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Buparlisib plus fulvestrant versus placebo plus fulvestrant in postmenopausal, hormone receptor-positive, HER2-negative, advanced breast cancer (BELLE-2): a randomised, double-blind, placebo-controlled, phase 3 trial

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Declaration of interests

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Summary

Background—Phosphatidylinositol 3-kinase (PI3K) pathway activation is a hallmark of endocrine therapy-resistant, hormone receptor-positive breast cancer. This phase 3 study assessed the efficacy of the pan-PI3K inhibitor buparlisib plus fulvestrant in patients with advanced breast

cancer, including an evaluation of the PI3K pathway activation status as a biomarker for clinical benefit.

Methods—The BELLE-2 trial was a randomised, double-blind, placebo-controlled, multicentre study. Postmenopausal women aged 18 years or older with histologically confirmed, hormone receptor-positive and human epidermal growth factor (HER2)-negative inoperable locally advanced or metastatic breast cancer whose disease had progressed on or after aromatase inhibitor treatment and had received up to one previous line of chemotherapy for advanced disease were included. Eligible patients were randomly assigned (1:1) using interactive voice response technology (block size of 6) on day 15 of cycle 1 to receive oral buparlisib (100 mg/day) or matching placebo, starting on day 15 of cycle 1, plus intramuscular fulvestrant (500 mg) on days 1 and 15 of cycle 1, and on day 1 of subsequent 28-day cycles. Patients were assigned randomisation numbers with a validated interactive response technology; these numbers were linked to different treatment groups which in turn were linked to treatment numbers. PI3K status in tumour tissue was determined via central laboratory during a 14-day run-in phase. Randomisation was stratified by PI3K pathway activation status (activated *vs* non-activated *vs* and unknown) and visceral disease status (present *vs* absent). Patients, investigators, local radiologists, study team, and anyone involved in the study were masked to the identity of the treatment until unblinding. The primary endpoints were progression-free survival by local investigator assessment per Response Evaluation Criteria In Solid Tumors (version 1.1) in the total population, in patients with known (activated or non-activated) PI3K pathway status, and in PI3K pathway-activated patients. Efficacy analyses were done in the intention-to-treat population. Safety was analysed in all patients who received at least one dose of study drug and had at least one post-baseline safety assessment according to the treatment they received. This trial is registered with ClinicalTrials.gov, number NCT01610284, and is currently ongoing but not recruiting participants.

Findings—Between Sept 7, 2012, and Sept 10, 2014, 1147 patients from 267 centres in 29 countries were randomly assigned to receive buparlisib (n=576) or placebo plus fulvestrant (n=571). In the total patient population (n=1147), median progression-free survival was 6·9 months (95% CI 6·8–7·8) in the buparlisib group versus 5·0 months (4·0–5·2) in the placebo group (hazard ratio [HR] 0·78 [95% CI 0·67–0·89]; one-sided p=0·00021). In patients with known PI3K status (n=851), median progression-free survival was 6·8 months (95% CI 5·0–7·0) in the buparlisib group *vs* 4·5 months (3·3–5·0) in the placebo group (HR 0·80 [95% CI 0·68–0·94]; one-sided p=0·0033). In PI3K pathway-activated patients (n=372), median progression-free survival was 6·8 months (95% CI 4·9–7·1) in the buparlisib group versus 4·0 months (3·1–5·2) in the placebo group (HR 0·76 [0·60–0·97], one-sided p=0·014). The most common grade 3–4 adverse events in the buparlisib group versus the placebo group were increased alanine aminotransferase (146 [25%] of 573 patients *vs* six [1%] of 570), increased aspartate aminotransferase (103 [18%] *vs* 16 [3%]), hyperglycaemia (88 [15%] *vs* one [<1%]), and rash (45 [8%] *vs* none). Serious adverse events were reported in 134 (23%) of 573 patients in the buparlisib group compared with 90 [16%] of 570 patients in the placebo group; the most common serious adverse events (affecting ≥2% of patients) were increased alanine aminotransferase (17 [3%] of 573 *vs* one [<1%] of 570) and increased aspartate aminotransferase (14 [2%] *vs* one [<1%]). No treatment-related deaths occurred.

Interpretation—The results from this study show that PI3K inhibition combined with endocrine therapy is effective in postmenopausal women with endocrine-resistant, hormone receptor-positive

and HER2-negative advanced breast cancer. Use of more selective PI3K inhibitors, such as α-specific PI3K inhibitor, is warranted to further improve safety and benefit in this setting. No further studies are being pursued because of the toxicity associated with this combination.

Introduction

Hormone receptor-positive tumours are the most common subtype of breast cancer,[1] for which endocrine therapy-based regimens form the backbone of treatment.[2–4] Recent studies have indicated that hormone receptor-positive breast cancer is not homogeneous, but rather is characterised by various genomic alterations that might affect treatment outcomes, providing opportunities for targeted therapies.[1,5] Activating *PIK3CA* mutations (encoding the p110α isoform of phosphatidylinositol 3-kinase [PI3K]) are often observed in hormone receptor-positive breast cancer and have been associated with disease progression and endocrine therapy resistance;[1,6–9] targeting PI3K is therefore a potential therapeutic strategy. Identification of patients with *PIK3CA* mutations who derive benefit from PI3K-targeted therapy could help guide treatment decisions. One of the non-invasive techniques that can be used for detection of *PIK3CA* mutations is circulating tumour DNA (ctDNA) analysis. ctDNA analysis provides a more accurate measure of mutational status and heterogeneity over time and treatment, compared with archival tumour tissue.[10,11]

Research in context

Evidence before this study

We searched PubMed for articles published between Nov 21, 2006, to Nov 21, 2016, using the terms “PI3K inhibitor” OR “PI3 kinase inhibitor” AND “metastatic breast cancer”, and identified 140 full-text original articles in English. Of these articles, 11 publications reported data from phase 1 or phase 2 clinical trials involving phosphatidylinositol 3-kindase (PI3K) inhibitors in breast cancer. No publication related to any phase 3 trial of a PI3K inhibitor in metastatic breast cancer was identified.

Added value of this study

To our knowledge, BELLE-2 is the first global phase 3 clinical trial assessing the safety and efficacy of a pan-PI3K inhibitor, buparlisib, in postmenopausal women with aromatase inhibitor-resistant, hormone receptor-positive and HER2-negative advanced breast cancer, which showed significantly longer progression-free survival with buparlisib and fulvestrant compared with placebo and fulvestrant. In this disease setting and in light of the emerging treatment landscape, a progression-free survival benefit of about 2 months observed in the overall population can be considered a clinically moderate improvement. However, many patients discontinued the study treatment early, mainly for tolerability reasons, resulting in short treatment exposure, particularly with buparlisib (median 1·9 months), which might have limited the potential benefit. Additionally, to our knowledge, this was the first randomised, phase 3 clinical trial to include a prospective analysis of progression-free survival in a subset of patients with ctDNA *PIK3CA* mutations. This subset of patients showed a clinically meaningful improvement in progression-free survival with buparlisib plus fulvestrant compared with fulvestrant alone.

