Effects of the Moderate CYP3A4 Inhibitor Erythromycin on the Pharmacokinetics of Palbociclib: A Randomized Crossover Trial in Patients With Breast Cancer

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Palbociclib is an oral inhibitor of cyclin-dependent kinases 4 and 6 used in the treatment of locally advanced and metastatic breast cancer, and is extensively metabolized by cytochrome P450 enzyme 3A4 (CYP3A4). A pharmacokinetic/pharmacodynamic relationship between palbociclib exposure and neutropenia is well known. This study aimed to investigate the effects of the moderate CYP3A4 inhibitor erythromycin on the pharmacokinetics of palbociclib. We performed a randomized crossover trial comparing the pharmacokinetics of palbociclib monotherapy 125 mg once daily (q.d.) with palbociclib 125 mg q.d. plus oral erythromycin 500 mg three times daily for seven days. Pharmacokinetic sampling was performed at steady-state for both dosing schedules. Eleven evaluable patients have been enrolled. For palbociclib monotherapy, geometric mean area under the plasma concentration-time curve from zero to infinity (AUC_{0-24h}), maximum plasma concentration (C_{max}), and minimum plasma concentration (C_{min}) were 1.46 × 10^3 ng•h/mL (coefficient of variation (CV) 45.0%), 80.5 ng/mL (CV 48.5%), and 48.4 ng/mL (CV 38.8%), respectively, compared with 2.09 × 10^3 ng•h/mL (CV 49.3%, *P* = 0.000977), 115 ng/mL (CV 53.7%, *P* = 0.00562), and 70.7 ng/mL (CV 47.5%, *P* = 0.000488) when palbociclib was administered concomitantly with erythromycin. Geometric mean ratios (90% confidence intervals) of AUC_{0-24h}, C_{max}, and C_{min} for palbociclib plus erythromycin vs. palbociclib monotherapy were 1.43 (1.24–1.66), 1.43 (1.20–1.69), and 1.46 (1.30–1.63). Minor differences in adverse events were observed, and only one grade ≥ 3 toxicity was observed in this short period of time. To conclude, concomitant intake of palbociclib with the moderate CYP3A4 inhibitor erythromycin resulted in an increase in palbociclib AUC_{0-24h} and C_{max} of both 43%. Therefore, a dose reduction of palbociclib to 75 mg q.d. is rational, when palbociclib and moderate CYP3A4 inhibitors are used concomitantly.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Exposure to palbociclib is increased when combined with a strong cytochrome P450 enzyme 3A4 (CYP3A4) inhibitor. No clinical trial with moderate CYP3A4 inhibitors was performed so far, while these moderate inhibitors are more frequently used in clinical practice. A higher palbociclib exposure is related to an increased risk of toxicity.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ This study aimed to investigate the effects of the moderate CYP3A4 inhibitor erythromycin on the pharmacokinetics of palbociclib.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ Palbociclib exposure was increased by more than 40% when administered concomitantly with erythromycin in this clinical trial.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ In case concomitant administration of palbociclib with a moderate CYP3A4 inhibitor is necessary, it is rational to reduce the palbociclib dose by 40%, i.e., to 75 mg q.d.

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INTRODUCTION

Palbociclib is an orally administered inhibitor of cyclin-dependent kinases 4 and 6, and is currently approved in combination with an aromatase inhibitor or fulvestrant for the treatment of hormone receptor-positive, human epidermal growth factor receptor 2 (HER2)–negative, locally advanced or metastatic breast cancer.[1–3] In the pivotal PALOMA2 (a study of palbociclib (PD 0332991) combined with letrozole vs. letrozole for first-line treatment of postmenopausal women with ER-positive, HER2-negative advanced breast cancer) study, patients receiving palbociclib plus letrozole as a first-line treatment had a significantly longer median progression-free survival compared with patients treated with letrozole alone (24.8 months vs. 14.5 months, hazard ratio 0.58, $P < 0.001$).[4] Similarly, the addition of palbociclib to fulvestrant was superior to fulvestrant alone in second or subsequent treatment lines in the PALOMA3 (palbociclib (PD 0332991) combined with fulvestrant vs. fulvestrant in hormone receptor-positive, HER2-negative metastatic breast cancer after endocrine failure) study (median progression-free survival 9.2 vs. 3.8 months, hazard ratio 0.42, $P < 0.001$).[5] The approved dose of palbociclib is 125 mg once daily (q.d.) in a 3-weeks-on/1-week-off dosing schedule.

