Real-World Pharmacokinetics and Pharmacodynamics of Everolimus in Metastatic Breast Cancer

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Abstract

Purpose

This study investigated the relationship between the pharmacokinetics and pharmacodynamics of everolimus in patients with metastatic breast cancer (mBC) in real-world practice.

Methods

Twenty-two patients with mBC treated with everolimus plus exemestane were enrolled. Blood everolimus concentrations were measured at outpatient visits. The inhibition of the mammalian target of rapamycin (mTOR) activity in peripheral blood mononuclear cells (PBMCs) was examined. The efficacy and safety endpoints were progression-free survival (PFS) and the cumulative incidence of dose-limiting toxicities (DLTs), respectively.

Results

Blood samples were obtained from 19 consenting patients. Everolimus did not completely inhibit mTOR activity in PBMCs at therapeutic concentrations (~56% maximal inhibition). The most common adverse event was stomatitis (any grade 77%). The trough concentration (C_{trough}) was significantly higher in patients experiencing DLTs than in those without any DLTs ($P = 0.030$). The optimal C_{trough} cutoff predicting DLT development was 17.3 ng/mL. The cumulative incidence of DLTs was significantly higher in patients with C_{trough} ≥17.3 ng/mL than in other patients (sub-hazard ratio 4.87, 95% confidence interval [CI] 1.53–15.5; $P = 0.007$). Furthermore, the median PFS was numerically longer in patients who maintained a steady-state C_{trough} below the threshold than in those who did not (327 days [95% CI 103–355 days] vs 194 days [95% CI 45 days–not estimable]; $P = 0.35$).

Conclusion

The suggested upper threshold for the therapeutic window of everolimus C_{trough} was 17.3 ng/mL. Pharmacokinetically guided dosing may improve the efficacy and safety of everolimus for mBC, warranting further investigation in a larger study.

Introduction

Everolimus, an orally active inhibitor of the mammalian target of rapamycin (mTOR), has been used as an immunosuppressive drug in solid organ transplantation [1]. Based on its antiangiogenic and antitumor activity, everolimus has been approved as single-agent therapy for advanced renal cell carcinoma (RCC), neuroendocrine tumors (NET) of pancreatic, gastrointestinal, or lung origin, and tuberous sclerosis complex (TSC)-associated subependymal giant cell astrocytoma (SEGA) as well as TSC-associated renal angiomyolipoma [2–5]. Everolimus is also used in combination with exemestane to treat hormone

receptor-positive, human epidermal growth factor receptor 2 (HER2)-negative metastatic breast cancer (mBC) after progression on nonsteroidal aromatase inhibitors [6].

Everolimus has a narrow therapeutic index and shows significant inter-individual pharmacokinetic variability when used as an immunosuppressant; therefore, routine therapeutic drug monitoring (TDM) is recommended to maintain a target blood everolimus trough concentration (C_{trough}) between 3 and 8 ng/mL [7,8]. On the other hand, for the treatment of TSC-associated SEGA only, the dose is adjusted based on TDM to attain a C_{trough} of 5–15 ng/mL [9]. The approved dosage of everolimus for other oncological indications is 10 mg orally once daily. This fixed dosing schedule is associated with clinically relevant toxicities, including severe stomatitis and non-infectious pneumonitis, which often necessitates dose modifications.

Everolimus is absorbed relatively rapidly, and the maximum drug concentration is reached between 1 to 2 h post-dose [10]. Everolimus is metabolized primarily in the gut and liver by cytochrome P450 (CYP) 3A4, 3A5, and 2C8 [10]. While everolimus is a substrate for the efflux pump P-glycoprotein (P-gp/ABCB1), it also inhibits P-gp and breast cancer resistance protein (BCRP/ABCG2) [11–13]. Genetic polymorphisms in genes encoding these drug-metabolizing enzymes and efflux transporters have no clinically relevant influence on everolimus pharmacokinetics in transplant recipients; however, the potential effects have not been fully characterized in cancer patients [14–18].

Regarding pharmacodynamics, everolimus binds to the intracellular FK506-binding protein 1A (FKBP1A) with two-fold less affinity than tacrolimus (FK506) [19]. This complex inhibits the kinase activity of mTOR, which inactivates the downstream p70S6 kinase (S6K) and increases the inhibitory binding of eukaryotic translation initiation factor 4E (eIF4E)-binding protein 1 (4E-BP1) to eIF4E; together these actions inhibit cell growth and metabolism as well as cell proliferation and survival [20]. It has been shown that the degree of inhibition of p70S6 kinase is identical in peripheral blood mononuclear cells (PBMCs) and simultaneously collected tumor tissue in cancer patients receiving CCI-779, another mTOR inhibitor, suggesting that PBMCs are a valid surrogate tissue for the pharmacodynamic monitoring of mTOR inhibitors *in vivo* [21].

The objectives of this study were as follows: (1) to characterize the pharmacokinetics and pharmacodynamics of everolimus in patients with mBC; (2) to investigate the potential impact of pharmacogenetics on everolimus exposure; and (3) to clarify the relationship between everolimus exposure and clinical outcomes in real-world practice.

Patients And Methods

Study design and patients

We conducted a single-center, prospective cohort study in the context of routine clinical practice rather than a clinical trial, with the aim of optimizing everolimus dosing through pharmacokinetic and pharmacodynamic analysis in patients with mBC. The protocol was approved by the institutional ethics

committee of Asahikawa Medical University (#14085). Written informed consent was obtained from each patient prior to participation in the study. Consecutive patients with mBC who started everolimus plus exemestane between June 2014 and December 2019 were prospectively enrolled. The baseline characteristics of the patients are summarized in **Table 1**.

