Cancer Chemother Pharmacol (2001) 47: 467–472
DOI 10.1007/s002800000250

ORIGINAL ARTICLE

**Matthijs E. C. van de Poll · Mary V. Relling
Erin G. Schuetz · Patricia L. Harrison · Walter Hughes
Patricia M. Flynn**

The effect of atovaquone on etoposide pharmacokinetics in children with acute lymphoblastic leukemia

Received: 14 July 2000 / Accepted: 16 November 2000 / Published online: 8 February 2001

Abstract *Purpose*: The use of trimethoprim/sulfamethoxazole in the prevention of *Pneumocystis carinii* pneumonia in patients with acute lymphoblastic leukemia (ALL) may cause undesirable adverse effects: fungal overgrowth, neutropenia, and drug resistance. A possible alternative is atovaquone, a hydroxynaphthoquinone with anti-*Pneumocystis carinii* activity. However, it is not known if atovaquone alters the disposition or adverse effects of antileukemic drugs. *Methods*: Using a crossover study design, we compared the pharmacokinetics of etoposide and its CYP3A4-formed catechol metabolite when given as a 300 mg/m^2 i.v. infusion following daily atovaquone versus trimethoprim/sulfamethoxazole in nine patients. *Results*: The area under the concentration time curve (AUC) of etoposide, etoposide catechol and the catechol to etoposide AUC ratio were slightly higher (a median of 8.6%, 28.4%, and 25.9%) following atovaquone as compared to trimethoprim/sulfamethoxazole ($P = 0.055$, $P = 0.031$ and $P = 0.023$), respectively. In vitro analysis in human liver microsomes showed modest inhibition of etoposide catechol formation in the presence of atovaquone. Using uptake of ^{3}H-vinblastine in L-MDR1 cells, atovaquone was shown to inhibit P-glycoprotein with an apparent Ki of 95.6 μ*M*. *Conclusions*: Although the effect of atovaquone on etoposide disposition was modest, in light of the fact that the risk of etoposide-related secondary acute myeloid leukemia has been linked to minor changes in schedule and concurrent therapy, we suggest caution with the simultaneous administration of atovaquone and etoposide, particularly if used with other CYP3A4/P-glycoprotein substrates.

Keywords Etoposide · Atovaquone · CYP3A4 · P-glycoprotein · Cotrimoxazole

M. E. C. van de Poll · M. V. Relling (✉) · E. G. Schuetz
W. Hughes
Department of Pharmaceutical Sciences,
St. Jude Children's Research Hospital,
332 North Lauderdale. Memphis,
TN 38105, USA
E-mail: mary.relling@stjude.org
Tel.: +1-901-4953663
Fax: +1-901-5256869

P. L. Harrison
Department of Biostatistics,
St. Jude Children's Research Hospital,
Memphis, TN, USA

W. Hughes · P. M. Flynn
Department of Infectious Diseases,
St. Jude Children's Research Hospital,
Memphis, TN, USA

M. E. C. van de Poll · M. V. Relling · E. G. Schuetz
College of Pharmacy, The University of Tennessee,
Memphis, TN, USA

P. M. Flynn
College of Medicine, The University of Tennessee,
Memphis, TN, USA

Introduction

One of the infections seen in chronically immunocompromised children is *Pneumocystis carinii* pneumonia (PCP). The standard agent for PCP prophylaxis in patients with acute lymphoblastic leukemia (ALL) is trimethoprim/sulfamethoxazole [12]. Trimethoprim/sulfamethoxazole prophylaxis, given daily or even 3 days per week, reduces the attack rate of about 20% to less than 1% [11]. However, adverse effects associated with trimethoprim/sulfamethoxazole, especially neutropenia and rash, hamper the use of the drug in some patients. The broad antibacterial activity of trimethoprim/sulfamethoxazole affects the ecology of the microbial flora, resulting in fungal overgrowth which may increase the risk of local and systemic fungal infections [11, 12], and could theoretically contribute to the development of antibiotic-resistant bacterial infections.