As palbociclib is extensively metabolized by cytochrome P450 enzyme 3A4 (CYP3A4), its exposure can be markedly affected by concomitant administration with CYP3A4 modulators.[1,3] In a previous drug–drug interaction study in 12 healthy male volunteers, coadministration of itraconazole, a strong CYP3A4 inhibitor, in a dose of 200 mg for 5 days resulted in an increase in the area under the plasma concentration-time curve from zero to infinity ($AUC_{0-\infty}$) and maximum plasma concentration (C_{max}) of 87% and 34%, respectively, after a single dose of palbociclib on Day 5.[1,3,6,7] Based on these data, it is recommended in the drug labels of both the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) to avoid concomitant use of strong CYP3A4 inhibitors, or otherwise to reduce the palbociclib dose to 75 mg q.d. instead of the standard dose of 125 mg q.d.[1,3]

No clinical studies have been performed to study the effects of moderate CYP3A4 inhibitors on palbociclib pharmacokinetics. Simulations with a physiologically-based pharmacokinetic (PBPK) model of palbociclib predicted that concomitant administration of palbociclib with moderate CYP3A4 inhibitors diltiazem and verapamil would lead to an increase in palbociclib AUC and C_{max} of 38% and 22% for verapamil, and 42% and 23% for diltiazem, respectively.[7] It has been concluded that the risk of drug–drug interactions for palbociclib coadministered with moderate CYP3A4 inhibitors is modest and that dose adjustments are thus not needed. However, we argue that a 40% increase in exposure could be clinically relevant, as higher palbociclib exposure is related to an increased risk of toxicity (i.e., neutropenia).[8,9] In the pivotal studies, 30% to 40% of patients needed a dose reduction due to toxicity.[4,5,10] Especially in these patients, concomitant administration with moderate CYP3A4 inhibitors could lead to increased adverse events. Based on these simulations, dose reductions to 75 mg q.d. or 100 mg q.d. (60% or 80% of the standard dose) might be a strategy to reduce the risk of toxicities, while maintaining adequate exposure. The effect of drug–drug interactions via CYP3A4 has thus far only been evaluated in single-dose studies in healthy male volunteers and PBPK simulations. Therefore, a drug–drug interaction study at steady-state concentration in real-life patients treated with palbociclib would provide the most essential and clinically relevant information. Moreover, this could serve as a showcase for other oral targeted therapies metabolized by CYP3A4 and other moderate CYP3A4 inhibitors.

Based on the above, we conducted a randomized pharmacokinetic crossover trial to study the effects of the moderate CYP3A4 inhibitor erythromycin on the pharmacokinetics of palbociclib in female breast cancer patients.

METHODS

Study design

We performed a prospective, multi-center, randomized clinical trial with a crossover design, according to the guideline of the FDA for drug–drug interaction studies.[11] **Figure 1** provides a schematic overview of the study design. Patients were randomized to start with either palbociclib 125 mg q.d. combined with erythromycin 500 mg three times daily (t.i.d.) (Arm A) or palbociclib monotherapy 125 mg q.d. (Arm B). Pharmacokinetic exposure was determined at both dosing schedules. Erythromycin was selected as a moderate CYP3A4 inhibitor, because this drug shows few side effects compared with other moderate CYP3A4 inhibitors. The selected dose was within the therapeutic range, and the dose was in agreement with other DDI studies where erythromycin was used.[12–15] Taking into account the duration-dependent inhibition of CYP3A4 by erythromycin and the mean elimination half-life of palbociclib of 29 hours, 1 week was considered to be sufficient to reach steady-state concentrations.[1,15] As erythromycin is inhibiting CYP3A4 irreversibly, it can take up to 1 week until CYP3A4 function is returned to baseline function after discontinuation of erythromycin.[16,17] Therefore, a washout period of 1 week followed by 1 week to reach new steady-state concentrations has been chosen. The crossover design of the study was chosen to evaluate potential effects of this washout on outcome. Erythromycin was administered for 7 days on either Day 1–7 or Day 15–21, depending on

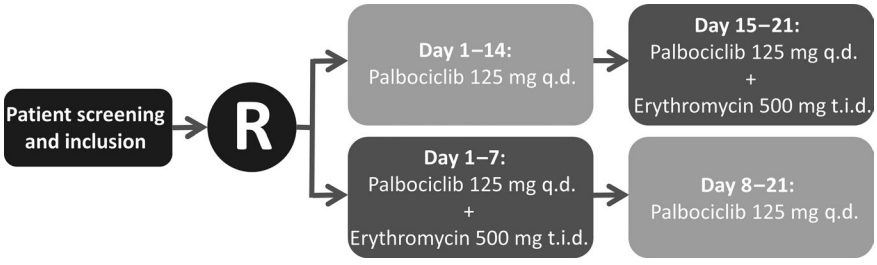

Figure 1 Schematic overview of clinical trial design. Pharmacokinetic sampling was performed at Day 7 and Day 21 of the study. R, randomization, q.d., once daily, t.i.d., three times daily.

randomization. Randomization was performed by block randomization in ALEA (FormsVision BV, Abcoude, The Netherlands). The block size was four, and blocks were only visible for a system administrator. Patients were instructed to take palbociclib at 8:00 a.m., and erythromycin t.i.d. at 8:00 a.m., 4:00 p.m., and 12:00 a.m., both palbociclib and erythromycin together with food (diet of own choice of the patient). Patients requiring a dose interruption or dose reduction or who discontinued treatment during the study were considered nonevaluable for the pharmacokinetic analyses and were replaced. At the end of the trial, palbociclib treatment was continued as part of standard care.