Implications of all the available evidence

Results from this study as well as others with PI3K inhibitors add to the growing evidence that targeting the PI3K pathway via dual-blocking strategies plays an important part in the treatment of hormone receptor-positive breast cancer and overcoming resistance to endocrine therapy. Furthermore, these results also support the hypothesis that endocrine resistance might be linked to PI3K pathway activation. ctDNA analysis seemed to be a more accurate and reliable method to assess the actual PI3K status compared with archival tissue because of possible changes in tumour biology over time. Assessment of *PIK3CA* mutations in ctDNA might help to select patients who could benefit from such treatment. Further efforts should focus on assessment of PI3K α-isoform-specific inhibition, which might be more effective than pan-PI3K inhibition, with a more tolerable toxicity profile, permitting prolonged administration at higher doses.

In early clinical studies, PI3K inhibitors have shown low single-agent activity.[12] Further preclinical and early clinical investigations showed that resistance can arise through enhanced oestrogen receptor pathway signalling, such as through *ESR1* mutations.[7,11,13] Combining PI3K inhibition with the oestrogen receptor antagonist fulvestrant can prevent oestrogen receptor activation, resulting in synergistic antitumour activity.[7,14] Thus, dual blockade of PI3K and oestrogen receptor pathways could restore treatment sensitivity and inhibit growth of endocrine therapy-resistant tumours. The phase 3 BOLERO-2 study showed that the mammalian target of rapamycin (mTOR) inhibitor everolimus plus the aromatase inhibitor exemestane is an effective treatment for hormone receptor-positive and human epidermal growth factor receptor (HER2)-negative advanced breast cancer;[3] however, mTOR's mechanism of activation is complex and only partially dependent on PI3K.[15,16]

Buparlisib (BKM120), an oral pan-PI3K inhibitor, targets all four isoforms of class 1 PI3K (α, β, γ, and δ).[17] Combined treatment with buparlisib and fulvestrant induced tumour regression in mice bearing oestrogen receptor-positive breast cancer xenografts.[14] In a phase 1 study, the combination was generally well tolerated and showed preliminary clinical activity in patients with oestrogen receptor-positive advanced breast cancer.[18]

The BELLE-2 study reported here assessed the efficacy and safety of buparlisib plus fulvestrant in postmenopausal women with aromatase inhibitor-resistant, hormone receptor-positive and HER2-negative advanced breast cancer, and investigated tumour PI3K pathway activation status as a biomarker for treatment response.

Methods

Study design and participants

The BELLE-2 trial was a randomised, double-blind, placebo-controlled, phase 3 trial. Eligible participants were postmenopausal women aged 18 years or older with a histologically or cytologically confirmed diagnosis of hormone receptor-positive and HER2-negative breast cancer, and availability of adequate tumour tissue. Patients must have progressed within 12 months of receiving aromatase inhibitor therapy in the adjuvant setting,

or within 1 month for metastatic or advanced disease, and have inoperable locally advanced or metastatic disease. Moreover, patients must have had radiological or objective evidence of recurrence or progression on or after the last systemic therapy before enrolment. Patients could receive any number of endocrine or hormonal lines of therapy before or after meeting the definition of refractory to aromatase inhibitors. Other anticancer therapies before or after progression on aromatase inhibitor therapy were allowed. Hormone receptor-positive and HER2-negative status were confirmed locally from the most recent tumour biopsy available. Patients had measurable disease, or non-measurable lytic or mixed bone lesions, per Response Evaluation Criteria In Solid Tumours (RECIST; version 1.1), adequate bone marrow and organ function assessed by routine biochemical and haematological laboratory tests, and Eastern Cooperative Oncology Group (ECOG) performance status of 2 or lower at baseline. Before treatment with fulvestrant, targeted PI3K–AKT–mTOR inhibition or more than one line of chemotherapy for advanced disease was not permitted. Patients receiving increasing or chronic treatment with corticosteroids or other immunosuppressants, and those receiving warfarin or other coumarin-derived anticoagulants, were also ineligible. The estimated life expectancy of eligible patients was about 20 months.

Patients with symptomatic CNS metastases and those with a concurrent malignancy or a malignancy within 3 years of study enrolment were excluded. Patients with a history of active major depression, bipolar disorder, obsessive-compulsive disorder, schizophrenia, suicidal attempt or ideation, homicidal ideation, or anxiety grade 3 or worse per Common Terminology Criteria for Adverse Events (CTCAE; version 4.03) at baseline were excluded, as were those with self-assessed depression (Patient Health Questionnaire [PHQ]-9 score ≥12) or anxiety (Generalised Anxiety Disorder [GAD]-7 score ≥15).

All patients provided written informed consent before enrolment. The study protocol and amendments were approved by an institutional review board or independent ethics committee at each site. The study was done in accordance with the guidelines for good clinical practice, local regulations, and the Declaration of Helsinki.

Randomisation and masking

Eligible patients were randomly assigned (1:1) to receive fulvestrant and buparlisib or fulvesterant and placebo, stratified by PI3K pathway status in tumour tissue (activated *vs* non-activated *vs* unknown) and visceral disease status (present *vs* absent). A validated, automated Interactive Response Technology (IRT) that included an interactive voice and web response system was used to randomly assign patients to treatment by allocating them random numbers that were in turn linked to different treatment groups. Randomisation was done with a block size of six for each group. Investigators provided identifying information for each patient at enrolment to register them into the IRT system, and each patient was assigned a unique seven-digit patient number, which they retained throughout their participation in the study; these numbers were generated to ensure treatment assignment was unbiased and concealed from patients and investigators. A patient randomisation list was produced by the IRT provider using a validated system to automate the random assignment of patient numbers to randomisation numbers. Each randomisation number was linked to a treatment group and a unique medication number. A separate treatment randomisation list

was produced by Novartis Drug Supply Management with a validated system to automate the random assignment of treatment numbers to treatment packs containing each study drug. Randomisation numbers were not communicated to investigators. The study was double-blinded, so patients, investigators, local radiologists, the study team, and anyone involved in the study were not aware of the treatment group allocation. Premature unmasking of study drug assignment was only allowed in case of an emergency. The identity of the treatments was concealed by the use of investigational drugs (buparlisib or placebo) that were identical in appearance and packaging, labelling, and schedule of administration. An external independent statistical group not involved in the trial conduct prepared periodic safety reports and interim futility reports for the data monitoring committee.

Procedures

All eligible patients were enrolled into a 14-day run-in treatment phase to allow initiation of fulvestrant 500 mg dosing on day 1 of cycle 1. The PI3K pathway activation status was determined (via Sanger sequencing of the *PIK3CA* gene and immunohistochemistry assessment of phosphatase and tensin homologue protein expression) during this period at a Novartis-designated laboratory. Patients were categorised into three groups: PI3K pathway activated (any mutation detected by Sanger sequencing in *PIK3CA* exons 1, 7, 9, or 20; or loss of phosphatase and tensin homologue [PTEN] expression [<10% of cells with expression level 1+ by immunohistochemistry, and none with level >1+]), PI3K pathway non-activated (no *PIK3CA* mutations detected and detectable PTEN expression), and PI3K pathway unknown (uninterpretable assessment for *PIK3CA* and PTEN, with the other marker non-activated). During the run-in phase, all patients received intramuscular fulvestrant 500 mg on days 1 and 15 of cycle 1, and on day 1 of subsequent 28-day cycles. Patients were then randomly assigned (1:1) on day 15 of cycle 1 to receive continuation of fulvestrant at the same dose and schedule (500 mg on day 1 of each cycle) plus either oral buparlisib (100 mg once daily, starting from day 15 of cycle 1) or matching placebo, starting on day 15 of cycle 1. Novartis Drug Supply Management (Basel, Switzerland) or its designee provided buparlisib and placebo as 10 mg and 50 mg hard gelatin capsules as individual patient supply, packaged in bottles. Fulvestrant was supplied according to local practice and regulation. There was no specific recommendation regarding the sequence of administration of buparlisib or placebo and fulvestrant.