Because of these and other possible unintended effects of trimethoprim/sulfamethoxazole, a drug with equal efficacy and fewer adverse effects is desirable. Therapeutic and prophylactic efficacy of atovaquone

have been documented for PCP [3, 8]. It has no antibacterial activity to disrupt the normal microbial flora, no suppressive effect on hematopoiesis, and a low rate of adverse effects [12]. Thus, atovaquone is a potential candidate to replace trimethoprim/sulfamethoxazole as PCP prophylaxis in children with ALL. However, the potential interaction of atovaquone with antileukemic agents has not been previously studied.

Atovaquone is structurally related to naphthoquinones which have been shown to inhibit microsomal cytochrome P450 (CYP) activities [7, 31]. There are limited data indicating modest interactions between atovaquone and CYP substrates [17]. Several anticancer drugs which are commonly used in front-line ALL continuation therapy, such as etoposide, vincristine and probably dexamethasone and prednisone, are substrates for CYP3A4 as well as P-glycoprotein [20, 21, 22, 28]. Drug interactions with etoposide could occur if atovaquone were a P-glycoprotein substrate or inhibitor. To some extent etoposide can serve as an indicator CYP3A/P-glycoprotein substrates, with lack of interaction with etoposide being indicative of the likely lack of interaction with other CYP3A substrates. Therefore the objective of this pilot study was to determine whether or not atovaquone affects etoposide pharmacokinetics.

Methods

Patients and drug administration

Children with newly diagnosed ALL or non-Hodgkin lymphoma (NHL) were enrolled on this crossover study at St. Jude Children's Research Hospital. The study was approved by the institutional review board and informed consent was obtained from the parent or guardian and (when appropriate) the patients. The study was conducted when patients were about 1 year into continuation therapy on one of two front-line treatment protocols, Total Therapy Study XIIIB or NHL-13, both of which included identical continuation therapy. Etoposide was administered, concurrently with cytarabine, as a 300 mg/m^2 2-h intravenous infusion during weeks 46 and 54. For the week preceding etoposide administration, chemotherapy consisted of daily 6-mercaptopurine and one low dose (40 mg/m^2) of methotrexate. Patients were randomized to receive oral trimethoprim/sulfamethoxazole 150/750 mg/m^2 per day in two divided daily doses or daily atovaquone 45 mg/kg per day in two divided daily doses during week 45. In week 53, patients were crossed over to the alternate PCP prophylaxis drug regimen (Fig. 1). Atovaquone was given as the micronized suspension and trimethoprim/sulfamethoxazole as a liquid suspension. During weeks 46 and 54, blood (3 ml) was obtained in heparinized tubes containing ascorbic acid before and at 2, 3, 4.5, 6 and 24 h after the dose of etoposide. The plasma was stored at –70ºC until the samples were analyzed in duplicate by HPLC. Both courses for a patient were assayed in the same run, and the intra-assay coefficient of variation for etoposide and etoposide catechol were $<0.7\%$ and 2.1%, respectively [2].

Pharmacokinetic analysis

A first-order two-compartment structural model was fitted to the data using a Bayesian estimation algorithm as implemented in ADAPTII [5]. The prior parameter estimates (variances) were: V_c 9.69 l/m^2 (17%), k_{cp} 0.032 h^{-1} (63%), k_{pc} 0.038 h^{-1} (22%), $k_{vp\text{-}cat}$ 0.019 h^{-1} (70%), $k_{e,cat}$ 1.20 h^{-1} (41%) and $k_{e,total}$ 0.325 h^{-1} (34%). Volumes and intercompartmental rate constants for etoposide and catechol were assumed to be equivalent [23]. The area under the plasma concentration time curve ($AUC_{0-\infty}$) was estimated for etoposide and etoposide catechol from simulated concentrations using each individual set of estimated pharmacokinetic parameters. Clearance was estimated as dose/$AUC_{0-\infty}$.

