Cancer Chemother Pharmacol (2012) 69:25–33
DOI 10.1007/s00280-011-1670-4

ORIGINAL ARTICLE

Gene polymorphisms, pharmacokinetics, and hematological toxicity in advanced non-small-cell lung cancer patients receiving cisplatin/gemcitabine

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Received: 10 March 2011 / Accepted: 3 May 2011 / Published online: 18 May 2011

Abstract
Background This study quantified the impact of drug pathway-associated genetic variants on the pharmacokinetics (PK) of gemcitabine and cisplatin in patients with advanced non-small-cell lung cancer (NSCLC).
Methods Thirty-seven patients with advanced NSCLC were sampled for plasma concentrations of gemcitabine, difluoro-deoxy uridine (dFdU), intracellular gemcitabine triphosphates (dFdCTP), and unbound platinum concentrations after gemcitabine 1,250 mg/m^2 i.v. followed by cisplatin 75 mg/m^2. We analyzed 13 germline single nucleotide polymorphisms and one deletion—glutathione S-transferase (GST) M1—within six drug pathway-associated genes (GSTM1, GSTP1, cytidine deaminase (CDA), solute carrier (SLC) 28A1, SLC28A2, and deoxycytidine kinase). PK models were fitted to the data using nonlinear mixed-effects modeling, and genetic data were tested on drug PK and hematological toxicity.
Results Patients carrying the nonsynonymous CDA SNP 79A >C (CDA*2) had a 21% lower gemcitabine clearance as compared to wild-type patients (outcomes and complications.0.0009), but the risk for chemotherapy-associated neutropenia (61% vs. 32%, $P = 0.07$) and severe neutropenia (17% vs. 5%, $P = 0.26$) was not significantly higher. Other gene polymorphisms were not associated with drug PK parameters or hematological toxicity. The known functional mutant variant CDA*3 was not found in any of the patients.
Conclusions Although the mutant CDA*2 allele results in an increased exposure to gemcitabine in Caucasian patients, this study gives no definite conclusion on the clinical relevance of this finding. Further studies should look into the relationship between CDA genotypes, plasmatic CDA activity, and clinical outcome in patients receiving gemcitabine-based chemotherapy.

Keywords Gemcitabine · Cisplatin · Pharmacogenetics · Lung cancer · Predictor · Drug modeling

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Introduction

The combination of gemcitabine and cisplatin is a standard first-line chemotherapy regimen for patients with advanced

non-small-cell lung cancer (NSCLC), with a significant 10% improvement of overall mortality compared to other platinum-containing regimens [13]. Gemcitabine (2′,2′-difluorodeoxycytidine; dFdC) is a synthetic pyrimidine analog with broad-spectrum activity against several solid tumors [15]. Gemcitabine is deaminated by cytidine deaminase (CDA) to its main metabolite 2′,2′-difluorodeoxyuridine (dFdU) [1]. Gemcitabine enters the cell by means of nucleoside transporters and becomes activated through an intracellular transformation catalyzed by deoxycytidine kinase (dCK) to the final triphosphate metabolite (dFdCTP) in a rate-limiting reaction [1, 15]. The primary elimination route of gemcitabine is deamination by CDA into dFdU, with subsequent excretion into the urine. Cisplatin (cis-dichlorodiammineplatinum) undergoes aquation and binding to macromolecules such as DNA, resulting in adduct formation, with unbound cisplatin being considered the pharmacologically active form.

Pharmacogenetics has shown that gene variants can influence drug pharmacokinetics and clinical outcome [24]. Limited data suggest that genetic variants in CDA are of potential clinical relevance in Japanese solid cancer patients receiving gemcitabine in combination with platinum salts or 5-fluorouracil [19]. Additionally, marked differences have been found in the frequency of gemcitabine pathway-associated genetic variants within Caucasians [12], Asians [17], and between Caucasians and African-Americans [8]. This study aims to quantify the impact of drug pathway-associated genetic variants on the pharmacology and hematological toxicity of cisplatin/gemcitabine chemotherapy in patients with advanced NSCLC.

Materials and methods

Patient population, blood sampling, and bioanalysis

This is a prospective, noninterventional study carried out at the Netherlands Cancer Institute in Amsterdam (The Netherlands) as part of a larger multicenter study that assessed the impact of genetic variants on the clinical outcome in patients with advanced NSCLC. Thirty-seven patients (23 men and 14 women) with advanced NSCLC were included between November 2005 and November 2006. Mean patient age was 56 years, 27 patients had stage IV disease, 10 patients stage IIIB disease. Histopathology showed adenocarcinoma in 13 patients (35%), squamous-cell carcinoma in two patients (5%) and NSCLC not otherwise specified in 22 patients (59%). All patients had adequate renal and hepatic function.

