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Title:

A randomized phase II study evaluating palbociclib in addition to letrozole as neo-adjuvant therapy in estrogen-receptor positive early breast cancer: PALLET trial

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Pfizer Inc. had no material role in the design, data collection, data analysis, or data interpretation of the PALLET study.

Abstract

Purpose:

CDK4/6 inhibitors are used to treat ER+ metastatic breast cancer (BC) in combination with endocrine therapy. PALLET is a phase II randomized trial evaluating the effects of palbociclib with letrozole combination as neo-adjuvant therapy.

Patients and methods:

Postmenopausal women with ER+ primary BC and tumors ≥2.0cm were randomized 3:2:2:2: A: letrozole (2.5mg/d), 14 weeks; B: letrozole, two weeks then palbociclib+letrozole to 14 weeks; C: palbociclib, two weeks then palbociclib+letrozole to 14 weeks; D: palbociclib+letrozole, 14 weeks. Palbociclib was given 125mg/d PO on a 21-days-on, 7-days-off schedule. Core-cut biopsies were taken at baseline, two, and 14 weeks. Co-primary endpoints for letrozole vs palbociclib+letrozole groups (A vs B+C+D) were: change in Ki67 (IHC) between baseline and 14 weeks and clinical response (ordinal, ultrasound) after 14 weeks. Complete cell-cycle arrest (CCCA) was defined as Ki67≤2.7%. Apoptosis was characterized by c-PARP.

Results:

307 patients were recruited. Clinical response was not significantly different between palbociclib+letrozole vs letrozole groups (p=0.20; CR+PR 54.3% vs 49.5%). PD was 3.2% vs 5.4% respectively. Median log-fold change in Ki67 was greater with palbociclib+letrozole vs letrozole (−4.1 vs −2.2; p<0.001) in the 190 (61.9%) evaluable patients corresponding to a geometric mean change of −97.4% vs

−88.5%. More patients on palbociclib+letrozole achieved CCCA (90% vs 59%, p<0.001). Median log-fold change (suppression) of c-PARP was greater with palbociclib+letrozole vs letrozole (−0.80 vs −0.42; p<0.001). More patients had grade ≥3 toxicity on palbociclib+letrozole (49.8% vs 17.0%; p<0.001) mainly due to asymptomatic neutropenia.

Conclusion:

Adding palbociclib to letrozole significantly enhanced the suppression of malignant cell proliferation (Ki67) in primary ER+ BC, but did not increase the clinical response rate over 14-weeks possibly related to concurrent reduction in apoptosis.

Introduction

The use of endocrine therapy for the treatment of hormone receptor positive (HR+) breast cancer (BC) is a seminal example of successfully targeted cancer treatment. Nonetheless, endocrine therapy resistance either *de novo* or acquired remains a challenge in patients with both early and advanced BC[1-4]. One approach to reverse resistance to standard endocrine therapy has been to target an alternative pathway.

The cyclin-dependent kinases, CDK4 and CDK6, promote progression from G1 phase to the S phase of the cell cycle. Inhibition of these kinases leads to decreased proliferation of estrogen-receptor positive (ER+) tumors and reverses endocrine resistance in some patients. The CDK4/6 inhibitor, palbociclib (Ibrance, Pfizer), has demonstrated considerable activity when combined with other endocrine therapies in patients with metastatic BC in both first-line and second-line settings[5-8], with recent results showing prolonged overall survival in the second-line setting[9]. Large phase III adjuvant BC trials with palbociclib and other CDK4/6 inhibitors are ongoing (PALLAS - NCT02513394, PENELOPE-B - NCT01864746, MONARCH-E - NCT03155997).

In early BC the use of neo-adjuvant therapy is an attractive option to facilitate breast conservation and, critically, enables assessment of *in vivo* biomarkers to identify proof of principle activity or to predict responsive or resistant subgroups of tumors[10,11]. The achievement of a pathological complete response (pCR) in HR+ cancers to chemotherapy is much less common than in other subtypes of BC. . A recent meta-analysis reported similar clinical responses and achievement of breast conservation in HR+ BC with neo-adjuvant endocrine therapy compared with combination chemotherapy, but with lower toxicity[12]. As such, strategies to further improve response to neo-adjuvant endocrine therapy in HR+ cancers are more

relevant than using chemotherapy. In HR+ disease, decrease in the proliferation marker Ki67 from baseline in response to endocrine therapy has been validated as a marker of treatment benefit, with measurement of Ki67 after two weeks of endocrine therapy shown to improve the prediction of recurrence-free survival (RFS)[13,14]. Given the predominantly anti-proliferative effects of palbociclib, suppression of Ki67 is a rational endpoint for estimating whether there is efficacy in adding palbociclib to an aromatase inhibitor (AI) vs AI alone in the neo-adjuvant setting.