Patient population

Patients with histological or cytological proof of cancer with an indication for treatment with palbociclib (i.e., advanced breast cancer) at the standard dose of 125 mg q.d. were eligible for inclusion. Further inclusion criteria were aged ≥18 years, World Health Organization performance status of 0, 1, or 2, and adequate organ function per judgment of the treating physician.

Exclusion criteria were concomitant use of other medication that could influence the pharmacokinetics of palbociclib within 14 days or five half-lives of the drug (whichever was shorter) before start of the study, including (but not limited to) CYP3A4 inhibitors or inducers, or a QT duration corrected for heart rate (QTc) >450 milliseconds (or >480 milliseconds for patients with bundle branch block) because erythromycin may potentially prolong the QTc interval. Therefore, an electrocardiogram was performed at screening.

Pharmacokinetics

At Day 7 and Day 21 of the study, patients were admitted to the hospital and blood samples were collected for pharmacokinetic analyses. Timepoints were before dosing (directly before ingestion of palbociclib) and 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, and 24 hours post dosing (just before ingestion of a new palbociclib dose). At each timepoint, a blood sample was collected in a 3-mL K_2 EDTA tube and centrifuged directly after collection (1,500 g, 5 minutes, 4°C). Plasma was stored at −20 °C until analysis. Plasma palbociclib concentrations were quantified using a validated liquid chromatography–tandem mass spectrometry method.[18] This method was validated according to the EMA and FDA guidelines on bioanalytical method validation over a linear range of 50–1,000 ng/mL.[19,20]

Study end point

The primary objective of this trial was to study the effect of the moderate CYP3A4 inhibitor erythromycin on the pharmacokinetics of palbociclib, measured as AUC_{0-24h}, C_{max}, and minimum plasma concentration (C_{min}). As a secondary objective, the incidence and severity of adverse events (AEs) with and without erythromycin was compared, according to Common Terminology Criteria for Adverse Events (CTCAE), version 5.0.[21]

Safety assessments

Patients were instructed to use a diary to keep track of AEs. Recording of AEs, vital signs, and hematology and blood chemistry assessments were performed at Day 7 and Day 21 of the study. The incidence, severity, and start and end dates of all AEs were recorded and graded according to the CTCAE version 5.0.

Statistics

For the sample size calculation, it was assumed that concomitant administration with erythromycin would result in a 40% increase in palbociclib exposure, based on previous simulations. By assuming an intraindividual standard deviation of the difference of 50% between the two dosing schedules, 11 evaluable patients had to be included to obtain 80% power (one-sided $\alpha = 0.05$) to detect an increase of ≥40% in exposure. Pharmacokinetic parameters were calculated using noncompartmental analysis. AUC_{0-24h} was calculated using the linear/log trapezoidal method. C_{max} was defined as the highest measured concentration. C_{min} was defined as the mean value of the pre-dose and 24 hours post-dose concentration. AUC_{0-24h}, C_{max}, and C_{min} of palbociclib monotherapy and combined with erythromycin were compared using one-sided Wilcoxon signed rank tests because of the small sample size. The relative difference was calculated by dividing the value for the treatment with palbociclib plus erythromycin by the value for palbociclib monotherapy. Statistical analyses were performed using R version 3.6.3 (R Project, Vienna, Austria), and the geometric mean and confidence intervals were calculated using the Gmean function in the DescTools package.[22]

Ethics approval and consent to participate

The study was approved by the Medical Ethics Committee of The Netherlands Cancer Institute, Amsterdam. Participating centers were The Netherlands Cancer Institute and the Erasmus Medical Center (MC) Cancer Institute. Local approval was obtained in each participating center. The study was conducted in compliance with Good Clinical Practice and the Declaration of Helsinki. All patients provided written informed consent prior to inclusion in the trial. This study was registered in the Netherlands Trial Register (www.trialregister.nl, NL7549) and the EudraCT database (2018-004032-29). The full trial protocol can be accessed upon reasonable request by contacting the corresponding author.

RESULTS

Patient characteristics

Twelve female patients were enrolled in the study from April 2019 until May 2021. One patient withdrew informed consent before pharmacokinetic (PK) sampling at the second dosing schedule, and was excluded. Baseline characteristics of the evaluable patients are provided in **Table 1**. Median age was 59 years, and the median

Table 1 Baseline characteristics (*n* = 11)

Characteristic	*n* (%) or median (range)
Gender, female	11 (100%)
Age (years)	59 (36–79)
Tumor type	
Breast cancer	11 (100%)
Combination therapy	
Fulvestrant	9 (82%)
Anastrozole	1 (9%)
Letrozole	1 (9%)
WHO performance status	
0	8 (73%)
1	3 (27%)
Previous lines of systemic treatment in metastatic setting (number)	1 (0–6)
Previous systemic treatment	
Chemotherapy	11 (100%)
Antihormonal therapy	11 (100%)
Time on palbociclib at study inclusion (months)	10.1 (1.2–22.8)

WHO, World Health Organization.

time on palbociclib treatment before enrollment in the study was 10.1 months.

