although chemotherapy is currently still advisable in patients at risk for visceral crisis;[27] a setting in which palbociclib has not yet been assessed.

Biomarker and tumour biology can significantly change from the original primary tumour. To our knowledge, PALOMA-3 is the first registrational study to evaluate protocol-

mandated biopsy samples from patients who have relapsed with advanced disease, which allowed assessment of hormone sensitivity at the time of disease recurrence, rather than reliance on data derived at the time of initial diagnosis. Previous studies suggest a higher level of expression of hormone receptors has been associated with benefit to endocrine therapy, whereas patients with lower hormone-receptor expression derive less benefit.[2] Conversely, the benefit from palbociclib in breast cancer appears to be similar across the various levels of ER expression or clinical sensitivity to previously administered endocrine therapy. The demonstration that the benefit of palbociclib may be independent of the level of expression of hormone receptors in luminal breast cancer, strongly suggests that the mechanism of action of CDK4/6 inhibitors may be unrelated to established ER-associated resistance pathways but rather target a dependence of the luminal subtype breast cancer on CDK4/6, thus introducing a novel molecular target in patients with endocrine-positive disease.[2,11,13]

The PI3K/AKT/mTOR pathway is a major signalling pathway in normal and cancer physiology. Mutation in *PIK3CA* is a frequent genetic event in ER-positive breast cancer.[4] In primary breast cancer, *PIK3CA* mutations are associated with a lower rate of nodal and distant metastasis.[28,29] Preclinical assays suggested that these mutations confer a gain of function as measured by kinase activity supporting the clinical development of therapeutic agents targeting the related pathways.[28] In an attempt to exploit this observation, several PI3K inhibitors are in clinical development in hormone-receptor–positive breast cancer, but the results thus far have been disappointing. A retrospective, exploratory analysis of the BOLERO-2 study conducted in a subset of patients in the trial, showed no association between *PIK3CA* mutations and everolimus-

related benefit.[30] The study conclusions were hampered by the use of archival tissue, mostly from primary disease (mastectomy) that may have not reflected the molecular features of advanced disease. In the current study, we performed a targeted assessment of the most common *PIK3CA* mutations, using cfDNA molecular testing (plasma DNA derived *PIK3CA* analysis has high concordance with tumour *PIK3CA* status[20]) to reflect genetic events present in the cancer at the time of treatment.

The PALOMA-3 study represents the largest reported cohort of *PIK3CA* mutation analysis in plasma DNA in endocrine-resistant patients, with *PIK3CA* mutations detected at a rate comparable to that reported in the literature. We report that the *PIK3CA* mutational status was independent of the benefit of palbociclib plus fulvestrant with no interaction between genotype and treatment benefit. Patients with a *PIK3CA* mutation had numerically worse median PFS and lower ORR with palbociclib (table 2), but there was no significant interaction between *PIK3CA* mutation and magnitude of benefit from palbociclib (ORR interaction test, p=0·28).

The safety profile of palbociclib plus fulvestrant remained very favourable after a median 8·9-month follow-up; not only was it consistent with previously reported data, but it provided assurance with respect to the stability of the safety profile. Neutropenia is frequent, but the rate of febrile neutropenia is very low and higher than that observed with fulvestrant alone. Other symptomatic side effects were only modestly increased compared with those observed with fulvestrant plus placebo, and the rate of discontinuation due to adverse effects was low (occurring in only 14 (4%) of 347 of patients). This compares very favourably to discontinuation rates of other therapies currently approved for this patient population.

In conclusion, the current report of the PALOMA-3, prospective, phase 3 randomised study confirms a substantial efficacy of palbociclib combined with fulvestrant in patients with hormone-receptor–positive HER2-negative MBC. Palbociclib plus fulvestrant produced clinically relevant confirmed response rates that are potentially higher than those anticipated with chemotherapy in the hormone-resistant setting. Moreover, the efficacy of palbociclib was shown to be independent of the degree of hormone resistance, as measured by clinical parameters (sensitivity to previous endocrine therapy and number and type of previous therapies given), level of hormone-receptor expression, and *PIK3CA* mutational status in baseline cfDNA, all of which are factors commonly considered to be associated with endocrine resistance. We conclude that targeting CDK4/6 now represents a novel, effective, and safe therapeutic approach for the treatment of patients in an advanced setting, regardless of the degree of endocrine sensitivity, levels of ER or PR expression, and PI3K mutational status. The observed durable disease control that maintains quality of life and a high rate of objective responses across analysed subsets, represents a highly favourable approach.

Panel: **Research in context**

The management of hormone-receptor–positive HER2-negative MBC includes single-agent endocrine therapy or combination regimens. These treatments are usually selected on the basis of a defined clinical or biomarker-guided sequence. The current report is the only study to evaluate the benefit of the novel combination regimen of palbociclib and standard endocrine therapy with fulvestrant (± luteinizing hormone-

releasing hormone agonist) in relation to clinically defined molecular markers of endocrine sensitivity.

Evidence before this study

The PALOMA-3 study reported results at the interim analysis owing to the significant efficacy seen with palbociclib. The median duration of follow-up at the interim analysis was short, which limited both the ability to fully assess the benefit and risks of palbociclib as well as better selection of patient populations with recurrent disease that may differentially benefit from this novel treatment as addressed in this report.

Added value of this study

Palbociclib combined with fulvestrant is an effective therapeutic choice in hormone-receptor–positive HER2-negative MBC and the only combination with proven efficacy in multiple clinical settings. The current data demonstrate the benefit of the combination of palbociclib with fulvestrant, regardless of the degree of endocrine sensitivity, as assessed by clinical features (eg, previous endocrine sensitivity or exposure to chemotherapy). Moreover, palbociclib appears effective irrespective of hormone-receptor expression level or *PIK3CA* tumour mutational status. Palbociclib with fulvestrant induces frequent objective responses, although the response may take some months of therapy to manifest, and two thirds of patients treated with this combination achieve meaningful clinical benefit.

Implications of all the available evidence

This mature and final study analysis of PALOMA-3 provides the confirmatory evidence that palbociclib combined with fulvestrant is an effective option with favourable safety profile for the treatment of recurrent hormone-receptor–positive HER2-negative MBC regardless of the degree of endocrine sensitivity in this patient population.

Contributors

MCr, NCT, CHB, KZ, AD, YJ, SL, HI, NH, JR, and MK contributed to the design of the study and MK contributed to the study concept. NH was a steering committee member involved in all aspects of the trial and provided oversight. JR and DS contributed to patient recruitment, MCr and AD contributed to patient enrolment, and KPT contributed to the conduct of the study. IB, JR, S-AI, NM, SL, HI, NH, KZ, KPT, SV, DS, and YJ contributed to data collection, YJ contributed to data generation, and MC contributed to the study results. KZ, CHB, SL, KPT, JR, and SV contributed to data analysis. MCr, NCT, IB, JR, S-AI, NM, AD, SL, HI, NH, KZ, KPT, CHB, MK, SV, and DS contributed to data interpretation. DS and NM contributed to a review of the data, MCr, NT, S-AI, NM, MC, AM, SL, NH, KZ, KPT, CHB, SV, YJ, and DS contributed to the writing of the report, and CHB contributed to the structure of the figures and tables in the report, and KPT, NH, JR, and MK contributed to revisions of the manuscript. All authors contributed to review of the report and approved the final submitted version.