In general, treatment continued until disease progression (per RECIST version 1·1), unacceptable toxicity, death, or discontinuation for any other reason. As per the protocol, a patient could discontinue from study treatment for any of the following reasons: adverse event, loss to follow-up, non-compliance to study treatment, physician decision, pregnancy, progressive disease, protocol deviation, termination of the study by sponsor, technical problems, participant or guardian decision, or death. Treatment crossover from placebo to buparlisib was not permitted. Dose modifications were allowed in case a patient was unable to tolerate the protocol-specified dose. A maximum of three dose modifications implemented sequentially was allowed for buparlisib or placebo as follows: 80 mg/day continuously, 100 mg/day on 5 days of 7, and 80 mg/day on 5 days of 7. Dose reduction below 80 mg/day on 5 days of 7 was not allowed, and in case this modification was required for any patient, treatment was permanently discontinued for that particular patient. Once a

dose had been reduced during a treatment cycle, re-escalation was not permitted during any subsequent cycle. No dose modifications were allowed for fulvestrant (appendix). For buparlisib and placebo, dose interruptions for up to 28 days were allowed.

Tumour assessments (by CT or MRI) were done at screening and 6 weeks after the randomisation date, and every 8 weeks thereafter until disease progression, death, start of new antineoplastic treatment, loss to follow-up, or withdrawal of consent for efficacy follow-up. Imaging data used for tumour assessments were also collected centrally and were prospectively reviewed by a blinded independent central radiology committee. Other radiographic assessments included bone scan (done at screening), and brain scan, in case of clinical indication. The following laboratory assessments were done locally: haematology, biochemistry (full or partial), coagulation, fasting serum lipid profile, glucose, C peptide, lipase, glycosylated haemoglobulin (HbA_{1c}), and urinalysis. Laboratory assessments, including haematology and biochemistry, were done at baseline, weekly until day 22 of cycle 2, and on day 1 of subsequent cycles until the end of treatment. Safety monitoring was done regularly by haematology assessment, assessment of blood chemistry, including glucose monitoring and regular assessments of vital signs, physical condition, bodyweight, electrocardiogram (ECG), cardiac imaging, ECOG performance status, and assessments of patients' self-reported mood questionnaires. Adverse events were assessed and graded according to CTCAE version 4.03. A standard 12-lead ECG was done at screening, predose on day 15 of cycle 1, on day 1 of every cycle, and at the end of treatment. Safety was followed up for 30 days after study treatment discontinuation date. Two different patient self-reported mood questionnaires (PHQ-9 and GAD-7) were completed at screening, on day 15 of cycle 1, day 1 and day 15 of cycle 2 and cycle 3, and on day 1 of each subsequent cycle in addition to the end-of-treatment visit. After treatment discontinuation, patients were followed for survival every 3 months irrespective of their reason for discontinuation, except for those who withdrew consent, refused survival follow-up, or were lost to follow-up.

Plasma samples were collected from patients who were randomly assigned after a protocol amendment (mandatory blood collection for ctDNA analysis at study entry was implemented in June, 2013, as part of amendment 2 to the protocol; prior to this amendment, collection was optional, based on evidence supporting use of ctDNA to determine current tumour mutation status.[10,19,20] A pre-defined panel of 15 *PIK3CA* mutations in exons 1, 7, 9, and 20 (Arg88Gln, Arg93Trp/Gln, Lys111Glu/Asn, Gly118Asp, Glu365Lys, Cys420Arg, Glu542Lys, Glu545Gly/Lys, Gln546Lys, and His1047Arg/Leu/Tyr) was analysed by BEAMing technology.[10]

Outcomes

The primary endpoint was progression-free survival (according to local investigator assessment per RECIST version 1.1) in the total population (PI3K pathway activated, non-activated, and unknown), in the main study cohort (patients with known PI3K status: ie, PI3K activated and non-activated), and in the PI3K pathway-activated group. The primary endpoint in the total population was included as part of a protocol amendment done on July 9, 2014. There was no change to the existing primary and key secondary objectives of progression-free survival and overall survival in the main study cohort (patients with known

PI3K status) and PI3K pathway-activated subpopulation. The purpose of this amendment was to assess treatment effectiveness in the total population apart from the main study cohort. The proposed amendment added additional study hypotheses in the total population and implemented statistical testing in the total population if there was a statistically significant treatment effect in the main study cohort.

Progression-free survival was defined as time from randomisation date until the first documented progression or death due to any cause, whichever occurred first. Progression-free survival was also analysed with the central radiology assessment done by the blinded independent review committee.

The key secondary endpoints were overall survival, defined as time from randomisation to date of death due to any cause, in the total population, the main study cohort (patients with known PI3K status), and the PI3K pathway-activated group. Other secondary endpoints included overall response (defined as the proportion of patients who achieved an overall response of complete or partial response per RECIST version 1.1), clinical benefit (defined as the proportion of patients with a best overall response of complete or partial response or stable disease for ≥24 weeks per RECIST version 1.1), progression-free and overall survival in the PI3K pathway non-activated sub-population and PI3K-unknown cohort, pharmacokinetics, quality of life (based on global quality of life scale of the EORTC QLQ-C30 questionnaire and EORTC QLQ-BR23; mood questionnaires were part of the safety assessments and objectives), and safety (based on the frequency of adverse events and number of abnormal laboratory values that were outside predetermined ranges). The results of pharmacokinetics and quality-of-life assessments will be reported in another paper. For the primary endpoints progression-free survival, objective response rate, and ctDNA analysis, we show all subgroup analysis by PI3K status. Overall survival was an interim analysis, only done at the time of the primary endpoint; more detailed overall survival analysis will be done once the final number of overall survival events have been reached. Progression-free survival based on ctDNA *PIK3CA* mutation status at screening in a subset of patients was a prespecified exploratory endpoint.

Statistical analysis

The primary efficacy endpoint of progression-free survival in the total population, main study cohort, and PI3K pathway-activated group was analysed by log-rank tests (stratified by PI3K pathway and visceral disease status in the total population and main study cohort, and visceral disease status in the PI3K pathway-activated group). Median progression-free survival of 5 months was assumed for fulvestrant monotherapy.[21–23]

To conserve the overall type I error (one-sided significance level α=0·025) across primary endpoints, an α split with graphical gate-keeping approach was implemented (appendix p 2).[24,25] Statistical assumptions for testing the primary endpoints are shown in the appendix (p 9). A total of 589 progression-free survival events in the main study cohort were required to detect a hazard ratio (HR) of 0·67 with 91·8% power at a one-sided 0·02 level of significance. 230 progression-free survival events in the PI3K pathway-activated group were required to detect HR 0·60 with 93·6% power at a one-sided 0·01 level of significance. One preplanned interim futility analysis (done based on the data cutoff date of Jan 31, 2014)

using a γ spending function (γ=12·8) was done in the main study cohort after 261 (44·3%) of 589 progression-free survival events had been observed. Final progression-free survival analysis was done after the required number of events in the main study cohort and PI3K pathway-activated group had occurred, irrespective of events in the total population. Around 1200 patients in the total population and 842 in the main study cohort (a minimum of 334 with PI3K pathway-activated status) needed to be randomised, under the assumption 25–30% of patients had unknown PI3K pathway status. Sample size calculations were done using EAST 5.3.