Statistical analysis

The exact Wilcoxon signed ranks test was used to analyze differences in catechol, etoposide, and catechol over etoposide AUCs following atovaquone versus trimethoprim/sulfamethoxazole. The exact Mann-Whitney test was used to analyze the effect of order of administration of PCP prophylaxis treatment. All analyses were conducted using StatXact-4 Version 4.0.1.

In vitro studies with microsomes and P-glycoprotein

The effect of atovaquone on human microsomal metabolism of etoposide was studied using techniques previously described [1, 22]. Human livers were obtained from the Liver Tissue Procurement and Distribution System (Minneapolis, Minn.) and the Cooperative Human Tissue Network (Birmingham, Ala.). Etoposide 25 μ*M* (75H0402; Sigma Chemicals, St. Louis, Mo.) with or without atovaquone (GR151218X; Glaxo Wellcome, Research Triangle Park, N.C.) dissolved in methanol was added to each tube, and methanol was removed by evaporation under nitrogen. Buffer (0.1 M KPO_4, pH 7.4) and 0.2 mg human liver microsomes were added and allowed to mix at room temperature for 5 min. Final atovaquone concentrations were 0, 200, 500, 1000 μ*M* in a 250 μl incubation. Reactions progressed at 37ºC for 30 min with an NADPH regenerating system. Preliminary experiments failed to demonstrate catechol formation in the absence of NADPH or analytical interferences from atovaquone in the presence of NADPH. After incubation, the samples were extracted and analyzed in duplicate by HPLC for etoposide catechol [2].

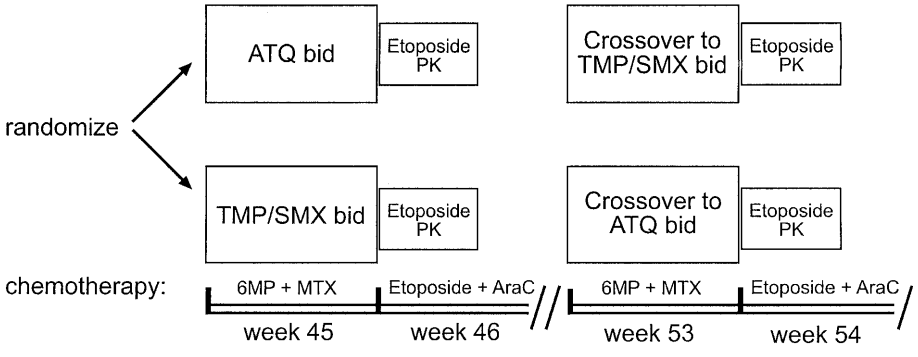

Fig. 1 Schema of study design. Etoposide pharmacokinetics (*PK*) were studied following daily atovaquone (*ATQ*) or after daily trimethoprim/sulfamethoxazole (*TMP/SMX*) in a randomized crossover study

P-glycoprotein substrate specificity

Atovaquone interactions with P-glycoprotein were determined as previously described [15] by measuring the effect of atovaquone concentration (0–200 μM) on the uptake of 0.5 μM ^{3}H-vinblastine into L-MDR1 cells [24].

Results

A total of 15 patients were enrolled on the study, but etoposide pharmacokinetics were evaluable after both atovaquone and trimethoprim/sulfamethoxazole in nine patients: seven on TXIIIB and two on NHL-13. The reasons for inevaluability were: inability to administer etoposide on schedule due to the myelosuppression of prior chemotherapy ($n=3$), failure to obtain blood samples ($n=2$), and noncompliance with PCP medications after enrollment ($n=1$). The median age (range) of seven male and two female patients was 7.0 years (3.1–16.7 years).

The median (range) systemic clearance of etoposide after atovaquone and trimethoprim/sulfamethoxazole was 35.4 ml/min per m^2 (21.8–44.7 ml/min per m^2) and 37.6 ml/min per m^2 (21.8–44.7 ml/min per m^2), respectively. The percentage changes in etoposide AUC, etoposide catechol AUC, and the catechol/etoposide AUC ratio after atovaquone compared to after trimethoprim/sulfamethoxazole are shown in Table 1. Etoposide AUC ($P=0.055$), etoposide catechol AUC ($P=0.031$), and catechol/etoposide AUC ratio ($P=0.023$) were increased following atovaquone administration (Fig. 2).

