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Pharmacokinetics and Pharmacogenetics of liposomal cytarabine in AML patients treated with CPX-351

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Running Title: CDA status impacts PK of liposomal Cytarabine

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Highlights:

- We studied pharmacokinetics and pharmacogenetics of CPX-351 in 9 leukemia patients.
- Release rate of free cytarabine from the liposome was greater than expected.
- Liposomal nanoparticles were transiently observed in patient's bone marrow.
- Cytarabine concentration in bone marrow was higher than in plasma.
- Cytidine deaminase status influences both pharmacokinetics and safety of CPX-351.

Keywords : liposomal cytarabine; cytidine deaminase, pharmacokinetics, drug release; modelling

Abstract

CPX-351 is a liposome encapsulating cytarabine and daunorubicin for treating Acute Myeloid Leukemia (AML) patients. To what extent differences in cytidine deaminase (CDA) activity, the enzyme that catabolizes free cytarabine in the liver, can affect the pharmacokinetics of liposomal cytarabine as well, is unknown.

We have studied the pharmacokinetics (PK) of released, liposomal and total cytarabine using a population-modeling approach in 9 adult AML patients treated with liposomal CPX-351. Exposure levels and PK parameters were compared with respect to the patient's CDA status (i.e., Poor Metabolizer (PM) vs. Extensive Metabolizer (EM)).

Overall response rate was 75%, and 56% of patients had non-hematological severe toxicities, including one lethal toxicity. All patients had febrile neutropenia. A large (>60%) inter-individual variability was observed on pharmacokinetics parameters and subsequent drug levels. A trend towards severe toxicities was observed in patients with higher exposure of cytarabine. Results showed that liposomal CPX-351 led to sustained exposure with reduced clearance (Cl = 0.16 L/h) and prolonged half-life ($T_{1/2}$ = 28h). Liposomal nanoparticles were observed transiently in bone marrow with cytarabine levels 2.3-time higher than in plasma. Seven out of 9 patients were PM with a strong impact on the PK parameters, i.e., PM patients showing higher cytarabine levels as compared with EM patients (AUC: 5536 vs. 1784 ng/ml.h), sustained plasma exposure ($T_{1/2}$: 33.9 vs. 13.7h), and reduced clearance (Cl: 0.12 vs. 0.29 L/h).

This proof-of-concept study suggests that CDA status has a major impact on cytarabine PK and possibly safety in AML patients even when administered as a liposome.

Introduction

Cytarabine and daunorubicin are the mainstay for treating Acute Myeloid Leukemia (AML). Those drugs are given following the canonical “7+3” regimen. To improve efficacy of this combination and reduce its side-effects, CPX-351 (Vyxeos®), a liposomal nanoparticle co-encapsulating both drugs in a 5:1 molar ratio, has been developed and recently approved for treating AML patients. This newly formulated combination is expected to enhance pharmacokinetics (PK) parameters and better target bone marrow. The high affinity of liposomal sphingolipids for proliferative myeloblasts should increase the antiproliferative efficacy of the encapsulated drugs, while sparing healthy cells, thus reducing systemic toxicity [1]. Registration studies with CPX-351 showed that plasma elimination half-life of cytarabine is prolonged as compared with standard drug, suggesting a reduced clearance [1]. We have previously demonstrated that CDA deficiency, a condition leading to impaired ability to detoxify nucleoside analogs in the liver, was associated with marked changes in the PK of standard cytarabine, with subsequent increased risk for severe/lethal toxicities but a trend as well towards prolonged survival [2]. Of note, in a previous study we observed that incidence of CDA deficiency was markedly higher in patients with blood disorders (i.e., 48%) than in patients with solid tumors (13.9%) or patients with no cancers (6.6%) [3]. This higher incidence of CDA deficiency in patients with leukemia calls for further investigating to what extent differences in CDA activity could have an influence in leukemia patients treated with nucleoside analogs. Liposomal drugs are expected to skip at least partly liver metabolism [4], but to what extent differences in CDA status should thus have a limited impact on the PK of liposomal cytarabine in CPX-351 patients remains to be demonstrated. In this proof-of-concept study, we have performed full PK monitoring of cytarabine in adult AML patients treated with the CPX-351 nanoparticles, to evaluate whether CDA has an influence on liposomal cytarabine clearance and subsequent exposure levels among individuals.

Material & Methods

Patients

This was an ancillary study to the clinical trial registered as EUDRACT #2017-A00070-53 on cytarabine pharmacogenetics and pharmacokinetics in adult patients with AML. The study was performed at La Conception University Hospital of Marseille, France, in accordance with the Declaration of Helsinki and the principles of Good Clinical Practices. Ethical Committee approval (*Comité de Protection des Personnes - Sud Méditerranée*, application file registered as 17.005), ANSM French health authority, and signed informed consents were obtained prior starting this study. Eligibility criteria was only to be ≥ 18 years old and be scheduled for liposomal CPX-351 for a newly diagnosed AML with myelodysplasia-related changes (AML-MRC) or therapy-related AML (AML-t). There were no exclusion criteria.

Treatment

Liposomal CPX-351 was administered according to drug label recommendations. For first induction cycles of treatment, equivalent of daunorubicin 44 mg/m² and cytarabine 100 mg/m² were administered via intravenous infusion over 90 minutes on day 1, 3 and 5.

Endpoints

The primary objective of this ancillary study was to estimate individual PK parameters and derive cytarabine systemic exposure parameters (i.e., C_{max} , C_{trough} , and AUC_{0-inf}) of patients treated with CPX-351 during the induction phase, with respect to CDA status. The secondary objectives were to estimate the accumulation of liposomal cytarabine in bone marrow and to tentatively associate PK parameters of liposomal cytarabine with clinical outcome.

Clinical outcome

Response was evaluated primarily by bone marrow examination. Complete remission (CR) was defined as bone marrow blasts <5%; absence of circulating blasts and blasts with Auer rods; absence of extramedullary disease; absolute neutrophil count $>1.0 \times 10^9/L$; platelet count $>100 \times 10^9/L$. Complete remission with incomplete hematologic recovery (CRi) was defined as all CR criteria except for residual neutropenia [$<1.0 \times 10^9/L$] or thrombocytopenia [$<100 \times 10^9/L$]. Treatment-related toxicities were monitored and graded using standard CTCAE-4 guidelines [5].