Declaration of interests

MK and DS are stockholders of Pfizer and MK and CHB are employees of Pfizer. CHB and YJ report other support from Pfizer during the conduct of the study, and YJ reports other support from Pfizer outside the submitted work. MK reports no financial activities or competing interests outside the submitted work. KZ reports personal fees from Pfizer, during the conduct of the study, and personal fees from Pfizer, outside the submitted work. AD reports grants and other support from Pfizer, outside the submitted work. SV has an advisory board role at Pfizer, and NCT has received advisory board honoraria and research funding from Pfizer, and NH reports personal fees from Pfizer. S-AI and SV report an advisory role for AstraZeneca, S-AI and HI report grants from AstraZeneca, and NM and HI report personal fees from AstraZeneca. HI reports grants from Chugai, and NM reports personal fees from Chugai, Kyowa-Hakko Kirin, and personal fees from Sanofi, outside the submitted work. SV reports an advisory role for Roche, S-AI reports an advisory role for Roche, outside the submitted work, and NH reports personal fees from Roche, outside the submitted work. SV and S-AI report an advisory role for Novartis, HI reports grants from Novartis, and NH reports personal fees from Novartis. HI reports grants from GSK, grants from Daiichi-Sankyo, and grants from Nihon Kayaku, outside the submitted work. HI and NM report personal fees from Eisai, and SV reports an advisory role at Eisai. HI reports grants from Eli Lilly and SV reports an advisory role at Eli Lilly. SV reports an advisory board role at Amgen, BMS, and Merck. NH reports personal fees from Celgene, personal fees from Genomic Health, and personal fees from Nanostring. MCr, IB, JR, MC, SL, KPT have not conflicts of interest to disclose.

Acknowledgments

This study was sponsored by Pfizer Inc. Fulvestrant was provided by AstraZeneca. DJS has received funding for the preclinical work on cyclin-dependent kinase 4/6 inhibition that generated translational concepts from the US Department of Defense Breast Cancer Innovator Award, the Revlon/UCLA Women's Cancer Research Program, and the Peter and Denise Wittich Breast Cancer Program.

We thank the patients who participated in the PALOMA-3 study and the investigators, study nurses, and site staff for their support of the trial. We also acknowledge the contributions of Alexandra Thiele, Ph.D., and Carla Giorgetti, Ph.D, full-time employees of Pfizer Inc, for the design of the study and development of the study protocol, and Patricia English, Ph.D., a full-time employee of Pfizer, for the analysis of *PIK3CA* mutation data. Editorial assistance was supplied by Kevin O'Regan, Ph.D., and Susan Reinwald, Ph.D., of Complete Healthcare Communications, LLC, and was funded by Pfizer Inc. Final approval of the manuscript rested solely with the authors.

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Figure Legends

Figure 1: Trial profile. cfDNA=circulating free DNA; PI3K= Phosphoinositide 3-kinase.

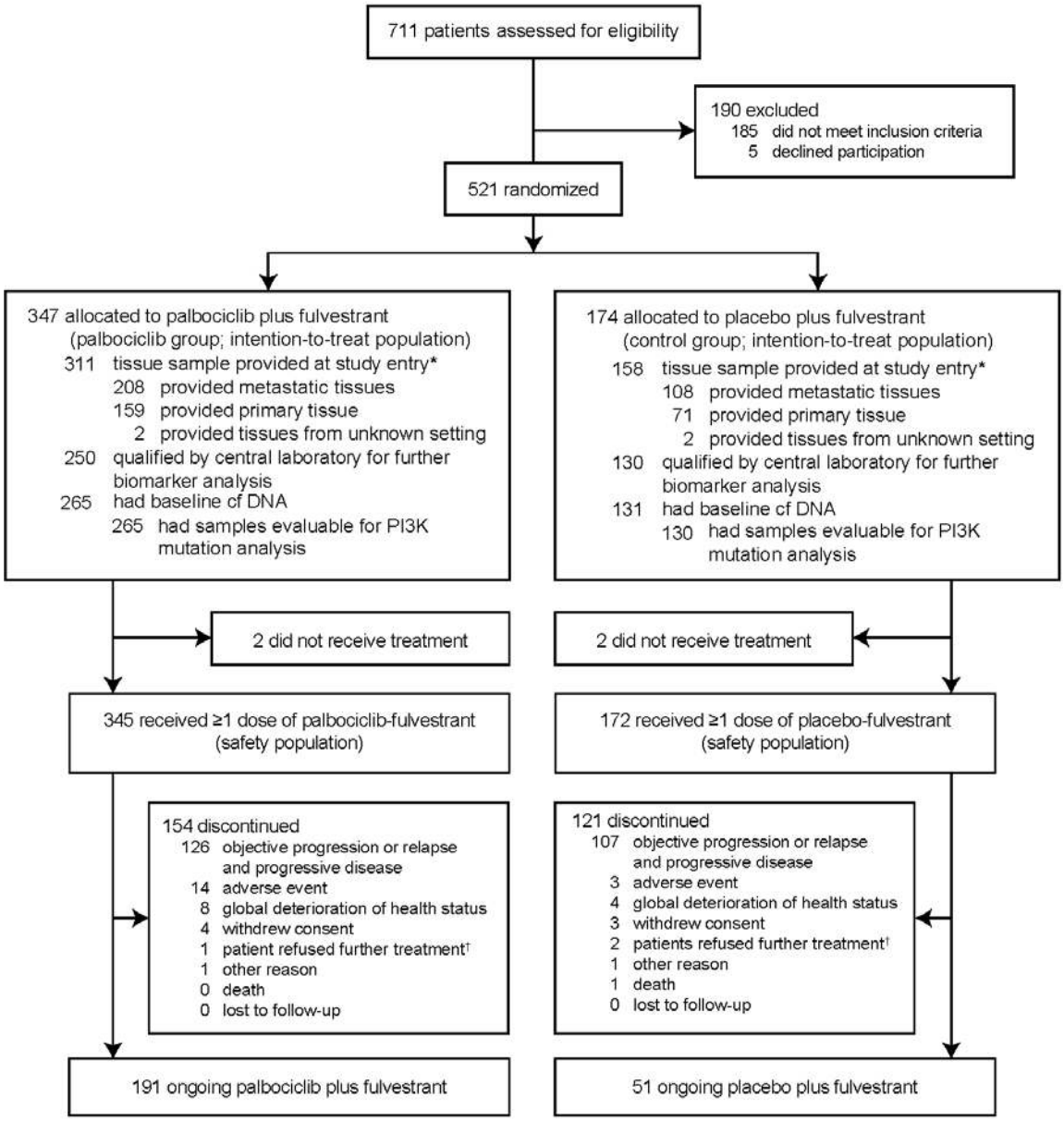
Figure 2: Progression-free survival – investigator assessed. (A) Intention-to-treat population (primary analysis). (B) Patients with no prior systemic therapy in the metastatic breast cancer setting. (C) Patients with at least one prior systemic therapy in the metastatic breast cancer setting. NE=not estimable; Prior systemic therapy=chemotherapy and/or endocrine.