Pharmacokinetics

Palbociclib exposure was higher, for all but one patient, when administered concomitantly with erythromycin (**Figure 2**, **Figure 3**, and **Table 2**) (no differences were observed between arms). For palbociclib monotherapy, geometric mean AUC_{0-24h}, C_{max}, and C_{min} were 1.46×10^3 ng•h/mL (coefficient of variation (CV) 45.0%), 80.5 ng/mL (CV 48.5%), and 48.4 ng/mL (CV 38.8%), respectively. When palbociclib was administered in combination with erythromycin, this resulted in an increase in AUC_{0-24h}, C_{max}, and C_{min} to 2.09×10^3 ng•h/mL (CV 49.3%, $P = 0.000977$), 115 ng/mL (CV 53.7%, $P = 0.00562$), and 70.7 ng/mL (CV 47.5%, $P = 0.000488$), respectively. Geometric mean ratios (90% confidence intervals) of AUC_{0-24h}, C_{max}, and C_{min} for palbociclib plus erythromycin vs. palbociclib monotherapy were 1.43 (1.24–1.66), 1.43 (1.20–1.69), and 1.46 (1.30–1.63), respectively. The elimination half-life of palbociclib was 29.8 hours (CV 42.0%) for palbociclib monotherapy, compared with 42.6 hours (CV 39.4%) for palbociclib plus erythromycin ($P = 0.00928$).

Treatment-related adverse events

An overview of all treatment-related AEs is provided in **Table 3**. Nine patients experienced one or more treatment-related AEs. No patients discontinued treatment and none required a dose reduction. Only one grade 3 toxicity (neutropenia) occurred during the treatment with palbociclib plus erythromycin.

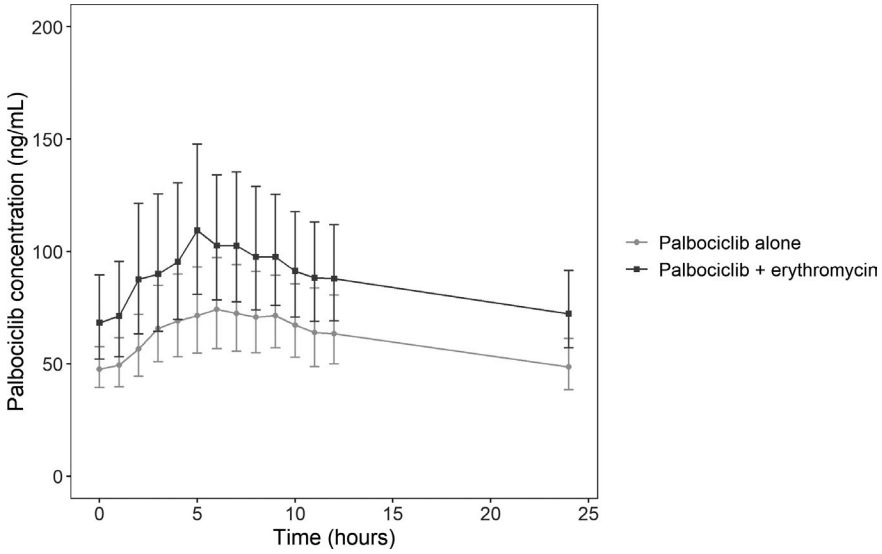

Figure 2 Palbociclib plasma concentration-time curves of palbociclib monotherapy or combined with the moderate CYP3A4 inhibitor erythromycin. Data are represented as geometric mean + 90% confidence interval. CYP3A4, cytochrome P450 enzyme 3A4.