CDA status determination

CDA activity was measured from serum using an ex-vivo enzymatic test based upon spectrophotometric method [6]. Results were expressed as U/mg of serum proteins. Patients were subsequently categorized as Poor Metabolizer (PM, i.e., CDA < 2 U/mg), Extensive Metabolizer (EM, i.e., CDA ≥ 2 U/mg) or Ultra-Rapid Metabolizer (UM, i.e., CDA > 6 U/mg)[2]. When informed consent for germinal genomic analysis was obtained, *CDA* gene was sequenced. Genomic DNA was isolated using blood sample collected in EDTA-tubes before administration of CPX-351, using QIA Symphony SP/AS (Qiagen France). Resulting DNA was quantitated, and purity checked using the NanoDrop TM 1000 spectrophotometer (Thermo Fisher Scientific, France). DNA samples were stored at -20°C in Tris-EDTA buffer until sequencing. Primers were designed for four exons of the *CDA* gene (data not shown) used for the PCR amplification. The human genome GRCh37/hg19 was used as a reference sequence. Sequencing was performed by SANGER method using the 3500 Dx Genetic Analyzer (Thermo Fisher Scientific, France). All sequences were next analyzed using the Variant Reporter Software 2.0.

PK blood sampling and bioanalysis

Blood for cytarabine assay was collected during the first induction phase on Day-5 90 minutes before the 3d administration, then 4, 6, 8, 24, 48, 72, 96 and 168 hours after administrating CPX-351. To separate released and encapsulated cytarabine, plasma was

filtered on Vivaspin® filters (Sartorius France), using an ultracentrifuge (Beckman Coulter France). Both free and encapsulated cytarabine plasma levels were next measured separately using a validated high-performance liquid chromatography with tandem mass spectrometry detection method (LC-MS/MS) [7]. Limit of Quantification was 1 ng/mL, and both precision and accuracy were below 15%. For assaying encapsulated cytarabine, liposomes were first destroyed by vortexing in methanol. Resulting solution was next 1/100 diluted in MilliQ water, and 100µl were further proceeded prior to LC-MS/MS analysis as previously described. Total cytarabine for each time-point was calculated as the sum between free and encapsulated cytarabine. Plasma concentrations were expressed as ng/mL. When myelogram was available on D15 or D30 for blast count, concentration of total cytarabine in the bone marrow was measured as well using the same LC-MS/MS technique.

Bone Marrow search for liposomes

Optical microscopy-analysis was performed on available bone marrow collected on D15 or D30 to search for liposomal nanoparticles. May-Grünwald Giemsa (MGG) staining was performed according to the manufacturer's instructions (Merck, France) and lipid staining was performed using Oil Red O kit (Biognost, Belgium) according to the manufacturer's instructions. Briefly, slides were fixed in 4% Formaldehyde and, after washing, slides were stained in Oil Red reagent working solution for 10 minutes. Nuclei were counterstained in Hematoxylin. Bone marrow previously collected from leukemia patients who were not treated with CPX-351 were used as negative control.

Evaluation of the predictive performance of cytarabine PopPK models

Model-based identification of PK parameters was performed on total cytarabine only since no population parameters were made available in the literature for free cytarabine released from liposomes or for liposomal cytarabine only. With respect to the small number of blood

samples made available, compartmental analysis for those two forms was not possible. Only two published popPK models were available for total cytarabine: the Nikanjam model, a one-compartment model fitted on PK data from a phase I study [8], and the Wang model, a two-compartment model built from pooled data from 3 clinical studies [9]. Model-based predictions of concentration were performed on Monolix® 2019R2 software (Lixoft, France) [10], taking into account patient demographics, biology data and PK observations, using a Bayesian data assimilation approach. Post-processing of Monolix® output was performed using R Statistical software (R Project, Austria)[11]. Selecting the best model included prediction- and simulation-based diagnostics through both numerical metrics and graphical methods as proposed by Sheiner and Beal [12]. The best model was further evaluated by *Simulation-based diagnostics*, which consisted in a normalized prediction distribution errors (NPDEs) analysis. The internal dataset was simulated 500 times with the inclusion of the inter-individual and residual unexplained variabilities. Diagnostic graphs and statistic tests were performed to examine whether the NPDE data followed a standard normal distribution.

Individual parameters estimation

Bayesian PK parameters estimates for each patient were calculated and used to derive individual concentration-time profiles for total cytarabine. Non-compartmental analysis was performed using PKanalix software (version 2019R2, Lixoft SAS, Antony, France) [13]. Maximum observed plasma concentration (i.e., C_{max}) and residual plasma levels on day 5 (i.e., C_{trough}) were determined directly from the individual data. Cumulative area under the plasma concentration-time curve from time zero to infinity (i.e., AUC_{0-inf}) was derived from the clearance and the dose. Regarding free cytarabine (i.e., released from the liposomes) and liposomal cytarabine, AUC was evaluated using trapezoidal rule, because no PK model for released cytarabine or encapsulated cytarabine was made available to estimate

individual parameters. The resulting AUC (AUC_{tau}) was truncated (i.e., from 96 to 144h) following recommendations from Wang et al. [9].

Statistical Considerations

With respect to the small sample size (9 patients), all statistical analysis were performed using Wilcoxon Exact test non-parametric testing on R Statistical software (R Project, Austria)[11].

Results

Patients' characteristics and CDA status

Nine adult patients hospitalized in La Conception University Hospital of Marseille, France, between January 2019 and January 2020, and treated with CPX-351 were recruited. This subset of patients (6F/3M) was 66 ± 13 years old (range: 37-76). Patients' characteristics are summarized in Table 1. Mean CDA value was 1.5 ± 1.7 U/mg. Seven patients were subsequently categorized as CDA deficient or poor-metabolizer (PM, mean CDA = 0.92 ± 0.51 U/mg) whereas two patients were considered as extensive-metabolizer (EM, mean CDA = 4.43 ± 2.0 U/mg). No ultra-rapid metabolizer (UM) patient was found.

Table 1. Baseline patients' characteristics

Characteristic	Overall, N = 9 ¹	CDA status ²		p-value ³
		EM, n = 2 ¹	PM, n = 7 ¹	
Age (years)	64 ± 12 (38, 77)	73 ± 4 (70, 76)	61 ± 12 (38, 77)	0.30
Body Weight (kg)	66 ± 9 (55, 79)	66 ± 15 (55, 76)	66 ± 8 (58, 79)	0.89
Body surface area (m ²)	1.74 ± 0.11 (1.59, 1.95)	1.73 ± 0.19 (1.59, 1.86)	1.75 ± 0.11 (1.65, 1.95)	0.89
Total bilirubin (µmol/L)	10.0 ± 7.1 (5.0, 28.0)	5.5 ± 0.7 (5.0, 6.0)	11.3 ± 7.7 (6.0, 28.0)	0.076
Aspartate amino transferase (IU/L)	26 ± 23 (10, 81)	46 ± 50 (10, 81)	20 ± 12 (11, 40)	>0.99
Alanine amino transferase (IU/L)	30 ± 29 (6, 75)	34 ± 33 (11, 57)	29 ± 30 (6, 75)	0.88
Gamma-glutamyl transferase (IU/L)	84 ± 118 (12, 385)	34 ± 26 (16, 53)	98 ± 133 (12, 385)	0.66
Alkaline Phosphatase (IU/L)	77 ± 32 (39, 148)	80 ± 1 (79, 80)	77 ± 37 (39, 148)	0.89