Here, we report the results of PALLET, a large, multi-national, neo-adjuvant randomized trial (NCT02296801, ISRCTN31243262), designed with co-primary endpoints examining the biological and clinical effects of neo-adjuvant letrozole with or without palbociclib for 14 weeks as primary treatment of ER+/HER2- early invasive BC.

Methods

Full details of the methodology are available in the supplementary material. In summary:

Trial Design and Patients

PALLET is a phase II randomized multicenter trial with parallel UK and North American protocols. Patients were recruited from 38 sites in the UK, USA, and Canada. Eligible patients were postmenopausal women with unilateral, operable, ER+, HER2- tumors measuring at least 2cm by ultrasound, with no evidence of metastatic disease. ER positivity and HER2 negativity were defined as per the ASCO/CAP guidelines[15,16] and were locally assessed.

Patients were randomized (3:2:2:2 ratio) to one of four treatment groups. Group A received letrozole alone for 14 weeks; Group B received letrozole for two weeks followed by palbociclib+letrozole to 14 weeks; Group C received palbociclib for two weeks followed by palbociclib+letrozole to 14 weeks; Group D received palbociclib+letrozole for 14 weeks (Figures S1 and S2). The parallel 4-group design with a two week change for groups B+C enabled the role of each drug alone, or in combination, in suppressing Ki67 to be assessed. Ki67 was centrally assessed. Treatment allocation was by computer generated random permuted blocks and stratified by geographic location: United Kingdom vs North America (USA and Canada) (see supplementary material for further details of randomization methods). Letrozole was given as 2.5mg/d PO continually and palbociclib was given as 125mg/d PO on a 21-days-on, 7-days-off schedule. Protocol specified dose modifications for palbociclib were recommended for various adverse events.

Procedures

Following randomization, patients visited clinic weekly for the first four weeks, then every other week until week 14. Follow-up visits were 30 days post trial treatment and 12 months after randomization. Assessments required at these visits are described in the protocol.

Core-cut biopsies and trial specific blood samples were taken at baseline (post-randomization), two weeks (prior to commencement of second drug for groups B+C), and 14 weeks or discontinuation of study therapy (within 48 hours of last dose of trial treatment).

Outcomes

Principal outcome analyses focused on changes between baseline and end of treatment (EoT) and compared letrozole (A) with palbociclib+letrozole (B+C+D). The co-primary endpoints were (i) clinical response (ultrasound- ECOG[17]) and (ii) change in the proliferation marker Ki67 (IHC). Secondary endpoints included pCR, changes in surgical intent, and safety. Additionally, changes in Ki67 between baseline and week two and week two to EoT were compared for groups in which treatment differed during each respective time period. Pre-specified exploratory biomarkers included c-PARP (apoptosis).

Statistical Analysis

The PALLET trial was powered (90%) using a conventional comparative design with alpha (α=5% overall) split between the two co-primary endpoints. Improved clinical response would be detected for palbociclib+letrozole over letrozole [CR: 31% vs 21%; PR: 57% vs 54%; SD 5% vs 15%; PR: 2% vs 5%] with 284 patients, α=4% and 90% power. With a 5% non-evaluable rate and 3:2:2:2 allocation ratio, the recruitment target was 306 patients. Improvement with decreased Ki67 from 80% in group A to 90% in groups B+C+D (log-fold change of -0.693; SD=1.5) would be detected with 279 patients with α=1% and 90% power. Interim analyses were planned at 25% and 50% of trial endpoint information and the trial would have terminated for futility at the second analysis if there was no evidence that either endpoint favored palbociclib.

Post-hoc analysis revealed that there were 279 evaluable clinical responses (93:186), which under the initial sample size specifications would give 88.1% power. Log-fold changes in Ki67 were available for 190 (61.9%) patients (65:125), to provide 75% power.

All patients were analyzed following the intention to treat approach. Clinical response was treated as an ordinal outcome and compared using the Mann-Whitney test in all patients with ECOG response data available at EoT. Changes in Ki67 and c-PARP were analyzed on the natural log fold scale in patients with biopsy data available at both baseline and EoT. As an exploratory analysis, complete cell cycle arrest (CCCA) at EoT (defined as a Ki67≤2.7%) was compared between groups using a logistic regression model adjusting for recruitment region and histological type.

Results

Between February 27, 2015 and March 8, 2018, 307 women were recruited; 166 from the UK (Table S1) and 141 from North America (Table S2) (Group A 103, Group B 68, Group C 69, Group D 67; Figure 1). Baseline demographic and clinical characteristics were similar across treatment groups (Table 1).

