

# Population Pharmacokinetics and Exposure-Response Analyses for CPX-351 in Patients With Hematologic Malignancies

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## Abstract

CPX-351, a dual-drug liposomal encapsulation of cytarabine and daunorubicin at a synergistic ratio, is approved in the United States for adults with newly diagnosed therapy-related acute myeloid leukemia or acute myeloid leukemia with myelodysplasia-related changes. Population pharmacokinetics analyses were performed using nonlinear mixed-effect modeling on pooled data from 3 clinical studies, and the impact of CPX-351 exposures on efficacy and safety was assessed. The pharmacokinetics of cytarabine and daunorubicin were described using 2-compartment models with linear elimination. None of the evaluated covariates had a clinically significant impact on plasma exposure to total cytarabine or daunorubicin, while bilirubin and formulation showed statistically significant effects on pharmacokinetic parameters of cytarabine and daunorubicin, respectively. In patients with mild/moderate renal impairment or serum bilirubin  $\leq 3$  mg/dL, plasma exposures to cytarabine and daunorubicin following CPX-351 were within the variability range for patients with normal kidney function or serum bilirubin levels. Exposure-response analysis demonstrated that better efficacy outcomes were associated with higher CPX-351 exposure quartiles. Early mortality rates in all CPX-351 exposure quartiles were lower vs the 7 + 3 control group, and lower mortality rates were associated with higher exposure quartiles. A trend toward greater frequency of grade 3 treatment-emergent adverse events (but not grade 4/5 events) was observed at higher CPX-351 exposure quartiles. Overall, the population pharmacokinetic analyses indicate no adjustments to the recommended dose and schedule of CPX-351 are warranted for patients with mild/moderate renal impairment or serum bilirubin  $\leq 3$  mg/dL. Results from the exposure-response analyses suggest the current CPX-351 regimen provides a favorable risk-benefit profile.

## Keywords

acute myeloid leukemia, cytarabine, daunorubicin, exposure-response, liposome, pharmacokinetics

Acute myeloid leukemia (AML) is a heterogeneous disease, with cases arising either de novo or developing as secondary AML, which evolves from an antecedent hematologic disorder or as a late complication of chemotherapy or ionizing radiation (therapy-related AML).<sup>1,2</sup> Cytarabine infused continuously for 7 days plus 3 days of an anthracycline, such as daunorubicin (7 + 3 regimen), has been a standard of care for AML induction therapy for decades.<sup>3,4</sup> However, outcomes with conventional induction chemotherapy remain suboptimal, especially among older patients and those with secondary AML.<sup>5,6</sup>

CPX-351 (Vyxeos, Jazz Pharmaceuticals, Inc., Palo Alto, California) is a dual-drug liposomal encapsulation of cytarabine and daunorubicin at a fixed 5 : 1 molar ratio<sup>7,8</sup> that was approved in 2017 by the US Food and Drug Administration for the treatment of adults with newly diagnosed therapy-related AML or AML with myelodysplasia-related changes.<sup>9</sup> Because cytarabine and daunorubicin have different pharmacokinetic profiles, the ratio between the 2 drugs can vary substantially over time when given as nonliposomal formulations.<sup>10</sup> The combined cytotoxic effects of cytarabine and daunorubicin could be synergistic, additive, or antagonistic.<sup>11</sup> CPX-351 liposomes were

designed to coordinate the release of cytarabine and daunorubicin, allowing the synergistic drug ratio of 5 : 1 to be maintained and delivered to leukemia cells. Additionally, in murine models, CPX-351 is taken up by leukemia cells to a greater extent than by normal bone marrow cells.<sup>7</sup>

Clinical antileukemia activity was observed in AML patients in a phase 1 study that established the recommended induction dose of daunorubicin 44 mg/m<sup>2</sup> and cytarabine 100 mg/m<sup>2</sup> over a 90-minute infusion on days 1, 3, and 5.<sup>12</sup> This dose and schedule were used in the subsequent phase 2 and phase 3 studies. In a randomized phase 2 study of patients aged 60 to

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75 years with newly diagnosed AML, higher remission rates were observed with CPX-351 (67%) compared with the 7 + 3 active control regimen (51%) in the overall study population ( $P = .07$ ), with improved median overall survival demonstrated in a subgroup analysis of patients with secondary AML (12.1 and 6.1 months, respectively; hazard ratio, 0.46;  $P = .01$ ).<sup>13</sup> Based on these results, a randomized phase 3 study was conducted to compare the efficacy and safety of CPX-351 with conventional 7 + 3 treatment in patients 60 to 75 years of age with newly diagnosed high-risk/secondary AML. In this pivotal study, treatment with CPX-351 significantly prolonged overall survival vs 7 + 3 (9.56 vs 5.95 months, respectively; hazard ratio, 0.69; 1-sided  $P = .003$ ).<sup>14</sup> When CPX-351 was compared with 7 + 3, event free survival (2.53 vs 1.31 months, respectively; hazard ratio, 0.74; 2-sided  $P = .021$ ) and rates of complete remission plus complete remission with incomplete recovery of platelets and neutrophils (47.7% vs 33.3%, respectively; 2-sided  $P = .016$ ) were also significantly improved. CPX-351 also enabled a greater proportion of patients to subsequently undergo hematopoietic cell transplantation compared with 7 + 3 (34.0% vs 25.0%; 2-sided  $P = .097$ ); an exploratory analysis of survival, landmarked from the time of hematopoietic cell transplantation, demonstrated a benefit with CPX-351 (hazard ratio, 0.46; 95% confidence interval [CI], 0.24–0.89; 1-sided  $P = .009$ ).<sup>15</sup> The safety profile of CPX-351 was generally consistent with that of 7 + 3, and early mortality rates appeared lower with CPX-351.<sup>14</sup>

Based on clinical pharmacokinetics data from phase 1, 2, and 3 studies and a population pharmacokinetics analysis of phase 1 data, CPX-351 is characterized by 2 key features: maintenance of a 5 : 1 molar ratio of cytarabine-daunorubicin in plasma and a longer half-life than 7 + 3 treatment.<sup>16</sup> Additionally, in contrast to other liposomal drugs, which typically deliver drug to target tissues and release the drug outside of cells, engulfment of intact CPX-351 liposomes is followed by subsequent intracellular release of cytarabine and daunorubicin.<sup>7</sup> Both encapsulated and released drugs are relevant to the antitumor effects of CPX-351. To account for all drug actions, total drug concentrations (encapsulated plus released drugs) were used in this population pharmacokinetics and exposure-response analyses.

A population pharmacokinetics analysis based on pooled data from approximately 200 patients across 3 clinical studies was conducted to assess potential sources of variability in pharmacokinetic parameters and to determine if baseline patient or disease characteristics warrant dose adjustments for CPX-351. Using the projected pharmacokinetic parameters from the population pharmacokinetic analysis, an exposure-response analysis was performed to understand the

pharmacokinetic and prognostic factors that might affect the efficacy and safety of CPX-351. An in-depth analysis was performed to assess the pharmacokinetics of CPX-351 based on a large sample size and explore relationships between drug exposure and efficacy and safety outcomes.

## Materials and Methods

### Study Design, Study Population, and Pharmacokinetic Sampling

The CPX-351 population pharmacokinetics analysis was based on data collected from 3 clinical studies: (1) a phase 1 dose-escalation study (Study 101) in patients with advanced hematologic malignancies, designed to assess the toxicity and maximum tolerated dose of CPX-351; (2) a phase 2 study (Study 206) of pharmacokinetics, pharmacodynamics, and safety in patients with documented AML or acute lymphocytic leukemia; and (3) a randomized, controlled, phase 3 study (Study 301) in patients with newly diagnosed high-risk/secondary AML. Safety and efficacy end points for these studies have been reported elsewhere.<sup>12,14,17</sup> In all 3 studies, complete remission was defined as <5% bone marrow blasts, an absolute neutrophil count  $\geq 1000/\mu\text{L}$ , and a platelet count  $>100\,000/\mu\text{L}$ . In Studies 206 and 301, complete remission with incomplete recovery was defined as <5% bone marrow blasts with an absolute neutrophil count  $<1000/\mu\text{L}$  or a platelet count  $<100\,000/\mu\text{L}$ .