Figure 3: Effect of subgroups on progression-free survival. ER=oestrogen receptor; PR=progesterone receptor. The blue boxes represent the hazard ratios with 95% CIs (horizontal lines); the size of each box is proportional to the size of the corresponding subgroup.

Figure 4: Subpopulation treatment effect pattern plot (STEPP) analysis to evaluate centrally assessed ER-positive and PR-positive expression levels. The x-axis represents the median H-score of (A) ER or (B) PR for patients in each of the subpopulations, There was no evidence of change in benefit for palbociclib with varying expression of ER (p=0·32) and PR (p=0·54).ER=oestrogen receptor; HR=hazard ratio; PR=progesterone receptor.

Figure 5: Progression-free survival of patients according to the presence of *PIK3CA* mutation(s). (A) Kaplan-Meier curve of all patients based on *PIK3CA* mutation status regardless of treatment assignment in the subset with evaluable cfDNA samples (n=395). Progression-free survival according to treatment for (B) *PIK3CA*-negative (n=266) and (C) *PIK3CA*-positive (n=129). cfDNA=circulating free DNA.

Figure 1: **Trial profile**



*The number of patients who provided tissues in more than one setting was 58 in the palbociclib group and 23 in the control group.
†For reasons other than adverse event.

Figure 2: **Progression-free survival – investigator assessed**

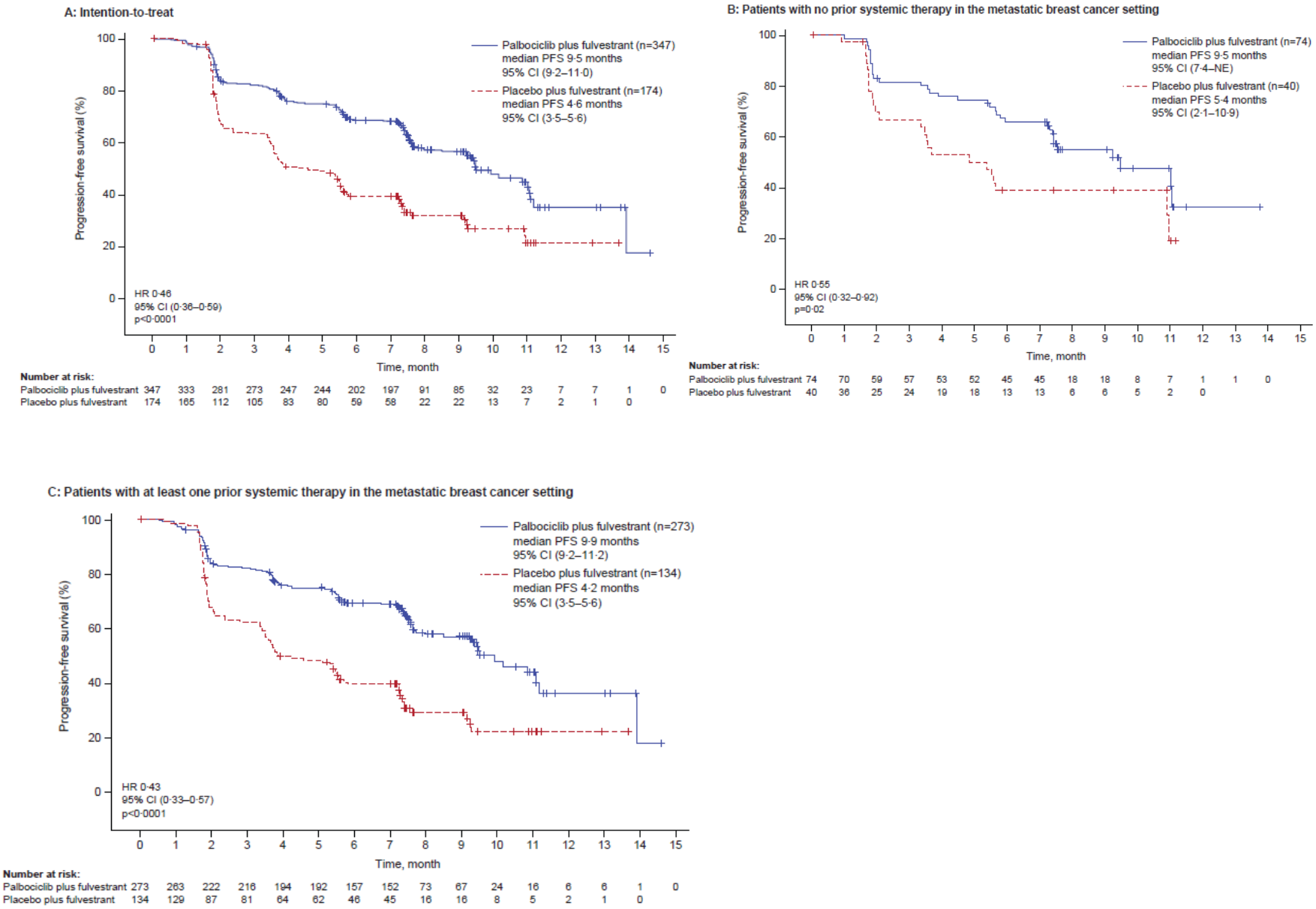


Figure 3: **Effect of subgroups on progression-free survival**

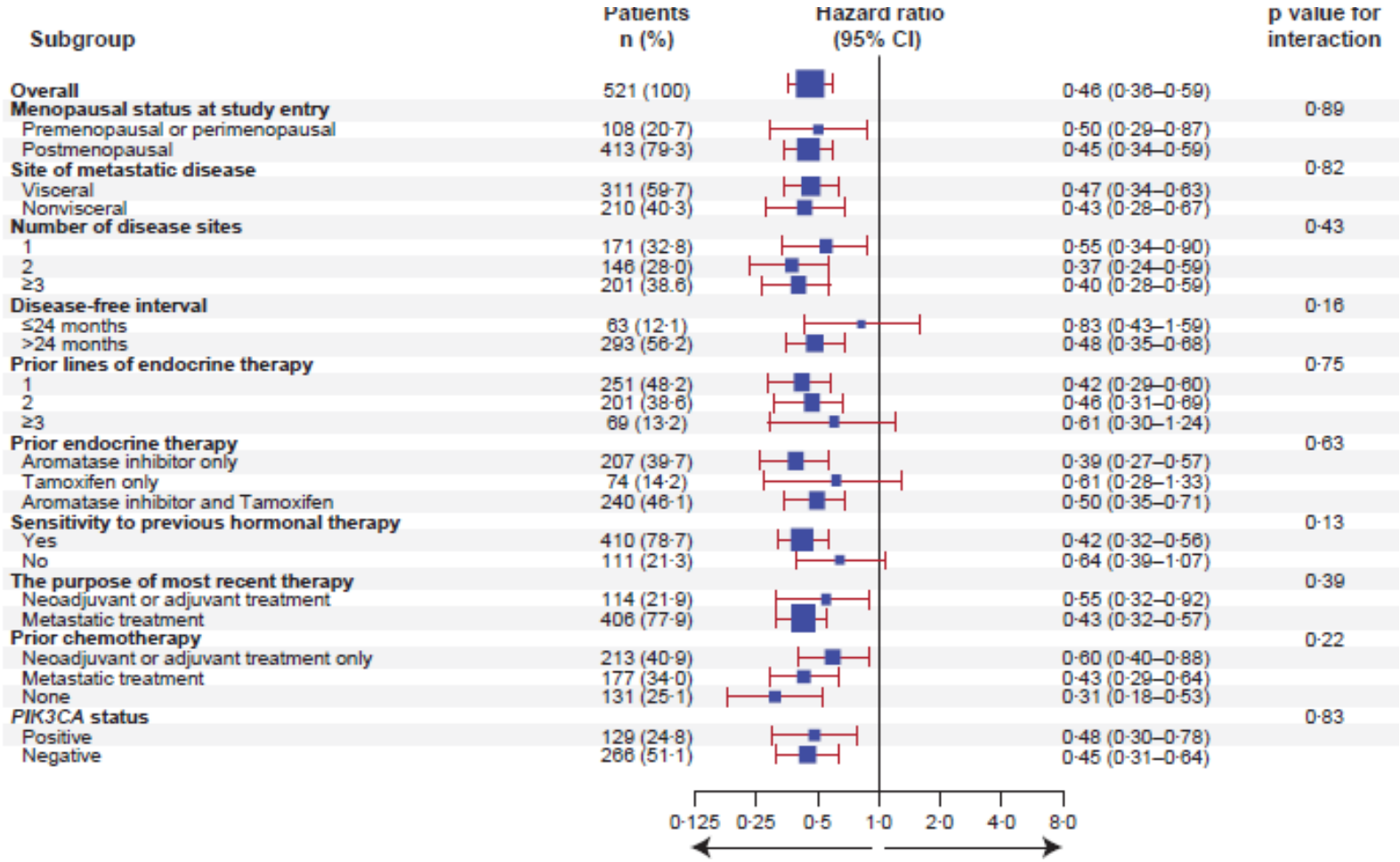


Figure 4. **Subpopulation treatment effect pattern plot (STEPP) analysis to evaluate centrally assessed ER-positive and PR-positive expression levels**

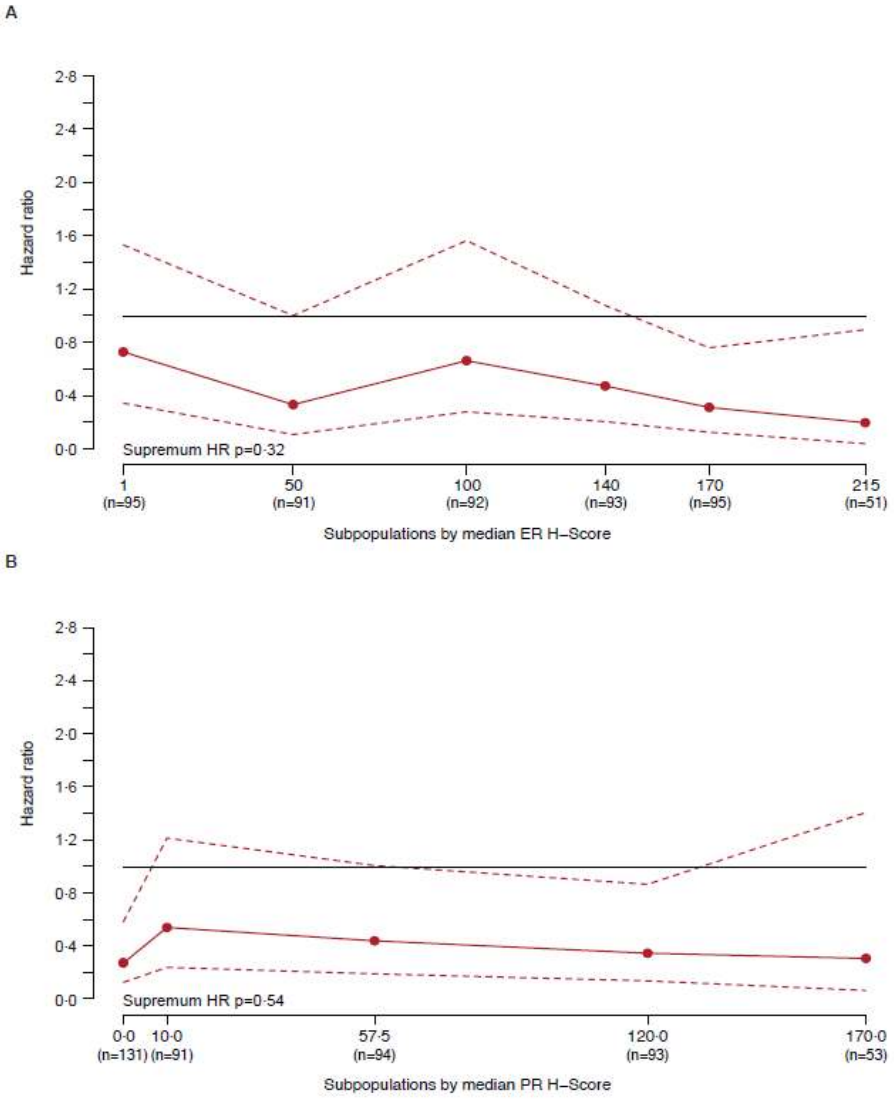


Figure 5: **Progression-free survival of patients according to the presence of *PIK3CA* mutation(s)**