Efficacy analyses were done in the intention-to-treat population, defined as all randomly assigned patients. Safety was analysed in all patients who received at least one dose of study drug and had at least one post-baseline safety assessment according to the treatment they received. For the primary and key secondary efficacy endpoints (progression-free survival and overall survival), all patients were included in the analysis as per the intention-to-treat principle. Median progression-free survival was estimated with the Kaplan-Meier method. A stratified Cox regression was used to estimate the HR, along with two-sided 95% CI. Proportional hazards assumption was verified using graphical plots. Progression-free survival was censored at last adequate tumour assessment date before the analysis cutoff date if no progression-free survival event was observed before the cutoff date. If the patient started a new antineoplastic therapy, the censoring date was the date of the last adequate tumour assessment before the initiation of the therapy, or before the data cutoff date, whichever occurred first. A missing adequate tumour assessment was defined as a tumour assessment not done at that assessment or an assessment with an unknown overall lesion response. If a progression-free survival event occurred after two or more missing assessments, progression-free survival was censored at the last adequate tumour assessment. The proportion of patients with an overall response and clinical benefit was summarised with 95% CIs based on the Clopper–Pearson method. All statistical analyses were done, and figures generated, with SAS version 9.4. Safety and interim futility analysis was monitored by an independent data monitoring committee; the study met predefined criteria to continue based on preliminary efficacy in an interim futility analysis (a data monitoring committee meeting to assess futility was held on April 17, 2014).

This study is registered with ClinicalTrials.gov, number NCT01610284.

Role of the funding source

The study was designed, conducted, and analysed by an academic steering committee including the sponsor (Novartis). Study drugs were provided by Novartis. The authors confirm adherence to the study protocol and vouch for the accuracy and completeness of data. BD, PU, SH, and CM had full access to the raw data. The corresponding author also had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between Sept 7, 2012, and Sept 10, 2014, 2025 patients were screened for eligibility, from 267 centres in 29 countries (appendix pp 5–9), of whom 847 were excluded for various

reasons (figure 1). The most common reasons for screen failure in around half of these screen failures was inadequate tumour tissue (poor quality or quantity, or both) required for PI3K pathway activation status testing, followed by inadequate bone marrow and organ function reserve. Among 1178 patients enrolled in the run-in phase, 31 discontinued from the study. 1147 patients were then randomly assigned to receive buparlisib and fulvestrant (buparlisib group; n=576) or placebo and fulvestrant (placebo group; n=571; figure 1). Baseline characteristics were well balanced between the treatment groups. A similar proportion of patients discontinued treatment in both the buparlisib group (481 patients) and the placebo group (475 patients) during the study.

Overall, median age was 62·0 years (IQR 54·0–69·0). Of 1147 patients, 1119 (98%) had ECOG performance status 0 or 1, 678 (59%) had visceral disease, 1142 (>99%) had received previous treatment with an aromatase inhibitor, 842 (73%) had previous chemotherapy in any setting (including adjuvant), and 847 (74%) and 318 (28%) had received previous endocrine therapy and chemotherapy for metastatic disease (table 1). PI3K pathway status in tumour tissue was activated in 372 (32%) of 1147 patients, non-activated in 479 (42%), and unknown in 296 (26%). Of 860 patients with known *PIK3CA* mutation status in tumour tissue, 276 (32%) harboured *PIK3CA* mutations (appendix p 10). Only 30 (3%) of 1147 tissue samples were from fresh biopsies; archival samples were acquired a median of 3·8 years (IQR 1·8–7·2) before study entry (appendix p 11). Of 587 patients with *PIK3CA* mutation status detected in ctDNA, 200 (34%) harboured *PIK3CA* mutations. Baseline characteristics were balanced between ctDNA subgroups (appendix p 12).

At the cutoff date for final analysis (April 29, 2015), 187 (16%) of 1147 patients remained on treatment. The most frequent reasons for treatment discontinuations in both groups were disease progression, adverse events, and patient or physician decision (figure 1). The median progression-free survival follow-up was 13·73 months (IQR 5·45–19·81) in the buparlisib group versus 14·32 (10·64–21·62) in the placebo group.

This study met its primary endpoint for significant improvement in progression-free survival with buparlisib versus placebo in the total population (n=1147): median progression-free survival was 6·9 months (95% CI 6·8–7·8) in the buparlisib group versus 5·0 months (4·0–5·2) in the placebo group (HR 0·78 [95% CI 0·67–0·89], one-sided p=0·00021; figure 2A, appendix p 14). The results from the multivariate Cox regression model, adjusting for prognostic clinical variables in the total population, showed a similar result in progression-free survival in favour of the buparlisib group (HR 0·78, 95% CI 0·67–0·91; p value not provided as it was a sensitivity analysis adjusting for prognostic factors, and the median is not affected because this is a multivariate analysis to assess the effect on HR). In the main study cohort (comprising all patients with known PI3K pathway status; n=851 [n=427 in the buparlisib group and n=424 in the placebo group]), improvement in progression-free survival in the buparlisib group versus the placebo group was significantly longer at one-sided α=0·02 (median 6·8 months [95% CI 5·0–7·0] in the buparlisib group *vs* 4·5 months [3·3–5·0] in the placebo group; HR 0·80 [95% CI 0·68–0·94], one-sided p=0·0033; appendix p 14, 18). However, in the PI3K pathway-activated group (n=372 [n=188 in the buparlisib group and n=184 in the placebo group]), there was no significant difference in progression-free survival between the groups according to predefined criteria at one-sided α=0·01

(median 6·8 months [95% CI 4·9–7·1] *vs* 4·0 months [3·1–5·2]; HR 0·76 [95% CI 0·60–0·97], one-sided p=0·014; figure 2B, appendix p 14). Subgroup analyses (pre-planned sensitivity analyses) were also done to assess the effect of buparlisib versus placebo on investigator-assessed progression-free survival in different patient subgroups (appendix p 3). Progression-free survival results assessed centrally were consistent with the local investigator's assessment (appendix p 15).

Overall survival data were immature; there were 281 (24%) deaths in the total population at the cutoff date (129 in the buparlisib group and 152 in the placebo group). There were 222 deaths in the main cohort and 98 in the PI3K-activated group. Follow-up for overall survival is ongoing. Given the data are immature and did not meet efficacy stopping boundary, we did not perform any subgroup analysis for overall survival (this will be done at the time of final overall survival, which will be reported elsewhere).

In the total population, the proportion of patients achieving an overall response was 11·8% (95% CI 9·3–14·7) in the buparlisib group versus 7·7% (5·7–10·2) in the placebo group and the proportion of patients achieving a clinical benefit was 43·8% (95% CI 39·7–47·9) versus 42·0% (37·9–46·2), respectively (appendix p 16). Similar proportions of patients achieving an overall response were seen in the main study cohort and PI3K pathway-activated group (appendix p 16). Because this was a large phase 3 study, wih several protocol-specified secondary endpoints, we focused on key efficacy and safety endpoints in this Article. Other secondary endpoints will be reported in a separate manuscript.