Study 101 (NCT00389428) was an open-label, single-arm, dose-escalation phase 1 study of CPX-351 in patients with advanced hematologic malignancies.<sup>12</sup> Eligible patients were required to have pathological confirmation of relapsed or refractory AML, acute lymphocytic leukemia, or myelodysplastic syndrome. Patients were permitted to receive up to 2 induction cycles of CPX-351 administered by 90-minute intravenous infusions on days 1, 3, and 5 of each cycle; patients who achieved a complete remission could receive a single consolidation cycle of CPX-351 administered at the same dose on days 1 and 3. The specific timing of reinduction and consolidation were at the investigator's discretion. Dose levels ranged from 3 units/m<sup>2</sup> (1.3 mg/m<sup>2</sup> daunorubicin and 3 mg/m<sup>2</sup> cytarabine) to a maximum of 134 units/m<sup>2</sup> (59 mg/m<sup>2</sup> daunorubicin and 134 mg/m<sup>2</sup> cytarabine).

Study 206 (NCT02238925) was an open-label, single-arm, phase 2 pharmacokinetic and pharmacodynamic study to assess the potential for QTc prolongation in adults aged 18 to 80 years of age with AML, relapsed or refractory acute lymphocytic leukemia, or myelodysplastic syndrome during treatment with CPX-351.<sup>17</sup> In the first induction cycle, CPX-351 100 units/m<sup>2</sup> (daunorubicin 44 mg/m<sup>2</sup> and cytarabine 100 mg/m<sup>2</sup>) was administered on days 1, 3, and 5; a

second induction cycle of CPX-351 100 units/m<sup>2</sup> on days 1 and 3 was permitted. Patients with a response of complete remission or complete remission with incomplete recovery could subsequently receive up to 4 cycles of consolidation with CPX-351 65 units/m<sup>2</sup> (daunorubicin 29 mg/m<sup>2</sup> and cytarabine 65 mg/m<sup>2</sup>) administered on days 1 and 3 of each cycle. The specific timing of reinduction and consolidation were at the investigator's discretion.

Study 301 (NCT00389428) was a phase 3, multicenter, open-label, and randomized study.<sup>14</sup> Patients 60 to 75 years of age with newly diagnosed high-risk/secondary AML were randomized 1 : 1 to receive CPX-351 or active control (standard of care 7 + 3, composed of cytarabine 100 mg/m<sup>2</sup>/day administered by 7-day continuous infusion [5 days for second induction and consolidation] with daunorubicin 60 mg/m<sup>2</sup> on days 1–3 [days 1–2 for second induction and consolidation]). Patients were stratified by age and AML subtype. The dose and schedule of CPX-351 in this study was similar to that described above for Study 206, except that patients could receive a maximum of up to 2 cycles of induction, and patients with complete remission or complete remission with incomplete recovery could receive up to 2 cycles of consolidation. If a patient received 2 cycles of induction with CPX-351, the second induction cycle was administered within 35 days of the first cycle. First and second cycles of consolidation could be given 35 to 75 days and 35 to 56 days from the start of the previous cycle, respectively.

In all 3 studies, pharmacokinetic data were obtained during the first induction cycle, in which CPX-351 was administered on days 1, 3, and 5; pharmacokinetic sampling schemes for each study are shown in Table S1. The pharmacokinetic-evaluable population was defined as all patients in Studies 101, 206, and 301 who received  $\geq 1$  dose of CPX-351, had reliable records for determining dose and sample collection times, and had  $\geq 1$  plasma concentration value.

The exposure-response analysis for efficacy was confined to patients in Study 301, while the exposure-response analysis for safety comprised patients from both the 206 and 301 studies. The exposure-response efficacy population included patients in the intent-to-treat population from the Study 301 who received  $\geq 1$  dose of study medication and were included in the pharmacokinetic-evaluable population. The exposure-response safety population included patients in the 206 and 301 studies who received  $\geq 1$  dose of study medication.

#### Bioanalytical Methods

Plasma concentrations of cytarabine and daunorubicin were determined by validated bioanalytical methods based on high-performance liquid chromatography in

conjunction with tandem mass spectrometry.<sup>16</sup> The bioanalytical procedures involved an initial liposomal rupture step that released encapsulated drug into the plasma; therefore, the assay measured "total" drug concentration (sum of encapsulated drug concentration plus free drug concentration). The range of detection was 1000 to 100 000 ng/mL for total cytarabine and total daunorubicin.

#### Efficacy and Safety Variables

For efficacy, overall survival, event free survival, and complete remission were included in the exposure-response analysis. For safety, incidence and severity of treatment-emergent adverse events (TEAEs), serious TEAEs, and TEAEs of special interest, as well as mortality (stratified by time from start of treatment), were evaluated in the exposure-response analysis. An exposure-response analysis was also conducted to evaluate the potential relationship between exposure and time to hematologic recovery.

#### Population Pharmacokinetic Modeling

Nonlinear mixed-effect modeling was performed using the mu-referencing method (SAEM/IMP methods with ITS preestimation) in NONMEM (Version VII [Level 7.3]; ICON plc, Dublin, Ireland). Data set preparation, exploration, and visualization of the data were performed using the statistical package in R (Version 3.2.5). Perl-Speak-NONMEM (PsN, Version 4.4.8) was used for modeling, stepwise analysis of covariates, and visual predictive checks.

The selection of appropriate models was based on prior studies of CPX-351.<sup>16,18</sup> Model evaluation and selection were assessed using a common model discrimination process,<sup>19</sup> including statistical criteria (eg, objective function value), as well as pertinent graphic representations of goodness of fit. Models had the following form:

$$C_{p_{ij}} = C(D_i, t_j, \theta_i) + \varepsilon_{ij} \quad (1)$$

$$\theta_i = (\theta_{i1}, \dots, \theta_{im})$$

where  $C_{p_{ij}}$  was the concentration at  $j$ th collection time for patient  $i$ ,  $D_i$  represented the dosing history for patient  $i$ ,  $\theta_i$  was the vector of  $m$  pharmacokinetic parameters for patient  $i$ , and  $\varepsilon_{ij}$  was the random error associated with  $j$ th concentration for patient  $i$ .

Between-subject variability in parameters was incorporated using a log-normal random effects (ETA) model of the following form:

$$\theta_i = \theta \times \exp(\eta_i)$$

where  $\theta$  was the typical population mean value,  $\theta_i$  was the individual value of the parameter, and  $\eta_i$  denoted

the interindividual random effect accounting for the deviation from the typical value for patient  $i$ . The  $\eta_i$ s were assumed to have a normal distribution with a zero mean and variance  $\omega^2$ .

The approximate percent coefficient of variation (%CV) was reported as:

$$\%CV \text{ (BSV)} = \sqrt{\exp(\omega^2) - 1} \times 100\% \quad (2)$$

Between-subject variability was also included on the residual unknown error model to reflect the fact that data from different individuals may have different information content, considering the very wide range of dose and concentrations observed in the current analysis.<sup>20</sup> Separate pharmacokinetic models were developed for cytarabine and daunorubicin, and extrinsic and intrinsic factors were evaluated as covariates. Extrinsic factors were CPX-351 dose level and formulation (frozen and lyophilized). Intrinsic factors included body weight, body mass index, body surface area (BSA), age, sex, race, white blood cell (WBC) counts, creatinine clearance, and markers of hepatic function (bilirubin, aspartate aminotransferase, and alanine aminotransferase). Additionally, categorical renal function (normal function, and mild, moderate, or severe renal impairment) and categorical bilirubin levels (<1.2 and 1.2-3 mg/dL) were included in the covariate analysis.

The covariate analysis was guided by assessing the relationship between ETA of pharmacokinetic parameters and covariates. Physiologically meaningful relationships were identified, and covariates of interest were included in the full model.<sup>21</sup> No model reduction step was performed, and all covariates were retained in the final population pharmacokinetic model as per Harrell.<sup>21</sup> Continuous covariates were included in the model using the follow equation:

$$\theta_i = \theta_{TV} \cdot \left( \frac{Cov_i}{Cov_{med}} \right)^{\theta_{eff}} \quad (3)$$

where  $\theta_i$  was the value of the parameter for patient  $i$ ,  $\theta_{TV}$  was the typical value of the pharmacokinetic parameter in the population,  $Cov_i$  was the value of the covariate for patient  $i$ ,  $Cov_{med}$  was the median value of the covariate, and  $\theta_{eff}$  represented the influence of the covariate on the parameter  $\theta_{TV}$ .

Categorical covariates were introduced into the model as follows:

$$\theta_i = \theta_{TV} \cdot \exp(Cov_i \cdot \theta_{eff}) \quad (4)$$

where  $Cov_i$  was 1 if applicable to each patient and was 0 otherwise.