Albumin (g/L)	33.5 ± 5.5 (23.2, 42.1)	31.0 ± 1.9 (29.6, 32.3)	34.2 ± 6.0 (23.2, 42.1)	0.33
C-Reactive Protein (mg/L)	65 ± 50 (2, 115)	106 ± 14 (96, 115)	53 ± 51 (2, 115)	0.22
Serum creatinine (μmol/L)	66 ± 28 (28, 124)	56 ± 40 (28, 85)	69 ± 28 (43, 124)	0.89
Glomerular filtration rate (mL/min/1.73m²)⁴	93 ± 25 (55, 119)	87 ± 41 (58, 116)	95 ± 22 (55, 119)	>0.99
Cytidine deaminase activity (UA/mg)	1.70 ± 1.77 (0.39, 5.89)	4.43 ± 2.06 (2.98, 5.89)	0.92 ± 0.51 (0.39, 1.87)	0.056

¹ Statistics presented: Mean ± SD (Range) or Frequency (%)

² PM, poor metabolizer; EM, extensive metabolizer; based on a CDA cutoff value of 2

³ Wilcoxon rank sum test; Wilcoxon rank sum exact test

⁴ eGFR was calculated according to CKD-EPI formula.

Specific informed consent required by French Biomedicine Agency to perform germline analysis was obtained in 7 out of 9 patients

Only 7 out of 9 patients provided specific informed consent for germinal genotyping and had subsequently their *CDA* gene sequenced. Results of *CDA* gene sequencing in those 7 patients are provided in Table 2. A total of 9 exonic and intronic SNPs were observed (i.e., c.-92A>G (rs602950), c.-31delC (rs3215400) and c.79A>C (rs2072671) in the first exon, c.435C>T (rs1048977) in the fourth exon, and c.267-77T>A (rs488991), c.324+71T>C (rs3738130), c.325-56G>A, (rs77158575), c.325-36G>A, (rs61781969) and c.325-23C>T (rs61781970) in intron). No difference in *CDA* genotypes was observed between EM and PM patients.

Table 2. Results from *CDA* sequencing

ID	CD A status	rs602950	rs3215400	rs2072671	rs488991	rs3738130	rs77158575	rs61781969	rs61781970	rs1048977
1	PM	AA	C-	AA	AA	TC	GA	GA	CT	CT
2	PM	AG	C-	AC	AA	TT	GG	GG	CC	CC
4	EM	AG	--	AC	AA	TT	GA	GA	CT	CT
5	EM	AG	--	CC	AA	TT	GG	GG	CC	CC
7	PM	AG	C-	AC	AA	TT	GG	GG	CC	CC
8	PM	AA	CC	AA	AA	TT	GG	GG	CC	CC
9	PM	AA	C-	AA	AA	TC	GA	GA	CT	CT

Abbreviation: ID, identifier; CDA, Cytidine Deaminase.

Cytarabine Plasma concentrations.

Plasma concentrations of total cytarabine, liposomal cytarabine and released cytarabine are displayed in Figure 1. The T_{max} of released cytarabine was delayed as compared with liposomal drug (C_{max} observed at 110h, i.e., 14h after the 3rd administration). Inter-patient variability ranged from 47 to 75% for released cytarabine concentrations, from 14 to 90% for liposomal cytarabine concentrations and from 31 to 74% for total cytarabine concentrations, depending on the sampling time (i.e., from -90 min to 168 h after the 3rd injection).

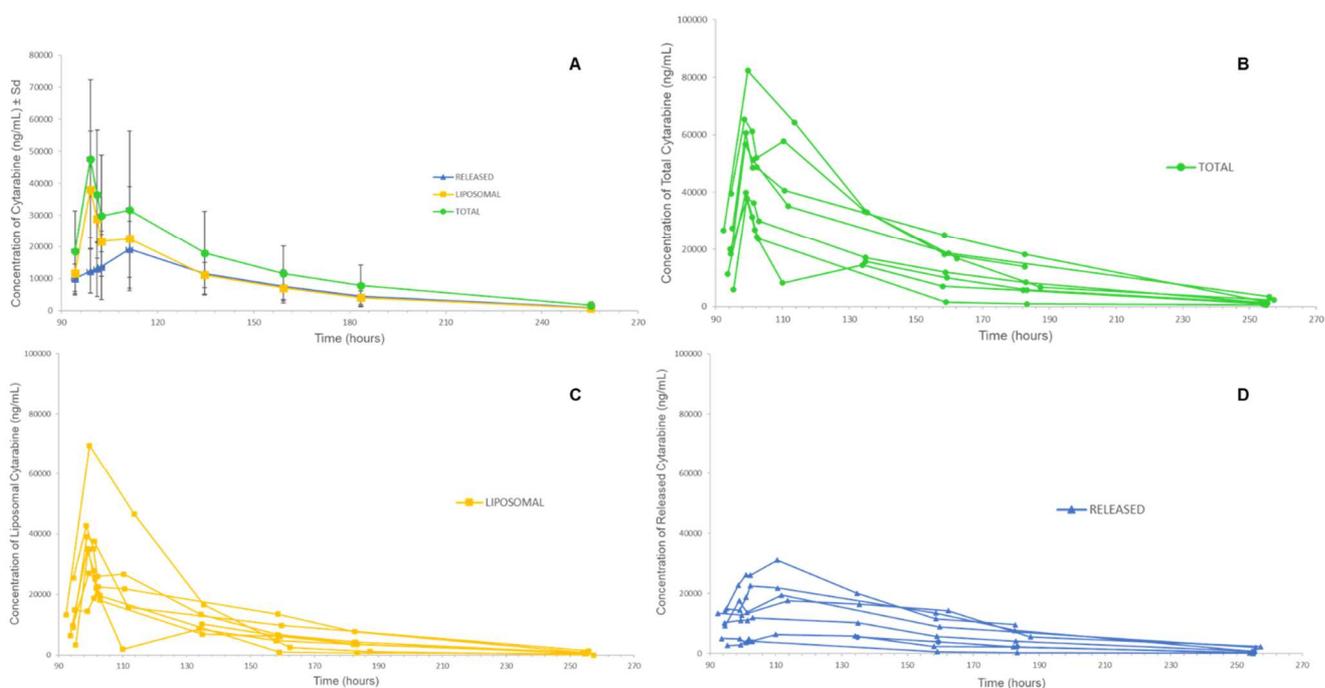


Figure 1. Mean concentration-time profiles of total, liposomal and released cytarabine after the 3rd injection of CPX-351. Administration started at T96h. 1A: mean values 1B: individual profiles for total cytarabine. 1C: individual profiles for liposomal cytarabine. 1D: individual profiles for released cytarabine.