The order of administration of atovaquone and trimethoprim/sulfamethoxazole affected the interaction with etoposide. The exact Mann-Whitney test indicated that the differences between the etoposide AUCs following atovaquone and those following trimethoprim/sulfamethoxazole for patients who received atovaquone first were greater than those of patients who received trimethoprim/sulfamethoxazole first (one-sided $P=0.016$), but overall, etoposide parameters did differ in those receiving atovaquone.

Etoposide catechol formation was inhibited by atovaquone (Fig. 3) in microsomes, with IC_{50} values of 256,

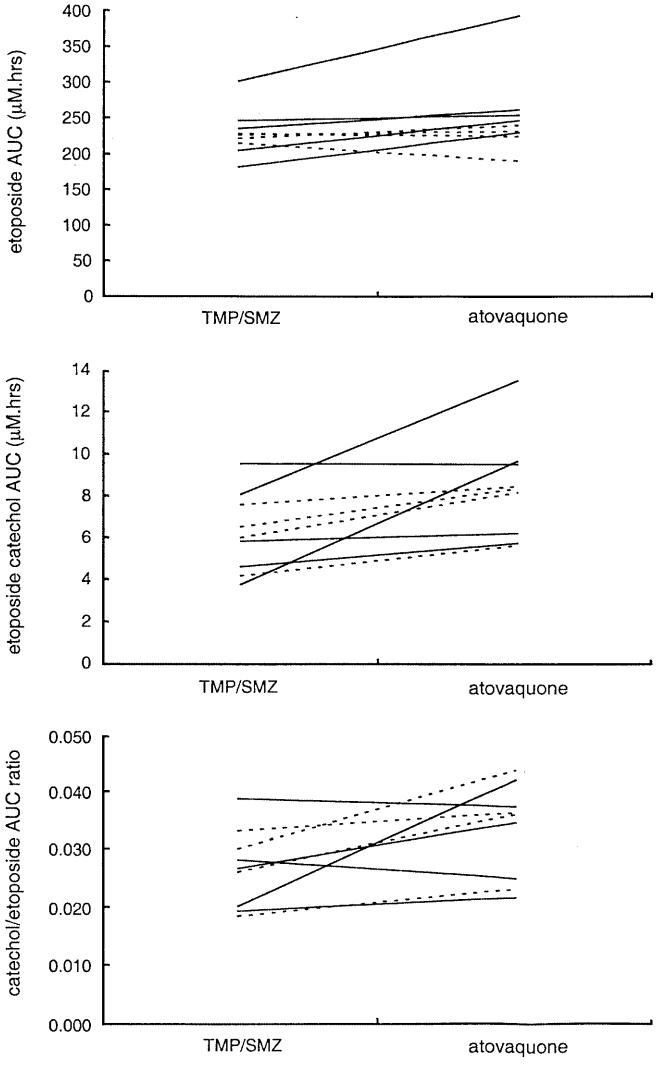

Fig. 2a–c Paired plots of (**a**) etoposide AUC, (**b**) etoposide catechol AUC, and (**c**) etoposide catechol/etoposide AUC ratio following trimethoprim/sulfamethoxazole and atovaquone administration. *Dotted lines* represent patients who received trimethoprim/sulfamethoxazole first; *solid lines* represent patients who received atovaquone first

Table 1 Change in plasma AUCs following atovaquone versus trimethoprim/sulfamethoxazole calculated as (value following ATQ–value following TMP-SMX)/value following TPM-SMX × 100 (*ATQ* atovaquone, *TMP-SMX* trimethoprim/sulfamethoxazole)