Overall, 253 (82.4%) patients completed 14 weeks treatment. In the letrozole group (A) this was 85% (n=88) compared with 81% (n=165) of patients receiving palbociclib+letrozole (B+C+D). The median percentage of scheduled letrozole received was 99% in all treatment groups. The median (IQR) percentage of the scheduled dose of palbociclib received in groups B, C, and D were 99.2% (82.9-100.0), 90.9% (67.8-100.0), and 97.4% (79.2-100.0), respectively. Palbociclib was interrupted/delayed in 21.6% (n=44) of patients, dose was reduced in 2.0% (n=4) of patients and treatment was interrupted/delayed and dose reduced in 15.2% (n=31) (Table S3).

Clinical response outcomes at EoT were available for 279 (90.8%) patients (Table 2). In the letrozole group (A), 46/93 (49.5%) achieved a complete or partial response compared to 101/186 (54.4%) with palbociclib+letrozole (B+C+D). There was no

evidence that the inclusion of palbociclib changed clinical response as measured by ultrasound (p=0.20).

The log-fold changes in Ki67 were available for 190 (61.9%) patients (Figure 2, Table 2). Reasons for non-availability of paired Ki67 results included missing and unevaluable samples (Table S4) with histological type and geographical region the only baseline characteristics differentiating availability. The median log-fold change in Ki67 between baseline and EoT was −2.2 (IQR: −3.4 to −1.0) in the letrozole group (A) compared with −4.1 (IQR: −5.0 to −2.8; one-sided p<0.001) in palbociclib+letrozole groups (B+C+D). This corresponds to a geometric mean change of −88.5% (95% CI: −92.3 to −82.9%), compared to −97.4% (95% CI: −98.1 to −96.4%). The geometric mean ratio was 0.16 (95% CI: 0.13 to 0.18; p<0.001). CCCA was observed in 38/65 (58.5%) patients in the letrozole group (A) compared to 113/125 (90.4%) in palbociclib+letrozole groups (B+C+D) (OR=6.83; 95% CI: 3.12 to 14.98; p<0.001).

Between baseline and week two there was a median (IQR) log-fold change in Ki67 with letrozole alone (A+B) of −1.3 (−2.9 to −0.7) compared with −3.1 (−4.1 to −1.5) in palbociclib alone (C) (p<0.001). The median (IQR) log-fold change in Ki67 at week two with palbociclib+letrozole (D) was −3.9 (−4.7 to −2.7; p<0.001) compared with groups who received letrozole alone for the first two weeks (A+B), and there was no significant difference between palbociclib alone (C) and palbociclib+letrozole (D) (p=0.06). At week two, CCCA was more common with palbociclib+letrozole than with palbociclib alone (D: 47/53 (89%; 95% CI: 76% to 96%) vs C: 44/61 (72%; 95% CI: 59% to 82%) (p=0.04). Between week two and week 14, there was a median (IQR) log-fold change in Ki67 of −0.1 (−1.1 to 0.4) with letrozole alone (A) compared with

−2.1 (−3.5 to −1.3; p<0.001), −0.4 (−2.1 to 0.0; p=0.12), and 0.0 (−0.1 to 0.9; p=0.08) with palbociclib+letrozole (B, C, D), respectively.

Pathological complete response (pCR) in the breast occurred infrequently and there was no evidence of a difference between letrozole (A) (1/87; 1.1%; 95% CI: 0.0 to 6.2) compared with palbociclib+letrozole (B+C+D) (6/180; 3.3%; 95% CI: 1.2 to 7.1; p=0.43). pCR in breast, axillary lymph nodes, and non-axillary sentinel nodes were found in 2/180 (1.1%; 95% CI: 0.0 to 4.0; p=1.00) patients receiving palbociclib+letrozole (B+C+D). There was no difference in the proportion of patients whose intended surgery changed from mastectomy at baseline to breast conservation at week 14 with letrozole (A) (13/92; 14.1%; 95% CI: 7.7 to 23.0%) compared with palbociclib+letrozole (B+C+D) (25/177; 14.1%; 95% CI: 9.4 to 20.1; p=1.00).