Determination of plasma exposure to free cytarabine and liposomal cytarabine

Because no popPK parameters were made available, it was not possible to identify the PK parameters of released cytarabine and liposomal cytarabine. Exposure levels were therefore estimated using graphical tools such as the trapezoidal rule.

Mean AUC (\pm SD) for free cytarabine was 682 ± 403 h.mg/L and mean AUC for liposomal cytarabine was 942 ± 305 h.mg/L.

Cytarabine bone marrow concentrations

Myelogram on D15 was available only for a single patient, all other patients being sampled on D30. Total cytarabine concentration measured in the bone marrow of this patient was 31 697 ng/mL. Plasma concentration for the same patient sampled at the same time was 13 876 ng/mL, i.e., a 2.3-times difference. Myelograms collected on D30 had all cytarabine levels below the limit of quantitation, including for the patient who had cytarabine in his bone marrow on Day-15 (data not shown).

Bone Marrow search for liposomal nanoparticles

Bone marrow smears revealed aplastic bone marrow with macrophages and plasma cells on Day 15. A small contingent of macrophages with empty cytoplasmic round vacuoles with heterogenous size were observed, and subsequent Oil Red O staining revealed lipidic nanoparticles in these macrophages, as well as many extracellular lipids. Bone marrow smears collected on Day 30 from the same patient presented no such lipidic inclusions anymore. Samples collected from patients who were not treated with CPX-351 and used as negative control showed no such lipidic inclusions (Figure 2).

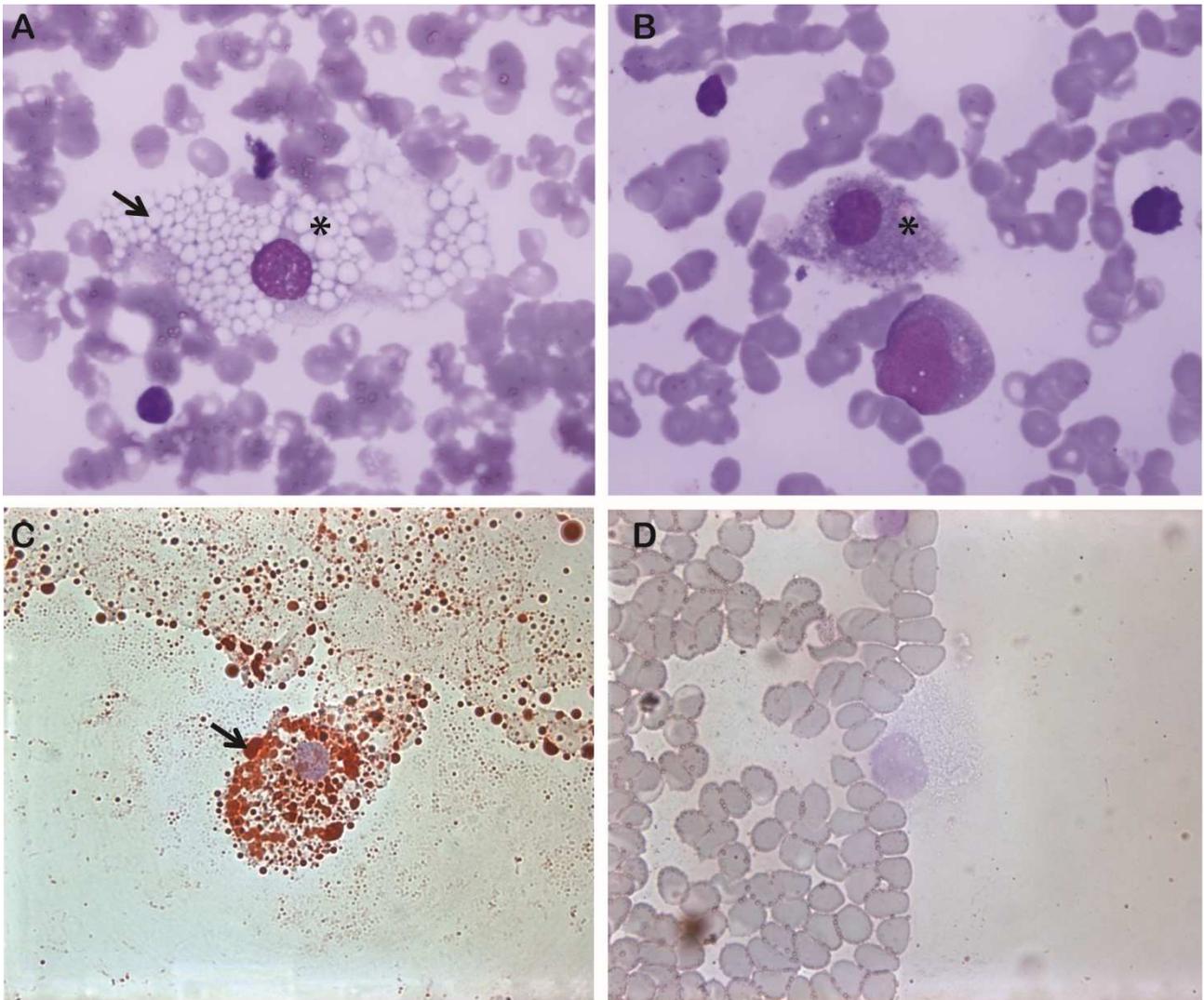


Figure 2. Optical microscopic-analysis (x100) performed after MGG (Figure 2A and 2B) and Oil Red O Kit (Figure 2C and 2D) colorations of bone marrow. Figure 2A and 2C: bone marrow sampled on D15 after CPX-351 administration. Figure 2B: same patient sampled on D30. Figure 2D: negative control (bone marrow from a patient not treated with CPX-351). On Day15, lipidic inclusions were observed in CPX-351 patient (2A, 2C) whereas no such lipidic inclusion was observed anymore on Day30 (2B). Similarly, no such lipidic inclusions were observed in patients not treated with CPX-351. Arrow: lipidic inclusion. Asterisk: macrophage.

Abbreviation: MGG, May-Grünwald Giemsa

Determination of Individual PK parameters and plasma exposure of total cytarabine

Comparative testing of the Nikanjam [8] and the Wang [9] models was done and showed that the Nikanjam's model had superior predictive performances. For instance, Bayesian predictions showed a small bias (mean prediction error of 5%) and the percentage of predicted values within $\pm 15\%$ interval of the observed concentrations (F15) was 60.3% for Nikanjam's model. Moreover, frequency histogram and Q-Q plot of NPDEs confirmed a normal distribution around each individual observation within the predictions of the model with a p-value, for Shapiro-Wilk normality test, higher than the predefined significance level (> 0.05). Individual PK parameters for total cytarabine plus mean parameters were therefore identified using the Nikanjam model [8] and are presented in Table 3.