For our prespecified exploratory analysis of progression-free survival based on *PIK3CA* mutation status in ctDNA, 446 patients had paired tumour and ctDNA samples (appendix p 13). Overall concordance of *PIK3CA* status in tumour tissue and ctDNA was 342 (77%) of 446. In 307 patients with *PIK3CA* wild-type tumour tissue, 243 (79%) had non-mutant ctDNA, and 64 (21%) had *PIK3CA* mutant ctDNA, potentially indicating tumour evolution between initial diagnosis and treatment. A significant difference in progression-free survival in the buparlisib group versus the placebo group was recorded in patients with ctDNA *PIK3CA* mutations (figure 3A), but not those with non-mutant ctDNA (figure 3B). The progression-free survival benefit in patients with ctDNA *PIK3CA* mutations was maintained in all subgroups, irrespective of the exploratory analysis by the Sanger sequencing mutation status in archival tissue (mutated, non-mutant, or unknown). In the 64 patients who had *PIK3CA* mutant ctDNA but non-mutant (ie, wild-type) *PIK3CA* status by Sanger in tissue (appendix p 13), the median progression-free survival was 4·6 months (95% CI 3·3–15·1) in the buparlisib group versus 1·5 months (1·4–5·1) in the placebo group (unstratified HR 0·58, 95% CI 0·32–1·05, p=0·036). No difference in progression-free survival was recorded in the 40 patients with ctDNA non-mutant but *PIK3CA* mutant status in tissue by Sanger sequencing (unstratified HR 1·18, 95% CI 0·49–2·85, p=0·646). 18 of 40 patients with non-mutant ctDNA harboured tissue mutations detected by Sanger sequencing that were not included in the BEAMing panel (appendix). Additionally, 36 of 199 patients with mutant ctDNA had unknown PI3K status by Sanger sequencing, potentially due to the lower sensitivity of the method (appendix). These patients also derived progression-free survival benefit (unstratified HR 0·44, 95% CI 0·18–1·10). In a post-hoc exploratory analysis of the ctDNA *PIK3CA*-mutant subgroup, the proportion of patients achieving an overall response

or clinical benefit was numerically greater in the buparlisib group than in the placebo group (appendix pp 4, 16).

Table 2 lists the most common adverse events that occurred during the trial (irrespective of whether or not they were judged to be related to treatment). Of note, in the buparlisib plus fulvestrant group, there was increased grade 1–4 hyperglycaemia, elevated alanine aminotransferase (ALT) and aspartate aminotransferase (AST), nausea, diarrhoea, rash, fatigue, and stomatitis compared with the placebo group. The most common grade 3–4 adverse events in the buparlisib group versus the placebo group were increased alanine amino-transferase (ALT; 146 [25%] of 573 patients *vs* six [1%] of 570), increased aspartate aminotransferase (AST; 103 [18%] *vs* 16 [3%]), hyperglycaemia (88 [15%] *vs* one [<1%]), and rash (45 [8%] *vs* none). The most frequently reported mood disorders were depression and anxiety, and these were more common in the buparlisib group than in the placebo group (table 2). Serious adverse events occurred in 134 (23%) of 573 patients in the buparlisib group compared with 90 (16%) of 570 patients in the placebo group; the most common (in ≥2% of patients) were increased ALT (17 [3%] of 573 *vs* 1 [<1%] of 570]) and increased AST (14 [2%] *vs* 1 [<1%]). Suicidal ideation was reported in three patients in the buparlisib group and two patients in the placebo group. No suicide attempts were reported. No treatment-related deaths occurred.

Median duration of treatment exposure overall was 4·2 months (IQR 2·3–9·6) in the buparlisib group versus 5·0 months (1·6–10·5) in the placebo group; median duration of exposure to buparlisib was 1·9 months (IQR 1·1–5·4; median relative dose intensity 93·2% [IQR 71·9–100]), whereas median duration of exposure to placebo was 4·4 months (IQR 1·4–10·2). Median relative dose intensity was not calculated for the placebo group because there were no dose reductions associated with fulvestrant. Buparlisib exposure was limited by higher frequencies of adverse events in this group than in the placebo group, leading to a higher incidence of dose interruptions (290 [51%] of 573 in the buparlisib group *vs* 81 [14%] of 570 in the placebo group), reductions (258 [45%] *vs* 32 [6%]), and discontinuations (222 [39%] *vs* 28 [5%]). The total proportion of patients reporting dose reduction or discontinuation, or both, was 401 (70%) of 573 in the buparlisib group versus 58 (10%) of 570 in the placebo group. In the buparlisib group, the most common adverse events leading to discontinuation were increased ALT (58 [10%] of 573]), increased AST (40 [7%]), hyperglycaemia (19 [3%]), depression (17 [3%]), and rash (16 [3%]). The most common adverse events leading to treatment discontinuation in the placebo group were fatigue, increased AST, arthralgia, and back pain (each three [0·5%] of 570). 24 on-treatment deaths occurred (12 [2%] in each group): nine (2%) in each group due to disease progression; three in the placebo group due to cerebrovascular accident, cerebral haemorrhage, and urosepsis; and three in the buparlisib group due to gastric ulcer haemorrhage, pneumonia, and septic shock. None of the on-treatment deaths were suspected to be related to study treatment.

Discussion

The results of this study show that the addition of buparlisib to fulvestrant significantly increased progression-free survival in postmenopausal women with aromatase inhibitor-

resistant, hormone receptor-positive and HER2-negative advanced breast cancer. These findings are consistent with the recently presented BELLE-3 results[26] wherein the addition of buparlisib to fulvestrant prolonged progression-free survival compared with the placebo plus fulvestrant group in postmenopausal women with hormone receptor-positive and HER2-negative advanced breast cancer who had received previous aromatase inhibitor and mTOR inhibitor treatment.

Our study met its primary endpoint in the total population and main cohort (patients with known PI3K pathway status), despite limited exposure to buparlisib caused by frequent discontinuations due to adverse events, which reduced treatment duration in the buparlisib group. This reduced exposure to buparlisib might have limited the efficacy of combination therapy. The most common psychiatric side-effects were depression and anxiety, which could be attributed to the high blood–brain barrier-penetrating properties of buparlisib.[17,27] These effects are not usually seen with other PI3K inhibitors (eg, pictilisib, alpelisib, and taselisib).[28–30]

Identifying patients with *PIK3CA* mutations (with breast as well as other types of cancers) who derive benefit from PI3K-targeted therapy could help to guide treatment decisions. A non-invasive technique that can be used for detection of *PIK3CA* mutations is ctDNA analysis, which might provide a more accurate measure of mutational status and heterogeneity over time and treatment, compared with archival tumour tissue. Based on emerging evidence supporting the use of ctDNA to assess current tumour alteration status,[10,11,20] to our knowledge, this study is the first randomised, phase 3 clinical trial to include a prospective analysis of progression-free survival in patients with ctDNA *PIK3CA* mutations. These patients had especially poor outcomes on fulvestrant monotherapy, potentially because of endocrine therapy resistance mediated by aberrant PI3K pathway activation.[7,14] Combined buparlisib and fulvestrant treatment resulted in clinically meaningful improvements in progression-free survival, overall responses, and clinical benefit compared with fulvestrant alone in the ctDNA *PIK3CA* mutant (but not the non-mutant) subgroup. The progression-free survival benefit in the ctDNA *PIK3CA* mutant subgroup was maintained irrespective of the tumour tissues' mutation status by Sanger sequencing. Results from the BELLE-3 study[26] also suggested that treatment benefit of buparlisib in combination with fulvestrant was limited to patients with *PIK3CA*-mutant tumours, assessed either in primary tumour samples via PCR or in ctDNA collected at baseline. However, in our study, no statistically significant progression-free survival benefit with buparlisib was recorded in patients with PI3K-pathway activation in tumour tissue. This finding might be attributed to the fact that the progression-free survival in the PI3K pathway-activated subpopulation was tested at a stringent one-sided level of significance of 0·01, taking into consideration the multiple primary endpoints in this study as well as changes in the tumour biology or methodological differences in PI3K status assessment.