Analysis and reporting of the pharmacokinetic data were prespecified according to published guidelines [23, 27]. Eligibility criteria included patients with cytologically or histologically confirmed advanced NSCLC stage IIIB or IV receiving first-line gemcitabine/cisplatin chemotherapy, the presence of measurable or evaluable disease according to the Response Evaluation Criteria in Solid Tumors (RECIST) criteria, no radio- or chemotherapy for at least 3 weeks prior to study entry, WHO performance status 0–2 and provision of written informed consent. Treatment-related toxicity was graded according to the National Cancer Institute's Common Terminology Criteria for Adverse Events Version 3.0. The study was approved by the local ethical committee. Patients received gemcitabine 1,250 mg/m^2 as a 30-min intravenous infusion on days 1 and 8, and followed by cisplatin 75 mg/m^2 over 4 h on day 1, and repeated at three-weekly intervals for up to six cycles. Hematological assessments were performed at weekly intervals. PK analysis was performed on day 1 of the first treatment cycle. Samples for gemcitabine were collected at time points 10, 20, 30, 45 min, and 2 h after starting gemcitabine, added to tetrahydrouracil to block degradation by CDA, and analyzed as described previously [25]. Gemcitabine intracellular triphosphates were additionally measured in peripheral blood mononuclear cells (PBMC) at time points 30 min and 2 h as described previously [10]. Samples for cisplatin were collected at time points 2, 4, 4½, and 6 h and measured as ultrafiltrated platinum as previously described [4]. Accuracy and precision for gemcitabine and cisplatin bioanalysis were ≤15% [4, 25], for gemcitabine triphosphates ≤20% [10].

Genetic analyses

The investigators performing genetic analyses (V.D., P.S.) were blinded to patient characteristics and clinical outcome. A 5-mL aliquot of EDTA blood was collected from each individual, and genomic DNA was isolated as described previously [3]. Genotypes examined and respective primer sequences are detailed in Table 1. DNA amplification was performed in a PTC-200 thermocycler (MJ Research, Waltham, MA, US). DNA sequencing was performed using the BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Nieuwerkerk a/d Ijssel, The Netherlands) on an ABI Prism 3100 DNA analyzer (Applied Biosystems) for 13 germline single-nucleotide polymorphisms (SNP) and one deletion (GSTM1) within the following six genes directly involved in the metabolism of gemcitabine/cisplatin: GSTM1, GSTP1, CDA, SLC28A1, SLC28A2, and dCK. For sequence alignment, the SEQSCAPE bioinformatics software (ver. 2.1; Applied Biosystems) was used. Hardy–Weinberg equilibrium was evaluated using the χ^2 test, and linkage disequilibrium analysis was performed using the GOLD software (ver. V.1.1.0.0.;Wellcome Trust Centre for Human Genetics, Oxford, UK).

Table 1 PCR primers and amplification conditions for the genotypes assessed

Gene	Mutation	Forward primer	Reverse primer
GSTM1	Deletion	5′-GAA CTC CCT GAA AAG CTA AAG C-3′	5′-GTT GGG CTC AAA TAT ACG GTG G-3′
GSTP1 (E6)	313A > G	5′-AAG CAG AGG AGA ATC TGG GAC TC-3′	5′-GGC CAG ATG CTC ACC TGG TC-3′
CDA (E1)	79A > C	5′-GTA CCA ACA TGG CCC AGA AG-3′	5′-GTG CCC ACC TTT ACC TTT GA-3′
CDA (E2)	208G > A	5′-TTC CTT CCT GCT TTG GAA TG-3′	5′-ATT GTT GCA ACC TGG CTT TC-3′
dCK (5′UTR)	−360C > G/−201C > T, −243G > T, −135G > C	5′-CTG CAG GTG ACG CCC TCT-3′	5′-GGG TGG CCA TTC CTT AGT CT-3′
dCK (E3)	261G > A, 300C > T, 364C > T	5′-TTG GGA AAG AGT ATG AGA AAG G-3′	5′-AAA CTT CAA ATG GCC ACG TA-3′
SLC28A1 (E15)	1543C > T, 1576G > A	5′-GGG GAT CAA GCT GTT TCT GA-3′	5′-GCA CCA CTT CAG TGT GTC TCA-3′
SLC28A2 (E4)	283A > C	5′-AGA AAT GGG ATG GAG TAA TTC TTG-3′	5′-TCA CCT TGA CAC AAG GCA AA-3′