Treatment

Everolimus was administered orally at 10 mg once daily in combination with exemestane (25 mg orally once daily). When severe adverse events were present, the everolimus dose was temporarily reduced or interrupted, followed by resumption at a reduced dose. Everolimus treatment was continued until the occurrence of disease progression, unacceptable toxicity, or patient refusal.

Blood samples

In the outpatient setting, we obtained blood samples immediately before each patient's morning dose to measure individual everolimus C_{trough} levels (i.e., C_0). Otherwise, blood samples were routinely collected from remnant blood specimens after laboratory testing at each visit. These *nontrough* blood samples, taken within approximately 4 h after the morning dose, were used to measure individual everolimus peak concentrations (C_{peak}). Blood samples for the concentrations C_1, C_2, C_3, and C_4 were defined as those obtained at 1 h ± 30 min, 2 h ± 30 min, 3 h ± 30 min, and 4 h ± 30 min post-dose, respectively. Additionally, blood samples taken in the post-absorption phase at 12 ± 2 h and 24 ± 2 h post-dose were used to determine C_{12} and C_{24}, respectively.

Pharmacokinetic assessment

Whole blood samples (150 µL) were deproteinized with 450 µL of methanol/0.2 M $ZnSO_4$ (70/30, v/v) containing the internal standard ascomycin. After vortex and centrifugation, the supernatant was treated with solid-phase extraction using Oasis HLB cartridges (Waters, Tokyo, Japan). The eluent was evaporated to dryness and reconstituted in 200 µL of 50% acetonitrile. After filtration, 50 µL was automatically injected into a *liquid chromatography–tandemmass spectrometry (LC–MS/MS) system. Chromatographic* separation was performed *with a C18 column heated at 65°C and a flow rate of 0.2 mL/min* (lower limit of quantification 1 ng/mL, run time 3.5 min). The isocratic mobile phase consisted of a mixture of 5% 10 mM ammonium acetate and 0.1% acetic acid in water and 95% 10 mM ammonium acetate and 0.1% acetic acid in acetonitrile. Analyses were performed in the multiple reaction monitoring mode at ion transitions *m/z* 976.4 ® 909.3 for everolimus and *m/z* 810.4 ® 756.7 for ascomycin. Inter- and intra-assay accuracies were ±10% with a precision (coefficient of variation) below 5%.

Genotyping

Genomic DNA was extracted from the peripheral blood of patients using NucleoSpin Blood QuickPure (Takara Bio, Kusatsu, Japan). Based on previous findings regarding pharmacogenetic determinants associated with everolimus metabolism and disposition, we examined *CYP3A4*22*, *CYP3A5*3*, and

ABCG2 421C>A polymorphisms [13,17,22]. Genotyping was performed using TaqMan SNP genotyping assays (Thermo Fisher Scientific, Tokyo, Japan).

mTOR activity in PBMCs

PBMCs were isolated from whole blood (approximately 2 mL) by Ficoll-Hypaque density gradient centrifugation. Cell counting and viability assessment were performed using the TC20 counter (Bio-Rad, Tokyo, Japan). PBMCs with viability exceeding 90% were used for the subsequent mTOR assay. After centrifugation at 400 x g for 2 min at 4 *°C*, the supernatant was discarded and the cell pellets were resuspended in 100 µL of ice-cold hypotonic lysis buffer containing protease and phosphatase inhibitor cocktails (Nacalai, Kyoto, Japan), and then stored at −80 *°C until analysis.*

The mTOR activity in the extracts of PBMCs was directly measured using the Kinase-linked immunosorbent assay (K-LISA) mTOR activity kit (CBA055, Calbiochem, USA) by assessing phosphorylation of the specific mTOR substrate p70S6 kinase at Thr^{389}, according to the manufacturer's protocol and a previous study [23]. A recombinant human mTOR enzyme (1362-end) with a specific activity of 186 U/mg (Lot# 2052551-D, Millipore, UK) was used for calibration (standard series, 0–250 ng). The protein concentration of each lysate was determined using the Coomassie Brilliant Blue R-250 Staining Solution (Bio-Rad, Tokyo, Japan) for normalization of mTOR activity. Finally, the mTOR activity in PBMCs was calculated relative to the baseline activity before the start of therapy in each patient.

Outcomes

The efficacy endpoints were progression-free survival (PFS) and overall survival (OS). For safety assessment, all adverse events were graded according to the Common Terminology Criteria for Adverse Events version 4.03. The cumulative incidence of dose-limiting toxicities (DLTs) leading to treatment discontinuation and dose interruption/reduction was estimated by adjusting for competing risks (e.g., death or treatment discontinuation due to disease progression) using the Fine and Gray model [24]. The data cutoff date was March 31, 2020.

Statistical analyses

The statistical significance of differences in non-parametric values between two groups was analyzed with the Mann–Whitney *U* test. A receiver operating characteristic (ROC) curve was constructed, and the area under the ROC curve (AUC_{ROC}) was calculated to estimate the optimal cutoff value of everolimus C_{trough} for predicting development of DLTs. The median PFS and OS were estimated using the Kaplan–Meier method, and the difference between two groups was examined using the Gehan–Breslow–Wilcoxon test. A two-sided $P < 0.05$ was considered statistically significant. All statistical analyses were performed using STATA software, version 16 (StataCorp LLC, Texas, USA).

Results

Patients and everolimus dose

Twenty-two patients with mBC who were treated with everolimus plus exemestane were enrolled (**Table 1**). The sample included one male patient. One female patient with advanced age (80 y) started everolimus treatment at 5 mg/day to minimize its toxic effects. Dose modifications of everolimus, specifically interruption and reduction due to DLTs, were performed in seven (32%) patients (*n* = 6 and 1, respectively). Everolimus treatment was discontinued because of disease progression (*n* = 10), adverse events (*n* = 8), surgery (*n* = 1), and patient refusal (*n* = 1). At the data cutoff date, everolimus therapy was ongoing in two patients. The median follow-up period was 494 days (range 102–1067 days).