Apoptosis, as measured by c-PARP, was a pre-specified exploratory biomarker with paired data available for 146 (47.6%) patients (Figure 3, Table 2). Other pre-specified exploratory biomarkers are under analysis but not yet available to report. The log-fold change in c-PARP between baseline and EoT was −0.42 (IQR: −0.99 to 0.20) with letrozole (A) compared with −0.80 (IQR: −1.35 to −0.29; one-sided p<0.001) with palbociclib+letrozole (B+C+D). Post-hoc analyses found that at week two there was a median (IQR) log-fold change in c-PARP with letrozole (A+B) of −0.1 (−0.6 to 0.2) compared with −0.3 (−0.8 to −0.1) with palbociclib (C) (p=0.004). The median (IQR) log-fold change in c-PARP at week two with palbociclib+letrozole (D) was −0.5 (−0.7 to 0.0) compared with letrozole (A+B) (p=0.07) and there was no evidence of a difference between palbociclib (C) vs palbociclib+letrozole (D) (p=0.47). Between week two and week 14, there was a median (IQR) log-fold change in c-PARP of −0.3 (−0.7 to 0.0) with letrozole (A) compared with −0.6 (−1.2

to −0.3; p=0.09), −0.3 (−1.0 to 0.1; p=0.72), and −0.3 (−0.7 to 0.1; p=0.82) in palbociclib+letrozole (B, C, D) groups, respectively. Any grade AE, irrespective of relationship to study treatment, was reported in 91% of patients with letrozole (A) and 99% of patients with palbociclib+letrozole (B+C+D). The majority of AEs were grade 1 or 2 (91%). Grade ≥3 AEs were reported in 17% of patients with letrozole (A) and 50% in palbociclib+letrozole groups (B+C+D) (p<0.001) (Table 3). In total, eight patients in palbociclib+letrozole groups (B+C+D) experienced ten events with CTCAE grade of 4 or 5. Of these, one patient experienced a grade 5 acute respiratory distress syndrome which was considered to be unrelated to letrozole or palbociclib.

Discussion

PALLET is the largest randomized trial of a CDK4/6 inhibitor in the neo-adjuvant setting, and demonstrates that the addition of palbociclib to letrozole markedly enhanced the suppression of malignant cell proliferation as assessed by Ki67. In addition, there was a significant increase in the number of patients who achieved CCCA in their tumor following 14 weeks of combination therapy compared with letrozole alone (90% vs 59%). Although the suppression of Ki67 in the first two weeks by palbociclib alone was significantly greater than by letrozole alone, the combination with palbociclib enhanced the proportion of patients achieving CCCA. In terms of toxicity, PALLET detected no new signals by the addition of palbociclib in patients with early stage primary BC.

The lack of difference in clinical response rate (54.3% vs 49.5%) is perhaps not a surprise given the cytostatic nature of endocrine based therapies, in contrast to similar neo-adjuvant trials using cytotoxic chemotherapies in triple negative BC or

targeted combinations in HER2+ BC[18]. In slower growing ER+ tumors, therapies with a predominantly anti-proliferative effect will yield a slower reduction in tumor size[19], especially over a short time-frame of 14 weeks. When using primary endocrine therapy to downstage ER+ BC, maximal tumor shrinkage may take at least 9-12 months[20]. We also demonstrate for the first-time (using c-PARP expression as a biomarker) that unlike chemotherapy, wherein apoptosis increases in addition to an anti-proliferative effect[21], CDK4/6 therapy in combination with an AI produces a greater suppression (not increase) in apoptosis compared with endocrine therapy alone. Measurement of c-PARP is only one of a number of approaches to assessing apoptosis *in situ.* It is notable that the decrease seen in the AI alone arm of PALLET is similar to that seen when using the TUNEL method in the IMPACT trial[22]. This reduction in cell death could also explain why overall tumor volume (i.e. clinical response) as determined by ultrasound, did not substantially change, nor did the surgical breast conservation rate, despite the markedly enhanced anti-proliferative effect. Indeed, these data are consistent with the PALOMA-2 (NCT01740427) study in advanced BC, in which the greatest clinical impact was seen in PFS (HR 0.58), rather than the best objective response rate (ORR) (55% vs 44%)[6,8]. Similarly, the ORR with abemaciclib+AI in the MONARCH-3 trial was 59% vs 44% with AI alone[23] and with ribociclib+AI in MONALEESA-2 the ORR was 52.7% vs 37.1% with AI alone[24], yet both studies also had highly significant improvements in PFS (HR 0.54 and 0.57, respectively). In early BC it remains to be seen whether the anti-proliferative differences seen in PALLET despite lack of change in ORR in the neo-adjuvant setting will translate into an impact on time to recurrence in the ongoing adjuvant studies.