PCR polymerase chain reaction, *GST* glutathione sulfotransferase, *CDA* cytidine deaminase, *dCK* deoxy-cytidine kinase, *SLC* solute carrier, *E* exon

Conventional statistical analysis

Mean values of drug clearance, area under the concentration–time curve (AUC), and intracellular gemcitabine triphosphate concentrations at two hours were compared between wild-type and mutant genotypes using the Student's *t* test or the Wilcoxon rank-sum test for genotypes with more than two categories (wild-type/heterozygous-/homozygous mutant). Similarly, the frequency of severe neutropenia (absolute neutrophil count <1 G/L) was compared between genotypes using Fisher's exact test for homogeneity between wild-type and mutant genotypes and the Wilcoxon rank-sum test for trend statistics in genotypes with more than two categories (wild-type/heterozygous-/homozygous mutant). The relationship between drug dose per m^2 and AUC of the drug (cisplatin/gemcitabine) was analyzed using the Spearman's rank correlation test). All tests of significance were two sided; $P < 0.05$ was considered significant. All conventional statistical analyses were performed using STATA 11.0 software (STATA Corp, College Station, Texas, US).

Population pharmacokinetic and pharmacogenetic model

Population PK analysis of the concentration–time data of gemcitabine and cisplatin was performed using the nonlinear mixed-effect modelling program (NONMEM) version VII [2]. NONMEM uses a maximum likelihood criterion to simultaneously estimate population values of fixed-effects parameters (e.g., drug clearance) and values of the random-effects parameters (e.g., interindividual variability). Log-transformed plasma drug concentrations were used together with the first-order conditional estimation method. Standard errors for all parameters were calculated using the COVARIANCE option, and individual Bayesian PK parameters were obtained with the POSTHOC option. Model selection was based on the minimum value of objective function (OFV), the precision of parameter estimates and by standard graphical plots. Concentration–time data of cisplatin were described using a linear two-compartment model. For gemcitabine, a four-compartment model with one peripheral compartment for gemcitabine and separate compartments for dFdU and intracellular triphosphates were used (Fig. 1), similar to what has been described by Tham and colleagues [21]. Linear transition from gemcitabine to its intracellular triphosphate pool was defined to be small (metabolic rate constant fixed to 10^{-6} times the metabolic rate constant for the transition from gemcitabine to dFdU) to avoid significant mass transport to the intracellular compartment. Subsequently, half-life of intracellular gemcitabine triphosphates was estimated. Proportional interindividual and

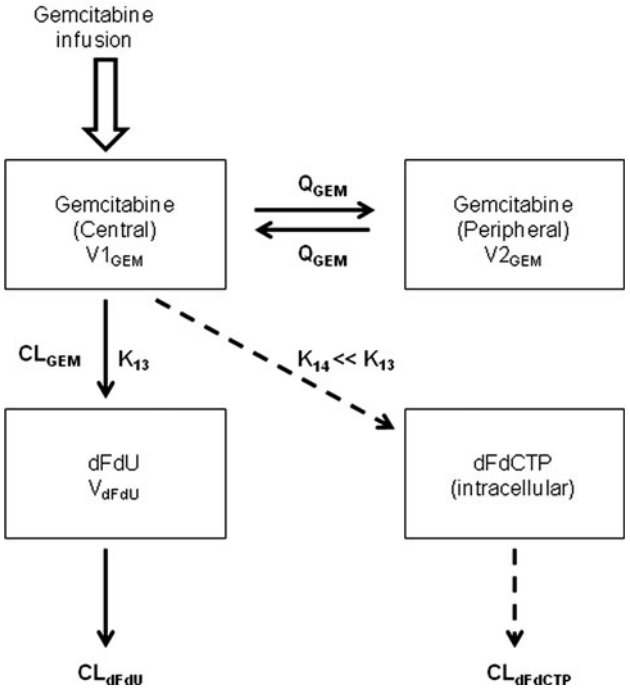

Fig. 1 Compartmental model of gemcitabine, difluoro-deoxy uridine (dFdU) and intracellular gemcitabine triphosphates (dFdCTP). CL_{GEM} clearance of gemcitabine, $V1_{GEM}$ volume of the central compartment of gemcitabine, Q_{GEM} intercompartmental clearance between the central and peripheral compartment of gemcitabine, $V2_{GEM}$ volume of the peripheral compartment of gemcitabine, CL_{dFdU} clearance of dFdU, V_{dFdU} volume of the central compartment of dFdU, CL_{dFdCTP} clearance of dFdCTP, K_{13} metabolic rate constant between the central compartment of gemcitabine and dFdU, K_{14} metabolic rate constant between the central compartment of gemcitabine and intracellular dFdCTP