Table 3. Individual Bayesian estimates and summary statistics of total cytarabine pharmacokinetic parameters and estimated exposures during induction cycle 1, following the FDA-approved dosing regimen^a.

Individual Parameters									
ID	Dose (mg)	Observed C _{trough} (mg/L)	CL (L/h)	V _c (L)	Predicted C _{trough} (mg/L)	C _{max} (mg/L)	AUC _{tau} (h.mg/L)	AUC _{0-inf} (h.mg/L)	t _{1/2} (h)
1	166	11.33	0.167	6.65	10.6	34.0	969.4	2990	27.7
2	168	19.95	0.125	6.34	15.1	39.9	1258	4024	35.2
3	178	27.36	0.083	5.27	23.9	56.1	1912	6410	43.9
4	183 ^a	-	0.377	6.42	-	-	-	971	11.8
5	158	5.94	0.211	4.76	4.7	35.8	740.4	2237	15.6
6	170	18.29	0.093	5.02	20.4	52.0	1683	5453	37.2
7	195	26.65	0.081	3.11	25.9	81.0	2345	7256	26.6
8	176	39.58	0.074	4.24	26.8	65.9	2178	7142	39.8
9	165	-	0.147	5.71	-	38.4	1084	3377	26.9

Mean Parameters		
PK Parameter	Value, Mean (CV%, n)	Median (min - max)
Observed C _{trough} (mg/L) ^b	21.3 (52.3%, n = 7)	20.0 (5.94 - 39.6)
CL (L/h)	0.151 (63.9%, n = 9)	0.125 (0.074 - 0.377)
V _c (L)	5.28 (21.8%, n = 9)	5.27 (3.11 - 6.65)
Predicted C _{trough} (mg/L)	15.6 (58.8 %, n = 9)	15.1 (1.7 - 26.8)
C _{max} (mg/L)	48.0 (35.6 %, n = 9)	39.9 (29.3 - 81.0)
AUC _{tau} (h.mg/L)	1404 (46.7 %, n = 9)	1258 (469.0 - 2345)
AUC _{0-inf} (h.mg/L)	4471 (48.7 %, n = 9)	4024 (1347 - 7256)
t _{1/2} (h)	29.4 (36.7%, n = 9)	27.7 (11.8 - 43.9)

Abbreviations: **C_{trough}**, trough plasma concentration on day 5; **CL**, systemic clearance; **V_c**, central volume of distribution; **C_{max}**, maximum observed plasma concentration; **AUC_{tau}** = AUC_{96-144h}, area under the plasma concentration-time curve from time 96 to 144 hours; **AUC_{0-inf}**, cumulative area under the plasma concentration-time curve from time zero to infinity; **t_{1/2}**, half-life.

^a Except for patient 4 (who received the dose on days 1 and 3 only), all patients received their doses on days 1, 3 and 5 of the treatment cycles.

^b Observed and estimated C_{trough} are defined by concentration of cytarabine measured 4 hours at maximum before the 3rd administration (from 92 to 96 hours after the initiation of treatment)

The estimated means for CL and Vc were 0.164 L/h and 5.39 L, respectively. Mean $AUC_{0-\infty}$ was 4465 h.mg/L. Inter-individual variability (IIV) expressed as CV% ranged between 20 and 61%, depending on the parameters. Comparison of median observed C_{trough} (19.1 mg/L) and predicted C_{trough} (17.9 mg/L) showed close values, thus confirming the good performance of the Bayesian predictor. Figure 3 displays the fit between simulated concentrations and measured concentrations of total cytarabine.

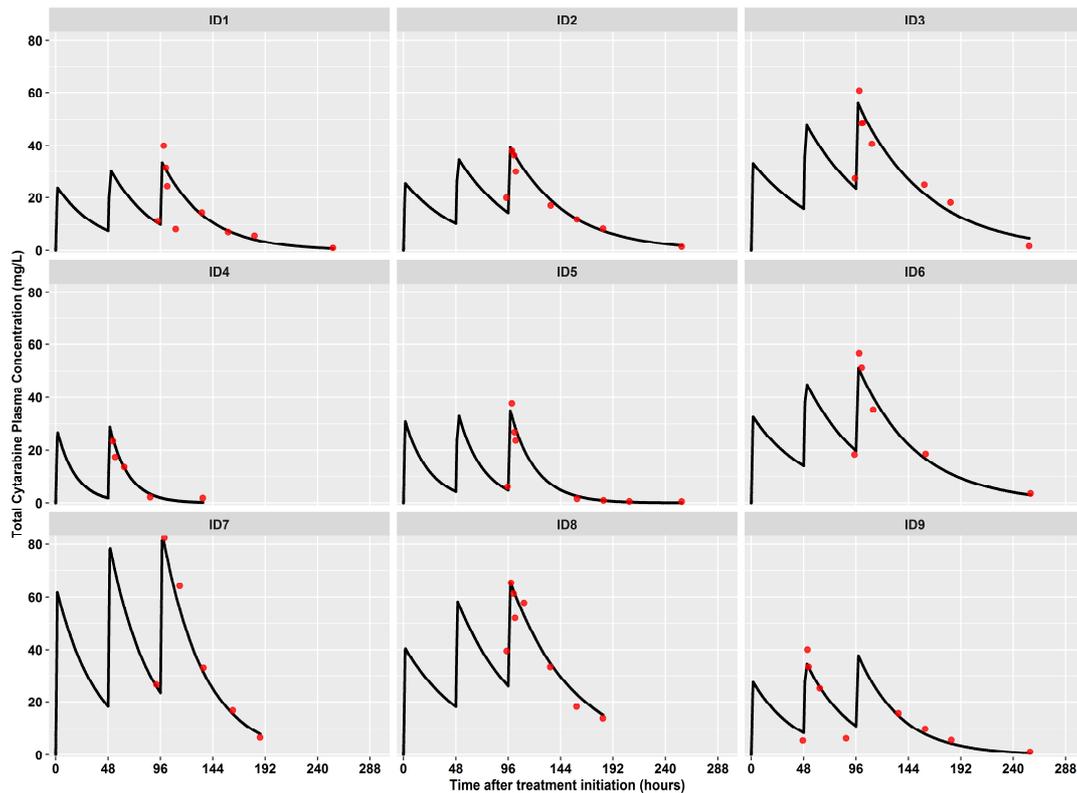


Figure 3. EBE-derived individual concentration-time profiles of total cytarabine. Red points represent PK observations.