The final population pharmacokinetic models for cytarabine and daunorubicin were used to predict concentration time profiles and clearance (CL) values for each

patient in the pharmacokinetic-evaluable population. The area under the curve from time zero to 48 hours after the dose on day 5 ( $AUC_{tau}$ ) was derived using the linear trapezoidal rule.

### Exposure-Response Analysis

The exposure-response analysis was performed using SAS (Cary, North Carolina), Version 9.3 for Windows. Patients randomized to receive CPX-351 in Studies 206 and 301 were only included in the exposure-response analysis if they were part of the pharmacokinetic-evaluable population. The exposure variables in the exposure-response analyses were the AUC values for cytarabine and daunorubicin in individual patients, which were available as output from the population pharmacokinetic analysis. There was a strong correlation between maximum concentration and area under the concentration-time curve (AUC; Figure S1), which suggests similar exposure-response relationships may be expected if maximum concentration values were used instead of AUC. Distinct exposure-response analyses were conducted using exposure data from cytarabine or daunorubicin as categorical (ie, separated into equivalent quantiles) or continuous variables. Because the results of these exposure-response analyses were similar for cytarabine and daunorubicin, results from the exposure-response analysis that used cytarabine exposure as a categorical variable (separated by quantiles) are presented in this article as representative, and results from the other analyses are reported in the Supporting Information.

**Exposure-Efficacy Analysis.** For the exposure-response analyses, when using exposure as a categorical variable, exposure effects on efficacy (overall survival or event free survival) were evaluated based on a stratified log-rank test of the exposure categories and pairwise  $P$ -values were provided to compare the CPX-351 exposure category with 7 + 3 treatment. Kaplan-Meier curves for CPX-351 and the 50th percentile of Kaplan-Meier estimates were used to estimate the median overall survival or event free survival, and 2-sided 95% CIs were provided for the 25th, 50th, and 75th percentiles.

A Cox proportional hazards regression analysis stratified by age and AML type was performed to provide the hazard ratio and 95% CIs for each exposure category, with reference to the 7 + 3 group for overall survival and event free survival. Cox proportional hazards regression analyses stratified by age and AML type were also conducted for overall survival and event free survival for each of the prespecified prognostic factors: sex, Eastern Cooperative Oncology Group (ECOG) performance status (0-2), cytogenetic risk (nonpoor and poor), WBC count (<20 or  $\geq 20 \times 10^9/L$ ), platelet count (<50 or  $\geq 50 \times 10^9/L$ ), hemoglobin level ( $\leq 9$  or

**Table 1.** Summary of Baseline Demographics and Characteristics in the Pharmacokinetic-Evaluable Population

	Study 101 (n = 38)	Study 206 (n = 26)	Study 301 (n = 131)	Total (N = 195)
Age (y), median (range)	62.5 (24–81)	67.0 (37–80)	68.0 (60–75)	67 (24–81)
Body weight (kg), median (range)	76.7 (38.9–156.5)	82.2 (41.9–133.3)	79.9 (48.9–138.9)	79.8 (38.9–156.5)
BSA (m <sup>2</sup> ), median (range)	1.94 (1.26–2.80)	1.94 (1.32–2.67)	1.95 (1.45–2.64)	1.94 (1.26–2.80)
Male sex, n (%)	26 (68.4)	14 (53.8)	79 (60.3)	119 (61.0)
Race, n (%)				
White	30.2 (84.2)	25 (96.2)	108 (82.4)	165 (84.6)
Black	2 (5.3)	1 (3.8)	6 (4.6)	9 (4.6)
Asian	4 (10.5)	0	6 (4.6)	10 (5.1)
Native American	0	0	1 (0.8)	1 (0.5)
Other	0	0	10 (7.6)	10 (5.1)
ALT (U/L), median (range)	28.0 (15–151)	20.0 (9–153)	23.0 (3–139)	24.0 (3–153)
AST (U/L), median (range)	28.0 (12–100)	20 (9–65)	22.0 (5–115)	23.0 (5–115)
ALP (U/L), median (range)	88 (34–319)	68 (32–164)	69.0 (21–284)	72.0 (21–319)
Bilirubin (mg/dL), median (range)	0.60 (0.2–1.8)	0.55 (0.2–1.4)	0.50 (0.1–2.5)	0.60 (0.1–2.5)
Bilirubin < 1.2 mg/dL, n (%)	37 (97.4)	23 (88.5)	119 (90.8)	179 (91.8)
Bilirubin 1.2–3 mg/dL, n (%)	1 (2.6)	3 (11.5)	12 (9.2)	16 (8.2)
Serum creatinine (mg/dL), median (range)	0.90 (0.60–1.60)	0.90 (0.48–1.55)	0.86 (0.34–2.02)	0.90 (0.34–2.02)
CrCL (mL/min), median (range)	79.6 (27.5–171.8)	86.4 (42.3–211.7)	85.8 (38.6–177.8)	85.3 (27.5–211.7)
CrCL ≥ 90 mL/min, n (%)	14 (36.8)	12 (46.2)	57 (43.5)	83 (42.6)
CrCL 60–89 mL/min, n (%)	20 (52.6)	8 (30.8)	55 (42.0)	83 (42.6)
CrCL 30–59 mL/min, n (%)	3 (7.9)	6 (23.1)	19 (14.5)	28 (14.4)
CrCL 15–29 mL/min, n (%)	1 (2.6)	0	0	1 (0.5)
WBCs (10 <sup>9</sup> /L), median (range)	3.2 (0.2–68.1)	3.7 (0.7–110.9)	3.4 (0.3–86.4)	3.4 (0.2–110.9)
Formulation, n (%)				
Frozen	38 (100)	0	0	38 (19.5)
Lyophilized	0	26 (100)	131 (100)	157 (80.5)

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; BSA, body surface area; CrCL, creatinine clearance; WBCs, white blood cells.

>9 g/dL), bone marrow blast percentage (<20%, 20%–40%, >40%–60%, or >60%), and *FLT3*-internal tandem duplication mutation status (yes or no). Prognostic factors with *P*-values ≤ .1 were included in multivariate Cox proportional hazards analyses for overall survival and event free survival. The final multivariate models retained the prognostic factors with *P*-values ≤ .05.

For complete remission, the number and percentage of responders, along with Clopper-Pearson 2-sided 95% CIs, were calculated for each exposure category. The effect of exposure was evaluated based on an extended Mantel-Haenszel mean score statistic (stratified), treating exposure categories as ordinal values. The resulting odds ratios, 95% CIs, and *P*-values were reported for each of the quartiles vs the 7 + 3 group.

Univariate and multivariate logistic regression analyses were conducted for complete remission and complete remission+complete remission with incomplete recovery rates for the prespecified prognostic factors, following the same principle as the univariate and multivariate Cox proportional hazard regression analyses.

**Exposure-Safety Analysis.** For the exposure-safety analysis, various safety parameters (including frequency, type, and severity of TEAEs and time to hematologic recovery) were evaluated against each exposure

category using summary statistics for continuous variables or frequencies for categorical variables. Time to recovery for each exposure category was evaluated based on Kaplan-Meier analyses.

## Results

### Population Pharmacokinetic Analysis

**Patient Population and Data Sets.** The pharmacokinetic-evaluable population consisted of 195 patients, from whom 2176 samples were obtained. The majority of patients (n = 157 [80.5%]) received 100 to 101 units/m<sup>2</sup> of CPX-351 for the first induction cycle. Baseline demographics and characteristics are shown in Table 1. The median age was 67 years (range, 24–81 years) and median body weight was 79.8 kg (range, 38.9–156.5 kg). At baseline, 83 (42.6%) patients had normal renal function; 83 (42.6%), 28 (14.4%), and 1 (0.5%) patients had mild, moderate, and severe renal impairment, respectively. The majority (n = 179 [91.8%]) of patients had bilirubin <1.2 mg/dL, and the remaining patients (n = 16 [8.2%]) had bilirubin ranging from 1.2 to 3 mg/dL.