intraindividual error models were used, with different intraindividual errors for gemcitabine, dFdU und intracellular triphosphates, respectively. Genetic variants were tested on their correlation with drug clearance, according to the following model:

$$\mathrm{CL_{drug}} = \theta_1 * \mathrm{GENE}^{(\theta 2)}$$

where θ_1* represents $\mathrm{CL_{drug}}$ in patients with the wild-type genetic variant, $\theta_1 \mathrm{EXP}^{(\theta 2)}$ $\mathrm{CL_{drug}}$ in patients with the mutated genetic variant. The difference in the OFV was evaluated after the introduction of a covariate into the model (forward inclusion), and the significance level was set at $P < 0.01$ that corresponds to a decrease in OFV of >6.7. Significant covariates were included into an intermediate model followed by a stepwise backward elimination procedure. Covariates remained in the model when elimination of the covariate caused an OFV increase of >7.9, corresponding to a P value of 0.005. To describe hematological toxicity, a semi-physiological model was fitted to individual neutrophile data as described previously [7]. The model includes a proliferation compartment, transit compartments mimicking cell maturation in bone marrow, and a compartment representing the pool of circulating cells. The average maturation or mean transition time (MTT) is the time a cell spents to pass from the proliferation stage to the circulation pool. The function by which drug concentrations affect the proliferation rate of circulating blood cells (E_{drug}) was modelled using a linear function, in which E_{drug} is represented by a slope factor (Slope) and drug concentration (c_{drug}), as follows:

$$k_{prol} = k_{tr} * FB - k_{tr} * (Slope * c_{drug}) \quad (1)$$

FB represents the feedback parameter $(ANC_{base}/ANC_t)^{\gamma}$, k_{prol} the proliferation rate constant, and k_{tr} the transition rate constant between the maturation compartments. Logarithmic transformation of both neutrophils and thrombocytes was used throughout model building together with the first-order conditional estimation (FOCE) method. Model-derived data simulations ($n = 1{,}000$) were performed to assess the quantitative effect of AUC_{GEM}, intracellular gemcitabine triphosphates, and gene polymorphisms on neutrophil time–concentration data. For this purpose, high AUC_{GEM} (highest vs. lower tertiles), high intracellular triphosphate concentration (highest versus lower tertiles), and mutant genotype carrier status (versus wild-type) were estimated on neutrophil slope parameter from the semiphysiological model as described above.

Results

Genetic analysis

The distribution of the various genotypes is outlined in Table 2. All polymorphisms were in Hardy–Weinberg equilibrium, and no significant linkage disequilibrium was found. The known functional mutant variant CDA*3 was not found in any of the patients. A single patient was carrier of the heterozygous linked dCK −360C > G/−201C > T polymorphism.

Population pharmacokinetic and pharmacogenetic model

Estimates of gemcitabine and cisplatin PK parameters are outlined in Table 3. Formation of intracellular gemcitabine triphosphates (dFdCTP) from gemcitabine was linear. Patients carrying the nonsynonymous CDA SNP 79A > C (Lys27Gln) (CDA*2) had a 21% lower CL_{GEM} compared to patients with wild-type CDA (152 vs. 193 l/min, $P < 0.0001$), and this translated into a 16% lower AUC_{GEM} in wild-type patients (41.5 vs. 49.2 μmol h/L, $P < 0.0001$). Intracellular gemcitabine triphosphates at 2 h were higher in carriers of the CDA*2 allele compared to wild-type

Table 2 Allele frequencies of the gene polymorphisms in 37 patients treated with cisplatin/gemcitabine

