Ipatasertib plus paclitaxel versus placebo plus paclitaxel as first-line therapy for metastatic triple-negative breast cancer (LOTUS): a multicentre, randomised, double-blind, placebo-controlled, phase 2 trial

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See Comment page 1293

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Summary

Background The oral AKT inhibitor ipatasertib is being investigated in cancers with a high prevalence of PI3K/AKT pathway activation, including triple-negative breast cancer. The LOTUS trial investigated the addition of ipatasertib to paclitaxel as first-line therapy for triple-negative breast cancer.

Methods In this randomised, placebo-controlled, double-blind, phase 2 trial, women aged 18 years or older with measurable, inoperable, locally advanced or metastatic triple-negative breast cancer previously untreated with systemic therapy were recruited from 44 hospitals in South Korea, the USA, France, Spain, Taiwan, Singapore, Italy, and Belgium. Enrolled patients were randomly assigned (1:1) to receive intravenous paclitaxel 80 mg/m² (days 1, 8, 15) with either ipatasertib 400 mg or placebo once per day (days 1–21) every 28 days until disease progression or unacceptable toxicity. Randomisation was by stratified permuted blocks (block size of four) using an interactive web-response system with three stratification criteria: previous (neo)adjuvant therapy, chemotherapy-free interval, and tumour PTEN status. The co-primary endpoints were progression-free survival in the intention-to-treat population and progression-free survival in the PTEN-low (by immunohistochemistry) population. This ongoing trial is registered with ClinicalTrials.gov (NCT02162719).

Findings Between Sept 2, 2014, and Feb 4, 2016, 166 patients were assessed for eligibility and 124 patients were enrolled and randomly assigned to paclitaxel plus ipatasertib (n=62) or paclitaxel plus placebo (n=62). Median follow-up was 10·4 months (IQR 6·5–14·1) in the ipatasertib group and 10·2 months (6·0–13·6) in the placebo group. Median progression-free survival in the intention-to-treat population was 6·2 months (95% CI 3·8–9·0) with ipatasertib versus 4·9 months (3·6–5·4) with placebo (stratified hazard ratio [HR] 0·60, 95% CI 0·37–0·98; p=0·037) and in the 48 patients with PTEN-low tumours, median progression-free survival was 6·2 months (95% CI 3·6–9·1) with ipatasertib versus 3·7 months (1·9–7·3) with placebo (stratified HR 0·59, 95% CI 0·26–1·32, p=0·18). The most common grade 3 or worse adverse events were diarrhoea (14 [23%] of 61 ipatasertib-treated patients *vs* none of 62 placebo-treated patients), neutrophil count decreased (five [8%] *vs* four [6%]), and neutropenia (six [10%] *vs* one [2%]). No colitis, grade 4 diarrhoea, or treatment-related deaths were reported with ipatasertib. One treatment-related death occurred in the placebo group. Serious adverse events were reported in 17 (28%) of 61 patients in the ipatasertib group and nine (15%) of 62 patients in the placebo group.

Interpretation Progression-free survival was longer in patients who received ipatasertib than in those who received placebo. To our knowledge, these are the first results supporting AKT-targeted therapy for triple-negative breast cancer. Ipatasertib warrants further investigation for the treatment of triple-negative breast cancer.

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Introduction

The PI3K/AKT signalling pathway plays a crucial part in carcinogenesis, promoting cell survival and growth.[1,2] AKT is the central node of the PI3K/AKT pathway.[3] Phosphatidylinositol (3,4,5)-triphosphate, a direct product of PI3K activity, promotes AKT trafficking to the cell membrane and association with other cell- signalling proteins.[4] Full activation of AKT occurs via phosphorylation at two threonine and serine residues, leading to phosphorylation and regulation of numerous cellular proteins, including mTORC1 and S6 kinase.

The PI3K/AKT signalling pathway is often activated in breast cancer, and has attracted interest as a target in triple-negative breast cancer.[5,6] Large-scale comprehensive genomic analyses have characterised the heterogeneous nature of triple-negative breast cancer, including a subgroup with genetic activation of the PI3K/AKT pathway through activating mutations in *PIK3CA* or *AKT1,* and alterations in *PTEN*.[7–9] Additionally, approximately half of triple-negative breast cancers have deficient expression of the tumour suppressor PTEN, which is associated with a higher degree of AKT pathway activation.[2,10]

Research in context

Evidence before this study

We searched PubMed to identify publications published between Jan 1, 2001, and March 31, 2017, that included the search terms "AKT", "PI3K", and "triple-negative breast cancer". We also searched PubMed for publications in the same period describing assessment of ipatasertib using the terms "ipatasertib" or "GDC-0068". We did not use any language restrictions in our search. No previous randomised trials have investigated the targeting of AKT or PI3K specifically in triple-negative breast cancer. Analyses of single-arm studies in mesenchymal and metaplastic triple-negative breast cancer have suggested a more pronounced response to a combination of an mTOR inhibitor, bevacizumab, and pegylated liposomal doxorubicin in patients with PI3K/AKT/mTOR pathway aberrations. A phase 1 study showed potent inhibition of AKT signalling with ipatasertib, with notable activity in metastatic breast cancer showing *PTEN* loss or *PIK3CA*/*AKT* mutations.

Added value of this study

To our knowledge, these are the first prospective trial results supporting AKT targeting in triple-negative breast cancer. Prespecified analyses in the population of patients with *PIK3CA*/*AKT1*/*PTEN*-altered tumours suggest efficacy of ipatasertib in this population.

Implications of all of the available evidence

Our results support future investigation of ipatasertib plus paclitaxel in diseases with high prevalence of PI3K/AKT pathway activation, particularly in patients with *PIK3CA*/*AKT1*/*PTEN*-altered tumours.

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See Online for appendix

Ipatasertib is a highly selective oral ATP-competitive small-molecule AKT inhibitor.[11] In cell line and xenograft models, ipatasertib showed activity in a broad range of cancer types, including prostate, breast, ovarian, colorectal, and non-small-cell lung cancers.[11] Sensitivity to ipatasertib tended to be associated with high phosphorylated AKT levels, PTEN protein loss or genetic mutations in *PTEN*, and *PIK3CA* mutations, whereas *KRAS* and *BRAF* mutations were typically associated with resistance to ipatasertib.[11] As PI3K/AKT pathway activation is relevant for survival during periods of mitotic stress,[12] the combination of ipatasertib and taxanes was explored. Preclinical studies showed synergy between ipatasertib and taxanes.[13] Analysis of on-study tumour biopsy samples from a phase 1 clinical study showed robust AKT pathway inhibition by ipatasertib at clinically achievable doses.[14]

Based on these findings and its mechanism of action, ipatasertib is under clinical assessment in cancers with a high prevalence of PI3K/AKT pathway activation. A phase 1 study[15] of single-agent ipatasertib in 52 pretreated patients with various tumour types, including breast cancer, showed an acceptable safety profile (characterised by gastrointestinal effects, asthenia or fatigue, and rash) and preliminary antitumour activity. Of note, many patients with disease stabilisation had PI3K/AKT pathway-activating alterations in their tumours. In breast cancer, the combination of ipatasertib (400 mg once daily, days 1–21) with paclitaxel 90 mg/m² per week (days 1, 8, and 15), repeated every 28 days, was well tolerated and showed radiographic responses in the phase 1b PAM4983g study.[13]

We report results of a randomised phase 2 trial investigating the addition of ipatasertib to paclitaxel as first-line therapy for metastatic triple-negative breast cancer.

Methods

Study design and participants

LOTUS is a randomised, double-blind, placebo-controlled, phase 2 trial. Patients were enrolled at 44 hospitals in South Korea, the USA, France, Spain, Taiwan, Singapore, Italy, and Belgium (appendix pp 2–3).