A limitation of our study was that exposure to buparlisib was restricted because of a high rate of treatment discontinuations due to adverse events in the combination therapy group (as observed in other clinical studies). PI3K α-isoform-specific inhibition might therefore be more effective than pan-PI3K inhibition with a more tolerable toxicity profile, permitting prolonged administration at higher doses.[29,31] Ongoing phase 3 randomised trials of PI3Kα

inhibitors include SOLAR-1 (NCT02437318; alpelisib and fulvestrant in hormone receptor-positive and HER2-negative advanced breast cancer, with a prospective analysis of *PIK3CA* mutations in ctDNA) and SANDPIPER (NCT02340221; taselisib and fulvestrant in *PIK3CA*-mutant, hormone receptor-positive and HER2-negative advanced breast cancer). Buparlisib is also being investigated for treatment of breast cancer in combination with other drugs such as letrozole, tamoxifen, and capecitabine in various clinical trials (eg, NCT01923168, NCT02404844, and NCT01300962). In addition to breast cancer, buparlisib has also been assessed in other indications such as head and neck cancer (BERIL-1, NCT01852292) and lung cancer (NCT01470209).

Approved therapies for postmenopausal patients with advanced hormone receptor-positive, HER2 negative breast cancer include the mTOR inhibitor everolimus with exemestane[3] or tamoxifen as a first-line treatment,[32] and the cyclin-dependent kinase (CDK)4/6 inhibitor palbociclib with letrozole or fulvestrant as second-line therapy.[4,33] The future role of PI3K inhibitors in cancer should be considered in light of these treatment options; triplet combinations of PI3Kα inhibitors with endocrine therapy, and CDK4/6 inhibitors (eg, ribociclib) are under investigation, and could potentially replace progressive chemotherapy in this indication.

This study shared a limitation common to biomarker studies: most tissue samples were from archival biopsies obtained several years before study entry. Archival tissue from the primary tumour might not accurately reflect the PI3K activation status at the time of study entry, which could change over time, especially after the patient has received multiple lines of previous therapy. Recent molecular profiling studies have shown high intratumoural heterogeneity in advanced breast cancers, with evidence of tumour evolution in response to several lines of antineoplastic therapy.[34–36] Thus, tissue-based PI3K activation status as determined in this trial might not optimally predict treatment benefit. As expected, we recorded a high concordance of *PIK3CA* wild-type status between ctDNA and tissue, because tumours are unlikely to lose acquired *PIK3CA* mutations (although several patients seemed to follow this pattern, potentially due to tumour heterogeneity or tissue sampling).[10] Concordance of PIK3CA mutant status might have been further affected by the time difference between tumour tissue and ctDNA sample acquisition, and differences in sequencing methods. Of note, progression-free survival benefit was recorded in all patients with ctDNA *PIK3CA* mutation, irrespective of the Sanger sequencing mutation status in archival tissue (mutated, non-mutated, or unknown; appendix). Our results therefore support the use of ctDNA analysis as a specific, sensitive, and minimally invasive technique to characterise current tumour status[10,19,20] The meaningful treatment benefit reported in patients with ctDNA *PIK3CA* mutations suggests that ctDNA analysis could be used to guide treatment decisions, especially when treatment response is expected to be limited (for example, intrinsic endocrine therapy resistance as a result of *PIK3CA* mutation). However, the results of our exploratory analysis need to be validated in suitably powered, prospective trials.

In conclusion, this study suggests that PI3K inhibition in combination with endocrine therapy provides clinically meaningful benefits to postmenopausal women with endocrine therapy-resistant, hormone receptor-positive and HER2-negative advanced breast cancer

harbouring ctDNA *PIK3CA* mutations. Further studies are warranted to validate ctDNA *PIK3CA* status as a predictive biomarker, and investigate how PI3Kα-specific inhibitors can be combined with complementary treatment regimens to provide the greatest clinical benefit in this setting. No further studies are being pursued owing to the toxicity associated with this combination.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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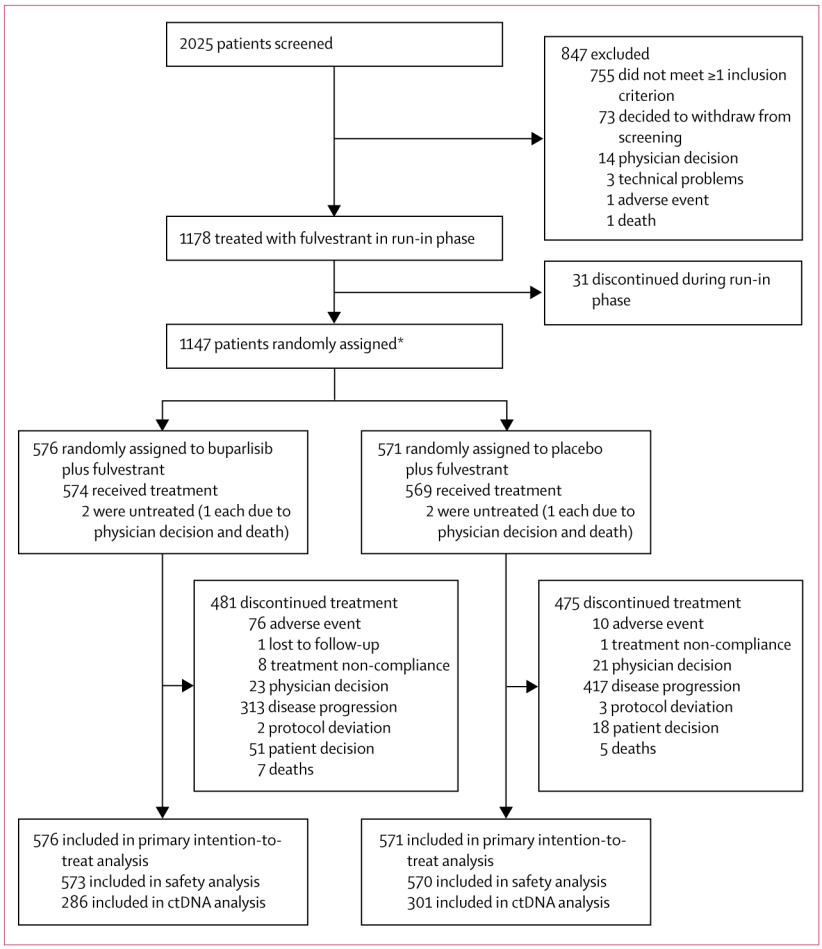

Figure 1. Trial profile

The safety analysis set is defined as patients who received at least one dose of study treatment and had one post-baseline safety assessment. Four patients (two from the buparlisib plus fulvestrant group and two from the placebo plus fulvestrant group) did not receive study treatment and were therefore excluded from the safety analysis. One patient was randomly assigned to the buparlisib plus fulvestrant group, but actually received treatment with placebo plus fulvestrant, so this patient was analysed as part of the placebo plus fulvestrant group for the safety analysis. ctDNA=circulating tumour DNA. PIK3=phospahtidylinositol 3-kinase. *587 of these patients submitted ctDNA for *PIK3CA* analysis.