Previous studies of neo-adjuvant endocrine therapy also demonstrated that suppression of Ki67, rather than clinical response, is a better indicator of therapeutic activity in ER+ early BC. In the IMPACT trial, no difference in clinical response rate was seen between anastrozole, tamoxifen, or the combination (37% vs 36% vs 39%)[25] following 3 months of therapy in 330 patients, yet significantly greater suppression of Ki67 was reported for anastrozole compared with tamoxifen at 12 weeks (81.6% vs 61.9%)[13,26]. These differences in Ki67 suppression were paralleled by the greater benefit from anastrozole vs tamoxifen or the combination of anastrozole and tamoxifen in the ATAC trial[27]. Furthermore, the log-fold reduction in Ki67 in IMPACT was a predictor of subsequent RFS in the adjuvant setting[13]. Similarly, the greater suppression of Ki67 by letrozole than tamoxifen in P024[28] paralleled the greater improvement in RFS with letrozole in the analogous BIG1-98 adjuvant trial (NCT00004205)[29]. When the different AIs were compared in Z1031 (NCT00265759)[14], the lack of difference in Ki67 suppression was supported by similar RFS between groups in the adjuvant studies MA-27 (NCT00066573)[30] and FACE (NCT00248170)[31]. More recently, the large UK POETIC trial (NCT02338310) confirmed that lack of suppression of Ki67 following two weeks of pre-operative AI predicted for a significantly worse 5-year relapse-free survival[32]. CDK4/6 inhibitors restrict passage through the cell cycle and are therefore, like endocrine agents, anti-proliferative. However, whether lack of Ki67 suppression after neoadjuvant CDK4/6 inhibitor therapy is similarly predictive is as yet unconfirmed.

The suppression of Ki67 in the first two weeks by palbociclib alone was significantly greater than by letrozole alone, a finding also reported recently in the small phase II pre-operative palbociclib (POP) trial (NCT02008734)[33]. However, in PALLET the 4-group design showed that the palbociclib+letrozole combination enhanced the

proportion of patients achieving CCCA in the first two weeks, and that addition of the AI maximizes Ki67 suppression.

In a previous small phase II study (NeoPalAna - NCT01723774) in 50 patients with ER+ early BC of different intrinsic subtypes, sequential biopsies were taken in patients initiated on anastrozole for four weeks, followed by the addition of palbociclib to study the further change or fall in Ki67[34]. The rates of CCCA with palbociclib and anastrozole were significantly higher (87%) than with anastrozole alone (26%), and biomarkers analysis suggested that response to palbociclib occurred independently of tumor grade, absence of PgR expression, or mutation in *p53*, *PIK3CA,* or *PTEN* genes, but was correlated with RB1 mutation status. Extensive gene and protein expression analyses are being undertaken in PALLET as exploratory endpoints. These will be correlated with anti-proliferative response, and could yield important information about predictive biomarkers for this class of therapy in the early BC setting, which can be tested in the adjuvant setting.

In NeoPalAna, it was reported that palbociclib's anti-proliferative effect diminished rapidly after treatment stopped in some patients, suggesting the need for continued therapy[34]. For this reason in PALLET, we aimed to ensure that the 14-week biopsy was taken during exposure to drug therapy, and excluded 2.6% of 14-week samples as they fell outside the 48 hour window since last drug dose taken. In addition, 13.0% of patients had an unevaluable sample which could reflect minimal cellularity in the core biopsy. Studies to look at the correlation between the 14-week samples with cellularity and Ki67 in the excised surgical sample are ongoing.

In the only other randomized neo-adjuvant trial of CDK4/6 inhibitors in ER+ early BC (NeoMONARCH-NCT02441946), 224 patients were randomized to either

anastrozole, abemaciclib (Verzenio, Eli Lilly), or the combination, with biopsies taken at baseline, two weeks and after 16 weeks of therapy[35]. The combination of abemaciclib+anastrozole was associated with a greater geometric mean fall in Ki67 at two weeks (−92.6% vs −63.2%), with a significant increase in CCCA (66% vs 14%). To date, biomarkers of response or resistance to abemaciclib have not been identified, although reports of induced histologic changes suggestive of tumor differentiation and increased lymphocytic infiltration were seen in some cases[35].

The incomplete availability of biopsy samples could potentially bias the biological findings for Ki67 and c-PARP. When EoT biopsies were not taken (n=38) this often occurred with incomplete treatment (n=29; 76%). Excluding these cases could overstate the proportion who responded. However, there were an approximately equal number of cases in which Ki67 was unevaluable due to scant tumor in the biopsy. A similar level of Ki67 suppression would be expected in these cases compared to the evaluable population so would not be expected to bias our findings. Other trials featuring Ki67 as an endpoint have observed similar evaluable proportions. In the NeoMONARCH study, 138/223 (61.9%) patients were evaluable for Ki67 compared to 190/307 (61.9%) in our trial. Analyses of Ki67 and c-PARP levels between baseline and week two and from week two to EoT in PALLET were conducted post-hoc and did not adjust for multiple testing so should be cautiously interpreted. Nonetheless, such findings match our expectations that adding palbociclib to letrozole would increase the suppression of cell proliferation.