Eligible patients were women aged 18 years or older, with Eastern Cooperative Oncology Group performance status 0 or 1, and locally advanced or metastatic triple-negative breast cancer (defined as <1% tumour cell expression of oestrogen and progesterone receptors and negative *HER2* status [fluorescence or chromogenic in-situ hybridisation {FISH/CISH} HER2/CEP17 ratio <2·0, or locally assessed immunohistochemistry 0 or 1+ {or 2+ but negative by FISH/CISH}]) not amenable to curative resection. Patients had to have measurable disease according to Response Evaluation Criteria in Solid Tumors (RECIST; version 1.1) and adequate haematological, renal, hepatic, and cardiac functions. A formalin-fixed paraffin-embedded tumour specimen was required from all patients for central analysis of PTEN expression before randomisation. The most recently obtained tumour sample was requested for submission, but a fresh biopsy sample was not required and primary tumour samples were acceptable.

Previous systemic therapy for locally advanced or metastatic disease was not permitted; however, previous (neo)adjuvant chemotherapy, radiotherapy, or chemoradiotherapy completed at least 6 months before the first dose was allowed. Patients were ineligible if they had known brain or spinal cord metastasis, ongoing grade 2 or worse peripheral neuropathy or grade 2 or worse uncontrolled or untreated hypercholesterolaemia or hypertriglyceridaemia, or active small or large intestine inflammation (such as Crohn's disease or ulcerative colitis).

All patients provided written informed consent before undergoing any study-specific procedures. Independent

institutional review boards at all participating centres approved the protocol and all study-related documents. The protocol is available in the appendix.

Randomisation and masking

Eligible patients were randomly assigned (1:1) to either ipatasertib plus paclitaxel or placebo plus paclitaxel by investigators using an interactive web-response system with an allocation sequence generated by Bracket Global LCC (Reading, UK). Randomisation was by stratified permuted blocks (block size of four). Randomisation was stratified by three criteria: previous (neo)adjuvant chemotherapy (yes *vs* no), chemotherapy-free interval (≤12 *vs* >12 months *vs* no previous chemotherapy), and central tumour PTEN status as assessed by immunohistochemistry (H score 0 *vs* 1–150 *vs* >150). In some cases, patients were randomly assigned before PTEN status was available; for stratification, these patients were assigned to the stratum with an H score more than 150. This approach was adopted because if patients were otherwise eligible and able to enrol on the study, we did not consider it ethically acceptable to delay their first-line treatment while waiting for centrally assessed PTEN status or if tissue samples were inadequate for central PTEN analysis. However, for stratified efficacy analyses, the actual PTEN status (if known) was used. The stratification factors of previous (neo)adjuvant chemotherapy and chemotherapy-free interval partly overlap. However, our intention was to try to balance the treatment groups in this heterogeneous treatment setting not only by sensitivity to previous (neo)adjuvant chemotherapy, but also according to tumour biology (depending on priming of the PI3K/AKT signalling pathway by previous chemotherapy) or clinical features of recurrence or de-novo stage IV disease that could be differentiated by previous (neo)adjuvant chemotherapy.

Placebo tablets were identical in shape and colour to the ipatasertib tablets. Investigators, patients, and the sponsor were masked to treatment assignment.

Procedures

Patients received intravenous paclitaxel 80 mg/m² on days 1, 8, and 15 of each 28-day cycle in combination with either oral ipatasertib 400 mg/day or placebo, administered on days 1–21 of each 28-day cycle. There is no standard paclitaxel schedule in metastatic breast cancer. Investigators indicated a strong preference for the 3 weeks on/1 week off schedule of paclitaxel 80 mg/m² per week when the LOTUS trial was designed. This schedule has been used in previous clinical studies[16,17] and maintains the cumulative dose intensity achieved with 175 mg/m² every 3 weeks (as recommended in the prescribing information). Treatment was continued until disease progression, intolerable toxicity, or withdrawal of consent. Ipatasertib or placebo could be temporarily interrupted for up to 4 consecutive weeks if patients had toxicity considered related to the study drug. Diarrhoea was managed with loperamide or according to institutional guidelines and standard of care, including but not limited to therapy with diphenoxylate and atropine, codeine, or octreotide. If symptoms persisted despite adequate (combination) antidiarrhoeal medications and dose interruptions, dose reductions were implemented. Ipatasertib (or placebo) was initially reduced to 300 mg/day, then to 200 mg/day, and was discontinued permanently at the third appearance of an adverse event requiring dose reduction. Paclitaxel dose modifications were implemented according to standard practice or institutional guidelines. The protocol suggested a reduction to 65 mg/m² at the first reduction and then permanent discontinuation if toxicity recurred. All patients who discontinued study therapy were allowed to receive subsequent anticancer therapy outside the study protocol. Disease progression that occurred after initiation of a new anticancer therapy was not collected per protocol; in such patients, progression-free survival was censored at the time of the last tumour assessment.

Tumours were assessed every 8 weeks by the investigators according to RECIST (version 1.1). After discontinuation of treatment, patients were followed up every 3 months for survival and subsequent anticancer therapies. Safety was assessed and graded according to National Cancer Institute Common Terminology Criteria for Adverse Events (version 4.0) on an ongoing basis until the study drug discontinuation visit (or resolution or stabilisation of ongoing related adverse events). Laboratory assessments (including haematology, fasting serum chemistry, coagulation, fasting lipid profile, and urinalysis) were done within 48 h before each study drug administration. Patient-reported outcomes (PROs) were assessed using the European Organisation for Research and Treatment of Cancer Core Quality of Life Questionnaire C30 (EORTC QLQ-C30), which includes 30 questions assessing five functional scales, three symptom scales, and six single items. Questionnaires were distributed by staff at the site and completed by the patient before study assessments or drug administration on day 1 of every cycle, at treatment discontinuation, and at tumour follow-up. Pharmacokinetic parameters of ipatasertib were assessed in all patients by sparse plasma sampling on day 1 of cycle 1 (0·5–2 h and 4–6 h after study drug administration) and on day 8 of cycle 1 (0–2 h and 2–5 h after study drug administration).

At screening, PTEN status was centrally assessed using antibody clone 138G6 (cat #9559, Cell Signaling Technology, Leiden, Netherlands; Targos Molecular Pathology GmbH, Kassel, Germany). Before the primary analysis, tumour tissue samples were assessed centrally by additional molecular assays to define the patient population with PTEN-low tumours (by immunohistochemistry; co-primary endpoint) and the patient population with PI3K/AKT pathway-activated tumours (secondary endpoint). For the co-primary endpoint, PTEN-low tumours were defined as those having

immunohistochemistry 0 in at least 50% of tumour cells using the Ventana immunohistochemistry assay (clone SP218; Spring Bioscience, Pleasanton, CA, USA). This assay was used instead of the one used to determine PTEN status for stratification because it had undergone a greater degree of technical validation and is being developed as a potential companion diagnostic assay for ipatasertib. The classification of PTEN-low tumours also adopted a scoring method based on quantification of the number of cells lacking expression, thus providing a more robust scale to measure the extent of complete loss of PTEN expression. The FoundationOne next-generation sequencing assay (Foundation Medicine, Cambridge, MA, USA)[18] was used to identify patients with PI3K/AKT pathway-activated tumours, defined as the presence of genetic *PTEN*-inactivating alterations or *PIK3CA/AKT1*-activating mutations (*PIK3CA* Arg88Gln, Asn345Lys, Cys420Arg, Glu542X, Glu545X, Gln546X, Met1043Ile, His1047X, or Gly1049Arg mutations, where X represents any change in aminoacid residue, or *AKT1* Glu17Lys mutations), referred to hereafter as *PIK3CA/AKT1/PTEN*-altered tumours.

Outcomes

The co-primary endpoints were investigator-assessed progression-free survival in the intention-to-treat population and progression-free survival in the subgroup of patients with PTEN-low tumours. Progression-free survival was defined as the interval between randomisation and the first occurrence of disease progression or death from any cause within 30 days of the last dose of study treatment (death on study). As specified in the protocol, patients who discontinued study treatment without documented disease progression were censored at the date of last tumour assessment before initiation of new anticancer therapy.

