

Pharmacology of CPX-351: A Nano-scale Liposomal Fixed Molar Ratio Cytarabine-Daunorubicin for Patients with Advanced Leukemia

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Introduction

Ratiometric dosing is an approach to optimizing the therapeutic efficacy of chemotherapy doublets by fixing the molar ratio of a combination within a drug carrier to ratios shown in vitro to maximize antitumor synergy and minimize antagonism. CPX-351 is a formulation of cytarabine and daunorubicin encapsulated at a 5:1 molar ratio within a liposomal carrier. The 5:1 molar ratio was shown in vitro to maximize antitumor efficacy across multiple leukemic and solid tumor cell lines and in animal model studies to be consistently more efficacious than conventional free-drug treatment with cytarabine and daunorubicin across a variety of molar ratios at MTD levels. The results of a phase I study of CPX-351 in patients with relapsed or refractory acute leukemias were presented at the 2008 ASH meeting and this report will focus on the pharmacokinetics and pharmacology results from that study.

Methods

Patients with advanced AML, ALL and high risk MDS were eligible. CPX-351 was given by 90 minute infusion on Days 1, 3 and 5 of each induction course. Second inductions were permitted with evidence of antileukemic effect and persistence of leukemia in a Day 14 bone marrow. The starting dose was 3 u/m² (1u = 1 mg cytarabine and 0.44 mg daunorubicin) and doses were doubled with each single patient cohort until evidence of drug effect was observed. Thereafter, 3-patient cohorts and 33% dose increments were continued until limiting toxicities signaled the end of dose escalation. Pharmacokinetic samples were collected on Days 1, 3 and 5 of the first induction course and analyzed for cytarabine, daunorubicin, Ara-U and daunorubicinol by validated LC-MS/MS methods.

Samples were collected at the following times (after the start of the infusion) during the first cycle:

- Prior to dosing on Day 1, at the mid-point and end of the infusion, then at 2, 4, 6, 8, 12 and 24 hours.
- Prior to dosing on Day 3, at the mid-point and end of the infusion.
- Prior to dosing on Day 5, at the mid-point and end of the infusion, then at 2, 4, 6, 8, 12, 24, 48, 72, 96, and 168 hours.

The methods for cytarabine and daunorubicin involved disruption of the liposome membrane to liberate the encapsulated drugs. Accordingly, these methods measured total cytarabine and daunorubicin; the sum of encapsulated and non-encapsulated drug in the plasma.

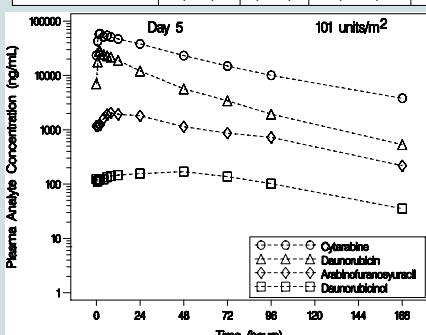
Pharmacokinetics

Maximum concentration (C_{max}), and the time of C_{max} (T_{max}) were taken directly from the observed data. The area under the concentration-time curve [AUC_(0-168h)] from time 0 to the time of the last observable concentration (C_{last}) was calculated using the linear trapezoidal rule. Noncompartmental pharmacokinetic analysis was performed on individual plasma CPX-351 concentration-time data using WinNonlin Professional (Version 5.2, Build 200701231637) Noncompartmental Analysis Program. Data manipulation was performed using Excel 2003 (11.8237.8221) SP3. All pharmacokinetic calculations were performed using published noncompartmental relationships¹. Nominal administration times and sample collection times were used for the analysis. Plasma concentrations below the lower limit of quantification (LLOQ) were set to zero for the noncompartmental analysis.