All 195 patients in the pharmacokinetic-evaluable population were included in the analysis. Of 2176 samples assayed, a total of 2052 and 2033 were included

**Table 2.** Population Pharmacokinetic Parameters of Cytarabine and Daunorubicin Following Intravenous Administration of CPX-351

Parameters	Total Cytarabine		Total Daunorubicin	
	Population Estimate <sup>a</sup>	BSV%	Population Estimate <sup>b</sup>	BSV%
CL, L/h	$0.107 \times (\text{BSA}/1.95)^{1.01} \times (\text{Bilirubin}/0.60)^{0.197}$	61.0%	$0.147 \times (\text{BSA}/1.95)^{0.829} \times (\text{Bilirubin}/0.60)^{0.0829} \times 0.760$ if frozen formulation	49.3%
V <sub>c</sub> , L	$4.92 \times (\text{BSA}/1.95)^{1.21}$	24.7%	$4.29 \times (\text{BSA}/1.95)^{1.12} \times 0.802$ if frozen formulation	23.7%
Q, L/h	$0.00165 \times (\text{BSA}/1.95)^{1.00}$	NA	$0.0294 \times (\text{BSA}/1.95)^{1.00} \times 0.576$ if frozen formulation	69.3%
V <sub>p</sub> , L	$0.129 \times (\text{BSA}/1.95)^{1.00}$	NA	$0.593 \times (\text{BSA}/1.95)^{1.00} \times 0.504$ if frozen formulation	86.9%
Error model (residual variability)	18.7%	83.1%	14.3%	102.7%

BSA, body surface area; BSV, between-subject variability; CL, systemic clearance; NA, not available (BSV% for Q and V<sub>p</sub> were not estimated due to high shrinkage on these parameters); Q, distributional clearance; V<sub>c</sub>, central volume of distribution; V<sub>p</sub>, peripheral volume of distribution.

<sup>a</sup>Pharmacokinetic parameters are calculated for a typical patient with BSA of 1.95 m<sup>2</sup> with baseline bilirubin of 0.60 mg/dL as shown in parentheses.

<sup>b</sup>Pharmacokinetic parameters are calculated for a typical patient with BSA of 1.95 m<sup>2</sup> who received the lyophilized formulation.

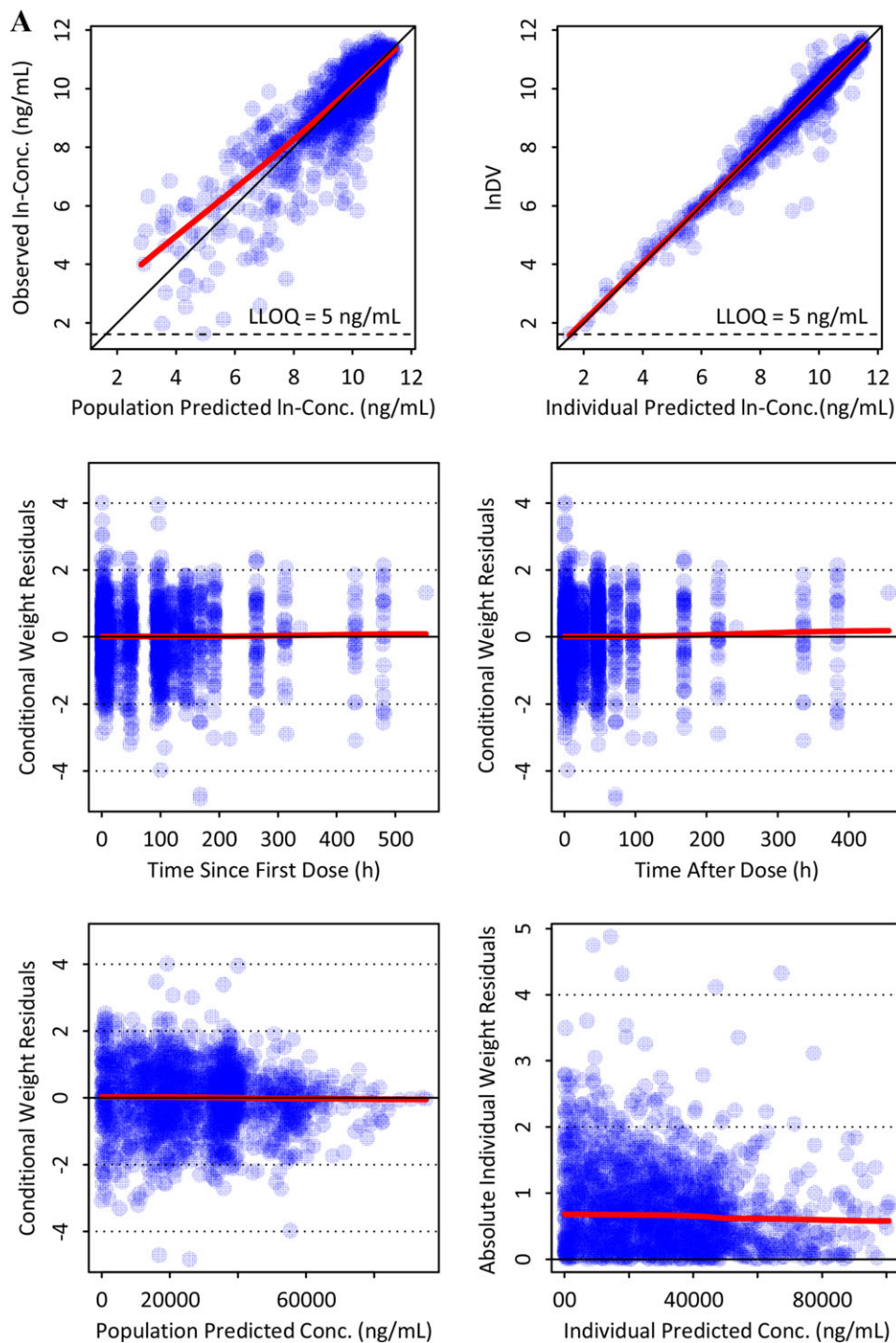
in the initial analysis for cytarabine and daunorubicin, respectively, and 2046 and 2023 were included in the final analysis, respectively. For each drug, 30 (1.4%) and 12 (<1%) samples were excluded due to missing and inconsistent sample collection records, respectively. A total of 82 (3.8%) and 100 (4.6%) samples had concentrations below the limit of quantification (5 ng/mL) for cytarabine and daunorubicin, respectively, and were set to missing. Six (0.3%) samples were excluded from the final cytarabine analysis, and 10 (0.5%) were excluded from the final daunorubicin analysis because they were considered outliers (ie, absolute conditional weighted residual >4).

**Population Pharmacokinetic Modeling of Cytarabine.** A 2-compartment model, with an OMEGA block on CL and central volume of distribution (V<sub>c</sub>), a residual error model (log additive with ETA), and an allometric model of BSA on CL, V<sub>c</sub>, peripheral clearance (Q), and peripheral volume of distribution (V<sub>p</sub>; estimated or fixed) was used as the base model for cytarabine. The relationship between ETA of pharmacokinetic parameters and covariates of interest are presented in Figure S2. Covariates were added to the model as described in the Methods section. Overall, the final population pharmacokinetics model for cytarabine included only the effect of bilirubin on CL. The final population pharmacokinetic parameters for total cytarabine are presented in Table 2. The CL, V<sub>c</sub>, and V<sub>p</sub> of cytarabine were mainly dependent upon BSA and consistent with the current dosing paradigm, where CPX-351 is dosed based on mg/m<sup>2</sup>. The effect of bilirubin on CL was statistically significant, but the relationship was very shallow, with an exponent of 0.197. These results suggest that patients with higher bilirubin values were

associated with a slightly faster CL of the liposome, since cytarabine is not expected to undergo hepatic elimination. Further, dose, formulation, and other intrinsic covariates did not exert a significant effect on the pharmacokinetic parameters of cytarabine. Additional pharmacokinetic parameters of cytarabine (eg, relative standard error [RSE], shrinkage) are presented in Table S2. Overall, pharmacokinetic parameters of cytarabine were robustly estimated, with RSE values <20% for all parameters, and ETA shrinkage was low.

The goodness-of-fit plots for population predicted and individual predicted concentrations vs observed concentrations of cytarabine are presented in Figure 1A; goodness of fit on a linear scale is presented in Figure S3. Population predicted and individual predicted concentrations of cytarabine were consistent with the observed data, with high and low concentration values evenly distributed around the line of identity. A slight bias was observed for population predicted low concentration values of cytarabine (ie, ln-transformed concentrations <6, corresponding to approximately <400 ng/mL). The goodness-of-fit plots, including conditional weighted residuals versus time, time after dose, and population predicted and individual predicted concentrations, demonstrated fair assessments of the model. A visual predictive check of predicted vs observed concentrations of cytarabine confirmed the adequate predictive performance of the final population pharmacokinetic model of cytarabine (Figure S4).