Secondary endpoints were investigator-assessed confirmed objective response (confirmed by a repeat assessment at least 4 weeks after the criteria for response are first met), duration of confirmed objective response (defined as the interval between first observation of a confirmed objective response and first observation of disease progression or death on study as assessed by the investigator), and overall survival in the intention-to-treat population and patients with PTEN-low tumours; efficacy (progression-free survival, confirmed objective response rate, duration of confirmed objective response, and overall survival) in patients with PI3K/AKT pathway-activated tumours; and safety (incidence, nature, and severity of adverse events). Additional objectives included assessment of pharmacokinetics; PROs for disease-related and treatment-related symptoms, patient functioning, and health-related quality of life; and further exploratory translational research. We also did post-hoc analyses of the clinical benefit (defined as either an objective response, or a best overall response of complete or partial response or stable disease together with a progression-free survival of 24 weeks or longer).

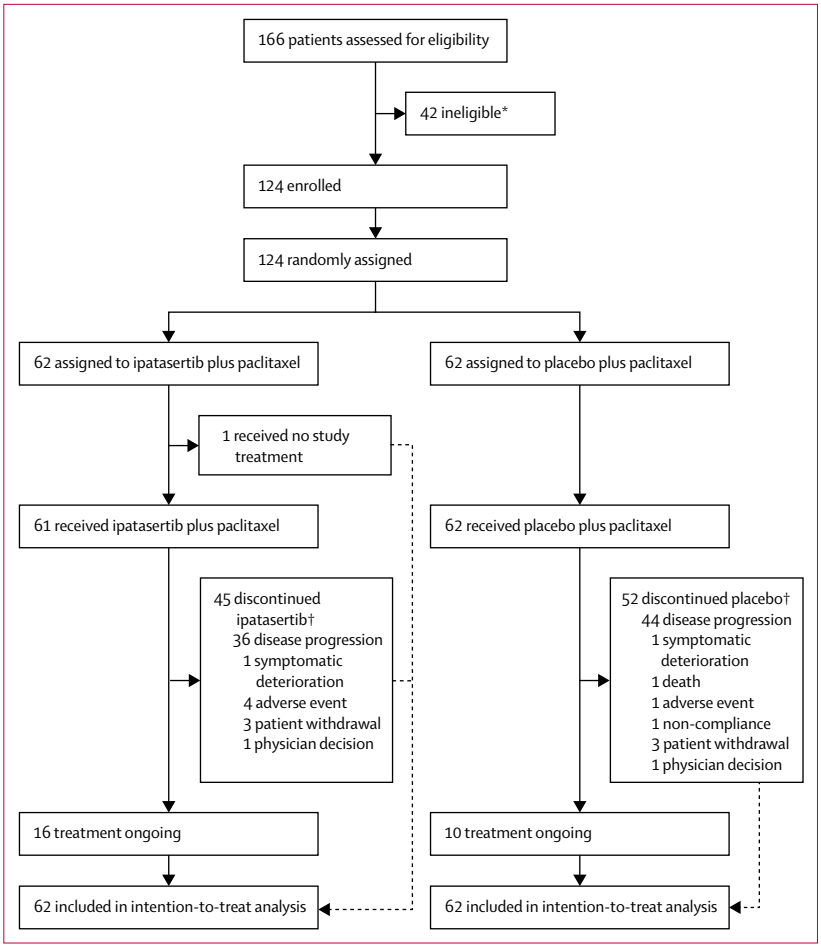

Figure 1: **Trial profile**
ECOG=Eastern Cooperative Oncology Group. LVEF=left ventricular ejection fraction. *The reasons for screen failure in 42 patients were: not meeting inclusion criteria (two signed informed consent, two ECOG performance status ≤1, one locally advanced or metastatic triple-negative breast cancer not amenable to curative resection, one measurable disease, and six adequate haematological and organ function), meeting exclusion criteria (one previous therapy for locally advanced or metastatic triple-negative breast cancer; two radiatiotherapy in previous 28 days; one major surgery, open biopsy, or significant traumatic injury in preceding 30 days; ten known brain or spinal cord metastasis; one New York Heart Association class II, III, or IV heart failure or LVEF <50%, or active ventricular arrhythmia requiring medication; one ongoing unstable angina or history of myocardial infarction in previous 6 months; one grade 3 uncontrolled or untreated hypercholesterolaemia or hypertriglyceridaemia; three congenital long QT syndrome or screening corrected QT interval ≥480 ms; three inability to comply with study and follow-up procedures; one other malignancy within 5 years; three potential contraindication); and 12 other reasons (more than one answer possible). †Five patients in the ipatasertib group and six in the placebo group received new anticancer therapy after discontinuing study therapy before disease progression. Further details of patients who discontinued without progression and received new anticancer therapy are provided in the appendix.

Statistical analysis

The planned sample size was 60 patients per group for a total of 120 patients overall to ensure 83 progression-free survival events for the primary analysis. As this hypothesis-generating trial was designed to assess safety and provide preliminary evidence of activity, it was not powered to detect minimal clinically meaningful differences between treatment groups at a significant α level of 5%. Instead, 90% CIs for the hazard ratio (HR) were calculated, anticipating that for clinically

	Intention-to-treat population		PTEN-low population		*PIK3CA/AKT1/PTEN*-altered tumour population	
	Ipatasertib plus paclitaxel (n=62)	Placebo plus paclitaxel (n=62)	Ipatasertib plus paclitaxel (n=25)	Placebo plus paclitaxel (n=23)	Ipatasertib plus paclitaxel (n=26)	Placebo plus paclitaxel (n=16)
Median age (years)	54 (44–63)	53 (45–63)	50 (44–63)	56 (46–65)	52 (44–63)	53 (46–60)
Age group						
18–40 years	10 (16%)	5 (8%)	4 (16%)	2 (9%)	5 (19%)	1 (6%)
41–64 years	40 (65%)	46 (74%)	18 (72%)	15 (65%)	18 (69%)	14 (88%)
≥65 years	12 (19%)	11 (18%)	3 (12%)	6 (26%)	3 (12%)	1 (6%)
Race						
Asian	28 (45%)	30 (48%)	10 (40%)	9 (39%)	16 (62%)	7 (44%)
White	26 (42%)	28 (45%)	12 (48%)	13 (57%)	8 (31%)	9 (56%)
Black or African-American	5 (8%)	3 (5%)	2 (8%)	1 (4%)	0	0
Other	3 (5%)	1 (2%)	1 (4%)	0	2 (8%)	0
ECOG performance status						
0	44 (71%)	36 (58%)	17 (68%)	15 (65%)	13 (50%)	9 (56%)
1	18 (29%)	22 (35%)	8 (32%)	7 (30%)	13 (50%)	7 (44%)
Missing	0	4 (6%)	0	1 (4%)	0	0
Previous (neo)adjuvant chemotherapy*	41 (66%)	40 (65%)	19 (76%)	15 (65%)	18 (69%)	10 (63%)
Anthracycline†	34 (55%)	34 (55%)	16 (64%)	12 (52%)	14 (54%)	7 (44%)
Taxane†	31 (50%)	34 (55%)	18 (72%)	14 (61%)	12 (46%)	7 (44%)
Chemotherapy-free interval (months)*						
≤12	18 (29%)	16 (26%)	8 (32%)	4 (17%)	7 (27%)	3 (19%)
>12	23 (37%)	24 (39%)	11 (44%)	11 (48%)	11 (42%)	7 (44%)
None	21 (34%)	22 (35%)	6 (24%)	8 (35%)	8 (31%)	6 (38%)
PTEN H score*						
0	10 (16%)	11 (18%)	9 (36%)	7 (30%)	8 (31%)	3 (19%)
1–150	27 (44%)	27 (44%)	10 (40%)	12 (52%)	6 (23%)	6 (38%)
>150	25 (40%)	24 (39%)	6 (24%)	4 (17%)	12 (46%)	7 (44%)
Histopathological subtype‡						
Ductal	59 (95%)	59 (95%)	25 (100%)	23 (100%)	24 (92%)	15 (94%)
Lobular	3 (5%)	1 (2%)	1 (4%)	0	2 (8%)	0
Tubular	1 (2%)	3 (5%)	0	0	1 (4%)	1 (6%)
Metastatic sites‡						
Lung	27 (44%)	32 (52%)	13 (52%)	14 (61%)	13 (50%)	9 (56%)
Liver	19 (31%)	17 (27%)	7 (28%)	6 (26%)	7 (27%)	5 (31%)
Lymph nodes	36 (58%)	38 (61%)	14 (56%)	18 (78%)	15 (58%)	12 (75%)
Bone	16 (26%)	17 (27%)	7 (28%)	10 (43%)	5 (19%)	8 (50%)

Data are n (%) or median (IQR). ECOG=Eastern Cooperative Oncology Group. IXRS=interactive web-response system. *Stratification factor, reported per IXRS. †Data not from IXRS. ‡More than one answer possible.