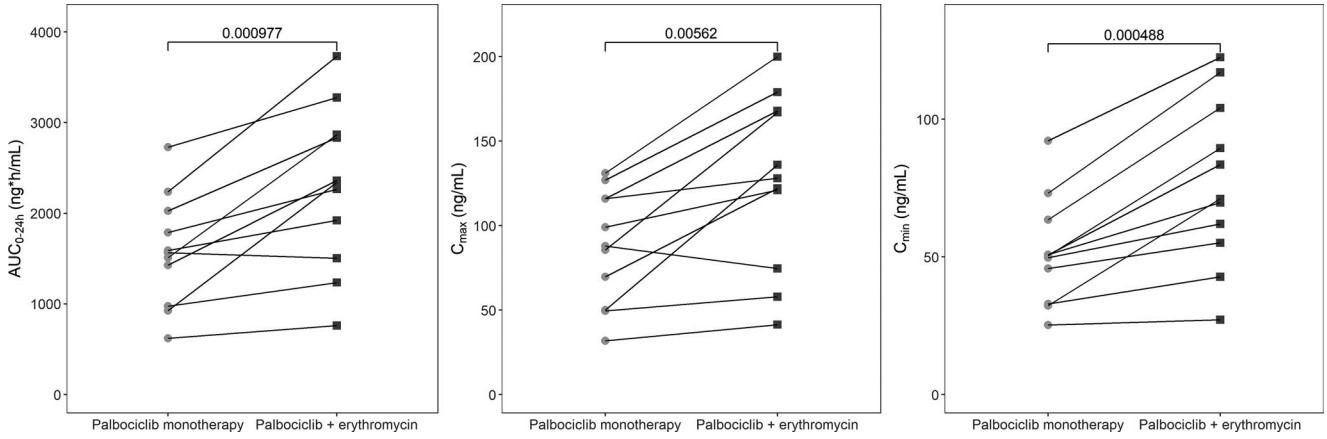

Figure 3 Plots of palbociclib AUC_{0-24h}, C_{max} and C_{min}, for palbociclib monotherapy and combined with the moderate CYP3A4 inhibitor erythromycin for each individual patient. Wilcoxon signed rank tests were performed to calculate *P* values (printed above the brackets). AUC_{0-24h} was calculated using the linear/log trapezoidal method. C_{max} was defined as the highest measured concentration for each dosing schedule. C_{min} was defined as the median value of the pre-dose and 24 hours post-dose sample. AUC_{0-24h}, area under the plasma concentration-time curve from 0 to 24 hours, C_{max}, maximum plasma concentration, C_{min}, minimum plasma concentration; CYP3A4, cytochrome P450 enzyme 3A4.

Table 2 Pharmacokinetic parameters of palbociclib with and without the moderate CYP3A4 inhibitor erythromycin

PK parameter	Palbociclib monotherapy	Palbociclib + erythromycin	Geometric mean ratio (90% CI)	*P* value[a]
AUC_{0-24h} (ng·h/mL)[b]	1.46×10^3 (45.0%)	2.09×10^3 (49.3%)	1.43 (1.24–1.66)	0.000977
C_{max} (ng/mL)[c]	80.5 (48.5%)	115 (53.7%)	1.43 (1.20–1.69)	0.00562
C_{min} (ng/mL)[d]	48.4 (38.8%)	70.7 (47.5%)	1.46 (1.30–1.63)	0.000488
$t_{1/2}$ (h)	29.8 (42.0%)	42.6 (39.4%)	1.43 (1.14–1.79)	0.00928

Pharmacokinetic parameters are expressed as geometric mean (CV%).
Administered doses were 125 mg q.d. for palbociclib and 500 mg t.i.d. for erythromycin.
AUC_{0-24h}, area under the plasma concentration-time curve from time zero to 24 hours; CI, confidence interval; C_{max}, maximum plasma concentration; C_{min}, minimum plasma concentration; CV, coefficient of variation; CYP3A4, cytochrome P450 enzyme 3A4; PK, pharmacokinetic; q.d., once daily; t.i.d., three times daily; $t_{1/2}$, elimination half-life.
[a]Wilcoxon signed rank tests were performed to calculate *P* values. [b]AUC_{0-24h} was calculated using the linear/log trapezoidal method. [c]C_{max} was defined as the highest measured concentration for each dosing schedule. [d]C_{min} was defined as the median value of the pre-dose and 24 hours post-dose sample.

DISCUSSION

Here, we reported the results of a prospective randomized cross-over study assessing the effects of the moderate CYP3A4 inhibitor erythromycin on the pharmacokinetics of palbociclib. Concomitant administration resulted in a significantly higher palbociclib exposure, with increases in AUC_{0-24h}, C_{max}, and C_{min} of 43%, 43%, and 46%, respectively, which is of clinical relevance. Minor differences in adverse events were observed, and only one grade 3 toxicity was observed, in this short period of time.

The observed effect size in the current study was in line with previous simulations for AUC_{0-24h}, but substantially larger for C_{max}, as earlier simulations with diltiazem and verapamil predicted an increase of ± 40% in AUC and ± 23% in C_{max}.[23] Notably, the effect on C_{max} as found in our study is even higher than the effect on C_{max} of the strong CYP3A4 inhibitor itraconazole (i.e., 34%).[1,6] Although no full explanation could be found for this discrepancy in effect size, this may partly be explained by the applied sampling schedule in the drug–drug interaction study with itraconazole (i.e., 2, 4, 6, 8, 12 hours post dose, instead of each hour up to 12 hours post dose in the current study, which may have missed the true C_{max}).[1,6]

The fact that a similar increase in AUC_{0-24h}, C_{max}, and C_{min} was observed (**Table 2**) suggests that the effect of erythromycin is—for an important part—determined by an increased bioavailability (i.e., via inhibition of intestinal CYP3A4). Yet, the elimination half-life was also significantly longer for palbociclib plus erythromycin compared with palbociclib monotherapy, which means that a lower clearance (i.e., via inhibition of hepatic CYP3A4) plays a role as well. The prolonged half-life of palbociclib when it is combined with erythromycin may imply that the washout period was shorter than five times the half-life. Still, the washout period was at least four times the half-life, which allowed for 94% of new steady-state. Most importantly, no difference in palbociclib PK when given as monotherapy was observed between treatment arms, and therefore, it could be concluded that the washout period was sufficient. Apart from being a moderate CYP3A4 inhibitor, erythromycin also inhibits P-glycoprotein (P-gp).[24] Theoretically, inhibition of P-gp could also explain the observed increase in bioavailability, as palbociclib is a substrate of P-gp.[25,26] However, a previous study in mice demonstrated that P-gp mainly restricted the brain penetration of palbociclib, whereas its oral bioavailability was only marginally affected.[26] Therefore, we expect the effect of P-gp inhibition on the palbociclib plasma concentrations in the current study to be minimal.

