RESEARCH PAPER

Pharmacokinetic and Pharmacogenetic Study of Etoposide in High-Dose Protocol (TI-CE) for Advanced Germ Cell Tumors

Sotheara Moeung[1,2] • Christine Chevreau[1] • Sabrina Marsili[1,2] • Christophe Massart[3] • Aude Fléchon[4] • Rémy Delva[5] • Gwenaëlle Gravis[6] • Jean-Pierre Lotz[7] • Jacques-Olivier Bay[8] • Marine Gross-Goupil[9] • Thomas Filleron[1] • Caroline Delmas[1,2] • Thierry Lafont[1,2] • Etienne Chatelut[1,2] (iD) • Fabienne Thomas[1,2]

ABSTRACT

BACKGROUND Etoposide dosing is based on body surface area. We evaluated if further dose individualization would be required for high dose (HD) etoposide within the TI-CE (taxol, ifosfamide, carboplatin, and etoposide) protocol.

METHODS Eighty-eight patients received 400 mg/m^2/day of etoposide as a 1-hour IV infusion on 3 consecutive days over 3 cycles as part of a phase II trial evaluating efficacy of therapeutic drug monitoring (TDM) of carboplatin in the TI-CE HD protocol. Pharmacokinetic (PK) data were analyzed using population PK model on NONMEM to quantify inter- and intra-individual variabilities. Relationship between etoposide exposure and pharmacodynamic (PD) endpoints, and between selected genetic polymorphisms and tumor response or toxicity were evaluated.

RESULTS The inter-patient, inter- and intra-cycle variabilities of clearance were 16%, 9% and 0.1%, respectively. The PK-PD relationship was not significant despite a trend toward higher etoposide exposure in patients responding to treatment. A significant correlation was found between exposure and extended neutropenia at cycle 3. A significant association between UGT1A1*28 polymorphism and late neutropenia was observed but needs further evaluation.

CONCLUSIONS The present study suggests that neither *a priori* dose individualization nor dose adaptation using TDM is required validating body surface area dosing of etoposide in the TI-CE protocol.

KEY WORDS etoposide · germ cell tumors · high-dose regimen · pharmacogenetics · pharmacokinetics

BACKGROUND

Etoposide is considered as an ideal agent for high-dose (HD) protocol due to its wide range of clinical antitumor activity at standard doses, steep dose-response curve, and few non-myeloid side effects (1). Since the late 1980s, HD etoposide with autologous bone marrow transplantation has become a component of several HD therapies (2–7) including the TI-CE protocol for advanced germ cell tumors (8).

Up to the present, the conventional dosing method based on body surface area (BSA) is used to calculate the dose for etoposide despite a minimal or no correlation, reported in several studies (9–11), between this covariate and its pharmacokinetic (PK) parameters. Furthermore, a substantial interindividual PK variability (coefficient of variation between 25% and 32% for etoposide clearance) has been observed with etoposide administered at regular doses (12–14). Several studies have therefore attempted to define *a priori* dosage adjustments based on renal function (13,15) or to further perform a PK-guided therapeutic drug monitoring (TDM) (16) for individualizing etoposide doses in those standard-dose regimens.

The TI-CE protocol corresponds to 2 cycles of paclitaxel (T) and ifosfamide (I) followed by 3 cycles of HD carboplatin (C) and etoposide (E). In this HD protocol, etoposide is given at the dose of 400 mg/m^2/day during 3 consecutive days. We have recently demonstrated, in a phase II trial, that Bayesian-

✉ Etienne Chatelut
chatelut.etienne@iuct-oncopole.fr

[1] Institut Claudius-Regaud, IUCT-Oncopole, Toulouse, France

[2] CRCT, Université de Toulouse, Inserm, Toulouse, France

[3] Oncologie Médicale, Institut Gustave Roussy, Villejuif, France

[4] Centre Léon Bérard, Lyon, France

[5] Institut De Cancérologie de l'Ouest Paul Papin, Angers, France

[6] Institut Paoli Calmettes, Marseille, France

[7] Hôpital Tenon, Paris, France

[8] CHU, Clermont Ferrand, France

[9] Hôpital Saint André, Bordeaux, France

based TDM of carboplatin in this protocol allowed an accurate control of inter-patient variability of its exposure (17). In the setting of any high-dose chemotherapy, a good control of the exposure to anticancer drug is required to avoid unacceptable toxicity or underexposure of patients, which may hamper treatment benefit.