Patient no.	Order of administration		Percent change		
	Week 46	Week 54	Etoposide AUC	Catechol AUC	AUC ratio
1	TMP-SMX	ATQ	2.3	11.8	9.1
2	TMP-SMX	ATQ	–11.6	28.4	46.7
3	TMP-SMX	ATQ	–0.9	36.8	38.5
4	TMP-SMX	ATQ	8.6	35.8	27.8
5	ATQ	TMP-SMX	11.6	24.6	15.8
6	ATQ	TMP-SMX	25.8	162.5	110.0
7	ATQ	TMP-SMX	20.6	6.7	–10.7
8	ATQ	TMP-SMX	3.7	–0.3	–5.1
9	ATQ	TMP-SMX	30.3	68.4	25.9
Median			8.6	28.4	25.9

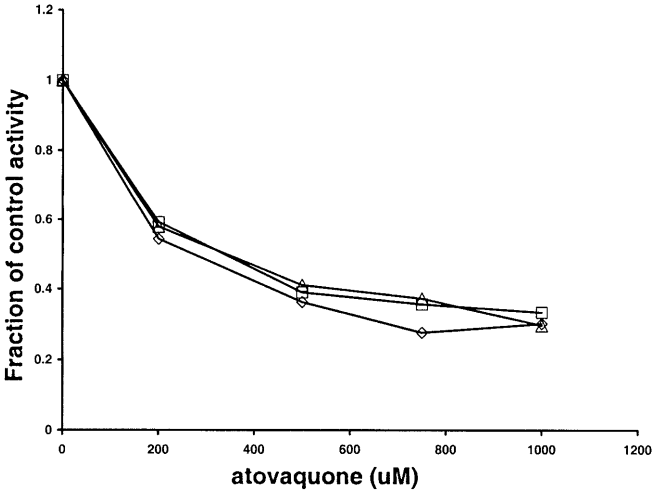

Fig. 3 Etoposide catechol formation expressed as fractions of control with various atovaquone concentrations in three different human liver microsomal preparations

309, and 324 μ*M*, respectively, in three different human hepatic microsomal preparations. The estimated K_i for reversal of ^{3}H-vinblastine accumulation into L-MDR1 cells was 95.6 μ*M*, which is generally higher than the K_i values that have been observed with known P-glycoprotein inhibitors (e.g. 0.15 μ*M* for cyclosporin and 2.4 μ*M* for verapamil, respectively) [15].

Discussion

We observed a modest but statistically significant higher etoposide and etoposide catechol exposure after daily atovaquone than after daily trimethoprim/sulfamethoxazole exposure. Scheduling these drugs in treatment protocols is of particular importance, because modest alterations in etoposide-containing therapy may alter the risk of etoposide-related secondary acute myeloid leukemia [18, 19, 23, 27, 32]. Hence, the systematic addition of possibly interacting drugs to existing etoposide regimens should be undertaken with caution. Although the median increased etoposide exposure (8.6%) following atovaquone was modest relative to the intrapatient coefficient of variation in etoposide exposure (12%) that has been reported in a small number of patients [16], it occurred in seven of nine patients, was more pronounced in a few patients (Fig. 2), and the increased exposure to catechol AUC was somewhat greater (median 28.4%) and occurred in eight of nine patients. Moreover, because additional CYP3A4/P-glycoprotein substrates are often given to patients with cancer, the potential for multiple competitive drug interactions must be considered. Thus, the effect of this dose of atovaquone was sufficient to dictate that caution be exercised before instituting its chronic introduction into etoposide-containing regimens.

