The lancet oncology 16.1 (2015): 25-35

The cyclin-dependent kinase 4/6 inhibitor palbociclib in combination with letrozole versus letrozole alone as first-line treatment of oestrogen receptor-positive, HER2-negative, advanced breast cancer (PALOMA-1/TRIO-18): a randomised phase 2 study

Richard S Finn, John P Crown, Istvan Lang, Katalin Boer, Igor M Bondarenko, Sergey O Kulyk, Johannes Ettl, Ravindranath Patel, Tamas Pinter, Marcus Schmidt, Yaroslav Shparyk, Anu R Thummala, Nataliya L Voytko, Camilla Fowst, Xin Huang, Sindy T Kim, Sophia Randolph, Dennis J Slamon

Summary

Background – Palbociclib (PD-0332991) **is** [present] an oral, small-molecule inhibitor of cyclin-dependent kinases (CDKs) 4 and 6 with preclinical evidence of growth-inhibitory activity in oestrogen receptor-positive breast cancer cells and synergy with anti-oestrogens. We **aimed** [past] to assess the safety and efficacy of palbociclib in combination with letrozole as first-line treatment of patients with advanced, oestrogen receptor-positive, HER2-negative breast cancer.

Methods – In this open-label, randomised phase 2 study, postmenopausal women with advanced oestrogen receptor-positive and HER2-negative breast cancer who **had not received** [past perfect] any systemic treatment for their advanced disease **were** [past] eligible to participate. Patients **were enrolled** [past] in two separate cohorts that **accrued** [past] sequentially: in cohort 1, patients **were enrolled** [past] on the basis of their oestrogen receptor-positive and HER2-negative biomarker status alone, whereas in cohort 2 they **were** also **required** [past] to have cancers with amplification of cyclin D1 (CCND1), loss of p16 (INK4A or CDKN2A), or both. In both cohorts, patients **were** randomly **assigned** [past] 1:1 via an interactive web-based randomisation system, **stratified** [past] by disease site and disease-free interval, to receive continuous oral letrozole 2.5 mg daily or continuous oral letrozole 2.5 mg daily plus oral palbociclib 125 mg, **given** [past] once daily for 3 weeks followed by 1 week off over 28-day cycles. The primary endpoint **was** [past] investigator-assessed progression free survival in the intention-to-treat population. Accrual to cohort 2 **was stopped** [past] after an unplanned interim analysis of cohort 1 and the statistical analysis plan for the primary endpoint **was amended** [past] to a combined analysis of cohorts 1 and 2 (instead of cohort 2 alone). The study **is** [present] ongoing but closed to accrual; these **are** [present] the results of the final analysis of progression-free survival. The study **is** [present] registered with the ClinicalTrials.gov, number NCT00721409.

Findings – Between Dec 22, 2009, and May 12, 2012, we randomly **assigned** [past] 165 patients, 84 to palbociclib plus letrozole and 81 to letrozole alone. At the time of the final analysis for progression-free survival (median follow-up 29.6 months [95% CI 27.9–36.0] for the palbociclib plus letrozole group and 27.9 months [25.5–31.1] for the letrozole group), 41 progression-free survival events **had occurred** [past perfect] in the palbociclib plus letrozole group and 59 in the letrozole group. Median progression-free survival **was** [past] 10.2 months (95% CI 5.7–12.6) for the letrozole group and 20.2 months (13.8–27.5) for the palbociclib plus letrozole group (HR 0.488, 95% CI 0.319–0.748; one-sided p=0.0004). In cohort 1 (n=66), median progression-free survival **was** [past] 5.7 months (2.6–10.5) for the letrozole group and 26.1 months (11.2–not estimable) for the palbociclib plus letrozole group (HR 0.299, 0.156–0.572; one-sided p<0.0001); in cohort 2 (n=99), median progression-free survival **was** [past] 11.1 months (7.1–16.4) for the letrozole group and 18.1 months

(13.1–27.5) for the palbociclib plus letrozole group (HR 0.508, 0.303–0.853; one-sided p=0.0046). Grade 3–4 neutropenia **was reported** [past] in 45 (54%) of 83 patients in the palbociclib plus letrozole group versus one (1%) of 77 patients in the letrozole group, leucopenia in 16 (19%) versus none, and fatigue in four (4%) versus one (1%). Serious adverse events that **occurred** [past] in more than one patient in the palbociclib plus letrozole group **were** [past] pulmonary embolism (three [4%] patients), back pain (two [2%]), and diarrhoea (two [2%]). No cases of febrile neutropenia or neutropenia-related infections **were reported** [past] during the study. Eleven (13%) patients in the palbociclib plus letrozole group and two (2%) in the letrozole group **discontinued** [past] the study because of adverse events.