Therefore, the objective of this ancillary pharmacokinetic study within this phase II TI-CE trial (17) was to quantify the etoposide PK inter- and intra-individual variability in order to evaluate if further dose individualization (*a priori* method or PK-guided TDM) of this drug would be required for this HD protocol. Furthermore, association between genetic variation of certain genes and treatment response or toxicity of etoposide was also investigated. This evaluation of the clinical pharmacology of etoposide is of best interest since all patients had roughly the same carboplatin exposure (i.e., overall AUC of 24 mg.min/mL for each cycle, or only 18 mg.min/mL in case of major toxicity during the previous cycle).

MATERIALS AND METHODS

Patients and treatment

Eighty-nine patients received etoposide as part of a phase II, multicenter trial (8 centers in France) evaluating efficacy of TDM of carboplatin in advanced germ cell tumor patients receiving the TI-CE HD protocol (ClinicalTrials.gov reference number NCT00864318). However, only eighty-eight patients were evaluable for the present analysis due to

unavailable etoposide concentration data in one patient. The baseline characteristics of the patients are shown in Table I. Among those 88 patients, 17 did not undergo the 3 planned cycles (i.e., they received only one or two cycles) because of unacceptable toxicity (6 patients), tumor progression (4 patients), death (3 patients) or other causes (4 patients).

Etoposide administration, blood sampling, drug assay

Etoposide was given in combination with carboplatin during the dose intensification phase at the dose of 400 mg/m^2/day for 3 consecutive days over 3 cycles with peripheral blood stem cell (PBSC) support. On each day, etoposide was administered as a 1-hour IV infusion in 1 L of saline solution immediately after the end of carboplatin's 1-hour infusion. In one center, the duration of the infusion was set at 4 hours according to a protocol specific to this site (n=10 patients). For each administration, three blood samples were collected before the start of infusion of etoposide, at the end of the infusion and at 3 hours after the end of infusion. These three sampling times correspond to the samples taken for the carboplatin PK analysis (17) in order to avoid multiple blood sampling in patients and were consistent to a previously published methodology (18). Plasma levels of etoposide were measured by reverse-phase high-performance liquid chromatography (HPLC) using teniposide as the internal standard after organic extraction according to a previously reported method (19) with ultraviolet detection at 229 nm. Intra- and inter-assay coefficients of variation were lower than 20%.

Table I Baseline Covariate Values of All the Patients and of the Patients used for Covariate Analysis

Covariates	All patients		Patients (n= 74) for covariate analysis*
	Mean (range)	n	Mean (range)
Age (years)	35 (20 - 56)	88	35 (20 - 56)
Body weight (kg)	75 (45 - 150)	88	76 (49 - 150)
Body surface area (m^2)	1.9 (1.5 - 2.9)	88	1.9 (1.5 - 2.9)
Serum creatinine (μM)	81 (46 - 180)	88	83 (46 - 180)
Ratio Body weight/Serum creatinine	1.0 (0.4 – 2.0)	88	1.0 (0.4 – 2.0)
Serum cystatin C (mg/L)	0.9 (0.5 - 1.6)	86	0.9 (0.5 - 1.6)
Aspartate transaminase (UI/L)	25 (6 - 155)	80	24 (6 - 155)
Alanine transaminase (UI/L)	38 (6 - 143)	80	38 (6 - 143)
Alkaline phosphatase (UI/L)	97 (40 - 282)	80	98 (40 - 282)
Total bilirubin (μM)	7 (1 - 46)	80	7 (1 - 29)
Lactate dehydrogenase (UI/L)	276 (103 - 1241)	79	271 (103 - 1241)
Gamma-glutamyl transpeptidase (UI/L)	60 (11 - 240)	80	61 (11 - 240)
Serum total protein (g/L)	65 (53 - 76)	80	65 (53 - 76)
Serum albumin (g/L)	37 (23 - 60)	79	37 (23 - 46)