The mechanisms underlying the drug interaction are unclear. In vitro studies have shown inhibition of catechol formation in human liver microsomes in the presence of atovaquone, with IC_{50} values in the 250–325 μ*M* range at etoposide concentrations of 25 μ*M*. Both drugs are highly plasma protein bound [9]. Peak etoposide plasma concentrations following a 300 mg/m^2 i.v. dose would routinely exceed 25 μ*M*, but concentrations as low as 1 μ*M* are associated with cytotoxicity [13]. Because atovaquone plasma concentrations at steady-state after administration of 45 mg/kg per day are expected to produce average concentrations of about 40 to 50 μ*M*, concentrations below those that were inhibitory in vitro, it is unclear whether inhibition of etoposide *O*-demethylation would be likely to occur from in vivo administration of atovaquone. What we observed in vivo was that both etoposide and catechol plasma concentrations were higher in the presence of atovaquone. The former observation was expected from the microsomal studies, but the latter observation is not explained by P450 inhibition. The reason for the higher catechol AUC is unclear.

One explanation for the drug interaction could be that atovaquone is a P-glycoprotein substrate. Such a substrate could block etoposide transport by P-glycoprotein [6, 25]. This could result in decreased biliary excretion, and thereby elevate etoposide and possibly also etoposide catechol AUCs. However, we did not find that atovaquone was a potent inhibitor of P-glycoprotein. Its Ki (95.6 μ*M*) was above the maximum plasma concentrations generally achievable clinically (40–50 μ*M*), indicating that plasma atovaquone is unlikely to affect the MDR1 systemically. In this study, etoposide was given intravenously and atovaquone was given orally. Thus, if intestinal secretion of etoposide were affected by gut P-glycoprotein (as has been found for vinblastine [29] and paclitaxel [26]), atovaquone in the gastrointestinal tract could inhibit etoposide secretion and thereby affect systemic pharmacokinetics. In addition, etoposide is also a substrate for other drug transporters such as MRP3 [14] and MRP1, and it is possible that atovaquone interacts with one or more of these transporters.

Of course, it is possible that trimethoprim/sulfamethoxazole caused etoposide and catechol concentrations to be lower than expected compared to no PCP prophylaxis and that atovaquone had no effect relative to placebo. Because we administer PCP prophylaxis to all children with leukemia, testing etoposide pharmacokinetics in the presence of placebo was not an option. In vitro studies have shown that CYP2C9 is responsible for the formation of the N_4-hydroxy metabolite of sulfamethoxazole [4]. Despite widespread use of sulfamethoxazole/trimethoprim, few clinically significant inhibitory interactions have been demonstrated with its use.

In conclusion, atovaquone administered at a dose of 45 mg/kg per day did modestly increase the etoposide catechol exposure in vivo. Because the interaction was minor, it is possible that by separating the coadministration of these agents by 1–2 days, an interaction could be completely avoided, as has been attempted with

atovaquone and azithromycin [17]. Whatever the mechanism of the interaction, because the *O*-demethylated metabolites of etoposide stabilize topoisomerase II-mediated DNA cleavage with a potency similar to that of the parent drug [10], have shown antiproliferative effects in some preclinical laboratory models [30], and have been suggested to contribute to etoposide's leukemogenesis, it would be prudent to avoid simultaneous administration of atovaquone and etoposide.

Acknowledgements We thank research nurse Ms. Diane Brand, and Mr. Ken Cox, Kazuto Yasuda and Dr. Lu-Bin Lan for technical assistance. This project was funded in part with federal funds from the National Institutes of Health, Bethesda, Md., Cancer Center CORE Grant nos. 21765, CA51001, ES 08658, and N01-DK-92310, a Center of Excellence Grant from the State of Tennessee, and American Lebanese Syrian Associated Charities (ALSAC).