Gene	Mutation	Wild-type[a]	Heterozygous[a]	Homozygous[a]	Missing[a]	Minor allele frequency (%)
GSTM1	Deletion	18		19	0	51.4
GSTP1 (E6)	313A > G	18	13	6	0	33.8
CDA (E1)	79A > C	19	18	0	0	24.3
CDA (E2)	208G > A	37	0	0	0	0
dCK (5′UTR)	−360C > G/−201C > T,	36	1	0	0	1.4
	−243G > T	37	0	0	0	0
	−135G > C	37	0	0	0	0
dCK (E3)	261G > A	37	0	0	0	0
	300C > T,	34	3	0	0	4.1
	364C > T	37	0	0	0	0
SLC28A1 (E15)	1543C > T	37	0	0	0	0
	1576G > A	10	18	8	1	47.2
SLC28A2 (E4)	283A > C	18	16	3	0	29.7

[a] Number of patients, *GST* glutathione sulfotransferase, *CDA* cytidine deaminase, *dCK* deoxy-cytidine kinase, *SLC* solute carrier, *E* exon, *n.a.* not available

Table 3 Population pharmacokinetic parameters

Parameter	Basic model			Covariate model			CDA*2 (79A > C)		
	Estimate	SE	IIV (%)	Estimate	SE	IIV (%)	Estimate	SE	(%/CL_{GEM})
Gemcitabine									
Pharmacokinetic	CL_{GEM} (L/h)	171	7.5	14.8	193	9.2	7.3	−21.4	6.2
Parameters	$V1_{GEM}$ (L)	11.6	3.0	47.3	12.6	3.3	54.5		
	Q_{GEM} (L/h)	135	17.9	23.6	126	24.0	21.6		
	$V2_{GEM}$ (L)	25.5	3.7	20.2	24.3	3.9	21.8		
	CL_{dFdU} (L/h)	17.7	1.2	10.1	17.7	1.2	10.5		
	V_{dFdU} (L)	30.8	3.3	25.3	30.6	4.4	24.9		
	HL dFdCTP (h)	3.9	1.2	60.5	3.9	1.3	62.7		
Residual error (%)	Gemcitabine	23.1			21.1				
	dFdU	18.2			18.0				
	dFdCTP	64.8			59.5				
Cisplatin									
Pharmacokinetic	CL_{CIS} (L/h)	13.1	5.6	19.7					
Parameters	$V1_{CIS}$ (L)	16.5	1.2	24.3					
	Q_{CIS} (L/h)	14.3	5.5	22.7					
	$V2_{CIS}$ (L)	482	183	–					
Residual error (%)	Cisplatin	16.4							

CL_{GEM} clearance of gemcitabine, $V1_{GEM}$ volume of the central compartment of gemcitabine, Q_{GEM} intercompartmental clearance between the central and peripheral compartment of gemcitabine, $V2_{GEM}$ volume of the peripheral compartment of gemcitabine, CL_{dFdU} clearance of dFdU, V_{dFdU} volume of the central compartment of dFdU, *HL* half-life, *dFdCTP* intracellular gemcitabine triphosphate, CL_{CIS} clearance of cisplatin, $V1_{CIS}$ volume of the central compartment of cisplatin, Q_{CIS} intercompartmental clearance between the central and peripheral compartment of cisplatin, $V2_{CIS}$ volume of the peripheral compartment of gemcitabine, *SE* standard error, *IIV* interindividual variability, *CDA* cytidine deaminase

carriers (291 vs. 218 μmol/L, $P = 0.05$). None of the other drug pathway-associated gene polymorphisms (GSTM1, GSTP1, SLC28A1, SLC28A2, dCK) was associated with the pharmacological parameters of either drug. Gemcitabine dose per m^2 was associated with AUC_{GEM} ($r = 0.33$, $P = 0.05$), but no association was found between cisplatin dose per m^2 and AUC_{CIS} ($r = 0.19$, $P = 0.26$). Goodness-of-fit plots between model-predicted