Table 1: **Baseline characteristics**

meaningful outcomes, the upper limit of the 90% CI would be less than 1. We report 95% CIs to be consistent with published literature. The primary analysis was intended to include 50 progression-free survival events in patients with PTEN-low tumours. Assuming 60% prevalence of PTEN-low tumours, we anticipated 83 progression-free survival events in the intention-to-treat population.

Efficacy analyses were based on all randomly assigned patients (intention-to-treat population). Analyses for the co-primary endpoints were stratified; the Cox proportional hazard model included the treatment group and three stratification factors as covariates. In this proof-of-concept study, the definition of progression-free survival for the primary endpoint was chosen with the aim of identifying antitumour activity closely related to

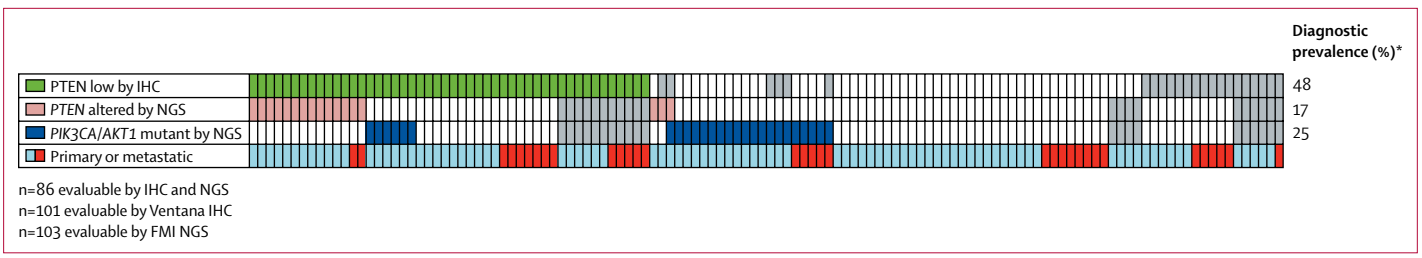

Figure 2: **Biomarker prevalence**
IHC=immunohistochemistry. NGS=next-generation sequencing. FMI=Foundation Medicine Inc. *Prevalence based on all available diagnostic data. Each vertical set of blocks represents an individual patient's tumour. Green blocks represent PTEN loss by IHC; pink blocks represent *PTEN*-altered by NGS; dark blue blocks represent *PIK3CA*/*AKT1*-mutant by NGS; grey blocks represent samples with no corresponding data available (assay failure or insufficient sample for testing). The bottom row shows whether samples are from primary (light blue) or metastatic (red) tumour sites.

study treatment. The risk of bias was reduced by the double-blinded, placebo-controlled trial design. However, recognising that in a poor-prognosis disease setting such as triple-negative breast cancer, this definition might lead to censoring of events related to disease progression, progression-free survival including death from any cause irrespective of time from last dose was included as a sensitivity analysis, otherwise using the same approach as for the primary analysis. All other analyses were not stratified; the only covariate in Cox proportional hazard models was the treatment group.

Safety analyses were based on all patients who received at least one dose of ipatasertib, placebo, or paclitaxel; patients were analysed based on the treatment actually received. PRO analyses were done on all patients in the intention-to-treat population with a baseline assessment and at least one post-baseline assessment. The full intention-to-treat set was used to assess compliance and completion rates, summarised at each timepoint by treatment group with reasons for missing data. Summary statistics of linear transformed scores were reported for all scales of the EORTC QLQ-C30 according to the EORTC scoring manual guidelines for each assessment timepoint. The mean change of the linear transformed scores from baseline (and 95% CI using the normal approximation) were reported. Changes from baseline of 10 points or more in PRO scores were defined as clinically meaningful.[19] Efficacy, safety, and PRO analyses were not adjusted for multiple comparisons.

Cumulative dose intensity for each study drug was calculated using the actual amount of study drug received in mg divided by the expected amount of study drug in mg. The expected amount of study drug was calculated based on treatment duration (the interval between the first and last administered doses of study drug) and the initial dose and schedule specified in the protocol.

Ipatasertib plasma concentration versus time data were pooled and analysed using a population-based pharmacokinetic (popPK) modelling approach. Non-linear mixed-effect modelling was used for the estimation of popPK parameters for ipatasertib. Covariates such as patient demographics, total protein, serum albumin, liver function tests, and serum creatinine were tested for significance on pharmacokinetic parameters of interest.

Analyses were done using SAS (version 9.4). An internal monitoring committee reviewed partly unblinded summaries of the safety data approximately 16 weeks after enrolment of the first 20 patients. After completion of enrolment and occurrence of approximately 40 progression-free survival events, the internal monitoring committee reviewed an interim safety and efficacy analysis.

	Ipatasertib plus paclitaxel (n=61)	Placebo plus paclitaxel (n=62)
Treatment duration (months)		
Ipatasertib or placebo	5·0 (3·5–7·8)	3·5 (1·6–5·4)
Paclitaxel	4·1 (3·2–7·2)	3·5 (1·5–5·1)
Cumulative dose intensity*		
Ipatasertib or placebo	99·0 (91·7–100·0)	100·0 (94·5–100·0)
Paclitaxel	100·0 (90·9–100·0)	100·0 (93·8–100·0)
Mean (SD) cumulative dose intensity*		
Ipatasertib or placebo	93·3 (11·0)	95·5 (11·9)
Paclitaxel	94·2 (11·8)	95·5 (11·3)
Treatment discontinued for adverse event		
Ipatasertib or placebo	4 (7%)†	1 (2%)‡
Paclitaxel	5 (8%)§	5 (8%)¶
Treatment interrupted for adverse event		
Ipatasertib or placebo	22 (36%)	12 (19%)
Paclitaxel	31 (51%)	30 (48%)
Dose reduced for adverse event		
Ipatasertib or placebo	13 (21%)	4 (6%)
Paclitaxel	23 (38%)	7 (11%)

Data are n (%) or median (IQR) unless otherwise stated. *Cumulative dose intensity for each study drug (ipatasertib, placebo, or paclitaxel) was calculated using the actual amount of study drug received in mg divided by the expected amount of study drug in mg. The expected amount of study drug was calculated based on treatment duration (the interval between the first and last administered doses of study drug) and the initial dose and schedule specified in the protocol. †Diarrhoea, asthenia, and vomiting (n=1), diarrhoea (n=1), tuberculosis (n=1), and embolism (n=1). ‡Cholestasis or cell death. §Hypoaesthesia (n=1), pharyngitis or tonsillitis (n=1), tuberculosis (n=1), back pain (n=1), and embolism (n=1). ¶Peripheral sensory neuropathy (n=2), neuropathy peripheral (n=1), cholestasis/cell death (n=1), neutrophil count decreased (n=1).