References

1. Blanco JG, Harrison PL, Evans WE, Relling MV (2000) Human cytochrome P450 maximal activities in pediatric versus adult liver. Drug Metab Dispos 28:379
2. Cai X, Woo MH, Edick MJ, Relling MV (1999) Simultaneous quantitation of etoposide and its catechol metabolite in human plasma using high-performance liquid chromatography with electrochemical detection. J Chromatogr B Biomed Sci Appl 728:241
3. Chan C, Montaner J, Lefebvre EA, Morey G, Dohn M, McIvor RA, Scott J, Marina R, Caldwell P (1999) Atovaquone suspension compared with aerosolized pentamidine for prevention of *Pneumocystis carinii* pneumonia in human immunodeficiency virus-infected subjects intolerant of trimethoprim or sulfonamides. J Infect Dis 180:369
4. Cribb AE, Spielberg SP, Griffin GP (1995) N4-hydroxylation of sulfamethoxazole by cytochrome P450 of the cytochrome P4502C subfamily and reduction of sulfamethoxazole hydroxylamine in human and rat hepatic microsomes. Drug Metab Dispos 23:406
5. D'Argenio DZ, Schumitzky A (1979) A program package for simulation and parameter estimation in pharmacokinetics systems. Comput Programs Biomed 9:115
6. Deuchars KL, Ling V (1989) P-glycoprotein and multidrug resistance in cancer chemotherapy. Semin Oncol 16:156
7. Dubin M, Villamil SHF, Stoppani AOM (1990) Inhibition of microsomal lipid peroxidation and cytochrome P450 catalyzed reactions by B-lapachone and related naphthoquinones. Biochem Pharmacol 39:1151
8. El-Sadr WM, Murphy RL, Yurik TM, Luskin-Hawk R, Cheung TW, Balfour HHJ, Eng R, Hooton TM, Kerkering TM, Schutz M, van der Horst C, Hafner R (1998) Atovaquone compared with dapsone for the prevention of *Pneumocystis carinii* pneumonia in patients with HIV infection who cannot tolerate trimethoprim, sulfonamides, or both. Community Program for Clinical Research on AIDS and the AIDS Clinical Trials Group. N Engl J Med 339:1889
9. Fleming RA, Evans WE, Arbuck SG, Stewart CF (1992) Factors affecting in vitro protein binding of etoposide in humans. J Pharm Sci 81:259
10. Gantchev TG, Hunting DJ (1998) The ortho-quinone metabolite of the anticancer drug etoposide (VP-16) is a potent inhibitor of the topoisomerase II/DNA cleavable complex. Mol Pharmacol 53:422
11. Hughes WT, Rivera GK, Schell MJ, Thornton D, Lott L (1987) Successful intermittent chemoprophylaxis for *Pneumocystis carinii* pneumonitis. N Engl J Med 316:1627
12. Hughes W, Leoung G, Kramer F, Bozzette SA, Safrin S, Frame P, Clumeck N, Masur H, Lancaster D, Chan C (1993) Comparison of atovaquone (566C80) with trimethoprim-sulfamethoxazole to treat *Pneumocystis carinii* pneumonia in patients with AIDS. N Engl J Med 328:1521
13. Jensen PB, Roed H, Skovsgaard T, Friche E, Vindelov L, Hansen HH, Spang-Thomsen M (1990) Antitumor activity of the two epipodophyllotoxin derivatives VP-16 and VM-26 in preclinical systems: a comparison of in vitro and in vivo drug evaluation. Cancer Chemother Pharmacol 27:194
14. Kool M, van der LM, de Haas M, Scheffer GL, de Vree JM, Smith AJ, Jansen G, Peters GJ, Ponne N, Scheper RJ, Elferink RP, Baas F, Borst P (1999) MRP3, an organic anion transporter able to transport anti-cancer drugs. Proc Natl Acad Sci USA 96:6914
15. Lan LB, Ayesh S, Lyubimov E, Pashinsky I, Stein WD (1996) Kinetic parameters for reversal of the multidrug pump as measured for drug accumulation and cell killing. Cancer Chemother Pharmacol 38:181
16. Lowis SP, Pearson ADJ, Newell DR, Cole M (1993) Etoposide pharmacokinetics in children: the development and prospective validation of a dosing equation. Cancer Res 53:4881