Everolimus pharmacokinetics/pharmacogenetics and pharmacodynamics

Pharmacokinetic and pharmacogenetic data were available from 19 consenting patients, two of whom did not provide blood samples for determining everolimus C_{peak} levels. **Figure 1a** shows the distribution of blood everolimus concentrations at each time point during a 24-h dosing interval at steady state in patients receiving 10 mg/day of everolimus (total no. of observations 92). Overall, the maximum concentration of approximately 60 ng/mL appeared to be reached at 3 h post-dose, which was comparable with previous findings [25]. Large interindividual variability in C_{trough} was observed (8.3–36.8 ng/mL). Furthermore, everolimus showed considerable fluctuations in absorption, particularly within 2 h post-dose (C_1 7.8–107 ng/mL, C_2 6.7–85.3 ng/mL). Among four patients receiving 5 mg/day of everolimus due to toxicity, the median C_{peak} between 1 to 2 h post-dose at steady state was 10 ng/mL (range 3.0–28.5 ng/mL). This was lower than the median values predicted based on C_1 and C_2 at a dosage of 10 mg/day (20.7 and 27.1 ng/mL, respectively), suggesting nonlinear absorption. We calculated the average C_{trough} and C_{peak} with all the respective measurements available at steady state in each patient on 10 mg/day. No *CYP3A4*22* alleles were detected in the cohort, which was consistent with a previous observation in the Japanese population [26]. Additionally, the *CYP3A5* 6986G>A and *ABCG2* 421C>A polymorphisms did not influence C_{trough} or C_{peak} (**Supplementary Fig. S1**).

Pharmacodynamic data on mTOR activity in PBMCs were obtained in 11 patients with evaluable samples (total no. of observations 60). A significant correlation was observed between blood everolimus concentration and mTOR activity in PBMCs relative to baseline (R^2 = 0.65, P < 0.0001; **Fig. 1b**). Notably, everolimus did not completely inhibit mTOR activity in PBMCs at therapeutic concentrations at any point during the course of everolimus treatment (~56% maximal inhibition).

Everolimus toxicity and its relationship to drug exposure

The most frequently observed adverse event of any grade was stomatitis (77%, **Table 2**). No grade 4 or 5 toxicities were reported in this study. The most common grade 3 adverse events were stomatitis and anorexia (18% each), followed by fatigue (14%). Grade 3 hyperglycemia was observed in one patient, whereas there were no cases of hyperlipidemia. DLTs leading to treatment discontinuation included pneumonitis (*n* = 4), stomatitis, fatigue, urticaria, and muscle pain (one patient each). One patient

reported grade 3 dysgeusia requiring dose reduction. Dose interruption was required in patients with grade 3 non-hematological toxicities, including stomatitis and anorexia (*n* = 2 each) as well as herpes zoster and diarrhea (one patient each).

The average C_{trough} at steady state did not differ in patients with and without any grade of pneumonitis (median 9.4 ng/mL [*n* = 3] and 14.0 ng/mL [*n* = 16], respectively; *P* = 0.17). Notably, the average C_{trough} was significantly higher in patients with DLTs leading to treatment discontinuation and dose interruption/reduction than in those without any DLTs (*P* = 0.030; **Fig. 2a**). The optimal C_{trough} cutoff to predict development of DLTs was 17.3 ng/mL (AUC_{ROC} 0.79, 95% confidence interval [CI] 0.58–1.00; *P* = 0.031). Furthermore, as shown in **Figure 2b**, the cumulative incidence of DLTs was significantly higher in patients with an average C_{trough} ≥17.3 ng/mL than in other patients (sub-hazard ratio 4.87, 95% CI 1.53–15.5; *P* = 0.007).

Everolimus efficacy and its relationship to drug exposure

The disease control rate was 45% (10/22); two patients had a partial response and eight had stable disease per the Response Evaluation Criteria for Solid Tumors (RECIST) 1.1. The median PFS and OS were 308 days (95% CI 187–355 days) and not reached (95% CI 527 days–not estimable), respectively (**Fig. 3a**). The 1-y PFS and OS rates (95% CI) were 18% (3–44%) and 90% (65–97%), respectively. Although not statistically significant, as shown in **Figure 3b**, the median PFS was numerically longer in patients who maintained an average steady state C_{trough} below the threshold for DLTs (17.3 ng/mL) than in those who did not (327 days [95% CI 103–355 days] vs 194 days [95% CI 45 days–not estimable]; *P* = 0.35).

Discussion

There is accumulating evidence that shows a positive relationship between everolimus exposure and clinical outcomes in oncology settings [27]. The current study provides real-world evidence to support an association between everolimus C_{trough} and toxicity in patients with mBC. Deppenweiler et al. [28] previously reported that everolimus C_{trough} levels >26.3 ng/mL were associated with a four-fold increased risk of adverse events in patients with a variety of cancers, with a particularly high risk in those with breast cancer. In the present study, we determined that the optimal cutoff value of everolimus C_{trough} for predicting the development of DLTs was 17.3 ng/mL (**Fig. 2**), a finding comparable to that of a previous study that identified 19.2 ng/mL as the toxicity threshold in patients with mBC [29]. We also found that the average C_{trough} at steady state did not differ between patients with and without any grade of pneumonitis. This observation is consistent with those in earlier studies demonstrating no correlation between everolimus C_{trough} and the occurrence of pneumonitis [30,31]. Therefore, monitoring of blood everolimus concentrations may not help identify patients at increased risk of pneumonitis. Further development of reliable biomarkers in addition to serum KL-6 levels is necessary to improve the prediction of mTOR inhibitor–induced pneumonitis [31,32].