Interpretation – The addition of palbociclib to letrozole in this phase 2 study significantly **improved** [past] progression-free survival in women with advanced oestrogen receptor-positive and HER2-negative breast cancer. A phase 3 trial **is** [present] currently underway.

Introduction

Breast cancer **is** [present] a molecularly diverse disease with several defined molecular subgroups. Clinically, however, three therapeutic groups **are used** [present]: those classified as hormone receptor-positive (ie, oestrogen receptor-positive, progesterone receptor-positive, or both, with normal HER2 expression), those classified as HER2-positive, as defined by HER2 gene amplification or overexpression (about 45% of these cancers **can** also **have** [present] variable expression of oestrogen receptors, progesterone receptors, or both), and those classified as triple-negative by virtue of low or absent hormone receptors and the absence of the HER2 alteration.[1] More than 1.5 million new breast cancers **are reported** [present] worldwide each year, with roughly 60–65% of cases hormone receptor-positive, 20–25% HER2-positive, and 15–18% triple-negative.[2] Hormonally directed drugs including anti-oestrogens **have been** [present perfect] the mainstay of treatment for women with oestrogen receptor-positive breast cancers. However, some of these cancers **have** [present] de-novo resistance to this approach and even more **will** eventually **develop** [future] acquired resistance to these treatments and recur. At that point, patients often **receive** [present] chemotherapy that **has** [present] little activity in this setting and that **is** [present] often associated with clinically significant toxic effects.

New classes of molecularly targeted drugs **can affect** [present] the natural history of some subgroups of breast cancer such as HER2-positive disease.[3] However, until recently the addition of such drugs to anti-oestrogens **has not resulted** [present perfect] in similar improvements in hormone receptor-positive disease. This situation **changed** [past] with the approval of everolimus for aromatase inhibitor-resistant disease, which, when added to oestrogen blockade with exemestane, **resulted** [past] in an improvement in investigator assessed progression-free survival (hazard ratio [HR] 0.43, 95% CI 0.35–0.54; p<0·001) in oestrogen receptor-positive advanced disease.[4]

Dysregulation of the cell cycle **is** [present] one of the defined hallmarks of cancer[5] and several genetic alterations in key cell cycle regulatory proteins **have been described** [present perfect] in various cancers, including breast cancer.[5,6] The cyclindependent kinases (CDKs) **are** [present] a large family of serinem threonine kinases that together with their regulatory protein partners, the cyclins, **have** [present] a crucial role in the orderly and controlled progression through the cell cycle. Molecular changes in several of the genes controlling the cell cycle **have been reported** [present perfect] in various cancers, making them an attractive potential target for new treatments.[7] So far, several CDK-targeted drugs **have entered** [present perfect] clinical development, but none **have shown** [present perfect] significant activity in solid tumours and several **are** [present] associated with safety concerns.[8]

Palbociclib (PD-0332991) **is** [present] a reversible, oral, small molecule inhibitor of cyclin dependent kinases 4 and 6 (CDK4/6).[9] CDK4/6 and cyclin D **have** [present] a crucial role in the regulation of the G1/S transition through regulation of the phosphorylation state of pRb. When hyper phosphorylation of pRb occurs, it **causes** [present] release of transcription factors that then **allow** [present] the transition from G1 to S phase and progression of the cell cycle.[10] To investigate the therapeutic potential for palbociclib in breast cancer, we **tested** [past] its growth inhibitory effects preclinically in a large panel of human breast cancer cell lines and **identified** [past] potent activity in two therapeutic groups, those that **were** [past] oestrogen receptor-positive and those that **were** [past] HER2-amplified.[11] This activity **was associated** [past] with a major blockade of pRb hyper phosphorylation, resulting in a G1 arrest in sensitive cells. We also **noted** [past] that in combination with the anti-oestrogen drug tamoxifen, palbociclib **had** [past] synergistic growth inhibitory activity as well as efficacy in a model of acquired tamoxifen resistance.[11] We **noted** [past] similar findings in HER2-amplified breast cancer cell lines with trastuzumab used in combination with palbociclib.[11]