Table 2: **Treatment exposure (safety population)**

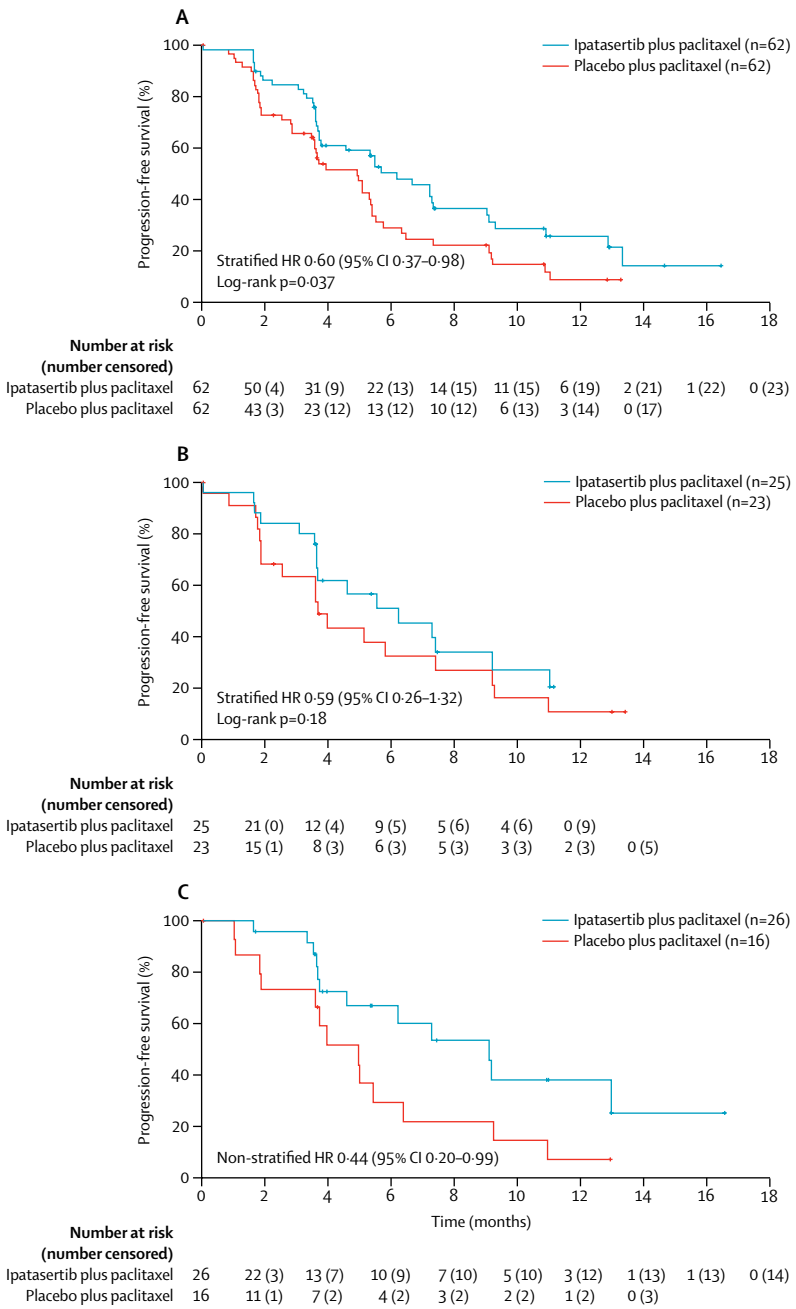

Figure 3: **Progression-free survival in the (A) intention-to-treat population, (B) PTEN-low subgroup, (C) *PIK3CA/AKT1/PTEN*-altered subgroup.**
HR=hazard ratio.

This trial is registered with ClinicalTrials.gov, number NCT02162719.

Role of the funding source

The funder of the study was involved in the study design, data collection, data analysis, data interpretation, and writing of the report, and gave approval to submit it for publication. All authors had full access to all the data in the study, were involved in writing the report, and approved the final version for submission. The first and second authors had final responsibility for the decision to submit for publication.

Results

Between Sept 2, 2014, and Feb 4, 2016, 166 patients were assessed for eligibility and 124 patients were randomly assigned to treatment with ipatasertib (62 patients) or placebo (62 patients; figure 1). One randomly assigned patient who received no study treatment was excluded from the safety analysis population. Baseline characteristics were generally balanced between treatment groups (table 1). Biomarker-assessable populations for both PTEN status and *PIK3CA/AKT1/PTEN* alterations showed similar baseline characteristics to the intention-to-treat population. In 11 patients (four randomly assigned to placebo and seven randomly assigned to ipatasertib), the PTEN status used for stratification differed from that used for analysis. Of these, five (two placebo, three ipatasertib) were classified as having a PTEN status of more than 150 at randomisation but their PTEN status in analyses was unknown, four (one placebo, three ipatasertib) were classified as having a PTEN status of more than 150 at randomisation but in the analyses, their PTEN H score was 1–150, and one (placebo) was classified with a PTEN status of 0 at randomisation but was classified with a PTEN H score of 1–150 in the analyses. The remaining patient (ipatasertib) was classified as having a PTEN status of more than 150 at randomisation but in the analyses her PTEN H score was 0.

Samples were centrally assessable for PTEN in 101 (81%) of 124 patients; in the remaining 23 (19%) patients, PTEN status could not be determined because of assay failure or insufficient sample for testing. Of these 101 assessable samples, 48 (48%) were classified as PTEN low, lower than the 60% predicted. Of the 103 patient samples assessed by next-generation sequencing, 42 (41%) had *PIK3CA/AKT1/PTEN*-altered tumours (appendix p 4). Of the 15 patients with *PTEN* genetic alterations by next-generation sequencing, and samples assessed for immunohistochemistry, 14 (93%) had loss of PTEN protein expression (figure 2). However, a substantial proportion of patients with loss of PTEN protein expression did not have a genetic alteration (figure 2). Of the 21 patients with activating mutations in *PIK3CA* and *AKT1* and samples assessed for immunohistochemistry, only six (29%) had PTEN loss by immunohistochemistry (figure 2). Prevalence of *PIK3CA/AKT1/PTEN* alterations did not differ between primary (n=76) and metastatic (n=27) samples (figure 2) or between samples that were collected before administration of (neo)adjuvant chemotherapy and samples collected from chemotherapy-naive patients (data not shown).

Treatment exposure is summarised in table 2. At the clinical cutoff date (June 7, 2016), the median duration of

	Number of events/patients		Median progression-free survival (95% CI), months		Non-stratified hazard ratio (95% Wald CI)
	Ipatasertib plus paclitaxel	Placebo plus paclitaxel	Ipatasertib plus paclitaxel	Placebo plus paclitaxel	
Age (years)					
<50	16/22	17/24	6·2 (3·6–9·1)	2·9 (1·8–5·4)	0·62 (0·31–1·24)
≥50	23/40	28/38	5·7 (3·7–12·9)	5·1 (3·7–6·3)	0·62 (0·35–1·09)
DFI since last chemotherapy (months)					
≤12	8/11	12/14	4·4 (1·9–7·3)	3·5 (1·6–3·9)	0·46 (0·16–1·36)
>12	16/31	20/28	9·1 (6·7–NA)	5·4 (3·6–7·3)	0·49 (0·25–0·95)
No prior chemotherapy	15/20	13/20	3·7 (3·6–9·0)	5·4 (2·8–9·1)	1·00 (0·46–2·17)
(Neo)adjuvant chemotherapy					
Yes	24/42	31/41	7·2 (5·5–9·3)	3·7 (2·9–5·1)	0·48 (0·28–0·81)
No	15/20	14/21	3·7 (3·6–9·0)	5·4 (2·8–9·1)	1·03 (0·48–2·20)
Targos IHC PTEN status (H-score)					
0	5/11	7/10	7·2 (5·5–NA)	1·8 (1·8–10·9)	0·21 (0·06–0·75)
1–150	23/30	22/29	3·7 (3·3–5·7)	5·1 (3·6–7·3)	1·01 (0·56–1·83)
>150	9/18	15/21	9·0 (5·5–NA)	3·7 (1·9–6·3)	0·36 (0·15–0·86)
Unknown	2/3	1/2	7·2 (1·6–7·2)	5·4 (NA)	0·82 (0·05–13·24)
Ventana IHC PTEN status (staining intensity)					
PTEN low	16/25	18/23	6·2 (3·6–9·1)	3·6 (1·9–7·3)	0·68 (0·35–1·35)
PTEN non-low	17/29	19/24	6·7 (3·7–12·9)	5·0 (2·9–5·5)	0·54 (0·27–1·07)
Unknown	6/8	8/15	5·7 (3·3–9·3)	5·1 (2·9–5·4)	0·51 (0·16–1·63)
NGS *PIK3CA/AKT1/PTEN*					
Altered	12/26	13/16	9·0 (4·6–NA)	4·9 (3·6–6·3)	0·44 (0·20–0·99)
Non-altered	21/28	23/33	5·3 (3·6–7·3)	3·6 (2·9–5·5)	0·76 (0·41–1·40)
Unknown	6/8	9/13	5·7 (3·2–7·3)	5·1 (1·7–11·0)	0·83 (0·29–2·42)
All	**39/62**	**45/62**	**6·2 (3·8–9·0)**	**4·9 (3·6–5·4)**	**0·62 (0·40–0·95)**