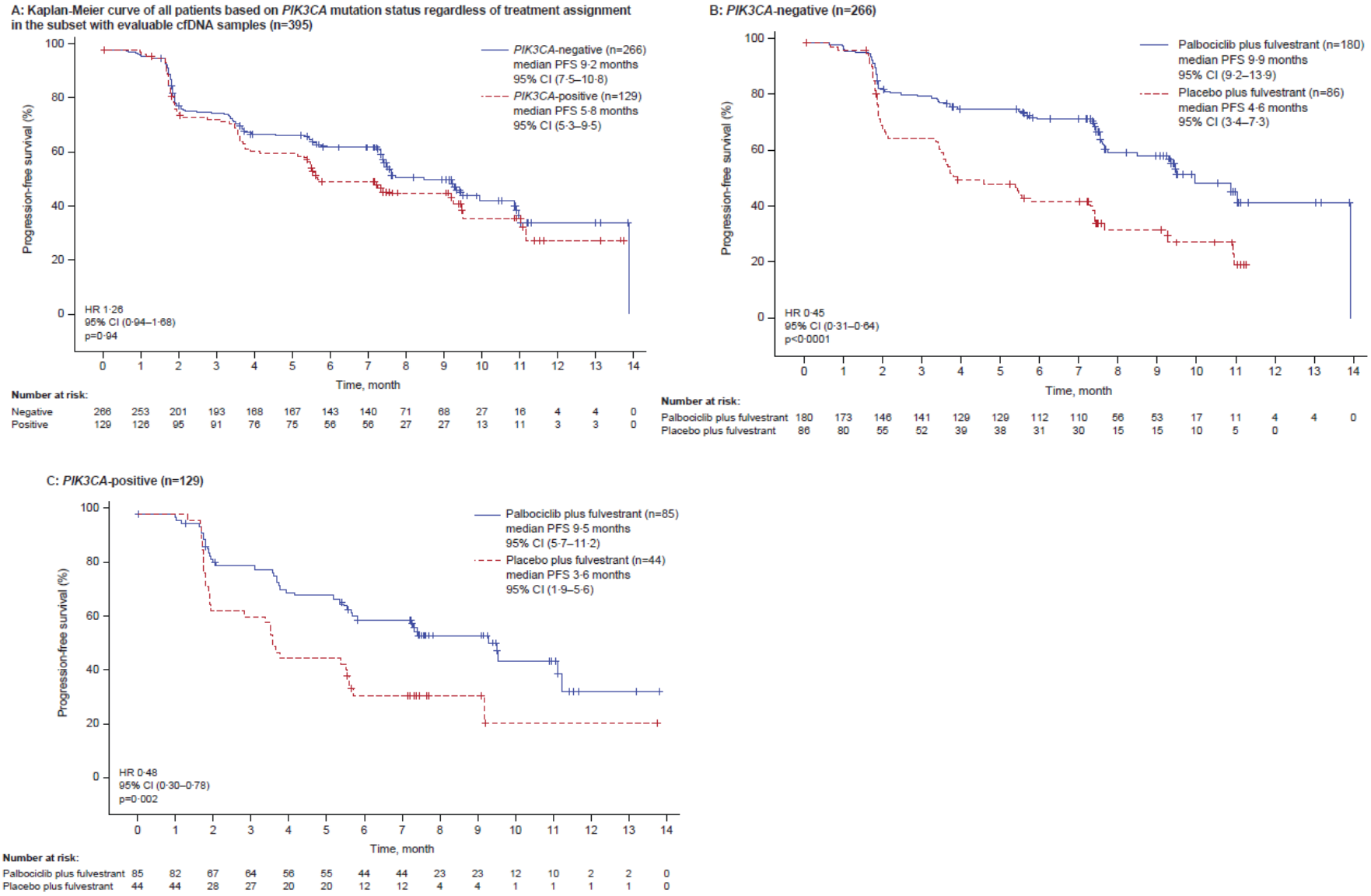


Table 1: **Clinical and pathologic characteristics of patients – intention-to-treat population and *PIK3CA* mutation analysis set**

	Intention-to-treat population		***PIK3CA* mutation analysis set[m]**			
			Palbociclib plus fulvestrant		**Placebo plus fulvestrant**	
	Palbociclib plus fulvestrant (n=347)	**Placebo plus fulvestrant (n=174)**	***PIK3CA*-positive (n=85)**	***PIK3CA*-negative (n=180)**	***PIK3CA*-positive (n=44)**	***PIK3CA*-negative (n=86)**
Age, years						
Median (range)	57 (30-88)	56 (29-80)	58 (33–88)	57 (31–87)	56 (39–77)	55 (29–80)
Race[a,b]						
White	252 (73%)	133 (76%)	56 (66%)	128 (71%)	32 (73%)	61 (71%)
Asian	74 (21%)	31 (18%)	24 (28%)	38 (21%)	10 (23%)	18 (21%)
Black and others	21 (6%)	10 (6%)	5 (6%)	14 (8%)	2 (5%)	7 (8%)
ECOG performance status						
0	206 (59%)	116 (67%)	46 (54%)	104 (58%)	31 (70%)	51 (59%)
1	141 (41%)	58 (33%)	39 (46%)	76 (42%)	13 (30%)	35 (41%)
Menopausal status						
Pre-/peri-menopausal	72 (21%)	36 (21%)	22 (26%)	31 (17%)	9 (20%)	17 (20%)
Postmenopausal	275 (79%)	138 (79%)	63 (74%)	149 (83%)	35 (80%)	69 (80%)
Nonmeasurable disease						
Bone	75 (22%)	36 (21%)	27 (32%)	29 (16%)	8 (18%)	11 (13%)
Others	4 (1%)	0	1 (1%)	2 (1%)	0	0
Measurable disease	268 (77%)	138 (79%)	57 (67%)	149 (83%)	36 (82%)	75 (87%)
Visceral disease[c]	206 (59%)	105 (60%)	41 (48%)	116 (75%)	33 (64%)	49 (57%)
Lung involvement	100 (29%)	45 (26%)	19 (22%)	59 (33%)	12 (27%)	22 (26%)

Liver involvement	127 (37%)	81 (47%)	27 (32%)	71 (39%)	25 (57%)	37 (43%)
Peritoneal involvement	2 (1%)	1 (1%)	0	0	0	0
Brain and/or pleural	4 (1%)	2 (1%)	1(1%)	3 (2%)	1 (2%)	1 (1%)
Prior lines of endocrine therapy						
1	160 (46%)	91 (52%)	33 (39%)	73 (41%)	24 (55%)	46 (53%)
2	140 (40%)	61 (35%)	19 (22%)	42 (23%)	4 (9%)	13 (15%)
≥3	47 (14%)	22 (13%)	13 (15%)	14 (8%)	6 (14%)	5 (6%)
Purpose of most recent treatment[d]	347 (100%)	173 (99%)	85 (100%)	180 (100%)	44 (100%)	86 (100%)
Adjuvant therapy	74 (21%)	40 (23%)	18 (21%)	41 (23%)	9 (20%)	18 (21%)
Treatment of advanced or metastatic breast cancer	273 (79%)	133 (76%)	67 (79%)	139 (77%)	35 (80%)	68 (79%)
Disease-free interval[e]	233 (67%)	123 (71%)	56 (66%)	129 (72%)	32 (73%)	58 (67%)
>24 months	192 (82%)	101 (81%)	46 (54%)	106 (59%)	28 (64%)	46 (53%)
12–24 months	30 (13%)	19 (15%)	4 (5%)	19 (11%)	3 (7%)	12 (14%)
<12 months	11 (5%)	3 (2%)	6 (7%)	4 (2%)	1 (2%)	0
Prior endocrine therapy						
Aromatase inhibitors	137 (39%)	70 (40%)	30 (35%)	73 (41%)	15 (34%)	35 (41%)
Tamoxifen	51 (15%)	23 (13%)	9 (11%)	26 (14%)	5 (11%)	12 (14%)
Aromatase inhibitors and Tamoxifen	159 (46%)	81 (47%)	46 (54%)	81 (45%)	24 (55%)	39 (45%)
Previous chemotherapy[f]						
Neoadjuvant or adjuvant	139 (40%)	74 (43%)	34 (40%)	82 (46%)	19 (43%)	32 (37%)