Based on these data, we **designed** [past] a clinical study to test the safety and efficacy of CDK4/6 inhibition by palbociclib in combination with anti-oestrogen drugs in oestrogen receptor-positive breast cancer. Initially, a single-arm, phase 1b study **was done** [past] to assess the safety of palbociclib given with letrozole in patients with oestrogen receptor-positive, HER2-negative, advanced breast cancer and to determine a recommended phase 2 dose of the combination.[12] The results **suggested** [past] a dose and schedule consisting of oral palbociclib 125 mg once daily for 3 weeks followed by 1 week of treatment in a 28-day cycle, combined with the standard dose of oral letrozole 2.5 mg once daily. No drug–drug interactions **were identified** [past] and the most common treatment-related adverse events **were** [past] neutropenia, leucopenia, and fatigue. Based on these clinical data, we **planned** [past] a randomised, open-label, phase 2 study to assess the safety and efficacy of the palbociclib and letrozole combination compared with letrozole alone in the first-line treatment of women with advanced oestrogen receptor-positive, HER2-negative breast cancer.

Methods

Study design and patients

In this international, phase 2, multicentre, open-label randomised study (PALOMA-1/TRIO-18), postmenopausal women (aged 18 years or older) with oestrogen receptor-positive, HER2-negative, advanced breast cancer **were recruited** [past] from 50 sites in 12 countries (Canada, France, Germany, Hungary, Ireland, Italy Russia, South Africa, South Korea, Spain, Ukraine, USA; appendix). Patients **were enrolled** [past] in two separate cohorts that **accrued** [past] sequentially: in cohort 1, patients **were enrolled** [past] on the basis of their oestrogen receptor-positive and HER2-negative biomarker status alone, whereas in cohort 2 they **were** also **required** [past] to have cancers with amplification of cyclin D1 (CCND1), loss of p16 (also known as INK4A or CDKN2A), or both. All patients **were required** [past] to have either locally recurrent disease not amenable to surgery or evidence of metastatic disease.

Oestrogen receptor status **was determined** [past] by routine immunohistochemistry and HER2 status **was assessed** [past] by either fluorescent in-situ hybridisation (FISH) or immunohistochemistry, with both eligibility markers **reported** [past] at enrolling sites. Oestrogen receptor status **was determined** [past] on the basis of either the original tissue staining or, if available, a biopsy from the recurrence. For enrolment into cohort 2 of the study, central laboratory determination of CCND1 amplification orp16 loss **was required** [past]. CCND1 amplification **was defined** [past] as a CCND1-to-chromosome enumeration probe (CEP) 11 ratio greater than 1.5 and p16 loss as a p16-to-CEP9 ratio less than 0.8. These cutoffs **came** [past] from an internal analysis of British Columbia Cancer Foundation data from 778 breast cancer cases that **showed** [past] the frequency of CCND1

amplification (more than three copies) and p16 loss (loss of heterozygosity) to be roughly 36% in patients with luminal B breast cancer. To establish definitions for the genomic changes used for cohort 2, we **used** [past] a four-colour FISH assay (CCND1-to-CEP11 and p16-to-CEP9) to analyse 113 breast cancer samples from a tumour bank and, using a cutoff of CCND1-to-CEP11 greater than 1.5 or p16-to-CEP9 less than 0.8, we **determined** [past] that 42 (37%) of 113 patients in this cohort **had** [past] CCND1 amplification, CDKN2A loss, or both. No previous treatment for advanced disease **was permitted** [past] and all patients **were required** [past] to have measurable disease by Response Evaluation Criteria in Solid Tumors (RECIST, version 1.0) or bone-only disease with an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1 and adequate organ function (as assessed by haematological and blood chemistry analyses). Patients **were excluded** [past] from study if they **had received** [past perfect] letrozole as either neoadjuvant or adjuvant treatment within the 12 months before study entry, **had received** [past perfect] any previous treatment for advanced breast cancer, **had** [past] brain metastasis, or **had** previously **been treated** [past perfect] with a CDK inhibitor. The study **was done** [past] in accordance with the International Conference on Harmonization and Good Clinical Practice standards. Institutional review board approval **was obtained** [past] from all participating institutions and patients **provided** [past] written informed consent before the start of any study-specific screening procedures.