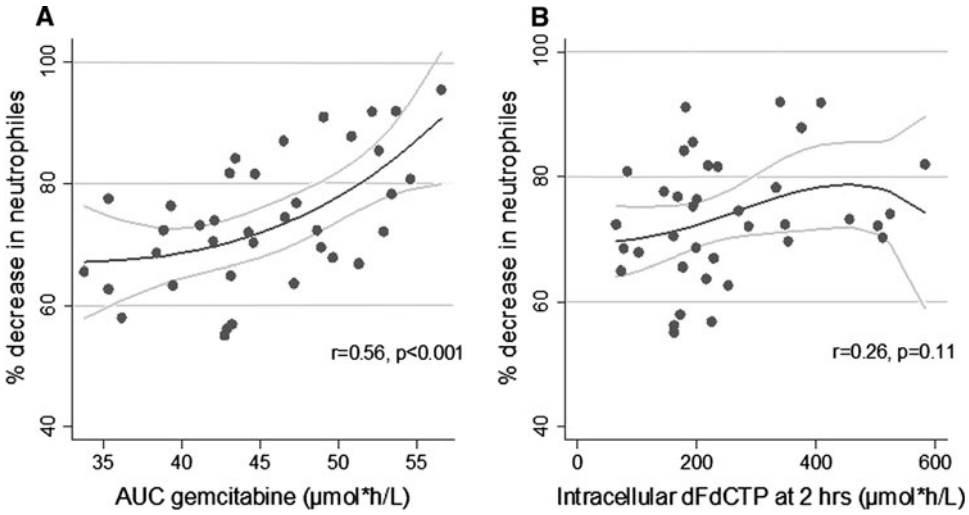

Fig. 2 Maximum individual neutrophil decrease (in %) in relationship to AUC_{gem} (**a**) and intracellular gemcitabine triphosphates (dFdCTP) (**b**) 2 h after the start of intravenous gemcitabine

and observed PK parameters supported the accuracy of the model (data not shown).

Hematological toxicity and pharmacogenetics

Severe neutropenia was found in one out of 19 patients with wild-type CDA and in three out of 18 carriers of the CDA*2 allele (5% vs. 17%, $P = 0.26$). There was a trend toward more frequent overall neutropenia in patients with wild-type CDA compared to CDA*2 carriers (61% vs. 32%, $P = 0.07$). Polymorphisms of dCK, SLC28A1, SLC28A2, and cisplatin pathway-associated gene polymorphisms were not associated with hematological toxicity. The semiphysiological model for neutrophils was successfully applied to gemcitabine concentration–time data, but not to cisplatin concentration–time data. The MTT for neutrophils was 153 h or 6.4 days (IIV 17.3%), the slope estimate for gemcitabine was 1.28 (IIV 44.2%), the coefficient of variation for neutrophils 39.6%, and γ as a component of the FB (Eq. 1) was 0.09. The average population nadir as derived from Bayesian estimates was 1,730 neutrophils/µl on day 13. The maximum percentage decrease in neutrophils is outlined against AUC_{GEM} and 2-h gemcitabine triphosphates in Fig. 2. AUC_{GEM} was significantly higher in patients experiencing leucocytopenia or neutropenia as compared to those without any leucocytopenia or neutropenia (48 vs. 43 µmol h/L, $P = 0.01$, 47 vs. 43 µmol h/L, $P = 0.02$, respectively). Median patient age was higher in patients experiencing any neutropenia (60 vs. 54 years, $P = 0.03$), and in patients experiencing any thrombocytopenia (62 vs. 54 years, $P = 0.008$) as compared to those not experiencing hematological toxicity. Within univariate analysis, a high AUC_{GEM} (OR 6.4, 95% CI 1.4–30.1) and male gender (OR 5.7, 95% CI 1.2–26.3) were significant predictors of neutropenia. Male gender was the only significant predictor of neutropenia within multivariate testing (OR 5.5, 95% CI 1.1–35.4). The association between AUC_{GEM}, 2-h gemcitabine triphosphates, CDA*2 genotype and individual simulated neutrophil curves is outlined in Fig. 3. The CDA genotype had the strongest impact on neutrophil nadir, with CDA*2 carriers experiencing a neutrophil drop to 17% from baseline as compared to 34% in wild-type carriers (Fig. 3c). Patients in the highest tertile for 2-h gemcitabine triphosphates experienced a neutrophil drop to 19% from baseline as compared to 34% in patients with low intracellular triphosphates (Fig. 3b), and patients in the highest tertile for AUC_{GEM} experienced a neutrophil drop to 27% from baseline as compared to 34% in patients with a low AUC_{GEM} (Fig. 3a).

Discussion

This study simultaneously investigated the population pharmacokinetics of gemcitabine, its metabolites dFdU and intracellular triphosphate, and cisplatin, and examined the effect of drug pathway-associated genetic variants on drug pharmacokinetics and hematological toxicity. Parameter estimates for gemcitabine and cisplatin using population pharmacokinetic modeling are in close agreement with previous data [11, 26]. Carriers of the mutant CDA*2 allele were found to have a 21% lower clearance of gemcitabine as compared to patients with wild-type CDA, and this resulted in more frequent severe neutropenia. Human CDA is involved in the salvaging of pyrimidines [6] and plays a key role in the detoxification of gemcitabine. Preclinical work demonstrated a lower activity of the CDA genetic variants 79A > C (CDA*2) [9] and 208G > A (CDA*3) [28] toward cytidine and cytarabine in a cellular expression system and compared to the CDA wild-type. In the study by Gilbert and colleagues, expression constructs for the