therapy only						
Treatment of metastatic disease (with or without adjuvant or neoadjuvant)	194 (56%)	113 (65%)	23 (27%)	54 (30%)	15 (34%)	30 (35%)
Previous sensitivity to endocrine therapy[g]						
Yes	274 (79%)	136 (78%)	69 (81%)	146 (81%)	38 (86%)	67 (78%)
No	73 (21%)	38 (22%)	16 (19%)	34 (19%)	6 (14%)	19 (22%)
ER and/or PR status confirmed by central laboratory testing[h]						
ER-positive and PR-positive						
≥median of distribution[i]	81 (23%)	40 (23%)	17 (20%)	44 (24%)	13 (30%)	19 (22%)
<median of distribution[j]	71 (20%)	29 (17%)	16 (19%)	38 (21%)	4 (9%)	18 (21%)
ER-positive or PR-positive						
≥median of distribution[k]	179 (52%)	100 (57%)	48 (56%)	88 (49%)	29 (66%)	48 (56%)
<median of distribution[l]	165 (48%)	90 (52%)	46 (54%)	80 (44%)	20 (45%)	48 (56%)
Central laboratory tested						
Median H-Score (IQR); Mean [SD]						
ER	110 (40–160); 107 [74]	114 (23–150); 99 [72]	95 (30–145); 96 [68]	118 (43–170); 111 [77]	130 (60–160); 112 [74]	100 (10–145); 94 [74]
PR	10 (0–100); 53 [68]	20 (0–100); 51 [62]	21 (0–110); 58 [68]	10 (0–110); 56 [72]	50 (2–80); 53 [51]	10 (0–100); 52 [67]
HER2	1 (negative-range); 2 [1]	2 (negative-range); 2 [1]	1 (1–2); 2 [1]	2 (1–2); 2 [1]	1 (1–1); 1 [<1]	2 (1–2); 2 [1]

Data are number (%), median (interquartile range [IQR]), or mean [SD]. ECOG=Eastern Cooperative Oncology Group; ER=oestrogen receptor; PR=progesterone receptor; SD=standard deviation.

[a]Based on self-report.

[b]In the placebo plus fulvestrant arm, *PIK3CA*-positive cohort does not add to 100% because of numerical rounding.

[c]Per protocol, visceral refers to lung, liver, brain, pleural, and peritoneal involvement and was a study stratification factor.

[d]Information for one patient's most recent therapy was not available/provided.

[e]Disease-free interval was defined as the time from diagnosis of primary breast cancer to first relapse in patients who received adjuvant therapy.

[f]The percentage for each interval was calculated based on the patients who received adjuvant therapy. Patients were counted for each treatment of metastatic disease (with or without neoadjuvant received). Treatment of metastatic disease included chemotherapy in the metastatic breast cancer setting.

[g]Based on randomisation.

[h]For classification of biomarkers' status (≥median of distribution, <median of distribution) the H-Score was used. The median was calculated based on the number of patients who were tested by central laboratory (250 patients in the palbociclib plus fulvestrant arm and 130 patients in the placebo plus fulvestrant arm).

[i]Any patient with ER+ ≥median and PR+ ≥median included.

[j]Any patient with ER+ <median and PR+ <median included.

[k]Any patient with ER+ ≥median or PR+ ≥median included.

[l]Any patient with ER+ <median or PR+ <median included.

[m]395-patients with baseline-circulating free DNA and evaluable for *PIK3CA* mutation analysis by the central laboratory.

	Palbociclib plus fulvestrant	Placebo plus fulvestrant	p value†	Palbociclib plus fulvestrant (*PIK3CA*-positive)	Placebo plus fulvestrant (*PIK3CA*-positive)	p value†	Palbociclib plus fulvestrant (*PIK3CA*-negative)	Placebo plus fulvestrant (*PIK3CA*-negative)	p value†

Table 2: **Summary of best overall tumour response by treatment and according to *PIK3CA* mutation status, investigator assessed**

	n	% (95% CI)*	n	% (95% CI)*		n	% (95% CI)*	n	% (95% CI)*		n	% (95% CI)*	n	% (95% CI)*	
Intention-to-treat	347		174			85		44			180		86		
Complete response ‡	0	0%	4	2·3%		0		0			0		4	4·7%	
Partial response ‡	66	19·0%	11	6·3%		13	15·3%	7	15·9%		53	29·4%	12	14·0%	
Stable disease	213	61·4%	94	54·0%		54	63·5%	21	47·7%		91	50·6%	38	44·2%	
Progressive disease	58	16·7%	57	32·8%		16	18·8%	15	34·1%		32	17·8%	28	32·6%	
Indeterminate	10	2·9%	8	4·6%		2	2·4%	1	2·3%		4	2·2%	4	4·7%	
Objective response rate	66	19·0% (15·0–23·6)	15	8·6% (4·9–13·8)		13	15·3% (8·4–24·7)	7	15·9% (6·6–30·1)		53	29·4% (22·9–36·7)	16	18·6% (11·0–28·4)	
Odds ratio		2·47 (1·36–4·91)			0·002		1·16 (0·38–3·95)			0·98		1·78 (0·92–3·66)			0·09
Clinical benefit response rate§	231	66·6% (61·3–71·5)	69	39·7% (32·3–47·3)		51	60·0% (48·8–70·5)	16	36·4% (22·4–52·2)		129	71.7% (64.5–78·1)	34	39.5% (29·2–50·7)	
Odds ratio		3·05 (2·07–4·61)			<0·0001		2·17 (0·93–5·04)			0·08		4·21 (2·35–7.76)			0·0001
Measurable disease	268		138			57		36			149		75		
Complete response‡	0	0	4	2·9%		0		0			0		4	5·3%	
Partial response‡	66	24·6%	11	8·0%		13	22·8%	7	19·4%		53	35·6%	12	16·0%	
Stable disease	143	53·4%	65	47·1%		30	52·6%	13	36·1%		63	42·3%	29	38·7%	
Progressive disease	51	19·0%	52	37·7%		12	21·1%	15	41·7%		29	19·5%	27	36·0%	
Indeterminate	8	3·0%	6	4·3%		2	3·5%	1	2·8%		4	2·7%	3	4·0%	

Objective response rate	66	24·6% (19·6–30·2)	15	10·9% (6·2–17·3)		13	22·8% (12·7–35·8)	7	19·4% (8·2–36·0)		53	35·6% (27·9–43·8)	16	21·3% (12·7–32·3)	
Odds ratio		2·69 (1·43–5·26)			0.001		1·18 (0·37–4·08)			0·96		2·10 (1·05–4·36)			0·04
Clinical benefit response rate[§]	171	63·8% (57·7–69·6)	50	36·2% (28·2–44·8)		31	54·4% (40·7–67·6)	12	33·3% (18·6–51·0)		103	69·1% (61·0–76·4)	29	38·7 (27·6–50·6)	
Odds ratio		3·10 (1·99–4·92)			<0·0001		2·12 (0·79–5·65)			0·16		4·09 (2·15–7.96)			<0·0001

Data are number (%) or (95% CI). RECIST=Response Evaluation Criteria in Solid Tumors (version 1.1).*CI was calculated using the exact (Clopper-Pearson) method. [†]Two-sided exact tests stratified by the presence of visceral metastases and sensitivity to prior hormonal therapy per randomisation. [‡]Confirmed response. [§]Clinical benefit response rate=complete response plus partial response plus stable disease equal to or more than 24 weeks.