Randomisation and masking

Patients **were** randomly **allocated** [past] 1:1 to receive either palbociclib plus letrozole or letrozole alone. The investigator or other member of the research staff **used** [past] an interactive web-based randomisation system to register and randomly assign patients with two stratifications factors: disease site (visceral, bone only, or other) and disease-free interval (greater than 12 months from the end of adjuvant treatment to recurrence vs. 12 months or less from the end of adjuvant treatment to recurrence or de-novo metastatic disease). The randomisation system **generated** [past] the random assignment of the two treatments in a block size of six for each of the stratification levels. Although this **was** [past] an open-label study, the randomisation codes **were** only **released** [past] at the time of interim and final analyses and crossover **was not allowed** [past] at any time.

Procedures

Patients randomly allocated to letrozole **received** [past] oral letrozole 2.5 mg once daily. Those allocated to palbociclib plus letrozole **received** [past] the same dose of letrozole plus oral palbociclib 125 mg, **given** [past] once daily for 3 weeks followed by 1 week off in 28-day cycles. Study treatment **continued** [past] until disease progression, unacceptable toxic effects, study withdrawal, or death. Dose interruptions and reductions **were allowed** [past] for management of toxic effects (appendix). Tumour assessments **were done** [past] locally at screening and every 8 weeks and **consisted** [past] of CT or MRI scan of the chest, abdomen, and pelvis; radiography for bone lesions (when applicable); and clinical assessment of cutaneous disease. Bone scans **were done** [past] at baseline and every 12 weeks. All patients with tumour responses **were required** [past] to have response confirmation no less than 4 weeks after documentation of the initial response report. Assessment of adverse events included incidence, severity (graded by the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0), timing, seriousness, and relatedness to study drug. Haematological and blood chemistry analyses **were done** [past] every 2 weeks for the first two treatment cycles and at the beginning of each cycle thereafter.

Outcomes

The primary endpoint **was** [past] investigator-assessed progression-free survival, **defined** [past] as the time from randomisation to radiological disease progression or death on study. Secondary efficacy endpoints **were** [past] objective response (by RECIST version 1.0), clinical benefit (as defined by the sum of complete plus partial responses and stable disease for 24 weeks or more), duration of response, and overall survival. Additional secondary endpoints **were** [past] safety and tissue and serum biomarker analyses. Finally, we also **assessed** [past] patient reported outcomes using the modified Brief Pain Inventory (Short Form; mBPI-sf) done on day 1 of each treatment cycle; the mBPI-sf **was used** [past] to capture whether palbociclib adds to the commonly reported adverse event seen with aromatase inhibitors (myalgias and joint pain).

Statistical analysis

We **used** [past] a two-part study design (ie, two sequential cohorts) to allow us to assess both the activity of the palbociclib plus letrozole combination and to determine whether further patient selection on the basis of additional biomarkers (CCND1 or p16) **was warranted** [past]. We **planned** [past] to enrol 60 patients (30 per treatment group) into cohort 1 to provide initial safety and efficacy (progression-free survival) data in patients with oestrogen receptor-positive, HER2-negative, advanced breast cancer. In cohort 2, we **planned** [past] to include 150 patients (75 per treatment group) who also **had** [past] CCND1 gene amplification or loss of p16. Cohort 1 **was intended** [past] to be an exploratory analysis, and the analysis of the primary endpoint **was** initially **intended** [past] to be based on cohort 2 only. Assuming 114 progression free survival events in cohort 2 and using a one-sided α of 0.10, a sample size of 150 **would have** [present] 80% power to detect an HR of 0.67 (palbociclib plus letrozole vs. letrozole alone), including one futility interim analysis. This HR would **represent** [past] a median progression-free survival of 9 months in the control group and 13.5 months in the experimental group.[13]