**Population Pharmacokinetic Modeling of Daunorubicin.** A 2-compartment model, with an OMEGA block on CL, V<sub>c</sub>, Q, and V<sub>p</sub>; a residual error model (log additive with ETA); and an allometric model of BSA on CL, V<sub>c</sub>,



**Figure 1.** Goodness-of-fit for the final model of cytarabine (A) and daunorubicin (B): predicted vs observed concentrations and weighted residuals. Blue circles represent observations, red lines represent locally weighted scatter plot smoothing (LOESS), and black lines represent identity lines. Based on data from all studies. LLOQ, lower limit of quantitation.

$Q$ , and  $V_p$  (estimated exponents) was used as the base model for daunorubicin. Covariates were evaluated and added to the final model of daunorubicin, as they were for the cytarabine model (Figure S5). Overall, the population pharmacokinetic model for daunorubicin only included the effect of bilirubin

on CL and formulation on all pharmacokinetic parameters (CL,  $V_c$ ,  $Q$ , and  $V_p$ ). Pharmacokinetic parameters of daunorubicin derived with final model are shown in Table 2. The CL,  $V_c$ , and  $V_p$  of daunorubicin were mainly dependent on BSA and consistent with the current dosing paradigm, where

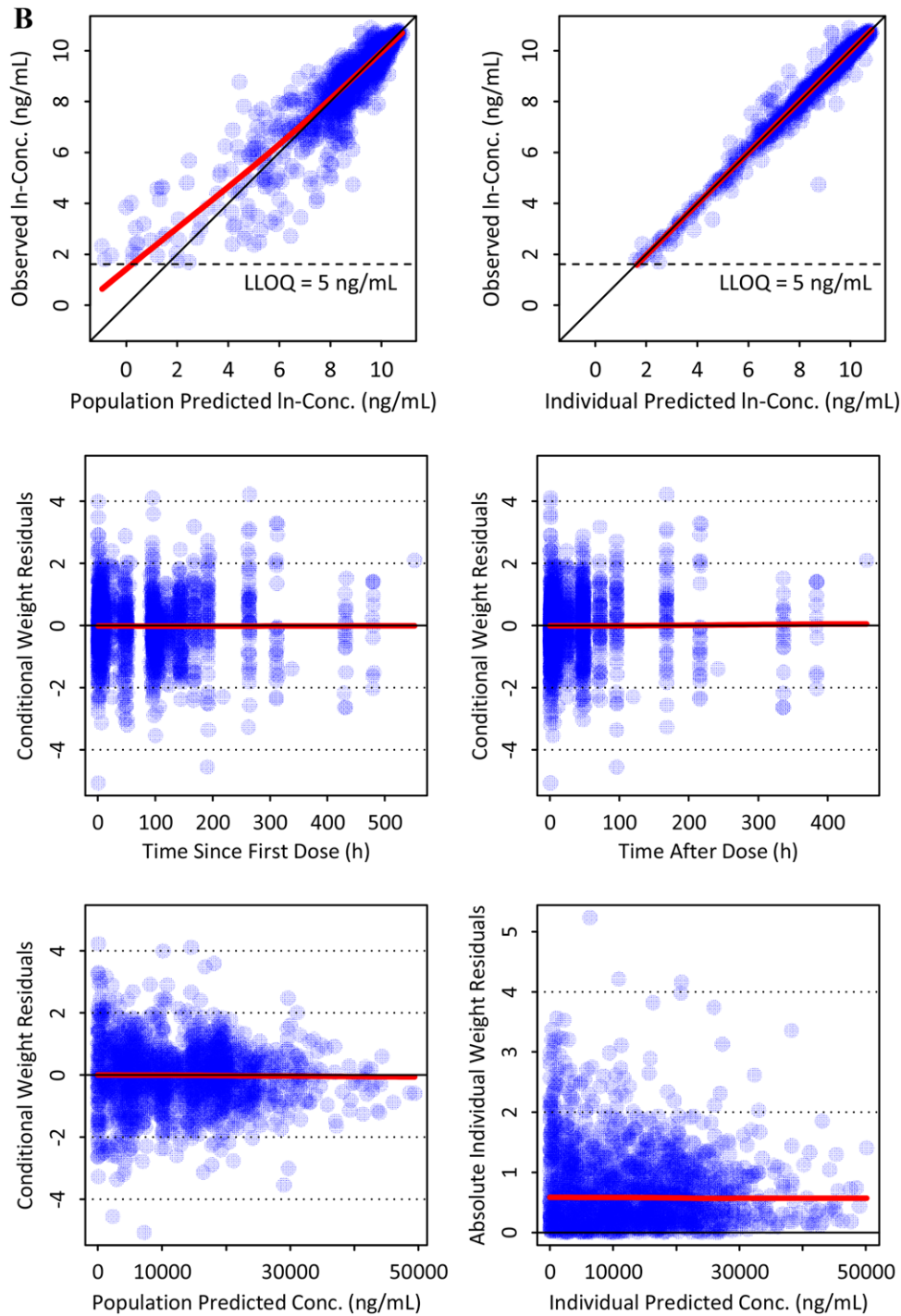


Figure 1. Continued.

CPX-351 is dosed based on  $\text{mg}/\text{m}^2$ . The effect of bilirubin on CL was very shallow, with an exponent of 0.0829. These results suggest that patients with higher bilirubin values were associated with a slightly faster CL of the liposome and/or daunorubicin, as daunorubicin is primarily eliminated through hepatic pathways. Further, dose and other intrinsic covariates

did not exert a significant effect on the pharmacokinetic parameters of daunorubicin. Additional pharmacokinetic parameters of daunorubicin (eg, RSE, shrinkage) are presented in Table S3. As with cytarabine, the pharmacokinetic parameters of daunorubicin were robustly estimated, with RSE values  $<20\%$  for all parameters, and ETA shrinkage was low.



**Table 3.** Predicted AUC<sub>tau</sub> for Cytarabine and Daunorubicin Among Renal Impairment and Bilirubin Categories

	AUC <sub>tau</sub> (μg h/mL)				
	Renal Impairment Categories (CrCL)			Bilirubin Categories	
	Normal (≥90 mL/min)	Mild (60–89 mL/min)	Moderate (30–59 mL/min)	Category 1 (<1.2 mg/dL)	Category 2 (1.2–3 mg/dL)
<b>Cytarabine</b>					
n	69	63	24	141	15
Mean (SD)	1784 (856)	2015 (943)	2145 (838)	1963 (911)	1645 (687)
%CV	48.0	46.8	39.1	55.4	45.4
Median	1637	1967	2278	1830	1425
Range	399–4261	455–5009	915–3744	399–5009	561–2999
95%CI	1582–1986	1782–2248	1809–2480	1813–2114	1297–1992
<b>Daunorubicin</b>					
n	69	63	24	141	15
Mean (SD)	572 (260)	640 (263)	677 (227)	623 (262)	540 (207)
%CV	45.5	41.0	33.5	42.1	38.2
Median	527	614	713	587	520
Range	139–1216	162–1161	347–1074	139–1219	222–980
95%CI	510–633	575–705	587–768	580–667	436–645

AUC<sub>tau</sub>, area under the curve during one dosing interval at steady state; CI, confidence interval; CrCL, creatinine clearance; CV, coefficient of variation for the arithmetic mean, expressed as a percentage; SD, standard deviation.

The goodness-of-fit plots for population predicted and individual predicted concentrations of daunorubicin were in agreement with the observed data, with high and low concentration values evenly distributed around the line of identity (Figure 1B); as with cytarabine, ln-transformed data are presented in Figure 1B and goodness of fit on a linear scale is presented in Figure S6. A slight bias was observed for population-predicted low concentrations of daunorubicin (ie, ln-transformed concentrations <6, corresponding to <55 ng/mL). The goodness-of-fit plots, including distribution of conditional weighted residuals vs time, time after dose, and population-predicted and individual-predicted concentrations, demonstrated fair assessments of the model (Figure 1B). The visual predictive check plot (Figure S7) confirmed the adequate predictive performance of the final population pharmacokinetic model of daunorubicin. Pharmacokinetic parameters of encapsulated vs nonliposomal cytarabine and daunorubicin are shown in Table S4.