* Patients having at least one missing baseline covariate value were excluded from covariate analysis

Genotyping

Patient genomic DNA was extracted from the whole blood samples according to the instructions of the EZ1 DNA blood kit and the EZ1 Advanced instrument (Qiagen, Chatsworth, CA). A total of 11 Single Nucleotide Polymorphisms (SNP) and genetic variations of 6 different genes involved in the transmembrane transport and metabolism of etoposide were studied (Table II). Genotyping of the patients was carried out by Taqman real-time PCR assay, sequencing or melting curve-based allele-specific PCR method. Taqman real-time PCR assay was performed according to manufacturer instructions (ThermoFischer). For UGT1A1 and ABCB1, regions of interest were amplified by PCR with Hotstart Taq Master mix (Qiagen) as described by the manufacturer and sequenced for SNP genotyping. A M13 primer sequence was incorporated at the 5' end of all PCR primers (shown in Table II) to facilitate subsequent DNA sequencing. PCR products were sequenced using the Big Dye Terminator v3.1 cycle sequencing kit (Applied Biosystems) with forward or reverse M13 primers. Products were analyzed using an ABI Prism 3100 genetic analyzer (Applied Biosystems). The presence of CYP3A5*1/CYP3A5*3 alleles was determined on genomic DNA with allele specific real time PCR as previously described by Yates and colleagues (20).

Pharmacokinetic analysis

Determination of individual clearance and AUC

The pharmacokinetic analysis was performed using non-linear mixed-effect modeling and Bayesian estimation on NONMEM software program (Version 7.2) to obtain individual PK parameters from a limited number of plasma samples according to a previously published methodology (18). Etoposide concentration-time data of all the patients on each day and each cycle of the treatment (3 concentrations x 3 days x 3 cycles) were simultaneously analyzed using a two-compartment PK model. The interindividual and the residual variability were assessed with log-normal model and proportional model respectively. The intra-patient variability of clearance (both intra- and inter-cycle variability) was evaluated using inter-occasion variability (IOV) model, and the first order conditional with interaction (FOCE-I) method was the estimation method. Individual values of actual clearance (CL) were obtained from the model as empirical Bayes estimates for each day of the 3 cycles of treatment. The Sampling Importance Resampling (SIR) procedure (with 20,000 final proposal samples and 2,000 resamples) was used to evaluate parameter uncertainty and to calculate the relative standard error (RSE) for the parameter estimates of the model. Daily actual AUC were then determined by dividing each daily

Table II Genetic Polymorphisms Selected for Pharmacogenetic Analysis with Corresponding Genotyping Methods

Gene	Polymorphism	Genotyping method	Taqman assay or primers
NR1I2 (PXR)	rs10934498	Taqman	C___1834252_10
	rs2472677	Taqman	C__26079845_10
	rs1523130	Taqman	C___9152783_20
	rs1523127	Taqman	C____263841_20
	rs3814055	Taqman	C__27504984_30
CYP3A4	rs35599367	Taqman	C__59013445_10
CYP3A5	rs776746	Melt curve analysis	Forward : 5'ACTGCCCTTGCAGCATTTAG3'
			Reverse (wild-type): TCCAAACAGGGAAGAGAAAT
			Reverse (*3): TCCAAACAGGGAAGAGAAAC
UGT1A1	rs8175347	Sequencing	Forward:5'-GCCAGTTCAACTGTTGTTGCC-3'Reverse:5'-CCACTGGGATCAACAGTATCT-3'
ABCB1 – Pgp (ABCB1)	rs1045642	Sequencing	Forward:5'-TTCAGCTGCTTGATGGCAAA-3'Reverse:5'-AGGCAGTGACTCGATGAAGG-3'
	rs2032582	Sequencing	Forward:5'-CAGGCTTGCTGTAATTACCC-3'Reverse:5'-TAGTTTGACTCACCTTCCCA-3'
ABCC3	rs4793665	Taqman	C__27829307_10

NR1I2 (PXR) nuclear receptor subfamily 1 group I member 2 (Pregnane X Receptor)

CYP3A4 cytochrome P450 family 3 subfamily A member 4

CYP3A5 cytochrome P450 family 3 subfamily A member 5

UGT1A1 UDP-glucuronosyltransferase family 1 member A1

ABCB1 ATP binding cassette subfamily B member 1

ABCC3 ATP binding cassette subfamily C member 3

etoposide dose by the corresponding actual CL value. The total actual AUC per cycle was obtained by the sum of the 3 daily actual AUCs of the same cycle.