However, an unplanned interim analysis of cohort 1 based on 31 progression-free survival events **was done** [past] when we **noted** [past] that almost twice as many patients in the control group **were coming** [past continuous] off the study because of disease progression. This interim analysis **showed** [past] clinically meaningful activity of the palbociclib plus letrozole combination compared with letrozole alone (HR 0.35, 95% CI 0.17–0.72, p=0.006). These preliminary results from cohort 1 also **suggested** [past] that further patient selection based on CCND1 amplification or p16 loss **was** [past] unlikely to further improve patient outcome over the use of oestrogen receptor and HER2 status alone (HR with CCND1 or p16 copy changes 0.37 [95% CI 0.10–1.40; p=0.13] vs. HR with no CCND1 or p16 copy changes 0.19 [0.05–0.67; p=0.0045]). As a result, we **stopped** [past] further enrolment into cohort 2 and **amended** [past] the statistical analysis plan such that the primary endpoint **would be analysed** [past] in cohorts 1 and 2 combined instead of cohort 2 alone. These study changes **were made** [past] prospectively without any efficacy results from cohort 2 and **were overseen** and **approved** [past] by the study steering committee. At the time enrolment was stopped, 165 patients **had been** randomly **assigned** [past perfect] (66 patients in cohort 1 and 99 patients in cohort 2). Based on the same original assumption that palbociclib plus letrozole **would increase** [present] progression-free survival from 9 months to 13.5 months compared with letrozole alone, this sample size **would have** [present] 80% power to detect an HR of 0.67 based on 114 progression-free survival events in the final analysis.

A second interim analysis **was added** [past] with these protocol amendments, which **was to be done** [past] when about half of the expected number of progression-free survival events across both cohorts (ie, about 57 of 114 total events) **had occurred** [past perfect]. At the time of the second interim analysis, 61 events **had occurred** [past perfect] and the HR for progression-free survival for the entire intention-to-treat population **was** [past] 0.37 (95% CI 0.21–0.63; one-sided p<0.0001). After these analyses **were done** [past], we **noted** [past] a substantial fall in the event rate over time and therefore

made [past] another adjustment to the final analysis plan such that the final analysis of progression-free survival **would be done** [present] when 95 progression-free survival events **had accumulated** [past perfect], giving greater than 98% power to detect an HR of 0.50 at a one-sided α of 0.10, or 75% power to detect an HR of 0.67.

We **adjusted** [past] the significance level for the final analysis for the interim analyses using the Lan-DeMets procedure with an O'Brien-Fleming stopping boundary. At the final analysis of progression-free survival, we **used** [past] a gatekeeping procedure for hypotheses testing in a hierarchical approach to further control for family-wise errors. This process **began** [past] with assessment of all randomly assigned patients (in cohorts 1 and 2 combined). If the null hypothesis **was rejected** [past], then the Holm procedure **would be used** [present] to test the same hypotheses for cohorts 1 and 2 as two separate studies. Using this approach, we **compared** [past] progression-free survival data between the treatment groups using a stratified log-rank test with stratification for site of disease, disease-free interval, and study cohort. We **estimated** [past] the HR using the Cox proportional hazards regression model; the proportionality of hazards assumption **was verified**[14] [past] and the results **were** [past] satisfactory.

To explore the effect of prespecified baseline prognostic factors on progression-free survival, we **did** [past] a multivariate analysis using the Cox regression model. The primary and secondary efficacy analyses **were done** [past] in the intention-to-treat population. The safety analyses **were done** [past] for all randomly assigned patients who received at least one dose of the study treatment. We only **controlled** [past] the type I error for the analysis of primary endpoint, not for any of the secondary endpoints. We **did** [past] seven prespecified sensitivity analyses for progression-free survival (unstratified analysis; analysis stratified per case report from data; including symptomatic deterioration as disease progression; including disease progression or death after 28 days of treatment discontinuation as disease progression; forced progression-free survival times to the planned assessment times; as-treated population analysis; and multivariate analysis). All statistical analyses **were done** [past] with the SAS version 9.2 or later.

The study **is** [present] registered with the ClinicalTrials.gov, number NCT00721409.

Role of the funding source

The funder **provided** [past] funding to the investigators for study design, conduct, treatment administration, and data collection. The study database **was held** [past] by the funder. The steering committee that oversaw the conduct of the study **consisted** [past] of the principal investigator (RSF), senior author (DJS), an independent statistician, two additional investigators, and three representatives from the funder (one clinician, one operations representative, and a statistician). The study steering committee **was involved** [past] in all discussions about study conduct. All authors **had** [past] unrestricted access to the raw and final study data, and **were** [past] responsible for data interpretation, preparation of the report, and the decision to submit for publication. The authors **attest** [present] to study completeness and the accuracy of the data and data analysis.