**Effects of Renal and Hepatic Impairment on the Pharmacokinetics of CPX-351.** The potential effects of renal and hepatic impairment on the pharmacokinetics of CPX-351 were examined in the population pharmacokinetic analyses for both cytarabine and daunorubicin. To this end, the final population pharmacokinetic models for cytarabine and daunorubicin were used to predict AUC<sub>tau</sub> for the 156 patients in the pharmacokinetic-evaluable populations of Studies 206 and 301 who were treated with the recommended induction dose of CPX-351 (100 units/m<sup>2</sup> [100 mg/m<sup>2</sup> cytarabine and 44 mg/m<sup>2</sup> daunorubicin] on days 1, 3, and 5).

The mean AUC<sub>tau</sub> for cytarabine and daunorubicin, respectively, was 20% and 18% higher in patients with moderate renal impairment versus patients with normal renal function. However, the differences in mean values for AUC<sub>tau</sub> were not significant when viewed in the context of %CV, which ranged from 39.1% to 48.0% for cytarabine and 33.5% to 45.5% for daunorubicin (Table 3). Similarly, the mean AUC<sub>tau</sub> for cytarabine and daunorubicin was slightly higher in patients with bilirubin levels <1.2 mg/dL than in those with bilirubin levels between 1.2 and 3.0 mg/dL (19% and 15% higher, respectively), but the differences were less than the observed %CV on AUC<sub>tau</sub> (cytarabine, 45.4%–55.4%; daunorubicin, 38.2%–42.1%) and were therefore not considered meaningful (Table 3). Taken together, these analyses do not suggest any associations between daunorubicin exposure and markers of renal or hepatic function.

#### Exposure-Response Analysis

**Patient Populations and Exposure Variables.** The exposure-response analysis population for efficacy consisted of 130 AML patients from Study 301 who received CPX-351 and had evaluable pharmacokinetic data, subdivided into cytarabine and daunorubicin exposure quartiles; these quartiles were compared with the 151 patients who received control treatment (7 + 3). Although the exposure-response analysis evaluated AUC for both cytarabine and daunorubicin, due to high retention of cytarabine and daunorubicin within CPX-351 liposomes the AUC for cytarabine and daunorubicin were strongly correlated (refer to Figure S1). Thus, the exposure-response analysis

produced nearly identical exposure-response relationships for the 2 drugs, and the exposure-response analysis presented here focuses on AUC for cytarabine. The median AUC values for cytarabine increased approximately 1.6-, 2.3-, and 3.4-fold, relative to the first quartile, in the second, third, and fourth quartiles, respectively. The percentage of patients who completed treatment in the first, second, third, and fourth quartiles were 6.1%, 15.6%, 15.2%, and 21.9%, respectively. The treatment completion rate in the first quartile was similar to that of the 7 + 3 arm (6.6%). There were no notable differences between the baseline demographics of the exposure quartiles (Table S5). Prognostic factors such as cytogenetic risk, WBC counts, bone marrow blast percentage, and *FLT3*-internal tandem duplication mutation were well balanced across exposure groups, including the 7 + 3 groups. However, ECOG performance status, platelet count, and hemoglobin levels were more variable across exposure groups (Table S5). For example, an ECOG score of 0 was observed in 12.1%, 18.8%, 18.2%, and 34.4% of the first, second, third, and fourth exposure quartiles, respectively. The incidence of favorable platelet count ( $>50 \times 10^9$  /L) was 27.3%, 25.0%, 42.4%, and 53.1% in the first, second, third, and fourth exposure quartiles, respectively, and the incidence of favorable hemoglobin level ( $>9$  g/L) was 30.3%, 28.1%, 36.4%, and 43.8% in the first, second, third, and fourth exposure quartiles, respectively.

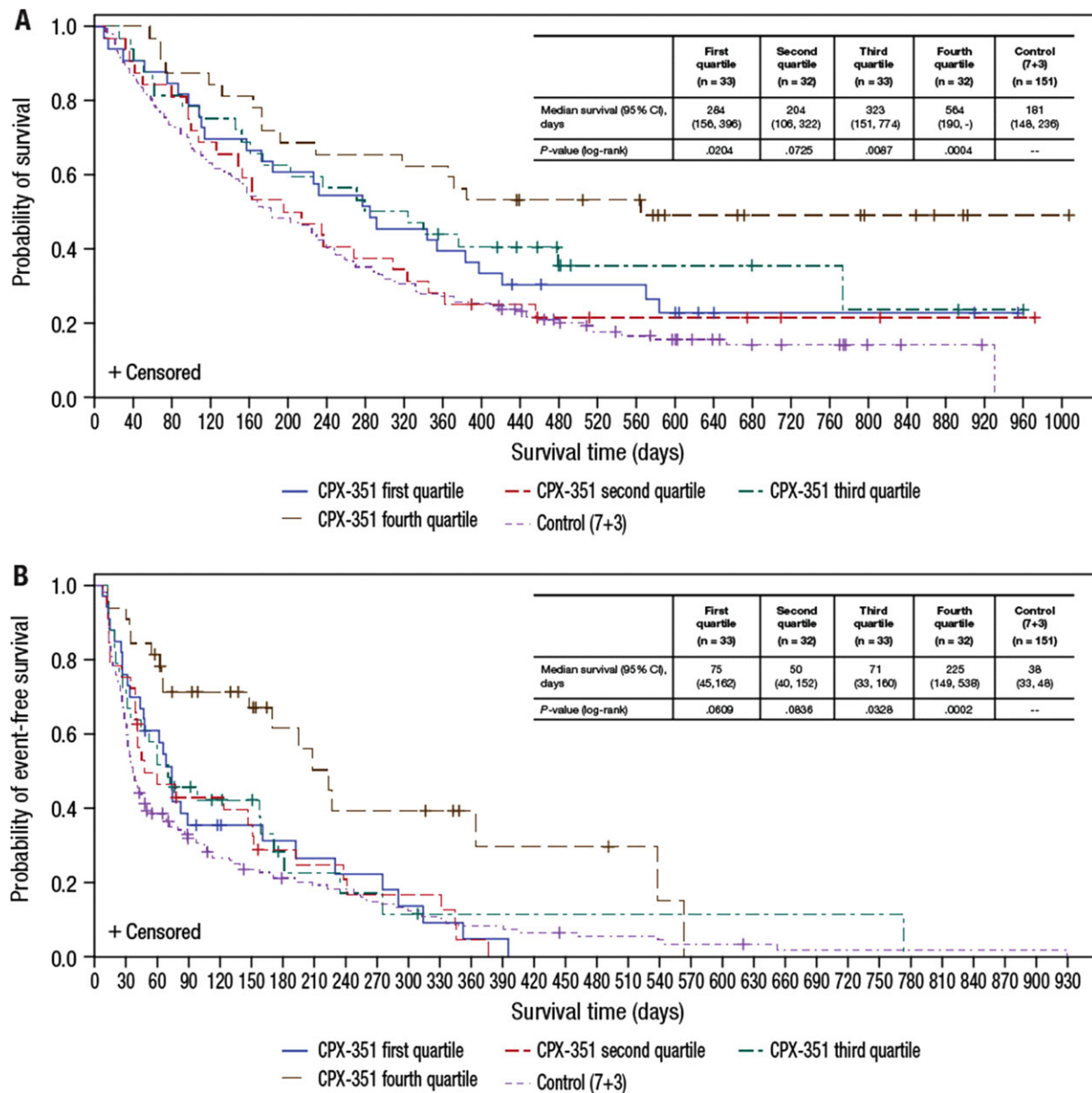
The safety-exposure population consisted of 156 patients from Studies 206 and 301 who received CPX-351 and had evaluable pharmacokinetic data, subdivided into cytarabine and daunorubicin exposure quartiles and compared with 151 patients who received 7 + 3. The majority of these patients had AML. The increase in median AUC relative to the first quartile was approximately 1.6-, 2.3-, and 3.3-fold in the second, third, and fourth quartiles, respectively. The percentage of patients who completed treatment in the first, second, third, and fourth cytarabine exposure quartiles was 5.1%, 15.4%, 12.8%, and 17.9%, respectively. There were no notable differences in baseline demographics between exposure quartiles (Table S6). Prognostic factors such as cytogenetic risk, WBC counts, bone marrow blast percentage, and *FLT3*-internal tandem duplication mutation were well balanced across exposure groups, including the 7 + 3 group. However, ECOG performance status, platelet count, and hemoglobin levels were more variable across exposure groups (Table S6). An ECOG performance status score of 0 was observed in 12.8%, 20.5%, 23.1%, and 30.8% of the first, second, third, and fourth exposure quartiles, respectively. The incidence of favorable platelet count ( $>50 \times 10^9$  /L) was 27.3%, 25.7%, 42.3%, and 52.8% in the first, second, third, and fourth exposure quartiles,

respectively, and the incidence of favorable hemoglobin level ( $>9$  g/L) was 30.3%, 28.6%, 34.6%, and 44.4% in the first, second, third, and fourth exposure quartiles, respectively.