Covariate analysis

Demographic and biological characteristics of each patient were considered as covariates to be tested to estimate their impact on PK parameters: age (in years), body weight (BW in kg), body surface area (BSA in m^2), serum creatinine (Scr in µM), serum cystatin C (CysC in mg/L), creatinine clearance calculated with Cockcroft-Gault equation (eGFR in mL/min), aspartate transaminase (ASAT in UI/L), alanine transaminase (ALAT in UI/L), alkaline phosphatase (ALP in UI/L), total bilirubin (BILI in µM), lactate dehydrogenase (LDH in UI/L), gamma-glutamyl transpeptidase (GGT in UI/L), total protein (PROT in g/L), albumin (ALB in g/L) as well as the patients' genotypes for the 11 selected SNPs and genetic variations. The influence of each covariate (including patients' genotypes) was separately evaluated on CL. Based on our previous work relative to etoposide pharmacokinetics (13), the ratio BW/Scr was also tested. The influence of PROT and ALB was examined on both central (V_1) and peripheral (V_2) volumes. Covariate analysis was carried out using allometric equation as follows:

$$TVP = \theta_1 \times (\text{quantitative covariate/mean covariate})^{\theta_2}$$

or $TVP = \theta_1 \times \theta_2^{\text{dichotomous covariate}}$

where θ_1 is the typical value of the PK parameter (TVP) for a patient with the mean covariate value and θ_2, the estimated influential factor for the covariate in the case of quantitative covariate. For dichotomous covariate taking either the value "1" or "0", θ_1 is the TVP for patients in the first category, and θ_2 represents the proportional increase or decrease of the TVP for the patient in the second category with respect to θ_1. In the case of genetic data, heterozygote patients were grouped with either those with homozygous mutant or homozygous wild-type genotype to form two categories.

Patients having missing baseline values for at least one covariate were excluded from the covariate analysis. Consequently, a dataset of only 74 patients was available for the covariate testing, and each patient of the dataset had different covariate values for each cycle of treatment except for genetic data. A covariate was considered having a significant relationship with a PK parameter if its inclusion in the model was associated with a decrease of the objective function value (OFV) of at least 3.84 (khi-2 test of difference with $p < 0.05$, one degree of freedom). The OFV is equal to minus twice the log likelihood of the data. This value is an indicator of the goodness-of-fit of the model. All the significant covariates were then included in an intermediate model. Afterwards, a stepwise backward elimination procedure was performed to keep in the model only covariates associated with an increase in OFV larger than 6.6 ($p < 0.01$, one degree of freedom).

A posthoc power analysis was performed to calculate the power associated with the analysis of PG covariates (SNPs) impact on CL.

Evaluation of dose individualization based on the final covariate equation

Evaluation of the final covariate equation was performed by calculating the total predicted dose per cycle (D_{pred}) for each patient using the clearance predicted (CL_{pred}) by the equation and the mean total actual AUC per cycle (considered as the "target AUC"): $D_{pred} = CL_{pred} \times$ mean actual AUC. For each cycle, the total AUC that each patient would have had was calculated using the D_{pred} and the actual CL.

Pharmacokinetic—pharmacodynamic relationship

Correlation between etoposide exposure and either treatment response or toxicity was evaluated using STATA software program. For the response evaluation, patients with complete response (CR) were considered as responders whereas patients with partial response, stable or progressive disease were classified as non-responders. Exposure's effect on tumor response was assessed by comparing the mean cycle-1 actual AUC or mean cumulative actual AUC between the responder and non-responder groups using student's t-test. The association between etoposide cumulative AUC and overall survival (OS) or progression-free survival (PFS) was evaluated using the Cox proportional hazards model.