CPX-351 exhibits 1st order elimination kinetics and minimal early phase distribution

Table 3: Mean (SD) Cytarabine, Daunorubicin, Arabinofuranosyluracil, and Daunorubicinol Pharmacokinetic Parameters on Day 5 Following Intravenous Administration of 101u/m² CPX-351 over 90 minutes on Days 1, 3 and 5

Analyte	C _{max} ng/mL (SD)	T _{max} hours (SD)	AUC _(0-168h) ng·hr/mL (SD)	t _{1/2} hours (SD)	C _{max} /Dose (ng/mL)/(mg/m ²) (SD)	AUC _(0-168h) /Dose (ng·hr/mL)/(mg/m ²) (SD)
Cytarabine	64608 (23230)	3.02 (2.25)	1851089 (934523)	36.9 (24.5)	640 (230)	18328 (9253)
Daunorubicin	30185 (6198)	1.87 (0.74)	666640 (209198)	25.2 (11.6)	680 (140)	15014 (4712)
Ara-U	2119 (789)	13.4 (12)	78411 (31709)	-	21 (7.81)	776 (314)
Daunorubicinol	180 (103)	27.7 (15.6)	7308 (4030)	-	4.05 (2.31)	165 (90.8)



PK Analysis shows:

- The log-linear elimination of both cytarabine and daunorubicin with minimal early phase distribution (Figure 1)
- Molar ratios for cytarabine:daunorubicin are maintained near 5:1 for greater than 24 hours across cohorts
- Elimination half-lives for both cytarabine and daunorubicin are not significantly different between day 1 and day 5 and between cohorts (24-134 units/m²)
- Mean elimination half-lives for cytarabine and daunorubicin are 36.9 hr and 25.2 hr, respectively, on day 5
- Cytarabine and daunorubicin exhibited linear dose pharmacokinetics parameters on Day 1 and 5
- Accumulation of cytarabine and daunorubicin on Day 5 relative to Day 1 was observed and consistent with the prolonged half-lives of both drugs

Cytarabine and Daunorubicin are Bioavailable

- Bioavailability was assessed for CPX-351 by measuring metabolite AUCs from 96-144hr (0-tau). (AUC_{ARAU} and AUC_{DAUNORUBICINOL})
- AUCs (0-∞) for free cytarabine & free daunorubicin as a result of their release from CPX-351 were calculated by dividing the total dose of cytarabine or daunorubicin from CPX-351 by the respective clearances reported for the conventionally administered drugs. (AUC_{ARAC} and AUC_{DAUNORUBICIN})
- The metabolite to drug AUC ratios were then calculated for each drug from CPX-351.
- These ratios were then compared to the ratios reported for conventionally administered drug.

Table 4: Bioavailability of CPX-351

AUC ratios	n	CPX-351 Ratio ±SEM	Conventionally Administered Drugs ^{2,3,4} Ratio ±SEM
AUC _{ARAU} / AUC _{ARAC}	37	108 ± 9.6	~100
AUC _{DAUNORUBICINOL} / AUC _{DAUNORUBICIN}	38	5.79 ± 0.63	4.74 ± 0.55

- Although total cytarabine and daunorubicin concentrations are markedly elevated for CPX-351, the metabolite to parent AUC ratios for both drugs are similar to those from conventionally administered drugs, indicating high systemic bioavailability.

References

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Demographics & Safety Profile of CPX-351

Table 1: Patient Demographics and Disposition:

	n	%
Gender	61	66
Female	16	34
Age (yr)	Median	62
<60yrs	26	55
>60yrs	21	45
Race	White	40
African American	3	6
Asian	4	9
ECOG	0	22
1	22	47
2	3	6
Diagnosis	AML	41
Secondary AML	3	6
ALL	3	6
Resp. to Last Line of Therapy	None	27
CR	20	43
Reason Off Study	Serious Adverse Event	8
Persistent Leukemia	26	55
Bone Marrow Transplant	2	4
Lost to Follow-up	1	2
Complete Remission	11*	23

* One of the BMT patients achieved a PR
† One of the CR patients transferred for BMT and is counted twice.