**Exposure-Response Analysis for Efficacy.** Patients in the fourth cytarabine exposure quartile had the longest survival compared with the other exposure groups (Figure 2A). The median survival time was 181 days in the 7 + 3 group and 284, 204, 323, and 564 days in the first, second, third, and fourth quartiles, respectively. Similar results were observed when exposure was treated as a categorical variable separated by tertiles (Figure S8A) or as a continuous variable (Table S7). Both univariate and multivariate Cox regression models were pursued to adjust for effects of prognostic factors on efficacy. In addition to cytarabine exposure quartiles, ECOG performance status, karyotype, and platelet category were included in the final model. When adjusted for these covariates, the hazard ratios (95%CI) vs 7 + 3 were 0.482 (0.280, 0.828), 0.710 (0.415, 1.215), 0.550 (0.325, 0.930), and 0.329 (0.181, 0.600) for the first, second, third, and fourth cytarabine quartiles, respectively (Table 4), indicating a lower risk of death for all CPX-351 exposure quartiles vs 7 + 3, with the lowest risk in the fourth quartile.

Patients in the fourth quartile for cytarabine exposure also had the longest median Event free survival (225 days) compared to 7 + 3 (38 days) and the first, second, and third quartiles of CPX-351 exposure (75, 50, and 71 days, respectively; Figure 2B). Results were consistent when the analysis of cytarabine exposure was performed on tertiles (Figure S8B). After univariate and multivariate Cox regression analysis, the prognostic factors karyotype and WBC count were included in the final model. When adjusted for these covariates, the hazard ratios (95%CI) vs 7 + 3 for Event free survival were 0.697 (0.440, 1.104), 0.817 (0.500, 1.337), 0.770 (0.483, 1.228), and 0.280 (0.158, 0.498) for the first, second, third, and fourth cytarabine quartiles, respectively (Table 4), demonstrating a lower risk of relapse/mortality across all CPX-351 exposure quartiles versus 7 + 3, with the lowest risk in the fourth quartile.

Consistent with overall survival and Event free survival, the complete remission rates 7 + 3 treatment, first, second, third, and fourth quartiles were 26.5%, 30.3%, 37.5%, 30.3%, and 59.4%, respectively (Table S8). After univariate and multivariate analyses, only karyotype was included in the final model. When adjusted for this covariate, the odds ratios (95%CI) for exposure groups with respect to 7 + 3 were 1.255 (0.509, 3.097), 1.904 (0.784, 4.624), 1.428 (0.586, 3.477), and 4.938 (2.062, 11.825) for the first, second, third, and fourth cytarabine quartiles, respectively. These odds ratios suggest a higher probability of reaching complete remission



**Figure 2.** Efficacy by cytarabine-exposure quartiles: overall survival (A) and event free survival (B). For the overall survival analysis, patients were censored at the date they were last known to be alive; for the event free survival analysis, patients were censored at the date of their last examination. P-values are for a comparison between each drug exposure quartile and 7 + 3. CI, confidence interval.

among all CPX-351 exposure quartiles versus 7 + 3, with the highest probability complete remission seen in the fourth exposure quartile.

**Exposure-Response Analysis for Safety.** The incidence of grade 3 to 5 TEAEs was 90.7% for the 7 + 3 group and 87.2%, 92.3%, 94.9%, and 97.4% for the first, second, third, and fourth cytarabine quartiles, respectively (Table 5). Analyses of TEAEs by tertile were consistent with the results from the quartile analysis (Table S9). Across exposure groups, TEAEs leading to discontinuation were rare (n = 1 each in the first and third quartiles; n = 2 in the 7 + 3 group). TEAEs

leading to death were similar across exposure groups (Table 5). There was a trend toward lower 60-day mortality with increasing cytarabine quartile (21.2% in the 7 + 3 group and 15.4%, 12.8%, 10.3%, and 7.7% for the first, second, third, and fourth quartiles, respectively). No relationship between exposure and cardiac or infection events was apparent (Table S10), although trends were noted with individual preferred terms, such as febrile neutropenia (71.5% in the 7 + 3 group and 59.0%, 74.4%, 76.9%, and 79.5% in the first, second, third, and fourth quartiles, respectively). A trend toward a greater incidence of rash (35.1% in the 7 + 3 group and 41.0%, 48.7%, 53.8%, and

**Table 4.** Multivariate Analysis of the Relationship Between Outcomes and Cytarabine Exposure

Factor	Factor Level	n/N (%)	Hazard Ratio (95%CI)	Overall P-Value
OS				
ECOG PS	0	64/262 (24.4)	0.429 (0.249–0.738)	.0084
	1	165/262 (63.0)	0.670 (0.428–1.051)	
	2	33/262 (12.6)		
Karyotype	Nonpoor	122/262 (46.6)	0.482 (0.340–0.682)	<.0001
	Poor	140/262 (53.4)		
Platelet category	$\leq 50 \times 10^9/L$	162/262 (61.8)	1.460 (1.039–2.052)	.0291
	$> 50 \times 10^9/L$	100/262 (38.2)		
Treatment quartile	Q1	32/262 (12.2)	0.482 (0.280–0.828)	.0006 <sup>a</sup>
	Q2	28/262 (10.7)		
	Q3	32/262 (12.2)		
	Q4	31/262 (11.8)		
	7 + 3	139/262 (53.1)		
Event free survival				
Karyotype	Nonpoor	122/263 (46.4)	0.485 (0.354–0.665)	<.0001
	Poor	141/263 (53.6)		
WBC category	$< 20 \times 10^9/L$	227/263 (86.3)	0.459 (0.298–0.706)	.0004
	$\geq 20 \times 10^9/L$	36/263 (13.7)		
Treatment quartile	Q1	32/263 (12.2)	0.697 (0.440–1.104)	.0006 <sup>a</sup>
	Q2	28/263 (10.6)		
	Q3	32/263 (12.2)		
	Q4	31/263 (11.8)		
	7 + 3	140/263 (53.2)		

CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group Performance Status; Q, quartile; WBC, white blood cells.

<sup>a</sup>Overall 2-sided P-value is from the Wald chi-square test.

**Table 5.** Summary of TEAEs

	CPX-351				Control 7 + 3 (n = 151)
	First Quartile (n = 39)	Second Quartile (n = 39)	Third Quartile (n = 39)	Fourth Quartile (n = 39)	
Any TEAEs, n (%)	39 (100)	39 (100)	39 (100)	39 (100)	151 (100)
Grade 3–5, n (%)	34 (87.2)	36 (92.3)	37 (94.9)	38 (97.4)	137 (90.7)
Grade 3, n (%)	18 (46.2)	23 (59.0)	24 (61.5)	26 (66.7)	92 (60.9)
Grade 4, n (%)	9 (23.1)	6 (15.4)	7 (17.9)	5 (12.8)	16 (10.6)
Grade 5, n (%)	7 (17.9)	7 (17.9)	6 (15.4)	7 (17.9)	29 (19.2)
Serious TEAEs, n (%)	21 (53.8)	20 (51.3)	22 (56.4)	23 (59.0)	65 (43.0)
TEAEs leading to discontinuation, n (%)	1 (2.6)	0	1 (2.6)	0	2 (1.3)
TEAEs leading to death, n (%)	7 (17.9)	7 (17.9)	6 (15.4)	7 (17.9)	29 (19.2)

TEAE, treatment-related adverse events.

59.0% in the first, second, third, and fourth quartiles, respectively) and bleeding events (59.6% in the 7 + 3 group and 71.8%, 71.8%, 84.6%, and 84.6% in the first, second, third, and fourth quartiles, respectively) was observed.

Among patients with complete remission, the median time for recovery of platelet count to  $\geq 100 \times 10^3/\mu L$  was prolonged with increasing cytarabine quartiles (30 days in the 7 + 3 group and 38, 35, 35, and 42 days in the first, second, third, and fourth cytarabine quartiles, respectively). The median time for neutrophil recovery to  $\geq 1000/\mu L$  was 28 days in the 7 + 3 group but did not appear to increase with increas-

ing cytarabine quartile (41, 37, 35, and 36 days in the first, second, third, and fourth quartiles, respectively) among patients achieving complete remission.