Results

Between Dec 22, 2009, and May 12, 2012, 165 women **were** randomly **assigned** [past], 84 to receive letrozole plus palbociclib and 81 to receive letrozole alone (figure 1). Baseline demographic characteristics and established prognostic factors of the intention-to-treat population **were** [past] generally well balanced, although there **were** [past] slight imbalances in disease site, disease-free interval, andprevious treatment (table 1). About half of the patients in each group **had** never **received**

[past perfect] either adjuvant or neoadjuvant systemic treatment. Conversely, a third of patients in each group **had received** [past perfect] previous antihormonal treatment, with half of these individuals **having** previously **received** [past perfect] aromatase inhibitors. As of the data cutoff for the final analysis (Nov 29, 2013), median follow-up **was** [past] 29.6 months (95% CI 27.9–36.0) for the palbociclib plus letrozole group and 27.9 months (25.5–31.1) for the letrozole group, with 19 (23%) of 84 patients in the palbociclib plus letrozole group and eight (10%) of 81 in the letrozole group remaining on treatment. At the time of the final analysis for progression-free survival, 41 progression-free survival events **had occurred** [past perfect] in the palbociclib plus letrozole group and 59 in the letrozole group. Median progression-free survival **was** [past] 20.2 months (95% CI 13.8–27.5) for the palbociclib plus letrozole group and 10.2 months (5.7–12.6) for the letrozole alone group (HR 0.488, 95% CI 0.319–0.748; one-sided p=0.0004; figure 2). For patients in cohort 1, median progression-free survival **was** [past] 26.1 months (95% CI 11.2–not estimable [NE]) for the combination and 5.7 months (95% CI 2.6–10.5) for letrozole alone (HR 0.299, 95% CI 0.156–0.572; one-sided p<0.0001; figure 2). For patients in cohort 2, median progression-free survival **was** [past] 18.1 months (95% CI 13.1–27.5) for the combination and 11.1 months (7.1–16.4) for letrozole alone (HR 0.508, 95% CI 0.303–0.853; one-sided p=0.0046; figure 2). The effect of the combination treatment relative to letrozole alone **was** [past] consistent across all demographic subgroups and patient baseline prognostic factors, apart from patients with disease recurrence 12 months or less from the end of adjuvant treatment, although this subgroup **is** [present] limited by small numbers in both groups (figure 3). The results from prespecified sensitivity analyses **were** [past] consistent with those of the main analysis (data not shown).

Table 2 **shows** [present] best responses to treatment. A greater proportion of patients in the palbociclib plus letrozole group than in the letrozole group **had** [past] an objective response to treatment, both in the intention-to-treat population (36 [43%, 95% CI 32–54] vs. 27 [33%, 23–45]; p=0.13) and in the population with measurable disease (36 [55%, 43–68] vs. 26 [39%, 28–52]; one-sided p=0.047). Similarly, a greater proportion of patients in the intention-to-treat population **achieved** [past] clinical benefit (68 [81%, 95% CI 71–89] vs. 47 [58%, 47–69]; one-sided p=0.0009). The median duration of response for patients who **had** [past] a complete or partial response was 20.3 months (95% CI 13.4–25.8) for the palbociclib plus letrozole group and 11.1 months (9.3–31.6) for the letrozole group. At the same time as the final progression-free survival analysis, we also **assessed** [past] overall survival. Median overall survival **was** [past] 37.5 months (95% CI 28.4–NE; 30 events) in the palbociclib plus letrozole group and 33.3 months (26.4–NE; 31 events) in the letrozole alone group (HR 0.813, 95% CI 0.492–1.345; two-sided p=0.42; figure 4).

The most common adverse events reported for the palbociclib plus letrozole group **were** [past] neutropenia, leucopenia, and fatigue (table 3). All 83 patients who received palbociclib plus letrozole **had** [past] at least one adverse event, compared with 65 (84%) of 77 who received letrozole alone. Despite the increase in all grades of neutropenia and leucopenia with palbociclib plus letrozole, no cases of neutropenic fever **were reported** [past]. Other adverse events (of any cause) that **were increased** [past] in the palbociclib plus letrozole group included anaemia, nausea, arthralgia, and alopecia, but most of these **were** [past] low grade (table 3). Of these adverse events, the difference between treatment groups **was** [past] significant only for anaemia (two-sided p<0.0001) and alopecia (two-sided p=0.0002). Serious adverse events that **occurred** [past] in more than one patient in the palbociclib plus letrozole group **were** [past] pulmonary embolism (three [4%] patients), back pain (two [2%]), and diarrhoea (two [2%]). No serious adverse events **occurred** [past] in more than one patient in the letrozole group.