Regarding the exposure–efficacy relationship, no significant difference in median PFS was reported between patients with everolimus C_{trough} >12.6 ng/mL versus <12.6 ng/mL who were also treated with exemestane [29]. Similarly, everolimus C_{trough} level was not a significant predictor of long-term efficacy in our patient cohort (**Fig. 3b**). These findings suggest that higher everolimus exposure will not translate into survival benefit, probably because of decreased tolerability due to toxicities such as severe stomatitis and anorexia (**Table 2**). Furthermore, the lack of survival benefit despite increased everolimus exposure may be explained by the fact that patients also received exemestane, which might have independently improved efficacy regardless of individual exposure to everolimus.

Overall, as shown in **Fig. 1a**, everolimus demonstrated variable absorption and marked variation in elimination. Although the number of patients was small, we found no indication that *CYP3A5* 6986G>A or *ABCG2* 421C>A polymorphisms influenced everolimus exposure (**Supplementary Fig. S1**), which is consistent with findings in previous studies [14–18]. Therefore, there may be no need to individualize the dose based on genetic polymorphisms in *CYP3A5* and *ABCG2*. Further investigations that consider non-genetic factors (e.g., food ingredients) [33] should be conducted to clarify the mechanisms underlying the variable, potentially nonlinear pharmacokinetic characteristics of everolimus in mBC.

In the present study, we directly measured mTOR activity in PBMCs as a marker of everolimus pharmacodynamics. Interestingly, the mTOR activity in PBMCs was not completely inhibited by everolimus at therapeutic concentrations (**Fig. 1b**). In a study of renal transplant recipients, Dekter et al. [23] showed similar results regarding the partial inhibition of mTOR activity by everolimus in PBMCs *in vivo* and *in vitro*. These observations may be explained by intracellular immunophilin FKBP1A potentially limiting mTOR inhibition by everolimus at high drug concentrations; this occurs with tacrolimus, which binds with the same immunophilin and incompletely inhibits calcineurin phosphatase activity at saturable concentrations [34].

Despite the fact that everolimus almost completely inhibits phosphorylation of the downstream S6 protein, partial inhibition of 4E-BP1 phosphorylation was previously observed in tumor samples and PBMCs from patients treated with everolimus [35,36]. Taking these findings and our results into account, partial rather than complete inhibition of the mTOR pathway by everolimus may be potentially beneficial for preventing life-threatening toxicities and/or recovering early from adverse drug reactions through dose reduction/interruption in patients receiving everolimus. Actually, no grade 4 or higher adverse events occurred in our patient cohort. On the other hand, given the fact that everolimus only partially inhibits mTOR, everolimus monotherapy might not exert adequate antitumor activity. Thus, in patients with advanced RCC who have already undergone one prior anti-angiogenic therapy, it seems reasonable to administer lenvatinib, a multikinase inhibitor, in combination with everolimus in order to increase response rates and survival [37].

Male breast cancer is a rare disease, accounting for less than 1% of all breast cancers [38]. In the present study, we enrolled one male patient with hormone receptor-positive, HER2-negative mBC who was treated with everolimus plus exemestane. Ten days after the start of therapy, the patient visited the emergency

room because of severe fatigue, leading to immediate discontinuation of everolimus treatment. On that day, the blood everolimus concentration more than 24 h after the last dose was 18.4 ng/mL, which was above the toxicity threshold identified in this study (17.3 ng/mL). The patient was subsequently treated with exemestane alone followed by high-dose toremifene, which was finally discontinued due to disease progression 5.5 months after he began receiving everolimus plus exemestane. Among patients with breast cancer, men had a numerically higher incidence of adverse events than women during everolimus treatment in real-world practice [39]. Potential gender differences in the exposure–toxicity relationship for everolimus should be investigated in a large population-based study that includes male patients with mBC.

Limitations of this study include its small sample size, lack of robust pharmacokinetic–pharmacodynamic profiling, and single-center design. Despite these limitations, the present study demonstrated that increased exposure to everolimus was associated with an increased risk of DLTs among patients with mBC. Through TDM, pharmacokinetically guided dosing of everolimus in routine clinical practice may help avoid the development of DLTs and improve efficacy as well as adherence to treatment in patients who receive everolimus for mBC.

Conclusions

The present study indicates that everolimus may exert antitumor activity without completely inhibiting mTOR activity in patients with mBC. Furthermore, our results suggest that the potential upper threshold for the therapeutic window of everolimus C_{trough} is 17.3 ng/mL, warranting further prospective investigation in a larger patient population.

Declarations

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Conflicts of interest: None to declare.

Ethics approval and consent to participate: The protocol of this study was approved by the institutional ethics committee of Asahikawa Medical University (#14085). The study was performed in accordance with the Declaration of Helsinki and its amendments. Written informed consent was obtained from each patient prior to participation in the study.

Consent for publication: All authors approved the final version of the manuscript.

Availability of data and material: The data supporting the findings of this study are available on request from the corresponding author. The data are not publicly available because of privacy or ethical restrictions.

Author contributions: Study concept and design: M.F., M.K.; acquisition, analysis, or interpretation of data: all authors; drafting of the manuscript: M.F.; critical revision of the manuscript for important intellectual content: all authors; statistical analysis: M.F.; acquisition of funding: M.F.; administrative, technical, or material support: K.I., M.K.; supervision: M.F.

Compliance with Ethical Standards

Disclosure of potential conflicts of interest: All authors have no conflicts of interest to declare.

Research involving human participants: The protocol of this study was approved by the institutional ethics committee of Asahikawa Medical University (#14085). The study was performed in accordance with the Declaration of Helsinki and its amendments.

Informed consent: Written informed consent was obtained from each patient prior to enrollment.