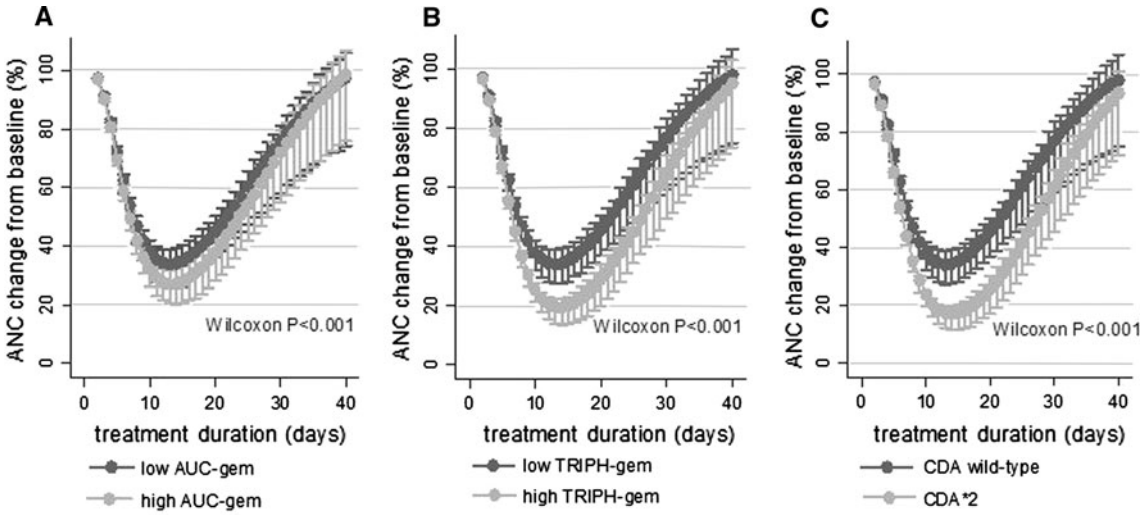

Fig. 3 Model-derived simulations ($n = 1{,}000$) of the relative neutrophil decrease over the course of 40 days in patients with a high AUC_{GEM} (highest tertile = *dark line*) versus low AUC_{GEM} (lower two tertiles = *gray line*) (**a**), patients with high intracellular gemcitabine triphosphates (TRIPH) (highest tertile = *dark line*) versus low intracellular gemcitabine triphosphates (lower two tertiles = *gray line*) (**b**), carriers of wild-type CDA (*dark line*) versus carriers of the CDA*2 allele (*gray line*) (**c**). *ANC* absolute neutrophil count

CDA 79A > C (Lys27Gln) nonsynonymous variant in mammalian cell lines showed a 34% lower activity of the CDA*2 genetic variant for the deactivation of gemcitabine [9]. However, this has not been confirmed by in vivo data of Ciccolini and colleagues, who studied CDA plasmatic activity in 108 adult patients [5]. Tibaldi and colleagues even found a higher CDA enzymatic activity in patients carrying the mutant CDA*2 allele [22]. Despite these controversial preclinical data, there is only one clinical study that assessed the quantitative impact of CDA genetic variants on gemcitabine pharmacokinetics in Caucasian patients as to our knowledge [14].

In Japanese patients, several clinical studies have assessed the frequency [20] and the impact of CDA genetic variants on the pharmacokinetics of gemcitabine and the occurrence of gemcitabine-associated toxicity [18, 19]. The studies by Sugiyama and colleagues showed a consistent association between the CDA*3 mutant variant, a decreased clearance of gemcitabine and an increased proportion of patients suffering from severe hematological toxicity. The earlier study included 256 Japanese patients with solid tumors and showed the CDA*3 mutant variant to be associated with a lower clearance of gemcitabine, and an increased incidence of neutropenia when patients were coadministered platinum-containing drugs or 5-fluorouracil [19]. In the second study, a population analysis was performed in 250 Japanese patients and a convincing gene-dose effect was found for CDA*3, in that heterozygous carriers of CDA*3 had a 17% decrease in the clearance of gemcitabine, and homozygous carriers were found to have a 64% decrease in the clearance of gemcitabine [18]. In the same study, mutant CDA*2 was not independently associated with the clearance of gemcitabine [18]. Overall, these data strongly support the clinical relevancy of the CDA*3 mutant variant in Asian (primarily Japanese) patients receiving gemcitabine chemotherapy.