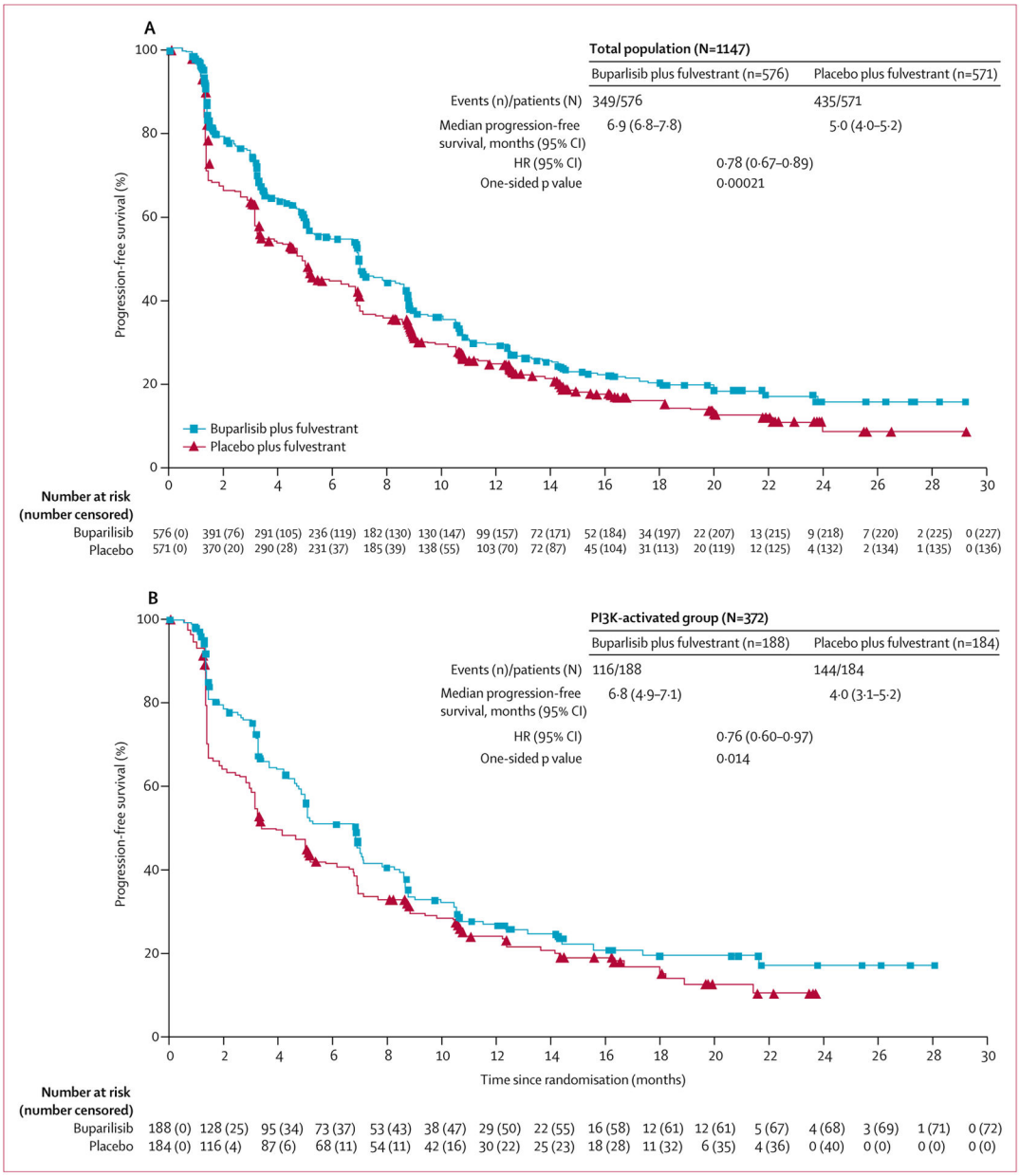

Figure 2. Progression-free survival

(A) Progression-free survival in the total population. (B) Progression-free survival in PI3K pathway-activated patients. HR=hazard ratio.