References

1. Budde K, Becker T, Arns W, Sommerer C, Reinke P, Eisenberger U, Kramer S, Fischer W, Gschaidmeier H, Pietruck F, ZEUS Study Investigators (2011) Everolimus-based, calcineurin-inhibitor-free regimen in recipients of de-novo kidney transplants: an open-label, randomised, controlled trial. Lancet 377:837–847
2. Motzer RJ, Escudier B, Oudard S, Hutson TE, Porta C, Bracarda S, Grünwald V, Thompson JA, Figlin RA, Hollaender N, Urbanowitz G, Berg WJ, Kay A, Lebwohl D, Ravaud A, RECORD-1 Study Group (2008) Efficacy of everolimus in advanced renal cell carcinoma: a double-blind, randomised, placebo-controlled phase III trial. Lancet 372:449–456
3. Yao JC, Shah MH, Ito T, Bohas CL, Wolin EM, Van Cutsem E, Hobday TJ, Okusaka T, Capdevila J, de Vries EG, Tomassetti P, Pavel ME, Hoosen S, Haas T, Lincy J, Lebwohl D, Öberg K; RAD001 in Advanced Neuroendocrine Tumors, Third Trial (RADIANT-3) Study Group (2011) Everolimus for advanced pancreatic neuroendocrine tumors. N Engl J Med 364:514–523
4. Franz DN, Belousova E, Sparagana S, Bebin EM, Frost M, Kuperman R, Witt O, Kohrman MH, Flamini JR, Wu JY, Curatolo P, de Vries PJ, Whittemore VH, Thiele EA, Ford JP, Shah G, Cauwel H, Lebwohl D, Sahmoud T, Jozwiak S (2013) Efficacy and safety of everolimus for subependymal giant cell astrocytomas associated with tuberous sclerosis complex (EXIST-1): a multicentre, randomised, placebo-controlled phase 3 trial. Lancet 381:125–132
5. Bissler JJ, Kingswood JC, Radzikowska E, Zonnenberg BA, Frost M, Belousova E, Sauter M, Nonomura N, Brakemeier S, de Vries PJ, Whittemore VH, Chen D, Sahmoud T, Shah G, Lincy J, Lebwohl D, Budde K (2013) Everolimus for angiomyolipoma associated with tuberous sclerosis

complex or sporadic lymphangioleiomyomatosis (EXIST-2): a multicentre, randomised, double-blind, placebo-controlled trial. Lancet 381:817–824

6. Baselga J, Campone M, Piccart M, Burris HA 3rd, Rugo HS, Sahmoud T, Noguchi S, Gnant M, Pritchard KI, Lebrun F, Beck JT, Ito Y, Yardley D, Deleu I, Perez A, Bachelot T, Vittori L, Xu Z, Mukhopadhyay P, Lebwohl D, Hortobagyi GN (2012) Everolimus in postmenopausal hormone-receptor-positive advanced breast cancer. N Engl J Med 366:520–529
7. Kovarik JM, Kahan BD, Kaplan B, Lorber M, Winkler M, Rouilly M, Gerbeau C, Cambon N, Boger R, Rordorf C, Everolimus Phase 2 Study Group (2001) Longitudinal assessment of everolimus in de novo renal transplant recipients over the first post-transplant year: pharmacokinetics, exposure-response relationships, and influence on cyclosporine. Clin Pharmacol Ther 69:48–56
8. Kovarik JM, Tedesco H, Pascual J, Civati G, Bizot MN, Geissler J, Schmidli H (2004) Everolimus therapeutic concentration range defined from a prospective trial with reduced-exposure cyclosporine in de novo kidney transplantation. Ther Drug Monit 26:499–505
9. Franz DN, Belousova E, Sparagana S, Bebin EM, Frost M, Kuperman R, Witt O, Kohrman MH, Flamini JR, Wu JY, Curatolo P, de Vries PJ, Berkowitz N, Anak O, Niolat J, Jozwiak S (2014) Everolimus for subependymal giant cell astrocytoma in patients with tuberous sclerosis complex: 2-year open-label extension of the randomised EXIST-1 study. Lancet Oncol 15:1513–1520
10. Kirchner GI, Meier-Wiedenbach I, Manns MP (2004) Clinical pharmacokinetics of everolimus. Clin Pharmacokinet 43:83–95
11. Crowe A, Lemaire M (1998) In vitro and in situ absorption of SDZ-RAD using a human intestinal cell line (Caco-2) and a single pass perfusion model in rats: comparison with rapamycin. Pharm Res 15:1666–1672
12. Chu C, Abbara C, Noël-Hudson MS, Thomas-Bourgneuf L, Gonin P, Farinotti R, Bonhomme-Faivre L (2009) Disposition of everolimus in mdr1a-/1b- mice and after a pre-treatment of lapatinib in Swiss mice. Biochem Pharmacol 77:1629–1634
13. Minocha M, Khurana V, Qin B, Pal D, Mitra AK (2012) Co-administration strategy to enhance brain accumulation of vandetanib by modulating P-glycoprotein (P-gp/Abcb1) and breast cancer resistance protein (Bcrp1/Abcg2) mediated efflux with m-TOR inhibitors. Int J Pharm 434:306–314
14. Lemaitre F, Bezian E, Goldwirt L, Fernandez C, Farinotti R, Varnous S, Urien S, Antignac M (2012) Population pharmacokinetics of everolimus in cardiac recipients: comedications, ABCB1, and CYP3A5 polymorphisms. Ther Drug Monit 34:686–694
15. Moes DJ, Press RR, den Hartigh J, van der Straaten T, de Fijter JW, Guchelaar HJ (2012) Population pharmacokinetics and pharmacogenetics of everolimus in renal transplant patients. Clin Pharmacokinet 51:467–480
16. Schoeppler KE, Aquilante CL, Kiser TH, Fish DN, Zamora MR (2014) The impact of genetic polymorphisms, diltiazem, and demographic variables on everolimus trough concentrations in lung transplant recipients. Clin Transplant 28:590–597