Influence of CDA status on the PK of cytarabine after CPX-351 administration

Although only two patients were categorized as EM on the CDA status, marked differences were observed between those patients and the rest of the subset when considering cytarabine PK parameters and exposure levels. As compared with PM patients, total cytarabine clearance was 142% higher in the EM patient (0.29 ± 0.12 vs. 0.12 ± 0.04 L/h, $p= 0.055$ Wilcoxon Exact test), plasma half-life was 60% shorter (13.7 ± 2.7 vs. 33.9 ± 6.9 h

$p= 0.055$ Wilcoxon Exact test), and exposure was consequently markedly reduced with 77% lower trough level (4.7 vs. 20.4 ± 6 L/h, $p= 0.071$ Wilcoxon Exact test), 66% lower AUC_{0-inf} (1784 ± 895 vs. 5536 ± 1784 h.ng/mL $p=0.055$ Wilcoxon Exact test), respectively. Figure 4 shows PK parameters and exposure levels depending on CDA status (i.e., PM vs. EM) for total cytarabine. The ratio: released/total cytarabine in plasma based upon respective their AUC's was 47% in PM patients and 18 % in EM patients. Of note, whereas inter-individual variability on clearance was 63% for the whole patients, this variability was reduced to 33 and 39% when considering PM and EM subsets, respectively.

Regarding free and liposomal cytarabine, plasma exposure (AUC and C_{max}) could not be determined for patient 4 and 9 due to missing samples. Free cytarabine AUC and C_{max} were 772 ± 356 h.ng/mL and 18.1 ± 8.6 mg/L, respectively, among PM patients. In EM patient, free cytarabine AUC and C_{max} were 144 h.ng/mL and 4.87 mg/L. Similarly, CDA PM patients showed higher exposure levels for the encapsulated form as compared with EM patients (988 ± 307 h.ng/mL vs. 668 h.ng/mL).

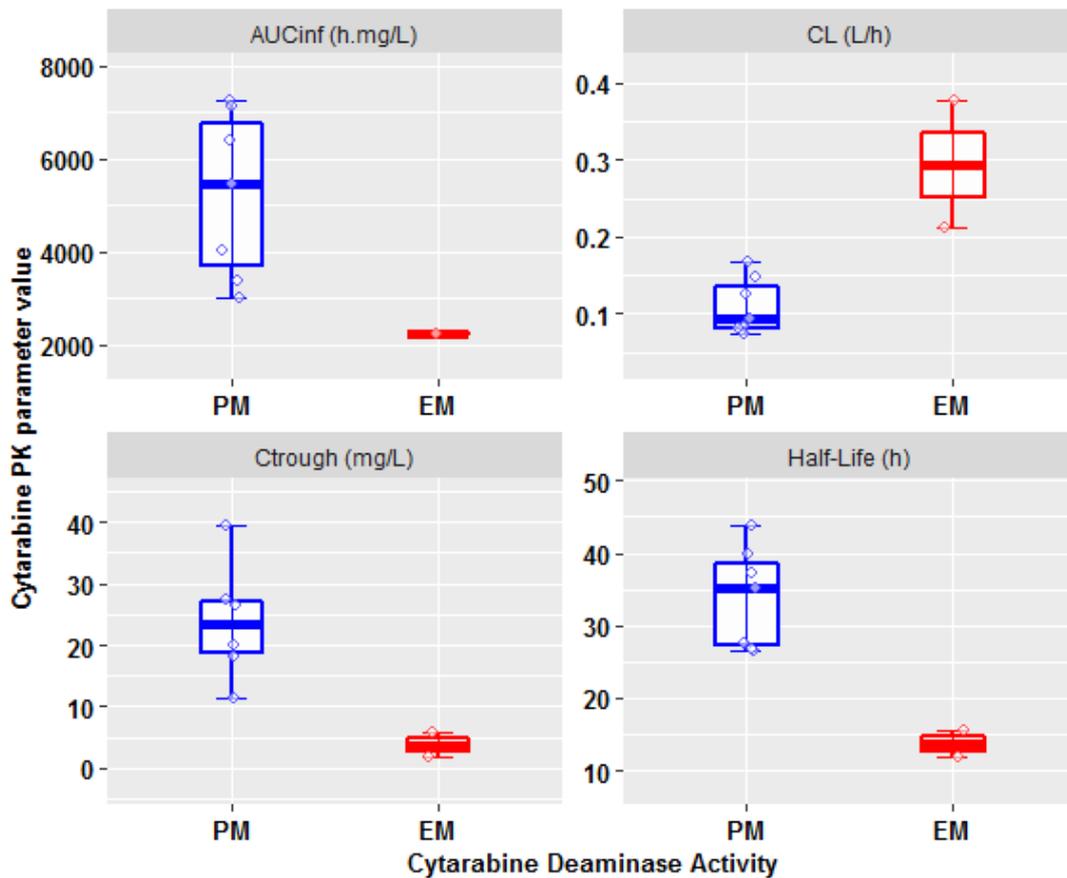


Figure 4. Exposure (AUC, trough levels) and PK parameters (Cl, Half-Life) of total cytarabine depending on CDA phenotype in patients.

Abbreviations: PM: poor metabolizer, EM: extensive Metabolizer; Ctrough, trough plasma concentration on day 5; CL, systemic clearance; AUCinf, cumulative area under the plasma concentration-time curve from time zero to infinity. In the box plots, the boundary of the box closest to zero indicates the 25th percentile, a line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile.

Exposure-effect relationships

Individual clinical outcome, both in terms of response and occurrence of toxicities, are summarized in Table 4.

Table 4. Clinical outcome: response and severe toxicities

ID	Efficacy	Grade 3 to 5 AEs (CTCAE 4.0)		
		Number of AEs, <i>n</i>	Non hematological AEs	Hematological AEs
1	CR	1	-	Febrile Neutropenia
2	PD	2	Hemorrhoid	Febrile Neutropenia
3	CR	3	Constipation, Toxic	Febrile

			death	Neutropenia
4	CR	1	-	Febrile Neutropenia
5	PR	2	Nausea	Febrile Neutropenia
6	PD	1	-	Febrile Neutropenia
7 ^a	-	1	-	Febrile Neutropenia
8	CR	3	Anorexia, toxidermia	Febrile Neutropenia
9	CR	4	Toxidermia, rectal bleeding, hemorrhoid	Febrile Neutropenia

SUMMARY DATA

OR	Value, n (%)
CR/PR	6/8 (75%)
PD	2/8 (25%)
Lost to follow-up	1/9 (11%)
Grade 3 to 5 AEs	Value, n (%)
Grade 0-2	9/9 (100%)
Grade 3-5	5/9 (56%)

Abbreviations: OR, Overall response; CR, Complete Response; PR, Partial Response; PD, Progressive Disease

^a Lost to follow-up patients.

One patient (#7) was lost to follow-up and could not be evaluated. In the remaining subset, ORR (i.e., CR + CRi) was 75% (i.e., 6 out of 8 assessable patients). Two patients showing progressive disease (PD) after induction phase failed also to respond next the consolidation cure and died due to complication of the disease. There was no significant difference between responders and non-responders with respect to total cytarabine AUC_{0-inf}, (3855 ±2418 vs. 4739 ±1010 h.mg/L, Wilcoxon-test: p=0.64).