In conclusion, the PALLET trial demonstrated that adding palbociclib to letrozole markedly enhanced suppression of malignant cell proliferation as measured by Ki67 expression, yet without an increase in tumor shrinkage as determined by clinical ultrasound. Correlating biomarkers of anti-proliferative response in the context of a

randomized neo-adjuvant study will be important in determining which patients may derive most benefit from CDK4/6 inhibitors in the ongoing adjuvant studies in early BC.

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Figure 1: CONSORT flow diagram

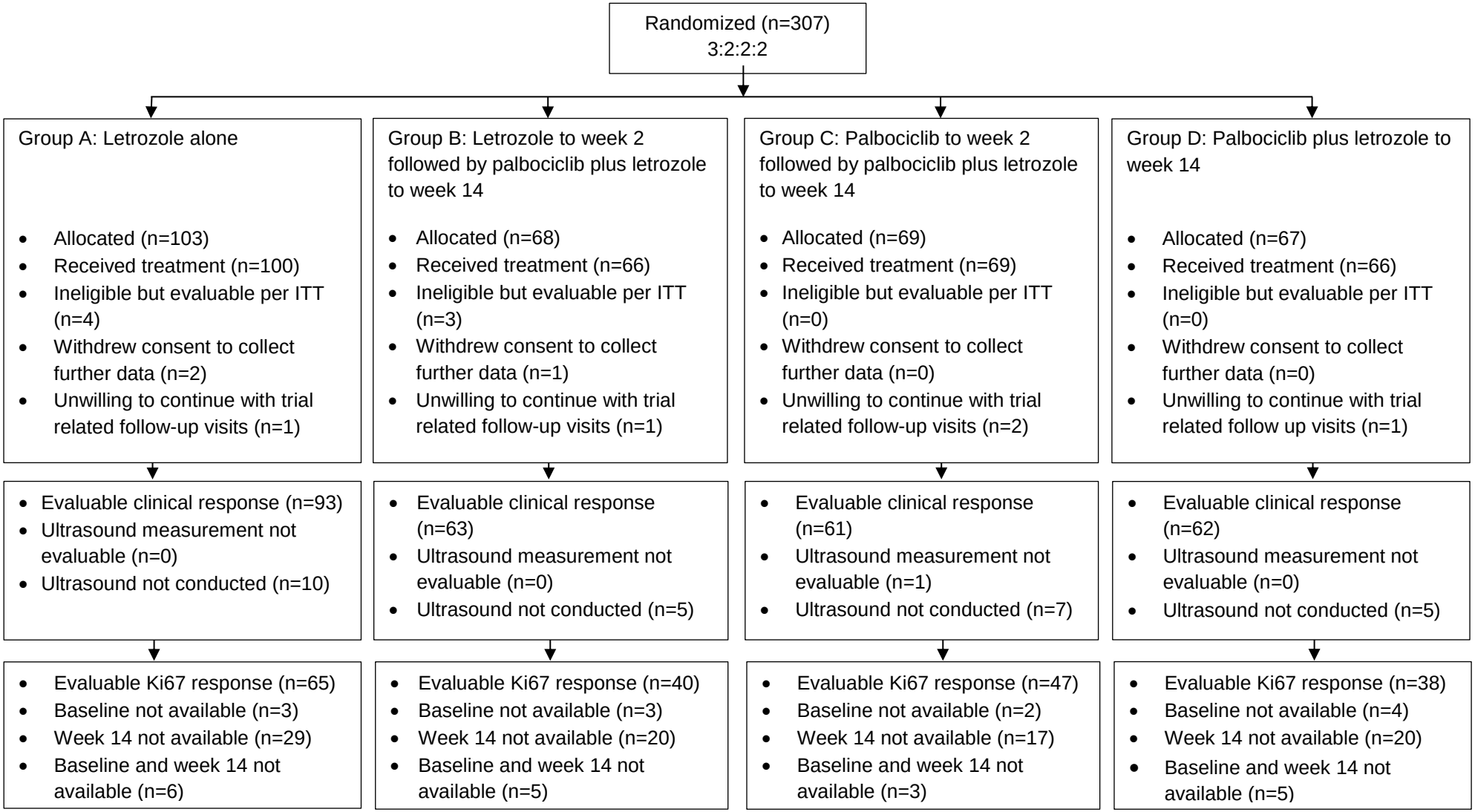

Figure 2: (A) Waterfall plot of log-fold change and percentage change in Ki67 between baseline and the end of treatment[1] and (B) spaghetti plots of individual trajectories of Ki67 by randomized treatment group