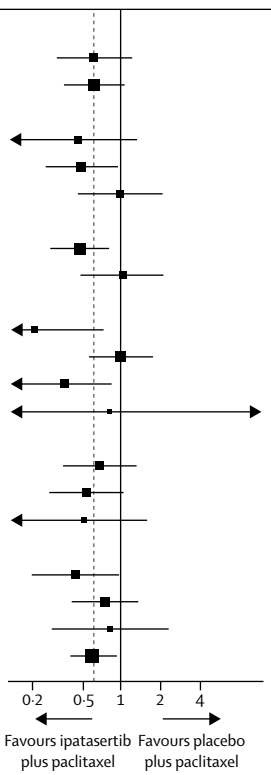

Figure 4: **Subgroup analysis of progression-free survival**
Non-stratified analysis. DFI=disease-free interval. IHC=immunohistochemistry. NA=not assessible. NGS=next-generation sequencing.

follow-up was 10·4 months (IQR 6·5–14·1) in the ipatasertib group and 10·2 months (6·0–13·6) in the placebo group. Among patients who discontinued study treatment before disease progression, the proportions subsequently receiving non-study systemic anticancer therapy were similar in the two treatment groups (five [8%] of 62 patients in the ipatasertib group and six [10%] of 62 patients in the placebo group). These patients were censored at the date of last tumour assessment before initiation of new anticancer therapy except for two patients (both with a progression-free survival event; appendix p 5).

The primary progression-free survival analysis was triggered by reaching approximately 83 progression-free survival events in the intention-to-treat population: 39 events in the ipatasertib group and 45 in the placebo group. One patient in each group died without evidence of progression; the remaining events were disease progression. Median progression-free survival was 6·2 months (95% CI 3·8–9·0) with ipatasertib versus 4·9 months (3·6–5·4) with placebo (stratified HR 0·60, 95% CI 0·37–0·98; log-rank p=0·037; figure 3A). In the sensitivity analysis, including all deaths from any cause, the stratified HR was 0·66 (95% CI 0·41–1·06; log-rank p=0·081); median progression-free survival was 5·9 months (95% CI 3·8–7·3) with ipatasertib versus 5·0 months (3·6–5·4) with placebo.

At the time of data cutoff, progression-free survival events had been documented in 34 (71%) of 48 patients in the PTEN-low population (16 [64%] of 25 in the ipatasertib group and 18 [78%] of 23 in the placebo group). In this population, median progression-free survival was 6·2 months (95% CI 3·6–9·1) with ipatasertib versus 3·7 months (1·9–7·3) with placebo (stratified HR 0·59, 95% CI 0·26–1·32, log-rank p=0·18; figure 3B).

Prespecified analyses in the subgroup of 42 patients with *PIK3CA/AKT1/PTEN*-altered tumours, after progression-free survival events in 12 (46%) of 26 patients in the ipatasertib group and 13 (81%) of 16 patients in the placebo group, showed median progression-free survival of 9·0 months (95% CI 4·6–not assessable) with ipatasertib versus 4·9 months (3·6–6·3) with placebo (non-stratified HR 0·44, 95% CI 0·20–0·99, log-rank p=0·041; figure 3C). In patients with *PIK3CA/AKT1/PTEN*-non-altered tumours, with progression-free survival events in 21 (75%) of 28 patients in the ipatasertib group and 23 (70%) of 33 patients in the placebo group,

	Intention-to-treat population		PTEN-low subgroup by Immunohistochemistry		*PIK3CA/AKT1/PTEN*-altered subgroup by next-generation sequencing	
	Ipatasertib plus paclitaxel (n=62)	Placebo plus paclitaxel (n=62)	Ipatasertib plus paclitaxel (n=25)	Placebo plus paclitaxel (n=23)	Ipatasertib plus paclitaxel (n=26)	Placebo plus paclitaxel (n=16)
Objective response	25 (40%)	20 (32%)	12 (48%)	6 (26%)	13 (50%)	7 (44%)
Duration of response (months)	7·9 (5·6–NA)	7·4 (3·9–9·2)	6·5 (4·4–NA)	7·5 (7·3–NA)	11·2 (5·6–NA)	6·1 (3·8–7·6)
Clinical benefit	30 (48%)	23 (37%)	14 (56%)	7 (30%)	14 (54%)	7 (44%)

Data are n (%) or median (95% CI). Objective response is per Response Evaluation Criteria in Solid Tumors version 1.1. Clinical benefit is defined as either an objective response, or a best overall response of complete or partial response or stable disease together with a progression-free survival of ≥24 weeks. NA=not assessable.

Table 3: **Summary of secondary efficacy endpoints**

the median progression-free survival was 5·3 months (95% CI 3·6–7·3) in the ipatasertib group versus 3·7 months (2·9–5·5) in the placebo group (non-stratified HR 0·76, 95% CI 0·41–1·40, p=0·36). Progression-free survival in selected subgroups, including the randomisation stratification factors, is shown in figure 4.

Further anticancer therapy was administered after disease progression to 30 (77%) of 39 patients in the ipatasertib group and 38 (84%) of 45 patients in the placebo group whose disease had progressed by the time of data cutoff. Overall survival results are immature, with deaths in nine (15%) of 62 patients in the ipatasertib group and 17 (27%) of 62 patients in the placebo group. The primary cause of death was disease progression in 22 (85%) of 26 patients. Secondary endpoints of objective response and duration of response, and the post-hoc assessment of clinical benefit are shown in table 3.

Median duration of response was similar in the two treatment groups for the intention-to-treat and PTEN-low populations, but was longer in ipatasertib-treated patients compared with placebo in the *PIK3CA/AKT1/PTEN*-altered subgroup.

The most common adverse events of any grade in the ipatasertib group were gastrointestinal effects (diarrhoea, nausea, and vomiting), alopecia, neuropathy, fatigue, and rash (table 4). These were typically grade 1 or 2 in severity. Grade 3 or worse adverse events occurred in 33 (54%) of 61 patients in the ipatasertib group and 26 (42%) of 62 patients in the placebo group (table 4). The most common individual grade 3 or worse adverse events in the ipatasertib group were diarrhoea, neutrophil count decreased, and neutropenia). The most common grade 3 or worse adverse events by grouped term (appendix p 6) were diarrhoea (14 [23%] of 61 ipatasertib-treated patients *vs* none of 62 placebo-treated patients), neutropenia (comprising neutropenia, neutrophil count decreased, and febrile neutropenia; 11 [18%] *vs* five [8%]), peripheral neuropathy (comprising peripheral sensory neuropathy, neuropathy peripheral, paraesthesia, hypoaesthesia, dysaesthesia, muscular weakness, neurotoxicity, and peripheral motor neuropathy; four [7%] *vs* three [5%]), fatigue or asthenia (three [5%] *vs* four [6%]), and pneumonia or lower respiratory tract infection (three [5%] *vs* none). Of note, there were no episodes of grade 4 diarrhoea and no reported cases of colitis. Serious adverse events were more common in the ipatasertib group (17 [28%] of 61 patients, predominantly infections and gastrointestinal effects) than in the placebo group (nine [15%] of 62 patients, predominantly infections). Four patients had adverse events resulting in death: one patient with pneumonia in the ipatasertib group (not considered related to study treatment) and three in the placebo group (one case each of cholestasis together with cell death [reported by the investigator as cytolysis (liver), both events assessed as related to placebo and paclitaxel]; metastatic breast cancer; and death from unknown cause 287 days after the last dose of the study drug).