17. Moes DJ, Swen JJ, den Hartigh J, van der Straaten T, van der Heide JJ, Sanders JS, Bemelman FJ, de Fijter JW, Guchelaar HJ (2014) Effect of CYP3A4*22, CYP3A5*3, and CYP3A combined genotypes on cyclosporine, everolimus, and tacrolimus pharmacokinetics in renal transplantation. CPT Pharmacometrics Syst Pharmacol 3:e100
18. Picard N, Rouguieg-Malki K, Kamar N, Rostaing L, Marquet P (2011) CYP3A5 genotype does not influence everolimus in vitro metabolism and clinical pharmacokinetics in renal transplant recipients. Transplantation 91:652–656
19. Schuler W, Sedrani R, Cottens S, Häberlin B, Schulz M, Schuurman HJ, Zenke G, Zerwes HG, Schreier MH (1997) SDZ RAD, a new rapamycin derivative: pharmacological properties in vitro and in vivo. Transplantation 64:36–42
20. Zou Z, Tao T, Li H, Zhu X (2020) mTOR signaling pathway and mTOR inhibitors in cancer: progress and challenges. Cell Biosci 10:31
21. Peralba JM, DeGraffenried L, Friedrichs W, Fulcher L, Grünwald V, Weiss G, Hidalgo M (2003) Pharmacodynamic evaluation of CCI-779, an inhibitor of mTOR, in cancer patients. Clin Cancer Res 9:2887–2892
22. Tang SC, Sparidans RW, Cheung KL, Fukami T, Durmus S, Wagenaar E, Yokoi T, van Vlijmen BJ, Beijnen JH, Schinkel AH (2014) P-glycoprotein, CYP3A, and plasma carboxylesterase determine brain and blood disposition of the mTOR Inhibitor everolimus (Afinitor) in mice. Clin Cancer Res 20:3133–3145
23. Dekter HE, Romijn FP, Temmink WP, van Pelt J, de Fijter JW, Smit NP (2010) A spectrophotometric assay for routine measurement of mammalian target of rapamycin activity in cell lysates. Anal Biochem 403:79–87
24. Fine JP, Gray RJ (1999) A proportional hazards model for the subdistribution of a competing risk. J Am Stat Assoc 94:496–509
25. Okamoto I, Doi T, Ohtsu A, Miyazaki M, Tsuya A, Kurei K, Kobayashi K, Nakagawa K (2010) Phase I clinical and pharmacokinetic study of RAD001 (everolimus) administered daily to Japanese patients with advanced solid tumors. Jpn J Clin Oncol 40:17–23
26. Okubo M, Murayama N, Shimizu M, Shimada T, Guengerich FP, Yamazaki H (2013) CYP3A4 intron 6 C > T polymorphism (CYP3A4*22) is associated with reduced CYP3A4 protein level and function in human liver microsomes. J Toxicol Sci 38:349–354
27. Falkowski S, Woillard JB (2019) Therapeutic drug monitoring of everolimus in oncology: evidences and perspectives. Ther Drug Monit 41:568–574
28. Deppenweiler M, Falkowski S, Saint-Marcoux F, Monchaud C, Picard N, Laroche ML, Tubiana-Mathieu N, Venat-Bouvet L, Marquet P, Woillard JB (2017) Towards therapeutic drug monitoring of everolimus in cancer? Results of an exploratory study of exposure-effect relationship. Pharmacol Res 121:138–144
29. Willemsen AECAB, de Geus-Oei LF, de Boer M, Tol J, Kamm Y, de Jong PC, Jonker MA, Vos AH, Grootjans W, de Groot JWB, Mulder SF, Aarntzen EHJG, Gerritsen WR, van Herpen CML, van Erp NP

(2018) Everolimus exposure and early metabolic response as predictors of treatment outcomes in breast cancer patients treated with everolimus and exemestane. Target Oncol 13:641–648

30. de Wit D, Schneider TC, Moes DJ, Roozen CF, den Hartigh J, Gelderblom H, Guchelaar HJ, van der Hoeven JJ, Links TP, Kapiteijn E, van Erp NP (2016) Everolimus pharmacokinetics and its exposure-toxicity relationship in patients with thyroid cancer. Cancer Chemother Pharmacol 78:63–71
31. Willemsen AECAB, Tol J, van Erp NP, Jonker MA, de Boer M, Meek B, de Jong PC, van Moorsel C, Gerritsen WR, Grutters JC, van Herpen CML (2019) Prospective study of drug-induced interstitial lung disease in advanced breast cancer patients receiving everolimus plus exemestane. Target Oncol 14:441–451
32. Willemsen AE, Grutters JC, Gerritsen WR, van Erp NP, van Herpen CM, Tol J (2016) mTOR inhibitor-induced interstitial lung disease in cancer patients: comprehensive review and a practical management algorithm. Int J Cancer 138:2312–2321
33. Kovarik JM, Hartmann S, Figueiredo J, Rordorf C, Golor G, Lison A, Budde K, Neumayer HH (2002) Effect of food on everolimus absorption: quantification in healthy subjects and a confirmatory screening in patients with renal transplants. Pharmacotherapy 22:154–159
34. Kung L, Halloran PF (2000) Immunophilins may limit calcineurin inhibition by cyclosporine and tacrolimus at high drug concentrations. Transplantation 70:327–335
35. Tabernero J, Rojo F, Calvo E, Burris H, Judson I, Hazell K, Martinelli E, Ramon y Cajal S, Jones S, Vidal L, Shand N, Macarulla T, Ramos FJ, Dimitrijevic S, Zoellner U, Tang P, Stumm M, Lane HA, Lebwohl D, Baselga J (2008) Dose- and schedule-dependent inhibition of the mammalian target of rapamycin pathway with everolimus: a phase I tumor pharmacodynamic study in patients with advanced solid tumors. J Clin Oncol 26:1603–1610
36. Wright KD, Yao X, London WB, Kao PC, Gore L, Hunger S, Geyer R, Cohen KJ, Allen JC, Katzenstein HM, Smith A, Boklan J, Nazemi K, Trippett T, Karajannis M, Herzog C, Destefano J, Direnzo J, Pietrantonio J, Greenspan L, Cassidy D, Schissel D, Perentesis J, Basu M, Mizuno T, Vinks AA, Prabhu SP, Chi SN, Kieran MW (2021) A POETIC Phase II study of continuous oral everolimus in recurrent, radiographically progressive pediatric low-grade glioma. Pediatr Blood Cancer 68:e28787
37. Motzer RJ, Hutson TE, Glen H, Michaelson MD, Molina A, Eisen T, Jassem J, Zolnierek J, Maroto JP, Mellado B, Melichar B, Tomasek J, Kremer A, Kim HJ, Wood K, Dutcus C, Larkin J (2015) Lenvatinib, everolimus, and the combination in patients with metastatic renal cell carcinoma: a randomised, phase 2, open-label, multicentre trial. Lancet Oncol 16:1473–1482
38. White J, Kearins O, Dodwell D, Horgan K, Hanby AM, Speirs V (2011) Male breast carcinoma: increased awareness needed. Breast Cancer Res 13:219
39. Sharma M, Duan Z, Zhao H, Giordano SH, Chavez-MacGregor M (2020) Real-world patterns of everolimus use in patients with metastatic breast cancer. Oncologist 25:937–942