(Attached as PDF)

[1] Five patients had a percentage increase greater than 125%

Figure 3: (A) Waterfall plot of log-fold change and percentage change in c-PARP between baseline and the end of treatment[2] (B) spaghetti plots of individual trajectories of c-PARP by randomized treatment group

(Attached as PDF)

[2] Five patients had a percentage increase greater than 125%

Table 1: Baseline demographic and clinical characteristics by randomized treatment group[3]

	Letrozole alone		Letrozole+Palbociclib from week 2		Palbociclib+Letrozole from week 2		Palbociclib+Letrozole		Palbociclib+letrozole regimen	
	Group A (N=103)		Group B (N=68)		Group C (N=69)		Group D (N=67)		Groups B, C and D (N=204)	
	Median	***IQR***	**Median**	***IQR***	**Median**	***IQR***	**Median**	***IQR***	**Median**	***IQR***
Age (years)	65.8	59.4-72.0	66.3	60.4-72.5	63.5	59.3-70.5	63.8	58.5-69.1	64.4	*59.5-71.1*
	n	***%***	**n**	***%***	**n**	***%***	**n**	***%***	**n**	***%***
Recruitment region										
UK	56	*42.4*	37	*54.4*	37	*53.6*	36	*53.7*	110	*53.9*
North America	47	*45.6*	31	*45.6*	32	*46.4*	31	*46.3*	94	*46.1*
Tumor grade										
Low	13	*12.6*	6	*8.8*	4	*5.8*	9	*13.4*	19	*9.3*
Intermediate	70	*68.0*	54	*79.4*	52	*75.4*	51	*76.1*	157	*77.0*
High	19	*18.5*	7	*10.3*	13	*18.8*	7	*10.5*	27	*13.2*
Not known	1	*1.0*	1	*1.5*	0	*0.0*	0	*0.0*	1	*0.5*
Histological type										
Ductal	74	*71.8*	49	*72.1*	46	*66.7*	45	*67.2*	140	*68.7*
Lobular	24	*23.3*	14	*20.6*	19	*27.5*	18	*26.9*	51	*25.0*
Mixed ductal and lobular	4	*3.9*	1	*1.5*	4	*5.8*	2	*3.0*	7	*3.4*
Mucinous	1	*1.0*	4	*5.9*	0	*0.0*	2	*3.0*	6	*2.9*
ER status										
Positive	103	*100.0*	68	*100.0*	69	*100.0*	67	*100.0*	204	*100.0*
PgR status										
Positive	74	*71.8*	47	*69.1*	41	*59.4*	53	*79.1*	141	*69.1*
Negative	15	*14.6*	10	*14.7*	15	*21.7*	7	*10.5*	32	*15.7*
Not determined	14	*13.7*	11	*16.2*	13	*18.8*	7	*10.5*	31	*15.2*
Surgical intent at baseline										
Partial mastectomy/lumpectomy	61	*59.2*	45	*66.2*	40	*58.0*	39	*58.2*	124	*60.8*

[3] See supplementary materials (results section) for information on the associations between baseline characteristics and availability of Ki67 results.

Total or modified radical mastectomy	39	*37.9*	20	*29.4*	25	*36.2*	24	*35.8*	69	*33.8*
Missing	3	*2.9*	3	*4.4*	4	*5.8*	4	*6.0*	11	*5.4*