Table 3: **Frequency of adverse events occurring in ≥10% patients in grades 1-2 and any adverse event occurring in grades 3, 4, and 5 in the palbociclib plus fulvestrant group (all causalities and all cycles)**

Adverse events*	Palbociclib plus fulvestrant (n=345)					Placebo plus fulvestrant (n=172)				
	All grades	Grades 1–2	Grade 3	Grade 4	Grade 5	All grades	Grade 1–2	Grade 3	Grade 4	Grade 5
Any adverse events	340 (99%)	85 (25%)	210 (61%)	41 (12%)	4 (1)	154 (90%)	113 (66%)	34 (20%)	4 (2%)	3 (2%)
Haematologic										
Neutropenia†	279 (81%)	56 (16%)	189 (55%)	34 (10%)	0	6 (3%)	5 (3%)	0	1 (1%)	0
Anaemia†	96 (28%)	86 (25%)	10 (3%)	0	0	19 (11%)	16 (9%)	3 (2%)	0	0
Leucopenia†	171 (50%)	76 (22%)	93 (27%)	2 (1%)	0	7 (4%)	5 (3%)	1 (1%)	1 (1%)	0
Thrombocytopenia†	73 (21%)	65 (19%)	6 (2%)	2 (1%)	0	0	0	0	0	0
Lymphopenia†	6 (2%)	4 (1%)	1 (<1%)	1 (<1%)	0	2 (1%)	1 (1%)	1 (1%)	0	0
Nonhaematologic										
Infections†	144 (42%)	137 (40%)	6 (2%)	1 (<1%)	0	52 (30%)	47 (27%)	5 (3%)	0	0
Fatigue	135 (39%)	127 (37%)	8 (2%)	0	0	49 (28%)	47 (27%)	2 (1%)	0	0
Nausea	112 (32%)	112 (32%)	0	0	0	47 (27%)	46 (27%)	1 (1%)	0	0
Headache	80 (23%)	78 (23%)	2 (1%)	0	0	33 (19%)	33 (19%)	0	0	0
Diarrhoea	74 (21%)	74 (21%)	0	0	0	32 (19%)	31 (18%)	1 (1%)	0	0
Constipation	66 (19%)	66 (19%)	0	0	0	27 (16%)	27 (16%)	0	0	0
Alopecia	58 (17%)	58 (17%)	0	0	0	11 (6%)	11 (6%)	0	0	0
Vomiting	58 (17%)	57 (17%)	1 (<1%)	0	0	25 (15%)	24 (14%)	1 (1%)	0	0
Hot flush	53 (15%)	53 (15%)	0	0	0	29 (17%)	28 (16%)	1 (1%)	0	0
Decreased appetite	52 (15%)	49 (14%)	3 (1%)	0	0	14 (8%)	13 (8%)	1 (1%)	0	0
Rash†	52 (15%)	50 (14%)	2 (1%)	0	0	9 (5%)	9 (5%)	0	0	0

Back pain	51 (15%)	47 (14%)	4 (1%)	0	0	29 (17%)	26 (15%)	3 (2%)	0	0
Cough	51 (15%)	51 (15%)	0	0	0	22 (13%)	22 (13%)	0	0	0
Arthralgia	49 (14%)	48 (14%)	1 (<1%)	0	0	27 (16%)	27 (16%)	0	0	0
Pain in extremity	43 (12%)	43 (12%)	0	0	0	21 (12%)	18 (10%)	3 (2%)	0	0
Stomatitis	43 (12%)	41 (12%)	2 (1%)	0	0	4 (2%)	4 (2%)	0	0	0
Dizziness	41 (12%)	40 (12%)	1 (<1%)	0	0	16 (9%)	16 (9%)	0	0	0
Dyspnoea	40 (12%)	39 (11%)	0	1(<1%)	0	14 (8%)	12 (7%)	2 (1%)	0	0
Pyrexia	38 (11%)	37 (11%)	1 (<1%)	0	0	9 (5%)	9 (5%)	0	0	0
Insomnia	33 (10%)	32 (9%)	1 (<1%)	0	0	12 (7%)	12 (7%)	0	0	0
Abdominal pain	27 (8%)	25 (7%)	2 (1%)	0	0	10 (6%)	9 (5%)	1 (1%)	0	0
Upper respiratory tract infection	27 (8%)	25 (7%)	2 (1%)	0	0	12 (7%)	12 (7%)	0	0	0
Musculoskeletal pain	26 (8%)	25 (7%)	1 (<1%)	0	0	12 (7%)	11 (6%)	1 (1%)	0	0
Aspartate aminotransferase increased	24 (7%)	15 (4%)	9 (3%)	0	0	8 (5%)	5 (3%)	3 (2%)	0	0
Injection site pain	22 (6%)	21 (6%)	1 (<1%)	0	0	17 (10%)	17 (10%)	0	0	0
Depression	21 (6%)	19 (6%)	2 (1%)	0	0	10 (6%)	9 (5%)	1 (1%)	0	0
Hypertension	21 (6%)	14 (4%)	7 (2%)	0	0	4 (2%)	3 (2%)	1 (1%)	0	0
Alanine aminotransferase increased	19 (6%)	13 (4%)	6 (2%)	0	0	6 (3%)	6 (3%)	0	0	0
Bone pain	17 (5%)	15 (4%)	2 (1%)	0	0	7 (4%)	5 (3%)	2 (1%)	0	0
Pain	17 (5%)	16 (5%)	1 (<1%)	0	0	14 (8%)	12 (7%)	2 (1%)	0	0
Abdominal distension	16 (5%)	15 (4%)	1 (<1%)	0	0	8 (5%)	8 (5%)	0	0	0