Diarrhoea typically occurred during the first cycle of ipatasertib (median time to onset 5 days) but some cases were observed in later cycles. Diarrhoea led to discontinuation of ipatasertib in two (3%) of 61 patients, dose reduction of ipatasertib in eight (13%), and temporary interruption of ipatasertib in four (7%). Anti-diarrhoeal drugs (predominantly loperamide) were administered in 39 (64%) of 61 patients in the ipatasertib group and six (10%) of 62 patients in the placebo group. At data cutoff, almost all episodes of diarrhoea had resolved.

There was high compliance with PRO assessment questionnaires: more than 90% of patients in each treatment group completed at least one item of the EORTC QLQ-C30 at each cycle (appendix p 7). In both treatment groups, mean change from baseline scores for most functional scales (cognitive, physical, and social) and the global health status/quality-of-life domain were not clinically meaningful according to the predefined threshold of a 10-point change from baseline (appendix pp 8–9). Similarly, no clinically meaningful changes were observed for most of the disease-related and treatment-related symptom scales (appetite loss, constipation, dyspnoea, nausea and vomiting, insomnia, pain, and financial difficulties) up to and including cycle 5. However, in the ipatasertib group, a clinically meaningful improvement in emotional functioning was observed at

	Ipatasertib plus paclitaxel (n=61)				Placebo plus paclitaxel (n=62)			
	All grades	Grade ≥3	Grade 3*	Grade 4*	All grades	Grade ≥3	Grade 3*	Grade 4*
All	61 (100%)	33 (54%)	27 (44%)	5 (8%)	60 (97%)	26 (42%)	20 (32%)	3 (5%)
Diarrhoea	57 (93%)	14 (23%)	14 (23%)	0	12 (19%)	0	0	0
Alopecia	33 (54%)	0	0	0	29 (47%)	0	0	0
Nausea	30 (49%)	1 (2%)	1 (2%)	0	21 (34%)	1 (2%)	1 (2%)	0
Vomiting	17 (28%)	2 (3%)	2 (3%)	0	14 (23%)	0	0	0
Peripheral sensory neuropathy	16 (26%)	3 (5%)	3 (5%)	0	10 (16%)	2 (3%)	2 (3%)	0
Fatigue	16 (26%)	2 (3%)	2 (3%)	0	21 (34%)	4 (6%)	4 (6%)	0
Rash	16 (26%)	1 (2%)	1 (2%)	0	12 (19%)	0	0	0
Asthenia	15 (25%)	2 (3%)	2 (3%)	0	6 (10%)	0	0	0
Myalgia	15 (25%)	0	0	0	15 (24%)	0	0	0
Neutropenia	13 (21%)	6 (10%)	4 (7%)	2 (3%)	15 (24%)	1 (2%)	0	1 (2%)
Decreased appetite	13 (21%)	0	0	0	11 (18%)	0	0	0
Stomatitis	11 (18%)	1 (2%)	1 (2%)	0	5 (8%)	0	0	0
Constipation	11 (18%)	0	0	0	10 (16%)	0	0	0
Dizziness	11 (18%)	0	0	0	9 (15%)	0	0	0
Insomnia	11 (18%)	0	0	0	8 (13%)	0	0	0
Neuropathy peripheral	10 (16%)	0	0	0	14 (23%)	1 (2%)	1 (2%)	0
Dermatitis acneiform	10 (16%)	0	0	0	5 (8%)	0	0	0
Neutrophil count decreased	9 (15%)	5 (8%)	3 (5%)	2 (3%)	9 (15%)	4 (6%)	3 (5%)	1 (2%)
Headache	9 (15%)	1 (2%)	1 (2%)	0	12 (19%)	0	0	0
Abdominal pain	9 (15%)	0	0	0	7 (11%)	0	0	0
Pyrexia	9 (15%)	0	0	0	6 (10%)	0	0	0
Arthralgia	9 (15%)	0	0	0	6 (10%)	0	0	0
Anaemia	8 (13%)	2 (3%)	2 (3%)	0	8 (13%)	2 (3%)	2 (3%)	0
Dyspepsia	8 (13%)	0	0	0	6 (10%)	0	0	0
Pruritus	8 (13%)	0	0	0	5 (8%)	0	0	0
Nasopharyngitis	8 (13%)	0	0	0	5 (8%)	0	0	0
Cough	7 (11%)	0	0	0	8 (13%)	0	0	0
Pneumonia	3 (5%)	3 (5%)†	2 (3%)	0	0	0	0	0
Febrile neutropenia	3 (5%)	2 (3%)	1 (2%)	1 (2%)	0	0	0	0
Hypertension	3 (5%)	1 (2%)	1 (2%)	0	3 (5%)	2 (3%)	2 (3%)	0
Bone pain	3 (5%)	0	0	0	2 (3%)	1 (2%)	0	1 (2%)
Hypocalcaemia	2 (3%)	1 (2%)	0	1 (2%)	0	0	0	0
Thrombocytopenia	1 (2%)	0	0	0	1 (2%)	1 (2%)	0	1 (2%)
Cholestasis	1 (2%)	0	0	0	1 (2%)	1 (2%)†	0	0
Death	0	0	0	0	1 (2%)	1 (2%)†	0	0
Pancytopenia	0	0	0	0	1 (2%)	1 (2%)	0	1 (2%)
Cell death	0	0	0	0	1 (2%)	1 (2%)†	0	0
Breast cancer metastatic	0	0	0	0	1 (2%)	1 (2%)†	0	0
Fever‡	0	0	0	0	1 (2%)	1 (2%)	0	1 (2%)

Adverse events in 10% or more of patients (any grade), all grade 3 or worse in more than one patient in either treatment group, or any grade 4 or 5. *Worst grade reported (eg, a patient who has an event at both grade 3 and grade 4 appears only in the grade 4 column). †Grade 5 in one patient (2%). ‡Unmapped (verbatim term shown).

Table 4: **Summary of adverse events in the safety population**

cycle 2, whereas a clinically meaningful worsening was observed for diarrhoea (cycles 2–5), fatigue (cycle 5), and role functioning (cycles 3–5). Scores from timepoints after cycle 5 are not described due to sample size attrition (in both groups, fewer than 50% of patients remained on treatment beyond cycle 5).

The plasma concentrations of ipatasertib obtained by sparse sampling in this study were consistent with known pharmacokinetic profiles and overall characteristics of ipatasertib and its metabolite G-037720. Exploratory analyses showed no relationship between ipatasertib exposure and incidence of diarrhoea, neutropenia, and neuropathy (data not shown).

Discussion

Results of the randomised, double-blind, placebo-controlled, phase 2 LOTUS trial show that adding ipatasertib to paclitaxel as first-line therapy for triple-negative breast cancer increased progression-free survival compared with that for placebo plus paclitaxel. The increase in median progression-free survival was quite modest in the intention-to-treat population and PTEN-low subgroup but more pronounced in predefined analyses of the patient population with *PIK3CA/AKT1/PTEN*-altered tumours characterised by next-generation sequencing. Overall, adverse events were consistent with previous experience, manageable, and reversible.

The 4·9-month median progression-free survival in the control group of the intention-to-treat population was within the range reported in subgroup analyses of patients with triple-negative breast cancer in previous randomised trials (4·6 months in the E2100 trial,[20] 5·5 months in the NU07B1 trial,[21] and 6·3 months in the MERiDiAN[22] trial). Of note, among patients in LOTUS who had previously received (neo)adjuvant chemotherapy, approximately a third had disease recurrence within 12 months of chemotherapy (25 [30%] of the 84 patients who had received prior chemotherapy), whereas such patients were excluded from the MERiDiAN trial.[22] Similarly, the 32% of patients achieving response in the control group of LOTUS seems to be consistent with the available data reported in the literature (21% with a higher starting dose of single-agent paclitaxel in a mixed population of triple-negative breast cancer and non-triple-negative breast cancer patients in the E2100 trial;[20] 28% with paclitaxel plus onartuzumab in a randomised phase 2 trial in triple-negative breast cancer predominantly in the first-line setting[23]).