Haematologic toxicity is expected after etoposide/carboplatin intensification cycles but should be overcome at day 14. It was then decided to focus on extended neutropenia and thrombocytopenia using the first available value of absolute neutrophil and platelet count between day 14 and day 21 after treatment (given on days 1, 2 and 3). These persistent haematologic toxicities were graded according to the Common Terminology Criteria for Adverse Events v3.0 (CTCAE). To evaluate the relationship between etoposide exposure and the presence of a persistent haematologic toxicity, at each cycle, mean actual AUC per cycle were compared between the patient groups having grade 0-2 late neutropenia or thrombocytopenia and those having grade 3-4 extended neutropenia or thrombocytopenia respectively using student's t-test.

Pharmacogenetic analysis

Pharmacogenetic analysis was performed using SNPassoc package on R software program (version 3.5.0). Deviation

of the genotype frequencies from those expected under Hardy–Weinberg equilibrium was assessed before any association study was carried out. The association between SNP genotypes and tumor response or cycle-1 late haematotoxicity was analyzed with the statistical significance set at $p < 0.05$.

RESULTS

Pharmacokinetic analysis

Determination of individual clearance

The two-compartment PK model describes the data accurately as shown by the visual predictive check (VPC) based on 500 simulations of the original data set using the final model (Fig. 1) and the observed residual variability (20.2%). The interindividual variability of CL was 15.9% and the inter-cycle variability was 9.1%. The intra-cycle variability for each cycle was very low (0.1%), so it was fixed at 0 for further analyses. PK parameters and their variabilities are presented in Table III. The mean actual CL (range) values were 31.2 mL/min (17.6 – 43.8), 31.0 mL/min (19.8 – 44.4), and 30.3 mL/min (22.0 – 43.2) for cycle 1, cycle 2, and cycle 3, respectively.
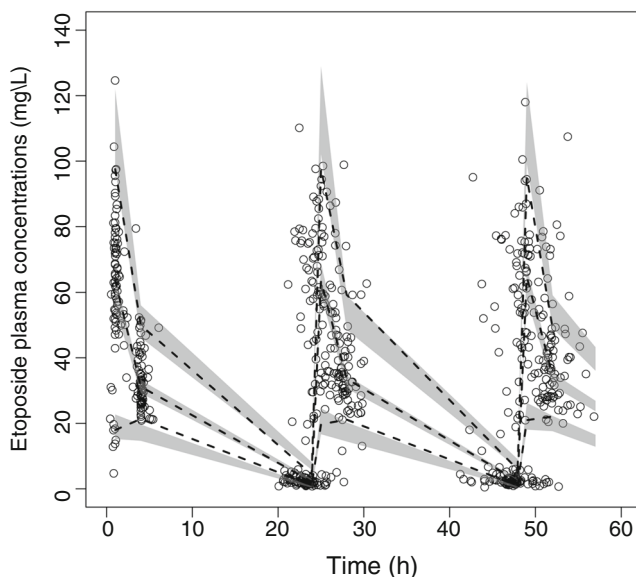

Fig. 1 Observed etoposide plasma concentrations (○) and results of Visual Predictive Check (VPC) based on 500 simulations of the original data set using the final model. The shaded areas represent the 95% confidence intervals of the 5th, 50th and 95th percentiles of the simulated concentrations. The dashed line represents the 50th percentile (median) and the 5th and 95th percentiles of the observed data.

Table III   Pharmacokinetic Parameters of Etoposide

Model parameters	Estimates [%RSE]	Shrinkage (%)
CL (mL/min)	30.2 [1.8]	-
V1 (L)	5.2 [10.2]	-
Q (L/h)	9.1 [17.2]	-
V2 (L)	7.2 [7.6]	-
IIV CL (%CV)	15.9 [9.1]	9.4
IIV V1 (%CV)	46.0 [9.8]	13.0
IIV Q (%CV)	58.0 [12.1]	30.0
IIV V2 (%CV)	28.1 [12.1]	24.5
Inter-cycle IOV CL (%)	9.1 [8.3]	24.4
Residual variability (%)	20.2 [2.4]	9.7

*%RSE percentage relative standard error obtained by SIR procedure; %CV percentage coefficient of variation ; IIV inter-individual variability; IOV inter-occasion variability