Gastroesophageal reflux disease	16 (5%)	14 (4%)	2 (1%)	0	0	3 (2%)	3 (2%)	0	0	0
Upper abdominal pain	14 (4%)	13 (4%)	1 (<1%)	0	0	13 (8%)	13 (8%)	0	0	0
Malaise	12 (3%)	9 (3%)	3 (1%)	0	0	7 (4%)	7 (4%)	0	0	0
Neck pain	11 (3%)	10 (3%)	1 (<1%)	0	0	6 (3%)	6 (3%)	0	0	0
Chest pain	8 (2%)	7 (2%)	1 (<1%)	0	0	11 (6%)	11 (6%)	0	0	0
Dehydration	8 (2%)	7 (2%)	1 (<1%)	0	0	2 (1%)	1 (1%)	1 (1%)	0	0
Pleural effusion	7 (2%)	5 (1%)	1 (<1%)	0	0	5 (3%)	4 (2%)	1 (1%)	0	0
Hypercalcaemia	5 (1%)	4 (1%)	1 (<1%)	0	0	2 (1%)	2 (1%)	0	0	0
Migraine	5 (1%)	4 (1%)	1 (<1%)	0	0	2 (1%)	1 (1%)	1 (1%)	0	0
Hyponatraemia	4 (1%)	2 (1%)	2 (1%)	0	0	2 (1%)	1 (1%)	1 (1%)	0	0
Cellulitis	3 (1%)	2 (1%)	0	1 (<1%)	0	0	0	0	0	0
Dental caries	3 (1%)	2 (1%)	1 (<1%)	0	0	2 (1%)	2 (1%)	0	0	0
Febrile neutropenia	3 (1%)	0	3 (1%)	0	0	1 (2%)	0	0	1 (1%)	0
Hyperglycemia†	3 (1%)	2 (1%)	1 (<1%)	0	0	4 (2%)	3 (2%)	1 (1%)	0	0
Pulmonary embolism†	3 (1%)	0	3 (1%)	0	0	0	0	0	0	0
Pneumonia	3 (1%)	2 (1%)	1 (<1%)	0	0	4 (2%)	3 (2%)	1 (1%)	0	0
Atrial fibrillation	2 (1%)	1 (<1%)	1 (<1%)	0	0	0	0	0	0	0
Increased blood bilirubin	2 (1%)	0	2 (1%)	0	0	4 (2%)	3 (2%)	1 (1%)	0	0
Deep vein thrombosis	2 (1%)	1 (<1%)	1 (<1%)	0	0	0	0	0	0	0
Device occlusion	2 (1%)	1 (<1%)	1 (<1%)	0	0	0	0	0	0	0
Erysipelas	2 (1%)	1 (<1%)	1 (<1%)	0	0	0	0	0	0	0
Disease progression	2 (1%)	0	0	0	2 (1%)	0	0	0	0	0

Hepatic failure	2 (1%)	0	1 (<1%)	0	1 (<1%)	0	0	0	0	0
Suicide attempt	2 (1%)	0	1 (<1%)	1 (<1%)	0	0	0	0	0	0
Viral infection	2 (1%)	1 (<1%)	1 (<1%)	0	0	1 (1%)	1 (1%)	0	0	0
Anogenital dysplasia	1 (<1%)	0	1 (<1%)	0	0	0	0	0	0	0
Bacteraemia	1 (<1%)	0	1 (<1%)	0	0	0	0	0	0	0
Breast mass	1 (<1%)	0	1 (<1%)	0	0	0	0	0	0	0
Cauda equine syndrome	1 (<1%)	0	1 (<1%)	0	0	0	0	0	0	0
Cholelithiasis	1 (<1%)	0	1 (<1%)	0	0	0	0	0	0	0
Chronic obstructive pulmonary disease	1 (<1%)	0	1 (<1%)	0	0	1 (1%)	0	1 (1%)	0	0
Disseminated intravascular coagulation	1 (<1%)	0	0	0	1 (<1%)	0	0	0	0	0
Drug-induced liver injury	1 (<1%)	0	1 (<1%)	0	0	0	0	0	0	0
Electrocardiogram QT prolonged	1 (<1%)	0	1 (<1%)	0	0	0	0	0	0	0
Endometrial cancer	1 (<1%)	0	1 (<1%)	0	0	0	0	0	0	0
General physical health deterioration	1 (<1%)	0	0	0	1 (<1%)	1 (1%)	0	1 (1%)	0	0
Granulocytopenia	1 (<1%)	0	1 (<1%)	0	0	0	0	0	0	0
Abnormal hepatic function	1 (<1%)	0	1 (<1%)	0	0	0	0	0	0	0
Obstructive hiatus hernia	1 (<1%)	0	1 (<1%)	0	0	0	0	0	0	0
Hyperkalaemia	1 (<1%)	0	1 (<1%)	0	0	6 (3%)	5 (3%)	1 (1%)	0	0

Intestinal obstruction	1 (<1%)	0	1 (<1%)	0	0	0	0	0	0	0
Abnormal liver function test	1 (<1%)	0	1 (<1%)	0	0	2 (1%)	1 (1%)	1 (1%)	0	0
Lower respiratory tract infection	1 (<1%)	0	1 (<1%)	0	0	0	0	0	0	0
Neutrophilia	1 (<1%)	0	1 (<1%)	0	0	0	0	0	0	0
Pleuritic pain	1 (<1%)	0	1 (<1%)	0	0	0	0	0	0	0
Psychotic disorder	1 (<1%)	0	0	1 (<1%)	0	0	0	0	0	0
Sedation	1 (<1%)	0	0	1 (<1%)	0	0	0	0	0	0
Syringomyelia	1 (<1%)	0	1 (<1%)	0	0	0	0	0	0	0
Increased troponin	1 (<1%)	0	0	1 (<1%)	0	0	0	0	0	0
Tumour ulceration	1 (<1%)	0	1 (<1%)	0	0	0	0	0	0	0
Wound infection	1 (<1%)	0	1 (<1%)	0	0	0	0	0	0	0

Data are number (%) unless otherwise noted; p values were not adjusted for multiple testing.

*****Adverse events graded in accordance with the maximum Common Terminology Criteria for Adverse Events, version 4.0, and MedDRA version 18.0. †Clustered preferred terms defined as follows: Anaemia is any event having a preferred term that equals to Anaemia or Haematocrit decreased or Haemoglobin decreased; Hypergycemia is any event having a preferred term that equals Blood glucose increased or Diabetes mellitus or Diabetes mellitus inadequate control or Glycosylated haemoglobin increased or Hyperglycaemia or Type 1 diabetes mellitus or Type 2 diabetes mellitus; Infections is defined as any event having a preferred term of the System Organ Class Infections and infestations; Leukopenia is any event having a preferred term that equals Leukopenia or White blood cell count decreased; Lymphopenia is any event having a preferred term that equals Lymphocyte count decreased or Lymphopenia; Neutropenia is any event having a preferred term that equals Neutropenia or Neutrophil count decreased; Rash is any event having a preferred term that equals Dermatitis or Dermatitis acneiform or Rash or Rash erythematous or rash maculopapular or Rash popular or Rash pruritic; Pulmonary embolism is any event having a preferred term that equals to Pulmonary artery thrombosis or Pulmonary embolism; Thrombocytopenia is any event having a preferred term that equals Platelet count decreased or Thrombocytopenia.

‡Grade 5 adverse events leading to death during the trial, or during the 28 days after the last dose of study medication, were not treatment-related toxicities