Regarding haematological toxicities, all patients had febrile neutropenia. A trend was observed between higher exposure levels to total cytarabine and non-hematological severe toxicities (C_{trough} 23.2 vs. 18.8 mg/L, AUC_{0-inf} 4638 ±2070 vs. 4167 ±2757 h.mg/L) (Figure 5). One toxic-death was observed (11%) due to colonic obstruction 40 days after initiating CPX-351. This patient was a 55-years old female with no comorbidities with PM phenotype (CDA = 0.64 U/mg, i.e., lower than the mean CDA value of 0.92 U/mg recorded

in PM patients). Consequently, total cytarabine clearance was 0.083 L/h with an estimated half-life of 43.9 days, i.e., the longest elimination half-life of all patients. Model-based estimation of AUC of total cytarabine was 2237 h.mg/L whereas exposures to released cytarabine and liposomal cytarabine estimated by trapezoidal rule were 1027 and 1091 h.mg/L, respectively, in this patient.

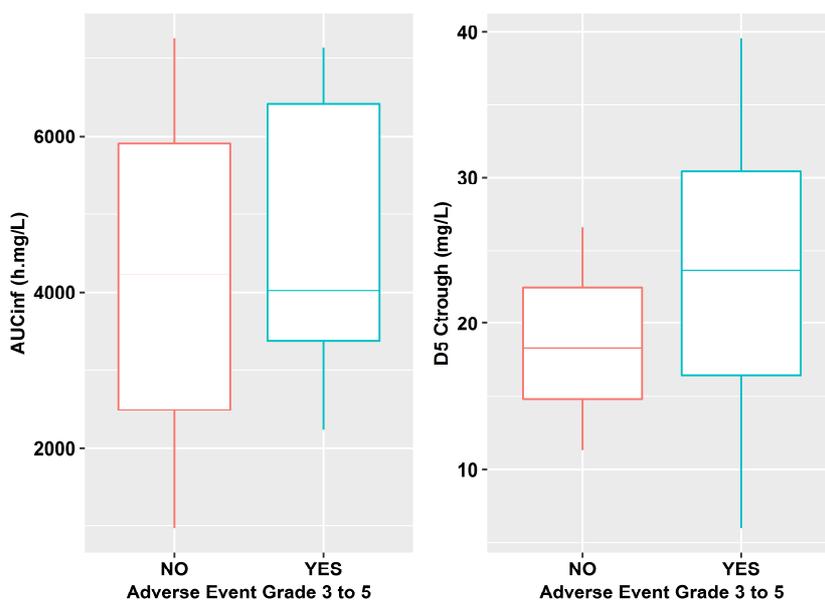


Figure 5. Exposure (AUC, trough levels on Days-5) in total cytarabine in patients with and without non-hematological severe toxicities.

However, with respect to the small sample size and the high inter-patient variability, this difference was not statistically significant (Wilcoxon test for both metrics: $p > 0.05$).

Similarly, exposure-response analysis on released and encapsulated cytarabine exposure levels demonstrated that none of those were predictive of efficacy or severe toxicities (Figure 6). Still for released cytarabine, as for total cytarabine, a trend was observed between exposure and severe toxicities ($AUC_{\tau} = 581 \pm 310$ h.mg/L for patients without severe toxicities vs. 759 ± 492 h.mg/L for patients with severe toxicities). However with respect to the small sample size, this difference was not significant.

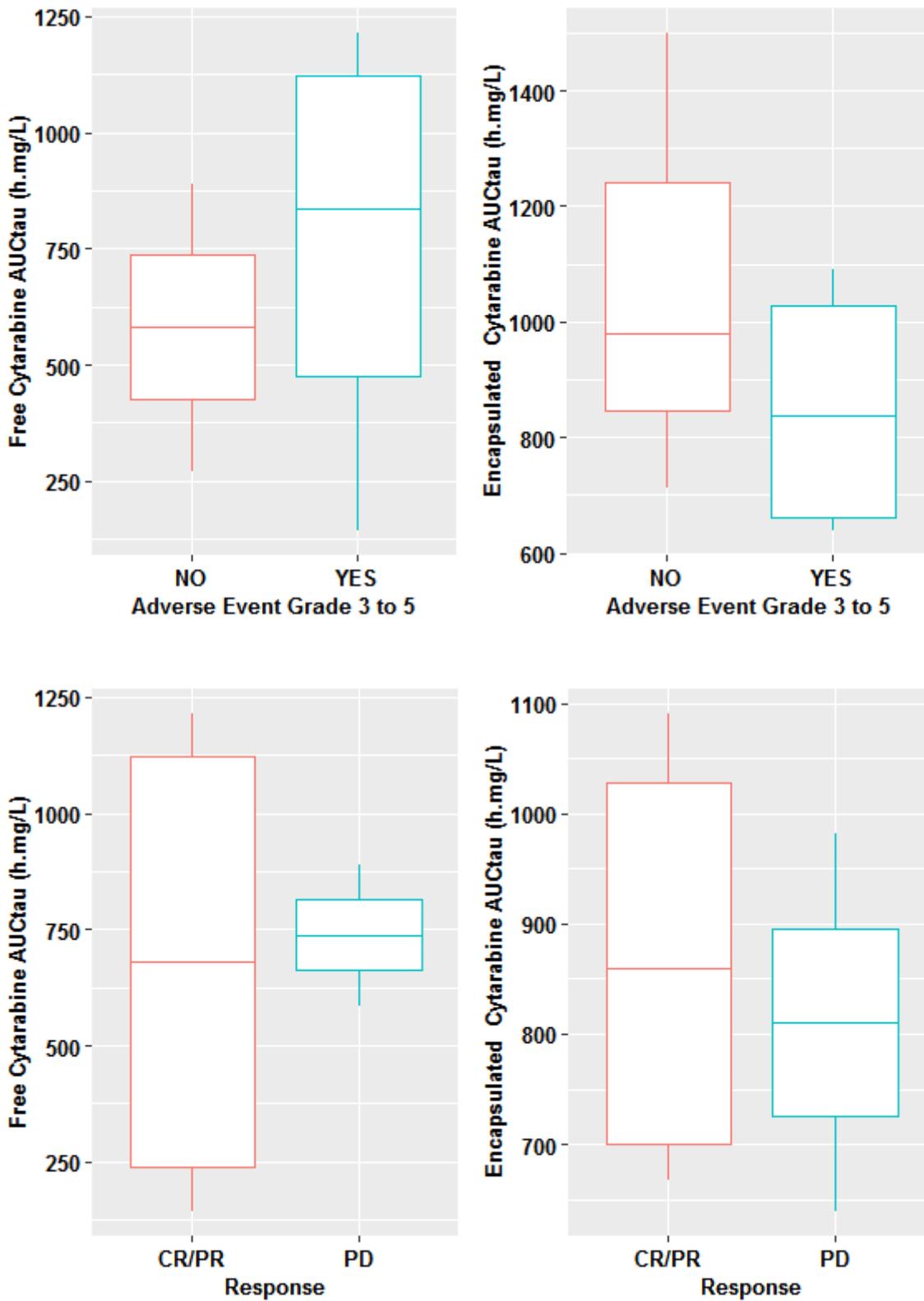


Figure 6. Relationships between exposure to released and liposomal cytarabine and clinical endpoints. Abbreviations: Progressive Disease (PD) and Complete Response/ Partial Response (CR/PR).