An important advantage of the drug–drug interaction study described here is that it was performed in the target population of (female) breast cancer patients. In the pivotal drug–drug interaction study with the strong CYP3A4 inhibitor itraconazole, only male healthy volunteers were included.[7] The subsequently performed PBPK simulations to predict the effect of moderate CYP3A4 inhibitors were based on the results found in the male subjects. To exclude the possibility of a gender effect, e.g., on CYP3A4 enzyme activity, this study was conducted in female patients, which are the patients using palbociclib in clinical practice.

Because of pharmacogenetic differences, the exposure to palbociclib could be different between patients. For CYP3A4 the polymorphism *CYP3A4*22* has been described by Wang *et al.*[27] In liver samples with a *CYP3A4*22* polymorphism ~ 15% of total CYP3A4 was nonfunctional, compared with 6% in wild-type liver samples. Because, in case of this polymorphism, still the majority of CYP3A4 will be functional, the genotype will have little effect on the extent of drug inhibition. Therefore, a meaningful comparison could be made between palbociclib monotherapy and palbociclib plus erythromycin combination therapy, without the need of prior pharmacogenetic analyses.

Neutropenia is the most common adverse event during palbociclib treatment. Higher palbociclib exposure has been related to an increased risk of neutropenia in previous studies.[8,9] It is, therefore, logical to assume that concomitant administration of palbociclib and moderate CYP3A4 inhibitors will result in a higher incidence of neutropenia, depending on dose and duration of concomitant administration of the inhibitor. The secondary outcome of the current study was to compare toxicities between the two dosing schedules (i.e., palbociclib monotherapy and palbociclib plus erythromycin). However, neutropenia is a cumulative toxicity that is most pronounced at the end of each palbociclib cycle. Therefore, comparisons of neutropenia between Day 7 and Day 21 of a cycle are not meaningful. Instead, comparisons could be made with previous palbociclib cycles, in which no moderate CYP3A4 inhibitors were used. However, only one grade 3 neutropenia was observed in our study, probably as a result of the short duration of erythromycin treatment of 7 days. The patient who experienced a grade 3

Table 3 Treatment-related AEs according to CTCAE v5.0

	Palbociclib monotherapy		Palbociclib plus erythromycin	
Adverse event	Any grade (*n*)	Grade ≥ 3 (*n*)	Any grade (*n*)	Grade ≥ 3 (*n*)
All patients				
Diarrhea	0	0	4	0
Nausea	0	0	2	0
Vomiting	0	0	1	0
Neutropenia	3	0	2	1
Total number of patients experiencing AEs	4	0	7	1
Patients in Arm A				
Diarrhea	0	0	3	0
Nausea	0	0	1	0
Vomiting	0	0	0	0
Neutropenia	3	0	0	0
Total number of patients experiencing AEs	3	0	3[a]	0
Patients in Arm B				
Diarrhea	0	0	1	0
Nausea	0	0	1	0
Vomiting	0	0	1	0
Neutropenia	0	0	2	1
Total number of patients experiencing AEs	1	0	4[b]	1

AEs, adverse events; CTCAE, Common Terminology Criteria for Adverse Events; Arm A started with palbociclib combined with erythromycin; Arm B started with palbociclib monotherapy.
[a]One patient experiencing both diarrhea and nausea; therefore total number of patients is lower than number of adverse events. [b]One patient experiencing both diarrhea and neutropenia; therefore total number of patients is lower than number of adverse events.

neutropenia at the end of the studied period had a grade 2 neutropenia at the end of her previous treatment cycles. Because of the short duration of concomitant use of a moderate CYP3A4 inhibitor, a meaningful comparison of toxicity could not be performed. However, as these patients had no indication to use a moderate CYP3A4 inhibitor, it was considered unethical to prescribe these drugs longer than necessary to reach steady-state concentrations. Since an exposure–toxicity relationship for palbociclib has already been described, the comparison between palbociclib monotherapy and palbociclib plus erythromycin based on PK was considered sufficient to give a dose recommendation for the interaction.