Tables

Table 1 Patient characteristics

Characteristic	*N* = 22
Sex, male/female, *n* (%)	1/21 (5/95)
Age, median (range), years	67 (36–80)
Body weight, median (range), kg	56 (39–73)
ECOG performance status, *n* (%)	
0	19 (86)
1	3 (14)
Number of previous therapies, *n* (%)	
1	4 (18)
2	5 (23)
³3	12 (55)
Unknown	1 (5)
Sites of metastases, *n* (%)	
Bone	16 (73)
Lung	11 (50)
Liver	9 (41)
Brain	2 (9)
Number of metastatic sites, *n* (%)	
1	10 (45)
2	5 (23)
³3	7 (32)

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Table 2 Adverse events

Toxicity	Any grade (%)	Grade 3 (%)
Stomatitis	77	18
Leukopenia	45	0
Fatigue	23	14
Anorexia	18	18
Diarrhea	18	5
Dysgeusia	18	5
Hyperglycemia	18	5
Pneumonitis	18	5
Rash	18	0
Paronychia	14	0
AST increased	9	0
g-GTP increased	5	5
Herpes zoster	5	5
Dry mouth	5	0
Dry skin	5	0
Hand–foot syndrome	5	0
Pruritus	5	0
Urticaria	5	0

AST aspartate aminotransferase, *g-GTP* g-glutamyl transpeptidase

Figures

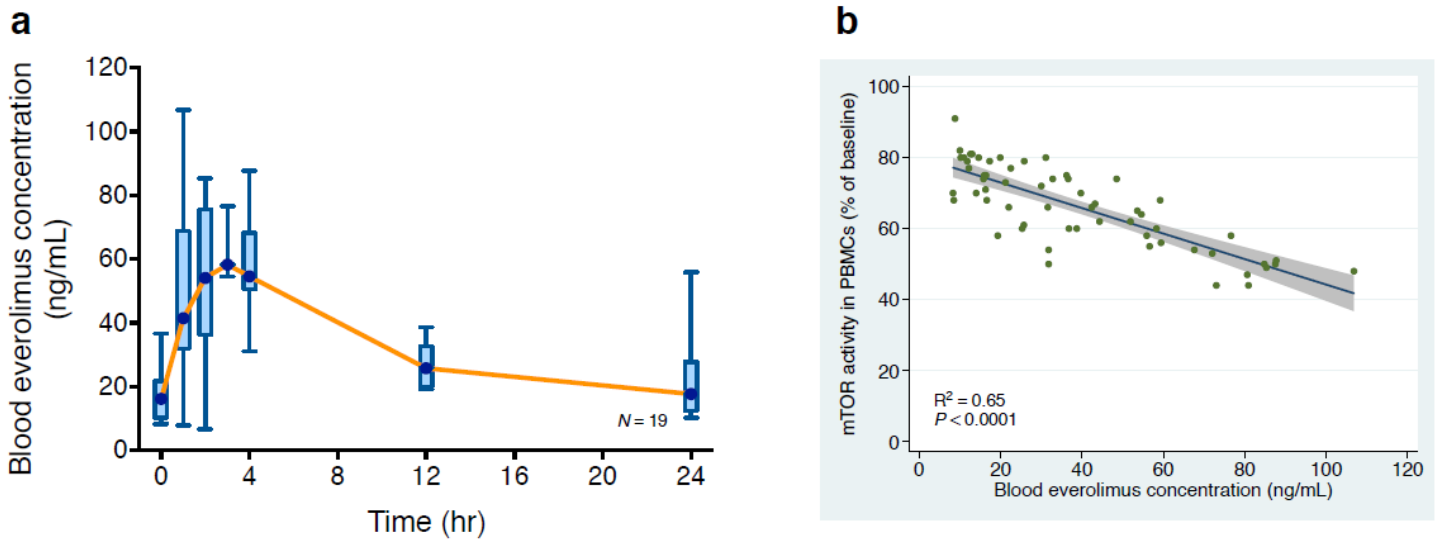

Figure 1

a Blood everolimus concentration–time profile in 19 patients. The box within the plot area indicates the median (circle) and the interquartile range of the data, with whiskers representing the minimum and maximum values. The medians are chronologically connected. b Correlation between blood everolimus concentration and the mammalian target of rapamycin (mTOR) activity in peripheral blood mononuclear cells (PBMCs). The grey bands around the regression line represent the 95% confidence intervals.