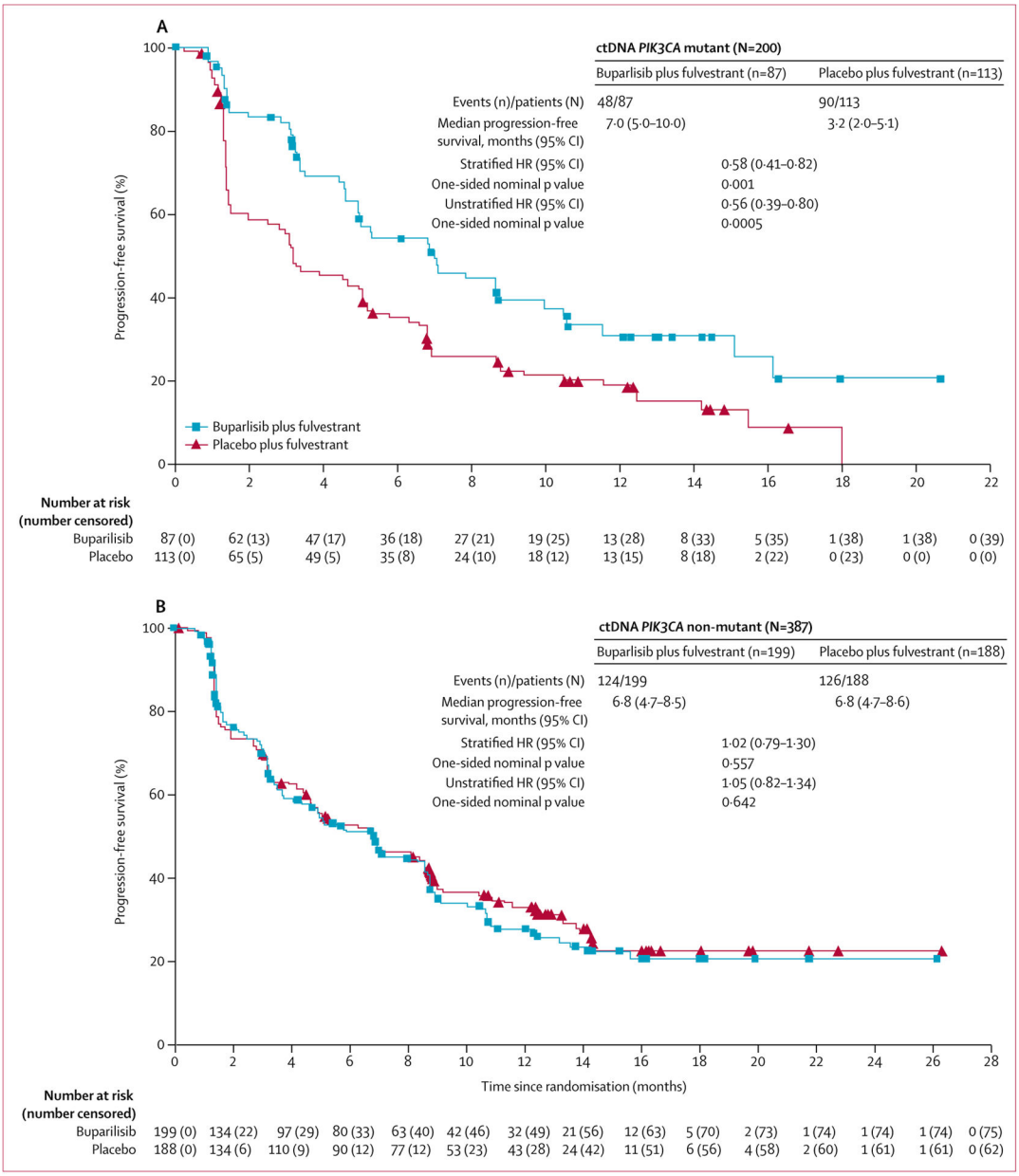

Figure 3. Progression-free survival by ctDNA *PIK3CA* mutant status

(A) Progression-free survival in ctDNA-mutant patients. (B) Progression-free survival in ctDNA wild-type patients. ctDNA=circulating tumour DNA. HR=hazard ratio.

Table 1

Baseline demographics and tumour characteristics of patients

	Buparlisib plus fulvestrant (n=576)	Placebo plus fulvestrant (n=571)
Age (years)	62·0 (55·0–69·0)	61 (54·0–68·0)
Race		
White	402 (70%)	376 (66%)
Asian	132 (23%)	153 (27%)
Black	5 (1%)	16 (3%)
Other	18 (3%)	7 (1%)
Unknown or missing	19 (3%)	19 (3%)
Previous treatment with aromatase inhibitor	574 (100%)	568 (99%)
ECOG performance status		
0	333 (58%)	344 (60%)
1	231 (40%)	211 (37%)
2	11 (2%)	16 (3%)
3*	1 (<1%)	0
Tumour histology		
Invasive ductal carcinoma	419 (73%)	424 (74%)
Invasive lobular carcinoma	76 (13%)	77 (13%)
Adenocarcinoma	49 (9%)	41 (7%)
Other	31 (5%)	28 (5%)
Unknown or missing	1 (<1%)	1 (<1%)
Presence of visceral disease	341 (59%)	337 (59%)
Previous therapy in metastatic setting		
Any endocrine therapy	418 (73%)	429 (75%)
0 previous lines of endocrine therapy	158 (27%)	142 (25%)
1 previous line of endocrine therapy	306 (53%)	301 (53%)
≥2 previous lines of endocrine therapy	112 (19%)	128 (22%)
Any chemotherapy	141 (24%)	177 (31%)
PI3K pathway status in tumour tissue†		
Activated	188 (33%)	184 (32%)
Non-activated	239 (41%)	240 (42%)
Unknown or missing	149 (26%)	147 (26%)
PIK3CA status in ctDNA‡		
Mutant§	87/286 (30%)	113/301 (38%)
Non-mutant§	199/286 (70%)	188/301 (62%)

Data are median (IQR) or n (%). ctDNA=circulating tumour DNA. ECOG=Eastern Cooperative Oncology Group. PIK3=phospahtidylinositol 3-kinase.

[*] Patient with an ECOG performance status of 3 at baseline was enrolled due to protocol deviation.

[†] PI3K activation was determined in all patients from tumour tissue submitted at screening and was defined by *PIK3CA* mutation or loss of phosphatase and tensin homologue expression.

[‡] Analysis of circulating tumour DNA was done in patients for whom samples were collected at screening (n=286 in buparlisib group, n=301 in placebo group); *PIK3CA* mutant is defined by an activating mutation in at least one of exons 1, 7, 9, or 20.

[§] Percentages calculated from the total number of circulating tumour DNA samples analysed.

Table 2

Adverse events

	Buparlisib plus fulvestrant (n=573)			Placebo plus fulvestrant (n=570)		
	Grade 1–2	**Grade 3**	**Grade 4**	**Grade 1–2**	**Grade 3**	**Grade 4**
Hyperglycaemia	159 (28%)	87 (15%)	1 (<1%)	43 (8%)	1 (<1%)	0
Increased ALT	84 (15%)	107 (19%)	39 (7%)	33 (6%)	6 (1%)	0
Nausea	212 (37%)	10 (2%)	0	124 (22%)	8 (1%)	0
Increased AST	111 (19%)	86 (15%)	17 (3%)	37 (6%)	16 (3%)	0
Diarrhoea	175 (31%)	21 (4%)	0	77 (14%)	6 (1%)	0
Rash	139 (24%)	44 (8%)	1 (<1%)	36 (6%)	0	0
Fatigue	155 (27%)	28 (5%)	0	127 (22%)	9 (2%)	0
Decreased appetite	162 (28%)	9 (2%)	0	62 (11%)	1 (<1%)	0
Depression	125 (22%)	21 (4%)	4 (1%)	49 (9%)	2 (<1%)	0
Anxiety	97 (17%)	30 (5%)	1 (<1%)	42 (7%)	5 (1%)	0
Stomatitis	112 (20%)	12 (2%)	0	34 (6%)	3 (1%)	0
Asthenia	99 (17%)	16 (3%)	0	54 (9%)	6 (1%)	0
Pruritus	76 (13%)	9 (2%)	0	32 (6%)	0	0
Dysgeusia	84 (15%)	0	0	21 (4%)	0	0
Vomiting	72 (13%)	12 (2%)	0	67 (12%)	7 (1%)	0
Headache	80 (14%)	1 (<1%)	0	73 (12%)	2 (<1%)	0
Weight decreased	76 (13%)	4 (1%)	0	22 (4%)	1 (<1%)	0
Cough	69 (12%)	1 (<1%)	0	59 (10%)	1 (<1%)	0

	Buparlisib plus fulvestrant (n=573)			Placebo plus fulvestrant (n=570)		
	Grade 1–2	Grade 3	Grade 4	Grade 1–2	Grade 3	Grade 4
Dizziness	68 (12%)	1 (<1%)	0	26 (5%)	0	0
Dry skin	65 (11%)	2 (<1%)	0	16 (3%)	1 (<1%)	0
Constipation	62 (11%)	0	0	63 (11%)	2 (<1%)	0
Insomnia	59 (10%)	1 (<1%)	0	48 (8%)	1 (<1%)	0
Arthralgia	49 (9%)	4 (1%)	0	70 (12%)	4 (1%)	0
Back pain	50 (9%)	3 (1%)	0	60 (11%)	5 (1%)	0

This table lists grade 1–2 adverse events reported in at least 10% patients in either group and all grade 3 and 4 events, irrespective of relation to study treatment. Adverse events were characterised and graded according to CTCAE version 4.03. 24 on-treatment deaths occurred (12 [2%] in each group): nine (2%) in each group due to disease progression; three in the placebo group due to cerebrovascular accident, cerebral haemorrhage, and urosepsis; and three in the buparlisib group due to gastric ulcer haemorrhage, pneumonia, and septic shock. None of the on-treatment deaths were suspected to be related to study treatment. ALT=alanine aminotransferase. AST=aspartate aminotransferase. CTCAE=Common Terminology Criteria for Adverse Events.