As palbociclib exposure increased by more than 40% when administered concomitantly with erythromycin, and palbociclib pharmacokinetics change in a dose-proportional manner,[1,3] it is rational to reduce the palbociclib dose by 40%, i.e., to 75 mg q.d., in case of concomitant administration with moderate CYP3A4 inhibitors, without fear for underdosing. For patients who already received prior dose reductions, e.g., due to toxicity, it could be considered to reduce the dose even further by switching to an every-other-day dosing schedule (as no smaller capsule size than 75 mg is currently available). Adjusting the dosing schedule to 5 days on/2 days off every 7 days with no weeks off therapy might also be possible, since it has been described that this alternative schedule leads to a better tolerability.[28] For strong CYP3A4 inhibitors, a dose reduction to 75 mg q.d. was recommended as well, while $AUC_{0-\infty}$ was increased by 87% in that case.[1,3,6] However, first of all that combination should be avoided according to the drug label. Secondly, 75 mg capsules are the lowest dose currently available in the market.

Next to palbociclib, there are other cyclin-dependent kinases 4 and 6 (CDK4/6) inhibitors available for the treatment of breast cancer.[2,29–32] However, these CDK4/6 inhibitors are also substrates of CYP3A4.[29,30] Combination with a strong CYP3A4 inhibitor increased the AUC of palbociclib by 1.9-fold, compared with an increase of 3.2-fold for ribociclib, and 1.7-fold to 2.5-fold for abemaciclib plus active metabolites (potency adjusted).[1,29–32] Complicating factors are the autoinhibition of CYP3A4 by ribociclib, and the metabolism of abemaciclib to active metabolites.[29–32] Since the effect of CYP3A4 inhibition on ribociclib is much larger than on palbociclib, the use of palbociclib is preferred if concomitant administration with a CYP3A4 inhibitor is necessary.[1,30] The effect of CYP3A4 inhibition on abemaciclib exposure seems comparable to the effect on palbociclib, but the effect of a moderate inhibitor on palbociclib is now studied in a clinical trial. Therefore, we recommend using palbociclib if concomitant administration with a CYP3A4 inhibitor is necessary.

To conclude, concomitant intake of palbociclib and the moderate CYP3A4 inhibitor erythromycin results in an increase in AUC_{0-24h} and C_{max} of palbociclib of both 43%, which is clinically relevant. Therefore, in case of concomitant use of palbociclib and

moderate CYP3A4 inhibitors, it is rational to reduce the palbociclib dose to 75 mg q.d., without fear of underexposure. This is especially relevant for the 30% to 40% of patients who need a dose reduction of palbociclib during regular treatment due to toxicity.[4,5,10] It should be considered to update the drug label of palbociclib to include these findings and recommendations, and add moderate CYP3A4 inhibitors to the list of potentially interacting drugs for CDK4/6 inhibitors.