27 (33%) patients in the palbociclib plus letrozole **had** [past] dose interruptions because of adverse events, compared with only three (4%) patients in the letrozole group. In the combination group, 37 (45%) patients **required** [past] a delay in the start of a subsequent treatment cycle because of an

adverse event and 33 (40%) patients **had** [past] a dose reduction. However, the mean relative dose intensity for palbociclib in the combination group **was** [past] 94% (SD 26).

Cycle delays and dose reductions **are** [present] not applicable to the letrozole group. The main reason for study discontinuation in both treatment groups **was** [past] disease progression (42 [50%] patients in the palbociclib plus letrozole group vs. 57 [70%] patients in the letrozole group). 11 (13%) patients in the palbociclib plus letrozole group and two (2%) patients in the letrozole group **discontinued** [past] the study because of an adverse event. Of these discontinuations, six (7%) patients in palbociclib plus letrozole group and two (2%) patients in the letrozole group **discontinued** [past] because of treatment-related adverse events. One death **occurred** [past] during the study in the palbociclib plus letrozole group because of disease progression; no treatment-related deaths **occurred** [past]. We **noted** [past] no significant differences in pain severity or the effect of pain on daily activities between the two treatment groups.

Discussion

The results of this open-label, phase 2 study **show** [present] that patients with oestrogen receptor-positive, HER2-negative advanced breast cancer **had** [past] significantly longer progression-free survival when treated with palbociclib and letrozole than when treated with letrozole alone. Additionally, the proportions of patients with an objective response and clinical benefit **were** [past] greater in the combination group than in the letrozole alone group. The study **was not powered** [past] to detect an overall survival advantage and few overall survival events **had occurred** [past perfect] at the time of this analysis; however, the initial data **suggest** [present] no detrimental effect on overall survival with the addition of palbociclib in the first-line setting (panel).

Hormone directed drugs **have been** [present perfect] the mainstay of treatment for advanced oestrogen receptor-positive breast cancer for more than four decades. Improvements in clinical outcomes **have occurred** [present perfect] with several drugs that target either specific hormone production (ie, ligands or the hormone receptor pathway), including tamoxifen, steroidal and non-steroidal aromatase inhibitors, and fulvestrant.[15] Despite efforts to further improve clinical outcomes for patients with oestrogen receptor-positive breast cancer with drugs that target other pathways thought to have a role in the development of resistance to hormone drugs, most results **have been** [present perfect] largely disappointing, including efforts to target the HER1 and HER2 pathways, angiogenesis, and IGFR.[16–18] Recently, however, targeting of mTOR, a crucial component of the PI3K pathway, with everolimus, used in combination with a steroidal aromatase inhibitor, **resulted** [past] in improved progression-free survival, although not overall survival, in patients with oestrogen receptorpositive, advanced disease who **had progressed** [past perfect] on antihormonal treatment.[4,19]

Our findings **need** [present] to be interpreted in the context of the limitations of the study design. Specifically, the study **is** [present] open-label and **did not use** [past] central radiology review to prospectively assess the primary endpoint, but rather a retrospective, masked, independent review after accrual **was completed** [past]. This analysis was limited by the fact that scans **were obtained** [past] retrospectively and **were not used** [past] to make on-treatment decisions. On-treatment decisions **were made** [past] on the basis of scan reviews at the individual study sites. We **noted** [past] some baseline imbalances based on the case report form data; however, sensitivity analyses including multivariate analysis to control for baseline factors consistently **showed** [past] treatment benefit in the combination group across all demographic and clinical subgroups.