## Discussion

This population pharmacokinetics analysis combined data from 3 clinical studies of CPX-351 in adults with hematologic malignancies to further characterize the pharmacokinetics of CPX-351 and to assess potential sources of pharmacokinetic variability that might warrant dose adjustments for special patient populations. It is hypothesized that the cytotoxicity of CPX-351

is exerted by cellular uptake of liposomes containing cytarabine and daunorubicin and the subsequent intracellular release of both drugs. Thus, the total plasma concentrations of cytarabine and daunorubicin are the most relevant analytes to the antileukemic effects of CPX-351. Circulating levels of free cytarabine and daunorubicin, and the active metabolite daunorubicinol, are less relevant. Hence, this population pharmacokinetic model is based on the assessment of total cytarabine and daunorubicin.

The final population pharmacokinetic models for cytarabine and daunorubicin were 2-compartment models with drug clearance from the central compartment. These models differ from the 1-compartment model derived by Nikanjam et al<sup>18</sup> using only data from Study 101, likely due to the more limited data from the Study 101 analysis because of smaller patient numbers and differences in sampling scheme. The pharmacokinetic parameters of free cytarabine and daunorubicin are markedly different from each other following administration of nonliposomal formulations.<sup>22</sup> Consistent with previous reports, the pharmacokinetic parameters of total cytarabine and daunorubicin were similar to each other when administered via CPX-351. Following CPX-351 administration, the majority (>99%, unpublished data) of circulating total cytarabine and daunorubicin remains encapsulated; thus, the pharmacokinetic parameters of total cytarabine and daunorubicin reflect those of CPX-351 liposomes. This resulted in substantially lower CL and, subsequently, higher and sustained plasma concentrations of total cytarabine and daunorubicin, as well as longer plasma half-lives. Further, the volumes of distribution of the total cytarabine and daunorubicin are small, as the CPX-351 liposomes remain largely confined to the vascular space.

Many intrinsic covariates were evaluated in the population pharmacokinetic models for total cytarabine and daunorubicin, including body weight, body mass index, BSA, age, sex, race, hepatic and renal function markers, and WBC counts. The inclusion of WBC counts in the models was based on the observation by Krogh-Madsen et al that baseline WBC count was a significant covariate for cytarabine and daunorubicin pharmacokinetics.<sup>22</sup> However, none of the evaluated intrinsic covariates had a clinically significant impact on plasma exposure to either total cytarabine or daunorubicin. In addition, an analysis based on model-predicted AUC<sub>tau</sub> values was performed to ensure possible relationships were not overlooked. Evaluation of the potential effects of renal or hepatic impairment on drug exposure was of particular interest, given that impaired renal or hepatic function can contribute to the variability in exposure to nonliposomal formulations of cytarabine and daunorubicin.<sup>23,24</sup> These analyses

did not suggest any associations between cytarabine or daunorubicin plasma exposure and markers of renal function. Although the effect was small, bilirubin was identified as a statistically significant covariate for clearances of total cytarabine and daunorubicin. As clearances of total cytarabine and daunorubicin are likely following the clearance of liposomes, this identification of bilirubin as a covariate suggested that, in patients with higher bilirubin levels, CPX-351 liposomes might be eliminated faster. Whether or not bilirubin level in AML patients is associated with higher mononuclear phagocytic system activity will need to be investigated, as this activity is assumed to mediate the clearance of CPX-351 liposomes. As shown in Table 3, there is no difference of AUC<sub>tau</sub> values in patients with bilirubin <1.2 mg/dL or 1.2 to 3 mg/dL; therefore, the effect of bilirubin on total drug exposures was not clinically significant. These findings suggest that dose modifications are not required for patients with baseline mild or moderate renal impairment, or for patients with elevated bilirubin up to 3 mg/mL. In this study, there were not sufficient data to assess the effects of severe and end-stage renal impairment, or of bilirubin levels >3 mg/mL; therefore, additional studies are needed to evaluate such effects. Extrinsic factors, such as dose, formulation (frozen vs lyophilized), and study phase, were also evaluated as potential sources of variability in pharmacokinetic parameters. Formulation had only minor effects on the pharmacokinetics of total daunorubicin, but not cytarabine (Table 2), and thus is unlikely to be of clinical importance. Study phase had no measurable effect on the pharmacokinetics of CPX-351.

The exposure-response analyses focused on patients who received the recommended dose of CPX-351 (100 units/m<sup>2</sup> [daunorubicin 44 mg/m<sup>2</sup> and cytarabine 100 mg/m<sup>2</sup>] for induction and 65 units/m<sup>2</sup> [daunorubicin 29 mg/m<sup>2</sup> and cytarabine 65 mg/m<sup>2</sup>] for consolidation). In general, better efficacy outcomes were observed in the higher exposure quartiles: the longest overall survival and Event free survival were associated with the highest exposure quartile, as were the highest rates of complete remission. Early mortality rates ( $\leq 30$  days or  $\leq 60$  days from treatment start) in all CPX-351 exposure quartiles were lower than those in the 7 + 3 treatment group. Death within 30 days of treatment occurred in few patients treated with CPX-351, and thus it was not possible to distinguish trends across exposure quartiles. However, there was a trend toward lower 60-day mortality in higher exposure quartiles. A trend toward a greater frequency of grade 3 to 5 TEAEs was observed at higher quartiles of CPX-351 exposure. This trend appeared to be driven by grade 3 TEAEs; a similar trend was not observed among grade 4 or grade 5 TEAEs. Rates of serious AEs were

also comparable across exposure quartiles. The limited effects of CPX-351 exposure on AE rates may be due, in part, to the large proportion of drug that remains encapsulated (>99% of cytarabine and daunorubicin). Overall, the results from exposure-response analysis suggest that the current CPX-351 regimen provides a favorable risk-benefit profile.

Univariate and multivariate analyses with prognostic factors were conducted to better understand the factors that might be relevant to the exposure-response analysis. Some prognostic factors, such as bone marrow blast count and WBC category, which were significant in the univariate analysis, were excluded after the multivariate analysis. However, multivariate analysis indicated that patients with lower ECOG scores, non-poor karyotype, or platelets  $\geq 50 \times 10^9$  /L tended to benefit more from CPX-351 treatment. Patients with poor prognostic factors (eg, adverse karyotype, ECOG scores >0, or platelets  $< 50 \times 10^9$  /L) tended to have lower drug exposures and less clinical benefit. One possible explanation is that CPX-351 liposomes are largely metabolized by the reticuloendothelial system (or mononuclear phagocyte system). Al-Matary and coworkers<sup>25</sup> recently reported an increase in accumulation of monocytes/macrophages in the bone marrow of AML patients and in the bone marrow and spleens of several AML mouse models, suggesting that the reticuloendothelial system may be upregulated in AML. This could, in turn, lead to greater clearance of CPX-351 and lower drug exposure in these patients. Additional work is needed to further investigate potential interactions between patient characteristics and CPX-351 exposure. This work will help to optimize the dose for patients who are predicted to have lower exposure at the standard dose, provided that the optimal dose is tolerable.

The population pharmacokinetic model was limited by the availability of data defining the terminal phase; thus, parameters associated with the peripheral compartment were less precisely described by the base model. In addition, the limited number of patients with severe renal impairment and the lack of patients with serum bilirubin levels >3 mg/dL provided insufficient data to evaluate the impact of severe renal and hepatic impairment on the pharmacokinetics of CPX-351. Drug release from liposomes was not assessed in this population pharmacokinetic analysis due to limited data on free drug measurement.

## Conclusions

Data from this comprehensive population pharmacokinetics analysis indicate that no adjustments to the recommended dose and dose schedule of CPX-351 are warranted for patients with mild or moderate renal impairment or serum bilirubin  $\leq 3$  mg/dL. The exposure-

response analysis indicates that the current CPX-351 regimen provides a favorable risk-benefit profile.

## Declaration of Conflicting Interests

Q.W. and K.B. are employees of and have stock ownership in Jazz Pharmaceuticals, Inc. G.V. and J.F.M. are employees of Certara, which was contracted by Jazz Pharmaceuticals, Inc. for the conduct of this work. J.G. is a former employee of and has stock ownership in Jazz Pharmaceuticals.

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## Data Sharing

All relevant data are provided within the article and supporting files. Queries about the data presented in this study may be directed to Qi Wang at Qi.Wang@jazzpharma.com.

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## Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.