Table 2: Endpoint by randomized treatment group

	Letrozole alone			Letrozole+Palbociclib from week 2			Palbociclib+Letrozole from week 2			Palbociclib+Letrozole			Palbociclib+Letrozole regimens		
	Group A (N=93)			Group B (N=63)			Group C (N=61)			Group D (N=62)			Groups B, C and D (N=186)		
	N		%	N		%	N		%	N		%	N		%
Clinical response															
Complete response	2		2.2	1		1.6	2		3.3	1		1.6	4		2.2
Partial response	44		47.3	30		47.6	33		54.1	34		54.8	97		52.2
Stable disease	42		45.2	30		47.6	25		41.0	24		38.7	79		42.5
Progressive disease	5		5.4	2		3.2	1		1.6	3		4.8	6		3.2
Pathological complete response	**Group A (N=87)**			**Group B (N=60)**			**Group C (N=60)**			**Group D (N=60)**			**Groups B, C and D (N=180)**		
pCR breast (any nodal status)	1		1.1	1		1.7	3		5.0	2		3.3	6		3.3
pCR breast & nodes	0		0.0	1		1.7	1		1.7	0		0.0	2		1.1
Log-fold change in Ki67	**Group A**			**Group B**			**Group C**			**Group D**			**Groups B, C and D**		
	N	**Med**	**IQR**	**N**	**Med**	**IQR**	**N**	**Med**	**IQR**	**N**	**Med**	**IQR**	**N**	**Med**	**IQR**
From baseline to week 14	65	-2.2	-3.4 – -1.0	40	-4.1	-5.1 – -2.7	47	-4.0	-5.1 – -3.0	38	-3.9	-5.0 – -2.9	125	-4.1	-5.0 – -2.8
From baseline to week 2	61	-1.3	-2.8 – -0.6	39	-1.3	-2.5 – -0.8	44	-3.1	-4.1 – -1.5	32	-3.9	-4.7 – -2.7	115	-2.8	-4.1 – -1.2
From week 2 to week 14	61	-0.1	-1.1 – 0.4	39	-2.1	-3.5 – -1.3	44	-0.4	-2.1 – 0.0	32	0.0	-0.1 – 0.9	115	-1.0	-2.2 – 0.0
Log-fold change in c-PARP	**N**	**Med**	**IQR**	**N**	**Med**	**IQR**	**N**	**Med**	**IQR**	**N**	**Med**	**IQR**	**N**	**Med**	**IQR**
From baseline to week 14	47	-0.4	-1.0 – 0.2	34	-0.9	-1.4 – -0.5	37	-0.8	-1.4 – -0.2	28	-0.6	-1.3 – -0.2	99	-0.8	-1.4 – -0.3
From baseline to week 2	42	-0.1	-0.5 – -0.3	31	-0.3	-0.7 – -0.1	36	-0.3	-0.8 – -0.2	23	-0.5	-0.7 – 0.0	90	-0.4	-0.7 – -0.1
From week 2 to week 14	42	-0.3	-0.8 – 0.0	31	-0.6	-1.2 – -0.3	36	-0.3	-0.8 – 0.1	23	-0.3	-0.7 – 0.1	90	-0.4	-0.9 – 0.0

Table 3: Most frequently occurring Adverse Events

The table contains the number of patients experiencing any grade or grade ≥3 as per MedDRA preferred term AEs. Sorted by most frequent AE of any grade occurring overall. Only AEs occurring in more than 10% of patients in Group A or in the palbociclib+letrozole groups are reported. Percentages within group based on the as-treated populations.

	Letrozole alone Group A (N=100)		Palbociclib+letrozole regimen Groups B+C+D (N=201)	
MedDRA coded AE preferred term	**Any grade n (%)**	**Grade ≥3 n (%)**	**Any grade n (%)**	**Grade ≥3 n (%)**
Fatigue	41 (41.0)	0 (0.0)	117 (58.2)	4 (2.0)
Neutrophil count decreased	2 (2.0)	0 (0.0)	110 (54.7)	82 (40.8)
Hot flush	40 (40.0)	0 (0.0)	54 (26.9)	0 (0.0)
Nausea	18 (18.0)	0 (0.0)	50 (24.9)	0 (0.0)
Arthralgia	26 (26.0)	0 (0.0)	37 (18.4)	1 (0.5)
Headache	21 (21.0)	0 (0.0)	37 (18.4)	0 (0.0)
White blood cell count decreased	1 (1.0)	0 (0.0)	49 (24.4)	12 (6.0)
Diarrhea	14 (14.0)	1 (1.0)	33 (16.4)	2 (1.0)
Constipation	10 (10.0)	0 (0.0)	26 (12.9)	0 (0.0)
Breast pain	12 (12.0)	0 (0.0)	20 (10.0)	1 (0.5)
Platelet count decreased	0 (0.0)	0 (0.0)	31 (15.4)	0 (0.0)
Dizziness	7 (7.0)	0 (0.0)	24 (11.9)	0 (0.0)
Alanine aminotransferase increased	7 (7.0)	0 (0.0)	23 (11.4)	8 (4.0)
Alopecia	3 (3.0)	0 (0.0)	26 (12.9)	0 (0.0)
Hypertension	11 (11.0)	8 (8.0)	15 (7.5)	9 (4.5)
Cough	3 (3.0)	0 (0.0)	21 (10.4)	0 (0.0)
Anemia	3 (3.0)	0 (0.0)	20 (10.0)	0 (0.0)
Epistaxis	2 (2.0)	0 (0.0)	20 (10.0)	0 (0.0)
Stomatitis	0 (0.0)	0 (0.0)	20 (10.0)	2 (1.0)
Depression	10 (10.0)	0 (0.0)	9 (4.5)	0 (0.0)
Pain in extremity	10 (10.0)	1 (1.0)	9 (4.5)	0 (0.0)
Myalgia	11 (11.0)	0 (0.0)	8 (4.0)	0 (0.0)