- 37 patients were recruited in 10 cohorts for dose escalation with additional patients entered to control tolerability and collect preliminary efficacy data for first relapse at the MTD resulting in a total of 47 patients treated on this study
- The majority of patients were male (66%), above 60 years of age (55%), white (85%), had ECOG PS of 0 or 1 (94%), had AML (94%), and failed to respond to treatment immediately prior to study entry.

• The MTD was exceeded at the 10th dose level (134 u/m²). Dose limiting toxicities included single patients with hypertensive crisis (grade 4), LV dysfunction (grade 3), and persistent cytopenias beyond day 56.

• The 9th dose level (101 u/m²) is recommended for Phase II use.

• Adverse events were similar to those expected for conventional cytarabine and daunorubicin treatment and included single cases of dose limiting hypertensive crisis, LV dysfunction; and persistent cytopenias at the 134u/m² dose level. At lower dose levels cytopenia-related complications including febrile neutropenia, various infectious events, and bleeding manifestations were prominent, expected, and accounted for most of the observed serious adverse events and a substantial fraction of the grade 3 and 4 adverse events.

• Persistent leukemia led to study discontinuation in 55% of study patients and accounted for 2/3 of the deaths observed during and after the study period.

• At study completion 1 of 3 ALL and 10 of 44 AML patients achieved CR, CRs and CRp were achieved at doses as low as 32 u/m², less than 1/3 the MTD.

• Among 22 AML patients treated in the ≥ 2nd salvage setting 2 CRs and 1 CRp were observed. Among 22 patients treated in the 1st salvage setting 7 CRs were observed including 6 CRs among 17 patients in first relapse.

Table 2: Non-hematological High Grade Adverse Events

Adverse Event	Cohort 9		TOTAL	
	101(MTD)	ALL		
Gr. 3	Gr. 4	n=36	%	
Infections				
Febrile Neutropenia	8	2	17	47
Bacteremia	7	0	9	25
Pneumonia	7	0	8	22
Sepsis/Septic Shock	4	0	5	14
UTI	0	0	1	3
Cellulitis	1	0	1	3
Clostridial Infection	1	0	2	6
Bleeding Events				
Intracerebral	0	0	1	3
GI bleeding	0	0	1	3
Mucosal/Skin	2	1	4	11
CNS				
Aphasia	0	0	1	3
Convulsion	0	0	1	3
Level of Consciousness	0	0	1	3
Metabolic				
Hypokalemia	2	1	5	14
Metabolic Acidosis	1	0	1	3
Hypophosphatemia	0	0	1	3
Skin				
Rash	1	0	3	8
Cardiovascular				
LV Dysfunction/Orthopnea	1	0	2	6
Pericardial Effusion	1	0	1	3
Atrial Fibrillation	0	0	1	3
Hypertension	0	0	2	6
Respiratory				
Dyspnea	2	0	3	8
ARDS	1	0	1	3
Hypoxia	0	0	2	6
Aspiration	0	0	1	3
Cough	0	0	1	3
Gastrointestinal				
Nausea/vomiting	0	0	1	3
Diarrhea	1	0	1	3
Mucositis	0	1	2	6
Renal				
Acute Renal Failure	1	0	2	6
General				
Fatigue	1	0	1	3
Pyrexia	0	0	3	8
Asthenia/Weakness	0	0	1	3

Discussion

The Phase 1 study established 101 u/m² as the maximal tolerated dose for Phase 2 use. Adverse events were similar to conventional cytarabine and daunorubicin treatment and were dominated by cytopenia related complications. In a population with the majority of subjects (81%) having prior exposure to cytarabine + daunorubicin, exposure to CPX-351 (a 5:1 molar ratio of liposome encapsulated cytarabine:daunorubicin) in the salvage setting produced a surprisingly high rate of complete remissions starting at dose levels as low as 32 u/m² (32 mg/m² cytarabine + 14 mg/m² daunorubicin). More precise estimates of efficacy are expected from the pair of randomized Phase 2 studies currently enrolling subjects.