When the trial was designed, it was anticipated that patients with PTEN-low tumours by immunohistochemistry might derive increased benefit from ipatasertib. This was hypothesised because a randomised phase 2 trial in metastatic castration-resistant prostate cancer showed that the effect of ipatasertib was more pronounced in the subgroup of patients with PTEN loss identified by immunohistochemistry or next-generation sequencing.[24] However, PTEN loss is only one of several mechanisms leading to activation of the PI3K/AKT pathway. In breast cancer, activating mutations in *PIK3CA* and *AKT1* are frequently observed, whereas in castration-resistant prostate cancer, these mutations are rare.[8,9,25] In our study population, a substantial proportion of patients with PTEN-low tumours by immunohistochemistry did not have a genetic alteration. This is consistent with previous reports of non-genetic loss of PTEN in triple-negative breast cancer.[26] In the LOTUS trial, the effect of ipatasertib in the subgroup of patients with PTEN-low tumours by immunohistochemistry was no greater than in those with PTEN-non-low tumours or in the intention-to-treat population. However, efficacy analysis in the population with *PIK3CA/AKT1/PTEN*-altered tumours supporting the study's secondary objectives showed an encouraging progression-free survival HR of 0·44 and an increase of 4·1 months in the median progression-free survival (median 9·0 months in the ipatasertib group *vs* 4·9 months in the placebo group). Duration of response results supported these findings. This difference in efficacy based on absence of expression of PTEN through non-genetic mechanisms compared with loss of PTEN function through mutations and copy-number loss could be a key difference in how PTEN loss might drive tumours and be PI3K/AKT-addicted in prostate versus breast cancers.

The most common adverse events were gastrointestinal, in particular diarrhoea. Most cases of diarrhoea were grade 1 or 2; grade 3 diarrhoea occurred in 23% of patients and there were no grade 4 cases. Diarrhoea was manageable and reversible, and only two patients discontinued ipatasertib because of diarrhoea. Of note, primary prophylactic antidiarrhoeal drugs were not specified as part of safety management guidelines in the protocol.

Although patients in the ipatasertib plus paclitaxel group had clinically meaningful worsening in patient-reported role function, diarrhoea, and fatigue, patients' overall global health status or quality of life was maintained up to and including cycle 5. There was also no clinically meaningful change in cognitive, physical, or social function scales or other symptom scales. Together, our results indicate that the tolerability of the ipatasertib plus paclitaxel regimen might allow rational combination with other carefully selected agents.

The similar pharmacokinetic profiles in this study and previous experience suggest that there was no paclitaxel–ipatasertib drug interaction that affected metabolism or clearance (as predicted from the phase 1b study and preliminary assessment by population pharmacokinetic methodology [Roche data on file]). Although exploratory analyses showed no clear relationship between ipatasertib exposure and incidence of diarrhoea, neutropenia, and neuropathy, assessment of the exposure–response relationship is difficult in a trial with only one dose level.

One of the main limitations of these results is the small sample size. The biomarker-selected population showing the most encouraging effect of ipatasertib includes only 42 patients; despite ours being prespecified analyses, our findings should be interpreted with caution and require prospective validation. Furthermore, although baseline characteristics in this population were generally balanced, randomisation was not stratified by next-generation sequencing results.

The prevalence of *PIK3CA/AKT1/PTEN* alterations was 41%. We observed no clinically significant difference in the prevalence of *PIK3CA/AKT1/PTEN* alterations in primary versus metastatic samples. We also saw no difference in alteration frequency between samples that were collected before administration of (neo)adjuvant chemotherapy and samples collected from chemotherapy-naive patients. However, this analysis does not preclude the possibility that these alterations might be enriched in patients with metastatic disease. The apparent absence of treatment effect in the population of patients who had received no previous chemotherapy (most of whom had de-novo stage IV disease at study entry) should be interpreted with caution due to the small sample size.

There have been few targeted therapy advances in the management of triple-negative breast cancer; chemotherapy (with or without the anti-angiogenic agent bevacizumab) remains the standard of care for these patients, who typically have a poor prognosis and no targeted treatment options. Randomised phase 3 trials specifically in triple-negative breast cancer have reported median progression-free survival of approximately 3–5 months and median overall survival of approximately 12 months with chemotherapy alone.[27,28] Ipatasertib in combination with paclitaxel is one of several novel strategies under assessment in randomised trials in triple-negative breast cancer[29] and the treatment landscape could change substantially in the near future. If emerging agents fulfil their potential, treatment decision making and sequencing could become increasingly complex, and biomarker selection is expected to play an important part in individualised therapy for the heterogeneous collection of diseases traditionally described as triple-negative breast cancer.

Our findings warrant further prospective investigation of ipatasertib in the population of patients with *PIK3CA/AKT1/PTEN*-altered tumours. Additional research in triple-negative breast cancer includes the randomised phase 2 FAIRLANE trial (NCT02301988), which is assessing the addition of ipatasertib to paclitaxel in the neoadjuvant setting.[30] Results from FAIRLANE might provide further information on patient selection, although, as in LOTUS, patients were not stratified by *PIK3CA/AKT1/PTEN*-altered tumours.

Although the development of ipatasertib to date has focused on triple-negative breast cancer, Lin and colleagues[11] observed similar sensitivity to ipatasertib in HER2-positive and hormone receptor-positive cell lines. Our results support future assessment of ipatasertib plus paclitaxel in diseases with high prevalence of PI3K/AKT pathway activation and in particular in patients with *PIK3CA/AKT1/PTEN*-altered tumours. A phase 3 trial in metastatic breast cancer is underway.

Contributors

S-BK, RD, SJI, MO, and CS were involved in the design of the study. S-BK, RD, S-AI, ME, SB, ART, SJI, MO, CS, and JB obtained the data. MJW, AVK, WYC, SMS, and DJM analysed the data. All authors interpreted the data, reviewed and revised the draft manuscripts, and approved the final version for submission.

Declaration of interests

S-BK reports research funding from Novartis, Sanofi Aventis, Kyowa Kirin Inc, and Dongkook Pharma Co, Ltd. RD reports honoraria for consultancy, advisory boards, and speaker engagements from Pfizer, Roche, Eisai, Merck, Novartis, and AstraZeneca and research funding from AstraZeneca. S-AI reports honoraria for advisory roles from Novartis, Hanmi, and Spectrum and a research grant from AstraZeneca. ME reports honoraria from Pfizer and Merck Sharp & Dohme, and research funding to his institution from Roche and Novartis. SB reports a contract (but no honoraria) for consulting and advisory roles for BMS and declares that her husband receives royalties associated with his position as a professor at the University of Washington and is the owner of the company All4cure. ART reports honoraria for an advisory board from AbbVie, and research funding (to institution) from Merck, Pfizer, and Genentech. SJI reports clinical research funding from Genentech. MO reports honoraria for consulting and advisory roles from Genentech/Roche. CS reports honoraria for consulting and advisory roles from Puma Biotechnology, Pfizer, and Roche and research funding (to her institution) from Genentech and AstraZeneca. MJW is an employee of Genentech, Inc, and holds shares in Roche and Ariad Pharmaceuticals. AVK, WYC, SMS, and DJM are employees of Genentech, Inc, and hold stock in Roche. JB reports personal fees and non-financial support from Roche/Genentech, during the conduct of the study; personal fees and non-financial support from Novartis, Eli Lilly, Chugai Pharamaceuticals, AstraZeneca, Sanofi-Aventis, and Bayer Pharmaceuticals; personal fees and stock from Auro Biosciences, Northern Biologics (f/k/a Mosaic Biomedicals), Infinity Pharmaceuticals, Juno Therapeutics, and Seragon; personal fees from Verastem; and stock from Aragon Pharmaceuticals and ApoGen Biotechnologies, outside the submitted work. Seragon was acquired by Roche/Genentech in 2014 and payments were made to acquire JB's equity interest in the company.

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