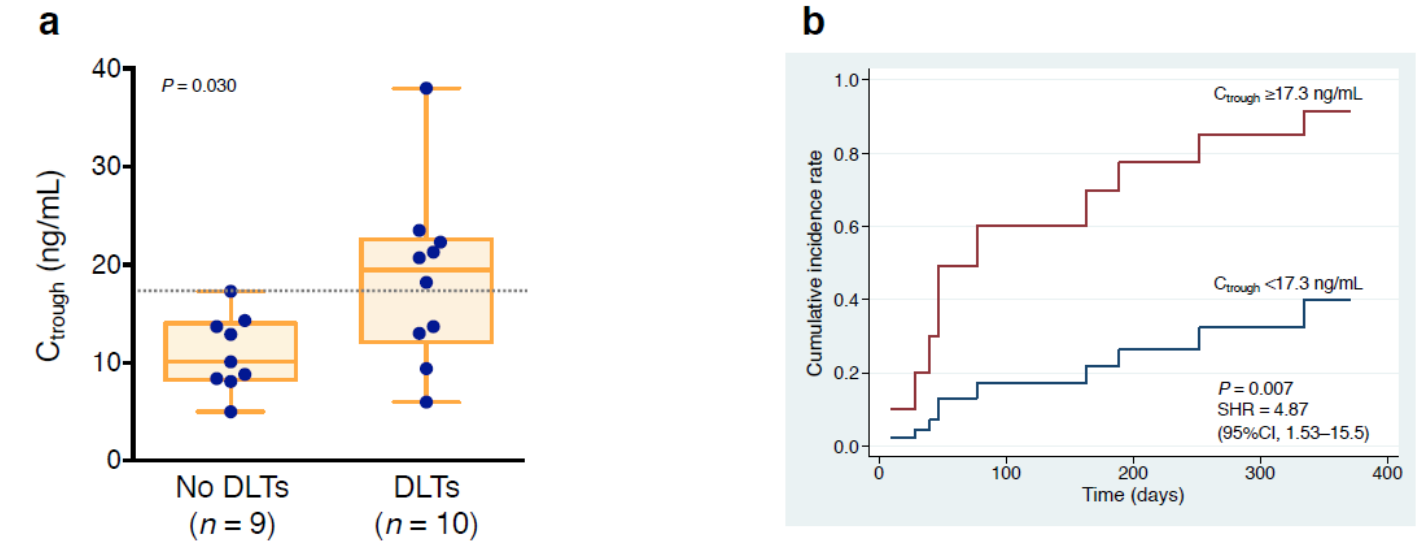

Figure 2

a Association between the average everolimus trough concentration (Ctrough) at steady state and the development of dose-limiting toxicities (DLTs). The boxes indicate the median and the interquartile range of the data; the whiskers represent the minimum and maximum values. The horizontal dotted line

indicates the optimal cutoff value (17.3 ng/mL) to predict the development of DLTs, as estimated by a receiver operating characteristic curve analysis. b Cumulative incidence of DLTs according to the average Ctrough at steady state. CI confidence interval, SHR sub-hazard ratio

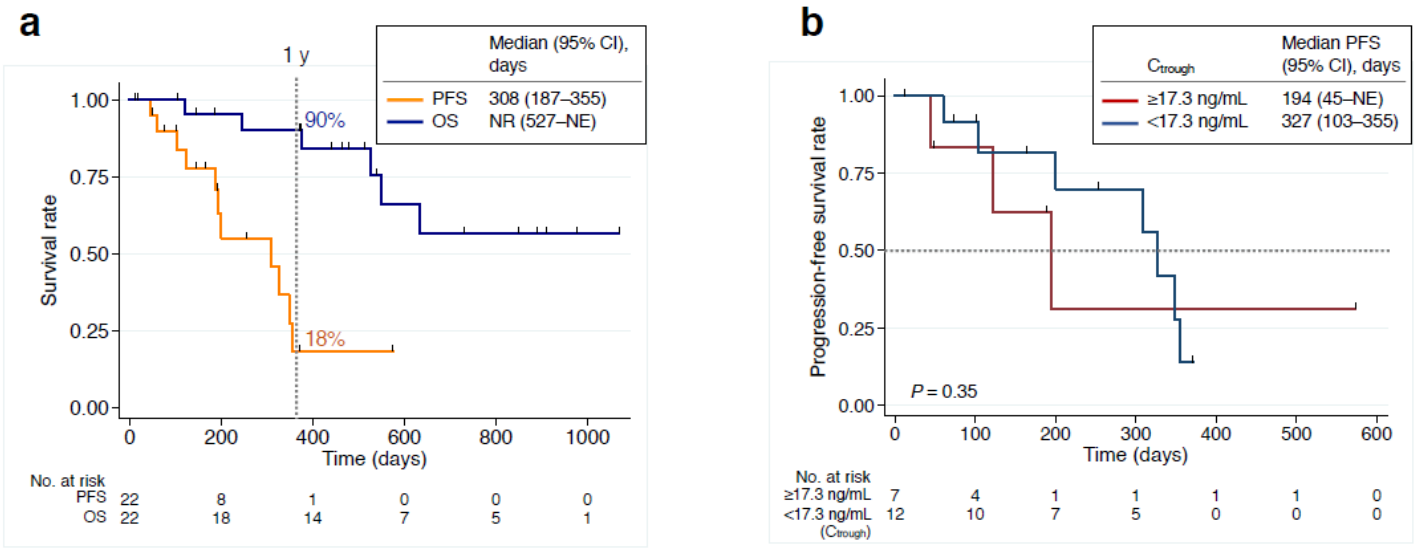

Figure 3

a Progression-free survival (PFS) and overall survival (OS). b PFS according to the average everolimus trough concentration (Ctrough) at steady state. CI confidence interval, NE not estimable, NR not reached