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REFERENCES

1. US Food and Drug Administration, Center for Drug Evaluation and Research. Palbociclib Clinical Pharmacology and Biopharmaceutics Review <https://www.accessdata.fda.gov/drugsatfda_docs/nda/2015/207103Orig1s000ClinPharmR.pdf> (2014).
2. Braal, C.L., Jongbloed, E.M., Wilting, S.M., Mathijssen, R.H.J., Koolen, S.L.W. & Jager, A. Inhibiting CDK4/6 in breast cancer with palbociclib, ribociclib, and abemaciclib: Similarities and differences. *Drugs* **81**, 317–331 (2021).
3. European Medicines Agency Committee for Medicinal Products For Human Use (CHMP). Palbociclib European Public Assessment Report <https://www.ema.europa.eu/en/documents/product-information/ibrance-epar-product-information_en.pdf> (2016).
4. Finn, R.S. *et al*. Palbociclib and Letrozole in Advanced Breast Cancer. *N. Engl. J. Med.* **375**, 1925–1936 (2016).
5. Turner, N.C. *et al*. Palbociclib in hormone-receptor-positive advanced breast cancer. *N. Engl. J. Med* **373**, 209–219 (2015).
6. Hoffman, J.T. *et al*. A phase I open-label, fixed-sequence, two-period crossover study of the effect of multiple doses of Itraconazole on Palbociclib (PD–0332991) pharmacokinetics in healthy volunteers. Proceedings of the 107th Annual Meeting of the American Assoc. for Cancer Research, New Orleans, LA, April 16–20, 2016. **76**, Abstract LB-196 (2016).
7. Yu, Y., Loi, C.-M., Hoffman, J. & Wang, D. Physiologically based pharmacokinetic modeling of palbociclib. *J. Clin. Pharmacol.* **57**, 173–184 (2017).
8. Flaherty, K.T. *et al*. Phase I, dose-escalation trial of the oral cyclin-dependent kinase 4/6 inhibitor PD 0332991, administered using a 21-day schedule in patients with advanced cancer. *Clin. Cancer Res.* **18**, 568–576 (2012).
9. Schwartz, G.K. *et al*. Phase I study of PD 0332991, a cyclin-dependent kinase inhibitor, administered in 3-week cycles (Schedule 2/1). *Br. J. Cancer* **104**, 1862–1868 (2011).
10. Finn, R.S. *et al*. 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. *Lancet Oncol.* **16**, 25–35 (2015).
11. US Food and Drug Administration. Guidance for industry: center for drug evaluation and research clinical drug interaction studies — cytochrome p450 enzyme- and transporter-mediated drug interactions <https://www.fda.gov/media/134581/download> (2020).
12. Lexicomp Erythromycin (systemic): Drug information <https://www.uptodate.com/contents/erythromycin-systemic-drug-information> (2021).
13. Kovarik, J.M., Beyer, D., Bizot, M.N., Jiang, Q., Shenouda, M. & Schmouder, R.L. Effect of multiple-dose erythromycin on everolimus pharmacokinetics. *Eur. J. Clin. Pharmacol.* **61**, 35–38 (2005).
14. Budha, N.R. *et al*. Evaluation of cytochrome P450 3A4-mediated drug-drug interaction potential for cobimetinib using physiologically based pharmacokinetic modeling and simulation. *Clin. Pharmacokinet.* **55**, 1435–1445 (2016).
15. Okudaira, T., Kotegawa, T., Imai, H., Tsutsumi, K., Nakano, S. & Ohashi, K. Effect of the treatment period with erythromycin on cytochrome P450 3A activity in humans. *J. Clin. Pharmacol.* **47**, 871–876 (2007).
16. Yang, J. *et al*. Cytochrome P450 turnover: regulation of synthesis and degradation, methods for determining rates, and implications for the prediction of drug interactions. *Curr. Drug Metab.* **9**, 384–394 (2008).
17. Chan, C.Y.S. *et al*. Derivation of CYP3A4 and CYP2B6 degradation rate constants in primary human hepatocytes: A siRNA-silencing-based approach. *Drug Metab. Pharmacokinet* **33**, 179–187 (2018).
18. Janssen, J.M. *et al*. Development and validation of a liquid chromatography-tandem mass spectrometry assay for nine oral anticancer drugs in human plasma. *J. Pharm. Biomed. Anal.* **174**, 561–566 (2019).
19. European Medicines Agency, Committee for Medicinal Products For Human Use (CHMP). Guideline on bioanalytical method validation <https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-bioanalytical-method-validation_en.pdf> (2012).
20. US Food and Drug Administration, Center for Drug Evaluation and Research. Guidance for Industry: bioanalytical method validation <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/bioanalytical-method-validation-guidance-industry> (2018).
21. National Cancer Institute, National Institutes of Health & US Department of Health and Human Services. Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 <https://ctep.cancer.gov/protocoldevelopment/electronic_applications/ctc.htm> (2017).
22. Signorell, A. et al. Package "DescTools" version 0.99.40 <https://cran.r-project.org/web/packages/DescTools/DescTools.pdf> (2021).
23. Yu, Y., Loi, C.-M., Hoffman, J. & Wang, D. Physiologically based pharmacokinetic modeling of palbociclib. *J. Clin. Pharmacol.* **57**, 173–184 (2017).
24. Eberl, S. *et al*. Role of P-glycoprotein inhibition for drug interactions: Evidence from *in vitro* and pharmacoepidemiological studies. *Clin. Pharmacokinet.* **46**, 1039–1049 (2007).
25. Raub, T.J. *et al*. Brain exposure of two selective dual CDK4 and CDK6 inhibitors and the antitumor activity of CDK4 and CDK6 inhibition in combination with temozolomide in an intracranial glioblastoma xenograft. *Drug Metab. Dispos.* **43**, 1360–1371 (2015).
26. De Gooijer, M.C. *et al*. P-glycoprotein and breast cancer resistance protein restrict the brain penetration of the CDK4/6 inhibitor palbociclib. *Invest. New Drugs* **33**, 1012–1019 (2015).
27. Wang, D., Guo, Y., Wrighton, S.A., Cooke, G.E. & Sadee, W. Intronic polymorphism in CYP3A4 affects hepatic expression and response to statin drugs. *Pharmacogenomics J.* **11**, 274–286 (2011).
28. Krishnamurthy, J. et al. A phase II trial assessing the safety of an alternative dosing schedule of palbociclib (palbo) in hormone receptor positive (HR+), HER2 negative (HER2-) metastatic breast cancer (MBC): Alt Dose Palbo. 2019 San Antonio Breast

Cancer Symposium, San Antonio, Texas, December 10–14, 2019. Abstract P1-19-13.
29. US Food and Drug Administration Center for Drug Evaluation and Research. Abemaciclib Multi-Discipline Review <https://www.accessdata.fda.gov/drugsatfda_docs/nda/2017/208716Orig1s000MultidisciplineR.pdf> (2017).
30. US Food and Drug Administration, Center for Drug Evaluation and Research. Ribociclib Multi-Discipline Review <https://www.accessdata.fda.gov/drugsatfda_docs/nda/2017/209092Orig1s000MultidisciplineR.pdf> (2017).
31. European Medicines Agency Committee for Medicinal Products For Human Use (CHMP). Abemaciclib European Public Assessment Report <https://www.ema.europa.eu/en/documents/product-information/verzenios-epar-product-information_en.pdf> (2018).
32. European Medicines Agency Committee for Medicinal Products For Human Use (CHMP). Everolimus European Public Assessment Report <https://www.ema.europa.eu/en/documents/product-information/afinitor-epar-product-information_en.pdf> (2014).