A pharmacodynamic model for the action of CPX-351 has emerged from preclinical studies using bone marrow-engrafting human leukemia xenografts. In this model, CPX-351 maintains high cytarabine and daunorubicin concentrations in the plasma for days after injection while preserving the 5:1 molar ratio. Furthermore, CPX-351 accumulates in the bone marrow compartment where it slowly releases the drugs and is selectively taken up by leukemia cells compared to normal bone marrow cells (See EHA 2009 Abstract #383).

The pharmacology data presented in Tables 3 and 4 and Figure 1, demonstrate that CPX-351 exhibits 1st order elimination kinetics and prolonged elimination half life. Multiple dose PK observations showed linear monoexponential plasma elimination of both cytarabine and daunorubicin and a very limited distribution phase. The C_{max} and AUC_(0-168h) of cytarabine and daunorubicin were linearly correlated with dose with evidence of drug accumulation between Day 1 and Day 5 for some dose groups. It must be remembered that the data in Table 4 and Figure 1 reflects predominantly encapsulated rather than free cytarabine and daunorubicin. Consequently, the marked increase in AUC and t_{1/2} for CPX-351 suggest the possibility that prolonged drug exposure may arise from free drugs released from the liposomes or from direct interactions with the liposome encapsulated agents.

The conclusion that CPX-351 bioavailability is likely to be high is based on comparing the ratio of the AUCs for cytarabine versus its metabolite Ara-U. Lillemark² reported that ratio to be approximately 100 following conventional cytarabine administration. Following CPX-351 administration a ratio of 108 was found, suggesting that most or nearly all of the encapsulated cytarabine was ultimately bioavailable. Similarly, Roberts³ reported that AUC daunorubicinol/AUCdaunorubicin to be 4.74 following conventional daunorubicin administration. CPX-351 produced a ratio of 5.79, again suggesting that release of drug from the liposome and its metabolism in tissue was similar to that of the conventionally administered drug. The bioavailability results suggest that most of the potential drug exposure from encapsulated drug is likely to be realized; with a likelihood that effective drug exposure will be extended by days beyond what is possible for conventionally delivered cytarabine and daunorubicin while maintaining the desired 5:1 drug ratio.

Conclusions

- The maximally tolerated dose for CPX-351 of 101 u/m² is recommended for use in Phase 2 studies
- Dose limiting toxicities were hypertensive crisis, LV dysfunction, and persistent cytopenias
- The CPX-351 safety profile consisted primarily of cytopenia-related adverse events. Alopecia and major gastrointestinal adverse events (≥ grade 3) were uncommon.
- Complete remissions were observed in both ALL (1 of 3 patients) and AML (10 of 44 patients). Remissions occurred in patients that were multiply relapsed, unresponsive to their immediate prior therapy, or in first relapse with short duration first CRs. CRs occurred at dose levels as low as 1/3 to 1/2 the MTD.
- CPX-351 maintains the desired 5:1 cytarabine:daunorubicin molar ratio for prolonged periods of time.
- CPX-351 exhibits log-linear elimination with substantial increases in AUC and t_{1/2} when compared with literature reports for conventional cytarabine and daunorubicin.
- CPX-351 appears to be bioavailable based on similar AUC_{metabolite}/AUC_{drug} ratios for both cytarabine and daunorubicin when compared to conventionally administered drug.
- Clinical evidence that CPX-351 increases the AUC and t_{1/2} of encapsulated cytarabine and daunorubicin with persistence of circulating liposomal drug in the plasma to Day 12 while maintaining drug bioavailability suggests that leukemic cells may be exposed to potentially cytotoxic drug levels during the first and second weeks of induction treatment.