Covariate analysis

During the separate evaluation of each covariate, those having significant influence on CL were BSA, BW, Scr, CysC, ALB, PROT, BILI, eGFR and the ratio BW/Scr. Genetic polymorphisms of the selected genes were not found to influence CL, and no covariate was significant on V_1 and V_2. The ratio BW/Scr allows a decrease in OFV greater than the sum of the decreases associated with BW and Scr evaluated separately or eGFR. Consequently, the ratio BW/Scr was instead included in the intermediate model with the other significant covariates on CL to perform the backward elimination. The significant covariates resulted from this procedure were the ratio BW/Scr and BILI with the equation ($\pm$ 95% confidence interval):

$$CL \ (mL/min) = 31.0 \ (\pm 1.2)$$
$$\times \ [(BW/Scr)/1.0]^{0.229(\pm 0.097)}$$
$$\times \ (BILI/7)^{-0.054(\pm 0.034)}$$

Due to the limited influential factor associated with BILI (i.e., the power is close to zero) and the negligible decrease of interindividual variability of CL when BILI was added to the ratio BW/Scr (from 13.6% to 13.3%), we decided not to retain this covariate. The model containing only the ratio BW/Scr was then re-evaluated on the whole dataset (88 patients) to obtain the final equation as follows:

$$CL \ (mL/min) = 30.8 \ (\pm 1.1) \times [(BW/Scr)/1.0]^{0.202(\pm 0.093)}$$

In comparison with the model without covariate, this model based on BW/Scr was associated with a significant decrease of the objective function value (i.e., -18.9, $p < 0.001$) and a decrease of the IIV on CL from 15.9% to 14.7%. A model based on the addition of a renal term including BW/Scr or

Table IV The Interindividual Variability (Expressed as Coefficient of Variation) of the Etoposide Plasma Exposure (AUC) Corresponding to Actual BSA-Dosing *vs.* Dosing Based on Body Weight/Serum Creatinine (BW/Scr)

Cycle (n patients)	BSA-dosing	Dosing based on BW/Scr	p value
Cycle 1 (88)	20.6%	18.4%	NS
Cycle 2 (78)	17.5%	16.1%	NS
Cycle 3 (71)	16.4%	15.0%	NS

NS not significant

eGFR and another term corresponding to the non-renal clearance has been also evaluated but it was not associated with a better adjustment than the above unique term.

In order to confirm that the lack of impact of genetic variables on CL was not due to a sample size issue, a post hoc power analysis was performed and showed that the power to identify a 20% variation of the CL (CV=15.9%) is 92.2% if 10 patients out of 70 have the variant genotype. This power value increases with the number of patients in the variant genotype group, to reach 99.8% in the case where the variant genotype group is composed of 35 patients.

Evaluation of dose individualization using covariates equation

Table IV compares the performance of etoposide dosing according to the above final equation based on BW/Scr *vs.* that of the BSA-dosing. The coefficient of variation corresponding to the dispersion of AUC values would not be significantly decreased by using the final equation.

Pharmacokinetic—pharmacodynamic relationship

Statistical comparison of mean actual AUC (cycle-1 and cumulative AUC) between responder and non-responder groups is shown in Table V. A trend toward higher exposure was observed in patients experiencing clinical response but the mean cycle-1 AUC or the mean cumulative AUC was not significantly different between the two groups. No association has been found between etoposide cumulative AUC and the OS or the PFS (p=0.78 and 0.29 respectively).

For haematologic toxicity, only the mean actual AUC of cycle 3 was found to be significantly higher (p=0.01) in the patients having grade 3-4 late neutropenia than those having grade 0-2 late neutropenia as shown in Table V.

Pharmacogenetic analysis

Genotype data were available for only 78 patients. No deviation of the genotype frequencies from those expected under Hardy–Weinberg equilibrium was observed. There was no significant association between polymorphism of each SNP with the response to treatment.

For haematotoxicity, a significantly (p=0.02) higher proportion of patients with grade 3-4 late neutropenia was observed among the patients carrying homozygous mutant genotype for UGT1A1 (i.e., having two UGT1A1*28 alleles) compared to heterozygous and wild-type patients (86% *versus* 40%) as shown in Table V.