These clinical results **are** [present] supported by preclinical data[11] that **provide** [present] a clear biological rationale for the development of palbociclib in this patient population. As with most other molecularly targeted drugs in oncology, the greatest gains **are** [present] often seen when rational and

appropriate patient selection **can be used** [present] prospectively. Preclinical data[11] with palbociclib **identified** [past] oestrogen receptor-positive breast cancer cells as one of the subtypes most sensitive to CDK4/6 inhibition, the other being HER2 amplification. Our results **provide** [present] clinical validation of these preclinical data and **support** [present] the further development of palbociclib for the oestrogen receptor-positive, HER2-negative subgroup of breast cancers. Genetic changes in cyclin D1 and p16 **are known** [present] to occur in breast cancer and might **have** [present] a role in the further selection of patients for treatment with a CDK4/6 inhibitor. In cohort 2, we **investigated** [past] the potential for these genetic changes to **be used** [present] to improve patient selection beyond use of oestrogen receptor-positive status alone. However, our results **did not** [past] substantiate this hypothesis. This analysis **confirmed** [past] that oestrogen receptor positivity **is** [present] currently the best and most effective predictive marker for the identification of patients likely to respond to CDK4/6 inhibition. Further biomarker research should **be designed** [present] to improve on oestrogen receptor-positive status as the selection biomarker is ongoing; however, in view of the large proportion of patients in our study who achieved a clinical benefit response (more than 80%), the benefit of additional biomarkers **could be** [present] difficult to ascertain.

Negative-selection biomarkers of resistance might **be** [present] more easily identified and **will** also **be assessed** [future] in ongoing and future molecular studies. One of the most important markers of sensitivity to palbociclib **is** [present] the presence of an intact Rb pathway; however, since pRb loss **is** [present] uncommon in oestrogen receptor-positive, HER2-negative breast cancers, it **was not used** [past] as a prospective independent biomarker for patient selection in the present study. Potentially, relative amounts of pRb (rather than its presence or absence) in the various breast cancer subtypes might **be** [present] of predictive importance and early preclinical data[11] **suggest** [present] that this possibility should be investigated.

When comparing median progression-free survival in the letrozole alone groups, we **noted** [past] a difference between cohorts 1 and 2, suggesting a potential predictive value for cyclin D1 gains, p16 loss, or both in determining response to letrozole alone. However, this finding **could** simply **be** [present] an artifact of the sample size in cohort 1, so further study **is** [present] necessary.

Since the initiation of this study, additional laboratory findings **have linked** [present perfect] CDK 4/6 inhibition to endocrine sensitivity in oestrogen receptor-positive breast cancer.[20,21] The Cancer Genome Atlas **has been used** [present perfect] to identify common changes in the Rb pathway in all breast cancer subtypes, including the luminal oestrogen receptor-positive, HER2-negative subgroup. However, our findings **suggest** [present] that limiting patient selection to those with defined genetic changes in the Rb pathway might **exclude** [present] a much larger group of patients that **could benefit** [present] from CDK4/6 inhibition. Additionally, the results of a recent phase 2, single-arm study[22] of palbociclib in patients with heavily pretreated advanced breast cancer **showed** [past] single agent activity in some patients with oestrogen receptor-positive, HER2-negative breast cancers.

Our results also **provide** [present] useful data for the safety profile of the combination of palbociclib and letrozole, suggesting that adverse events **are** [present] predictable and manageable. Neutropenia, although common, **was not accompanied** [past] by serious clinical outcomes and **is** likely **to be** [present] the result of an on-target effect of CDK4/6 inhibition on marrow progenitor cells. The absence of serious complications resulting from palbociclib-associated neutropenia probably **reflects** [present] a cytostatic rather than cytotoxic effect of the drug on bone marrow progenitor cells,[23] different from what **is seen** [present] with typical cytotoxic drugs. Additional analyses of the effect of palbociclib on quality-of-life measures **are** [present] ongoing in the context of phase 3 studies.

Taken together, the data from this study **provides** [present] a proof of concept for the activity and safety of CDK4/6 inhibition in advanced, oestrogen receptor-positive HER2-negative breast cancer. The improvement in progression-free survival **is** [present] substantial in this population and **is accompanied** [present] by manageable toxic effects. These data clearly **warrant** [present] further

investigation of the efficacy and safety of palbociclib in combination with hormonal blockade, both in patients with this subtype of breast cancer and in other cancer settings. A phase 3, double blind, placebo-controlled study (NCT01740427) in a similar patient population (n=650) with the aim of confirming the present phase 2 findings **is** now fully **enrolled** [present] and ongoing. Additionally, other phase 3 studies of palbociclib in combination with various anti-hormonal drugs in additional breast cancer settings **are** [present] now ongoing (NCT01942135 and NCT01864746).