DISCUSSION

Nanoparticles represent an appealing strategy to improve the efficacy/toxicity balance in oncology. However, only few entities have made their way from prototyping steps and experimental proof-of-concepts to clinical development, and even fewer of them have been finally approved for clinical use in patients with cancer. In this study, we present model-derived PK parameters of liposomal cytarabine in AML patients treated with liposomal CPX-351. Estimates of PK parameters on total cytarabine are consistent with those previously published with this nanoparticle during registration trials [8]. Here we observed a large inter-patient variability in PK parameters (i.e., up to 63% on clearance values) and exposure levels (i.e., >40% for AUC). This variability is much larger than the 30% variability previously reported with standard cytarabine [14]. This difference is consistent with the fact that liposomal drugs frequently show a higher PK variability than standard drugs, much probably due to differences in patients immunity [15,16]. Our results confirmed the sustained plasma exposure in cytarabine achieved in patients treated with liposomal CPX-351. Indeed, here mean cytarabine clearance was 99.9% lower when administered as liposomes as compared with standard cytarabine (i.e., 0.16 vs. 272 L/h) [8,17]. Similarly, mean plasma half-life of liposomal cytarabine was 28 h, i.e., 9 up to 28-times longer than the half-life of standard cytarabine reported elsewhere [18]. Although limited to a single patient, we have for the first time demonstrated that liposomal nanoparticles could be observed transiently in bone marrow two weeks after treatment by liposomal CPX-351. Quite remarkably, we found that cytarabine concentration in bone marrow on D15 was by far greater than plasma levels (i.e., 2.3-times higher). However, no more liposomes could be observed later (i.e., on D30) and cytarabine was not detectable anymore in bone marrow. Importantly, we found that the mean ratio between released cytarabine and total cytarabine was 47% in our patients, a figure markedly higher than the claims by the manufacturer with CPX-351 (i.e., 20%) [19]. Of note, here no clear PK/PD relationships could be demonstrated because efficacy was not associated with any of the metrics we collected regarding total cytarabine, released cytarabine or encapsulated

cytarabine levels. Non-hematological toxicities only showed a trend towards being more frequent in patients with higher exposures to total cytarabine. Similarly, a relationship was observed between severe toxicities and exposure to released cytarabine, although not statistically significant because of the small sample size.

Although this small number of patients in our study prompt us to be cautious, some new observations are to be highlighted. First, differences in CDA status could strongly influence the fate of liposomal CPX-351 in the body and participate to the large inter-patient variability we observed. CDA is the liver enzyme responsible for the detoxification of several nucleoside analogs widely used to treat a variety of cancers [20]. This enzyme is coded by a gene which is highly polymorphic with resulting impaired activity, possibly worsening clinical outcome with standard cytarabine and 5-azacytidine in leukemia patients [21,22]. Importantly, CDA PM status is a rare syndrome in patients with solid tumors but seems to be much more frequent in patients with hematological disorders [3]. In the present study, 7 out of 9 patients (i.e., 77%) were categorized as PM individuals, a remarkably high incidence probably due to chance since our previous studies showed that PM patients were rather around 50% in leukemia patients [3]. Here no clear genotype-to-phenotype relationships was found with CDA, an observation consistent with previous reports [6]. Still, CDA functional status could play a major role in the PK variability we report here. Indeed, the EM patients had 142% higher Cl values and 66% lower AUC_{0-inf} , with 60% shorter half-life than PM patients. Similarly, higher exposure to released cytarabine and liposomal cytarabine were observed in PM patients as compared with EM patients. Surprisingly, the difference in PK profiles between EM and PM patients was similar to that we observed previously with standard cytarabine [7], thus suggesting that encapsulation into liposomes does not allow CPX-351 to totally skip liver metabolism. Inter-individual variability on clearance was 33% in PM and 39% in EM patients, i.e., lower than the 63% measured in unsorted PM+EM patients. This suggests that differences in CDA status explain a major part of the high variability we observed with CPX-351. Finally,

CDA status also has an influence on the ratio: released/total cytarabine. This ratio was 47% in PM patients but only 19% in EM patients, much probably because the released cytarabine is catabolized much more quickly in EM individuals, making the free form decreasing as soon as it leaks from the nanoparticles. Overall, the high incidence of PM patients associated with higher and sustained plasma exposure plus bone marrow infiltration by lipidic nanoparticles could partly explain why ORR was 75% in our patients, a figure markedly higher than the 47.7% or CR + CRi reported in registration trials [23]. Similarly, this could explain why 100% of our patients had febrile neutropenia, an incidence much higher than the 60% reported with CPX-351 in registration trials [24,25]. Of note, the patient who experienced lethal toxicities was profoundly deficient in CDA with a markedly prolonged half-life of total cytarabine. In addition, in this patient exposure to released cytarabine was slightly higher than liposomal cytarabine (1091 h.mg/L vs. 1027 h.mg.L), thus suggesting that overexposure to the free, active drug, could explain the toxic death. There is a continuous effort today to develop precision medicine in oncology, i.e., by developing personalized dosing or scheduling with anticancer agents, provided that appropriate decision making tools are made available [26]. Despite its limitations due to the small sample size, the present study suggests that CDA phenotype could be used as a baseline biomarker to forecast the clearance of cytarabine in patients treated with CPX-351. Because severe non-hematological toxicities could be associated with PM status, CDA could thus be used to customize dosing *a priori* in frail patients such as elderly patients or patients presenting with several comorbidities, especially when profound deficiency in CDA is detected. Should exposure-response relationships be confirmed in further, larger studies, this could pave the way for CDA-based adaptive dosing strategies in the future. In addition to *a priori* tailoring of CPX-351 dosing, it is possible as well to identify individual PK parameters for total cytarabine using Bayesian estimate, based upon a couple of blood samples. This could be used to further predict plasma exposure over the repetitive administrations of CPX-351.

CONCLUSION

Our study suggests that CDA status could be a major cause for the variability observed in patients treated with liposomal CPX-351, with possible impact on safety. A trend between exposure and severe toxicities, including lethal toxicities, was observed. Further investigations on a larger cohort of patients should help understanding exposure-response relationships with CPX-351.

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