DISCUSSION

Etoposide plasma concentration data were adequately described by the two-compartment PK model with the value of the residual variability mainly limited to assay error. The population values of the PK parameters obtained from the model are similar to those reported in the literature for standard-dose protocol (12,16,18). However, the interpatient variability of CL (16%) was lower than that observed

Table V Correlation Between AUC (in mg.min/mL) and Treatment Response or Haematologic Toxicity and Impact of UGT1A1 Polymorphism on Haematotoxicity

Treatment response	Number of patients	AUC C1 Mean ± SD	Cumulative AUC Mean ± SD		
Responders	35	77.7 ± 16.2	223.7 ± 45.4		
Non-responders	43	71.1 ± 13.3	205.1 ± 39.5		
p-value		*0.054*	*0.058*		
Haematologic toxicity	AUC Cycle 1 Mean ± SD (n)	AUC Cycle 2 Mean ± SD (n)	AUC Cycle 3 Mean ± SD (n)	UGT1A1 (n)	
				*1/*1 & *1/*28	*28/*28
Grade 0-2 neutropenia	74.7 ± 15.4 (41)	73.7 ± 11.1 (37)	72.2 ± 10.9 (38)	34	1
Grade 3-4 neutropenia	73.3 ± 15.5 (35)	73.4 ± 12.4 (31)	81.1 ± 13.7 (19)	23	6
p-value	*0.69*	*0.90*	***0.01***	***0.02***	
Grade 0-2 thrombocytopenia	73.8 ± 15.9 (10)	70.9 ± 13.8 (8)	76.7 ± 15.5 (6)		
Grade 3-4 thrombocytopenia	75.2 ± 15.9 (63)	74.8 ± 12.9 (61)	75.2 ± 12.3 (50)		
p-value	*0.80*	*0.43*	*0.78*		

in other previous studies. This could be explained by the characteristics of the patients treated with this protocol; all were young adult males having adequate organ (renal and hepatic) function. This led to a limited interindividual variability of etoposide exposure as shown in Table IV.

Etoposide is known to be eliminated by renal route and hepatic metabolism. The result of our covariate analysis showed a limited impact of BILI. In other previous studies (13,15,21–23), no or weak correlations were found with regard to covariates which are indicators of liver function (i.e., ALB, PROT, BILI and liver enzymes). Having a low hepatic extraction ratio, etoposide clearance is dependent of both plasma unbound fraction (f_u) and intrinsic etoposide clearance (intrinsic CL) according to the following equation $CL = f_u$. intrinsic CL. The marginal influence of BILI on CL can be explained by the limited number of patients (n=2) with elevated bilirubin (i.e, > 17 µM) and, as described by Stewart and colleagues (22), by a contradictory effect of bilirubin increase on etoposide CL: a higher bilirubin level was associated with a higher etoposide f_u caused by competition between bilirubin and etoposide for albumin binding and a concomitant decrease of intrinsic CL. As demonstrated in the previous study (13), patients' characteristic having the most significant impact on etoposide CL was Scr corrected by the body weight. Nevertheless, the retrospective dose calculation using CL predicted from the final equation based on BW/Scr did not lead to a significant reduction of the AUC dispersion, indicating that the dose calculation method based on body surface area is valid for patients treated with this protocol.

The relationship between etoposide AUC and response was not significant. This is easily understandable regarding the limited interpatient variability in etoposide AUC and suggesting that efficacy is dependent on other factors than etoposide PK. However, it is interesting to note that the mean actual AUC (either the cycle-1 AUC or the cumulative AUC) was higher in the responder group than in the non-responder group despite the limited variability of etoposide exposure in this study. It can be concluded that etoposide exposure could be anyway, among others, a contributive factor to efficacy of this etoposide/carboplatin regimen. Regarding haematologic toxicity in cycle 3, the mean actual AUC of etoposide was significantly higher in the patient group having grade 3-4 late neutropenia than in the group having grade 0-2 late neutropenia (p=0.01). However, with the absence of significant difference of mean AUC between the two groups in the first 2 cycles, this result is not strong enough to encourage dose adaptation based on TDM. No correlation was found either between observed AUC and the degree of late thrombocytopenia in each cycle of treatment. One possible explanation is that the AUC used in our study corresponds to the total plasma etoposide, and it has been reported that exposure to unbound etoposide was found to correlate better with drug effect or haematologic toxicity than total plasma etoposide (24).