Data are more limited for Caucasian patients. Maring and colleagues recently studied 20 patients with lung cancer receiving gemcitabine 1'125 mg/m^2 [14]. While not statistically significant, the authors found a somewhat lower terminal half-life of gemcitabine in four patients being homozygous mutant for CDA*2 (0.16 h) as compared to eight patients being heterozygous mutant for CDA*2 (0.23 h), and compared to eight patients being wild-type for CDA (gemcitabine half-life 0.26 h). Finally, the predictive value of CDA*2 for clinical outcome was assessed in 65 chemotherapy-naïve patients with advanced NSCLC, receiving gemcitabine/cisplatin combination chemotherapy [22]. According to this study, overall survival was significantly higher in carriers of the wild-type CDA allele as compared to CDA*2 carriers, but this study did not perform pharmacokinetic drug assessment and the pathophysiological meaning of these results remain unclear [22]. Contrary to what has been described in Asian patients, mutant CDA*3 has not been detected in Caucasians [20]. While the allele frequency of CDA*3 was 0.5% in Koreans and 2.2% in Japanese patients, CDA*3 was not detected in Chinese-Americans, Caucasian-Americans or African-Americans. At the same time, the allele frequency of CDA*2 was 15.3% in Koreans,

32.7% in Caucasian-Americans, and 8.7% in African-Americans [20].

In the present study, no other association was found between drug pathway-associated genetic variants and gemcitabine/cisplatin pharmacokinetics or pharmacodynamics. The allele frequency of the linked promoter dCK −360C > G/−201C > T variant was 1.4%, in agreement with what we have found in Caucasians previously [12], and less frequent compared to Asian patients [16]. Mutant dCK −360C > G/−201C > T was found to result in increased levels of dCK mRNA as compared to the wild-type genotype, and this translated into a favorable treatment response in acute myeloid leukemia patients receiving cytarabine-based chemotherapy [16]. In the present study, the single patient heterozygous for dCK −360C > G/−201C > T had mild neutropenia and thrombocytopenia, but intracellular gemcitabine triphosphate levels were not increased. This is in agreement with previous results from Sugiyama and colleagues, confirming the lack of an association between genetic variants in the dCK gene and gemcitabine pharmacokinetics [18].

The present study provides some limited evidence for the clinical relevancy of the mutant CDA*2 allele in Caucasians with regards to gemcitabine elimination and gemcitabine-associated hematological toxicity. Additionally, high concentrations of intracellular gemcitabine triphosphates were also associated with increased neutrophil toxicity (Fig. 3b), while individual exposure to gemcitabine only had a small impact on neutrophil toxicity (Fig. 3a). Alternatively to polymorphisms of the CDA gene, plasmatic CDA activity has recently been shown to be associated with gemcitabine-associated severe toxicity in 64 patients with solid tumors [5]. However, data on the clinical relevancy of plasmatic CDA activity remain controversial (Sugiyama et al. JCO 2007). The strengths of the present study include the additional bioanalysis of the intracellular active compound gemcitabine triphosphate, the compartmental modeling of the concentration–time data of gemcitabine and metabolites, and the prospective inclusion of a homogenous population with all patients receiving equally dosed gemcitabine in combination with cisplatin. The gemcitabine/cisplatin doublet is frequently used in patients with lung and bladder cancer. Limitations of the study include the relatively low number of patients for performing a population PK analysis, with the potential for false-negative or false-positive results. Nevertheless, the final model performed reasonably good, supported by adequate data fit. The second important limitation of the study is the fact that plasmatic CDA activity was not analyzed, due to the fact that the potential clinical relevancy of plasmatic CDA activity was unclear at the time of study conception. In conclusion, the mutant CDA*2 allele results in an increased exposure to gemcitabine in Caucasian patients, but the present study gives no definite conclusion on the clinical relevance of this finding. Further studies should look into the relationship between CDA genotypes, plasmatic CDA activity, and clinical outcome in patients receiving gemcitabine-based chemotherapy.

Acknowledgments M. Joerger is supported by a fellowship grant funded by the European Society of Medical Oncology, by a Novartis-UICC Translational Cancer Research Fellowship funded by Novartis AG, and by a research grant from the Swiss National Science Foundation (PBBSB-102331).

Conflict of interest None of the authors has any former or present conflict of interest related to this study.

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