17. Ngo LY, Yogev R, Dankner WM, Hughes WT, Burchett S, Xu K, Sadler B, Unadkat JD (1999) Pharmacokinetics of azithromycin administered alone and with atovaquone in human immunodeficiency virus-infected children. The ACTG 254 Team. Antimicrob Agents Chemother 43:1516
18. Pedersen-Bjergaard J, Philip P, Larsen SO, Andersson M, Daugaard G, Ersboll J, Hansen SW, Hou-Jensen K, Nielsen D, Sigsgaard TC, Specht L, Osterlind K (1993) Therapy-related myelodysplasia and acute myeloid leukemia. Cytogenetic characteristics of 115 consecutive cases and risk in seven cohorts of patients treated intensively for malignant diseases in the Copenhagen series. Leukemia 7:1975
19. Pui C-H, Ribeiro R, Hancock ML, Rivera GK, Evans WE, Raimondi S, Head DR, Behm FG, Mahmoud MH, Sandlund JT, Crist W (1991) Acute myeloid leukemia in children treated with epipodophyllotoxins for acute lymphocytic leukemia. N Engl J Med 325:1682
20. Raderer M, Scheithauer W (1993) Clinical trials of agents that reverse multidrug resistance. A literature review. Cancer 72:3553
21. Relling MV (1996) Are the major effects of P-glycoprotein modulators due to altered pharmacokinetics of anticancer drugs? Ther Drug Monit 18:350
22. Relling MV, Nemec J, Schuetz EG, Schuetz JD, Gonzalez FJ, Korzekwa KR (1994) *O*-Demethylation of epipodophyllotoxins is catalyzed by human cytochrome P450 3A4. Mol Pharmacol 45:352
23. Relling MV, Yanishevski Y, Nemec J, Evans WE, Boyett JM, Behm FG, Pui CH (1998) Etoposide and antimetabolite pharmacology in patients who develop secondary acute myeloid leukemia. Leukemia 12:346
24. Schinkel AH, Wagenaar E, van Deemter L, Mol CA, Borst P (1995) Absence of the mdr1a P-glycoprotein in mice affects tissue distribution and pharmacokinetics of dexamethasone, digoxin, and cyclosporin A. J Clin Invest 96:1698
25. Smit JW, Schinkel AH, Muller M, Weert B, Meijer DK (1998) Contribution of the murine mdr1a P-glycoprotein to hepatobiliary and intestinal elimination of cationic drugs as measured in mice with an mdr1a gene disruption. Hepatology 27:1056
26. Sparreboom A, van Asperen J, Mayer U, Schinkel AH, Smit JW, Meijer DK, Borst P, Nooijen WJ, Beijnen JH, van Tellingen O (1997) Limited oral bioavailability and active epithelial excretion of paclitaxel (Taxol) caused by P-glycoprotein in the intestine. Proc Natl Acad Sci USA 94:2031
27. Sugita K, Furukawa T, Tsuchida M, Okawa Y, Nakazawa S, Akatsuka J, Ohira M, Nishimura K (1993) High frequency of etoposide-related secondary leukemia in children with non-hodgkin's lymphoma. Am J Pediatr Hematol Oncol 15:99

28. Tiberghien F, Loor F (1996) Ranking of P-glycoprotein substrates and inhibitors by a calcein-AM fluorometry screening assay. Anticancer Drugs 7:568
29. van Asperen J, Tellingen OV, Beijnen JH (2000) The role of MDR1A-p-glycoprotein in the biliary and intestinal secretion of doxorubicin and vinblastine in mice. Drug Metab Dispos 28:264
30. van Maanen JMS, DeVries J, Pappie D, et al (1987) Cytochrome P450-mediated *O*-demethylation: a route in the metabolic activation of etoposide. Cancer Res 47:4658
31. Weaver RJ, Dickins M, Burke MD (1993) Cytochrome P450 2C9 is responsible for hydroxylation of the naphthoquinone antimalarial drug 58C80 in human liver. Biochem Pharmacol 46:1183
32. Winick NJ, McKenna RW, Shuster JJ, Schneider NR, Borowitz MJ, Bowman WP, Jacaruso D, Kamen BA, Buchanan GR (1993) Secondary acute myeloid leukemia in children with acute lymphoblastic leukemia treated with etoposide. J Clin Oncol 11:209