Lastly, it should be emphasized that the limited interindividual variability in etoposide AUC in link with the rather homogeneous population and the fact that etoposide was combined with carboplatin may explained the lack of significant correlation between etoposide PK parameters and toxicity.

For certain drugs, the differences between patients in terms of response to treatment or toxicity can be attributed to genetic variations. The pharmacogenetic analysis in the present study showed that there is no association between the genetic polymorphism of the genes involved in etoposide pathway and the treatment response. However, for haematologic toxicity, UGT1A1*28 homozygous genotype was significantly associated with late neutropenia. UGT1A1 (UDP-glucuronosyl-transferase family 1 member A1) is a hepatic enzyme of the phase 2 metabolism which catalyses the glucuronidation of various endogenous and exogenous compounds including etoposide (25). UGT1A1*28 allele is characterized by the presence of an additional TA repeat in the TATA sequence of the UGT1A1 promoter [$(TA)_7TAA$ instead of $(TA)_6TAA$] which leads to a decreased transcriptional activity of the enzyme (26). Theoretically, the observed association can be explained by the accumulation of plasma etoposide due to the reduced elimination (i.e., decreased etoposide CL) in the homozygous mutant patients as reported for other neutropenia-inducing drugs or drug metabolites which are substrates of UGT1A1 (27,28). However, the influence of UGT1A1 on etoposide CL in our study was not significant when evaluated during the covariate analysis on NONMEM. Likewise, among the patients gradable for late neutropenia and having genotype data for UGT1A1 (n=64 patients), there was no significant difference between the mean actual CL of patients carrying 2 *28 alleles and those carrying 1 or 0 *28 alleles (32.1 mL/min vs. 31.4 mL/min respectively, p=0.85). As evoked earlier, this could be due to the competition between etoposide and plasma bilirubin with regard to protein binding in the presence of the decreased activity of UGT1A1. Indeed, among those patients gradable for neutropenia in our study, the median total bilirubin was higher in the homozygous mutant patients (11.1 µM ranging from 8 to 46 µM) than in those with heterozygous and homozygous wild-type genotypes (5.7 µM ranging from 1 to 28.5 µM). The reduced activity of the enzyme in homozygous mutant patients might result in the increase in total bilirubin (as bilirubin is one of UGT1A1's endogenous substrate) and the reduction of unbound etoposide clearance (or intrinsic clearance), which would then lead to the increase of etoposide plasma unbound fraction (f_u) and unbound concentration without apparent decrease of total systemic CL. As unbound etoposide is the active form of the drug, such an increase would then be accompanied by the observed increase in haematologic toxicity. Nevertheless, the present pharmacogenetic study serves only as an exploratory analysis, and the result needs to be confirmed using a wider population.

CONCLUSION

Despite the observed trend toward higher etoposide AUC in patients who responded to treatment, the association between AUC and response is not strong enough for us to recommend a target etoposide AUC for this protocol. So, neither *a priori* dose individualization based on clearance predicted from covariate equation nor dose adaptation using therapeutic drug monitoring is suggested for etoposide used in the TI-CE protocol, unlike carboplatin for which we have previously shown a benefit of TDM (17). Therefore, dose calculation according to body surface area remains valid for patients treated with this regimen.

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AUTHORS' CONTRIBUTIONS

Conception and design: C. Chevreau, E. Chatelut, F. Thomas, T Filleron

Study supervision: C. Chevreau

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): C. Chevreau, C. Massart, A. Fléchon, R. Delva, G. Gravis, J-P. Lotz, J-O. Bay, M. Gross-Goupil, S. Marsili, T. Lafont, C.Delmas

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): S. Moeung, C. Chevreau, T Filleron, E. Chatelut, F. Thomas,

Writing, review, and/or revision of the manuscript: all authors.

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