CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW

NDA 21-673

Drug name:	CLOLAR®
Generic name:	Clofarabine
Formulation:	1mg/mL solution for intravenous administration
Pediatric Indication:	Refractory or relapsed acute lymphoblastic leukemia (ALL) and acute myelogenous leukemia (AML) in children.
Current Submission:	NDA-NME
Applicant:	Ilex Products Inc. 4545 Horizon Hill Blvd. San Antonio, Texas 78229-2263
OCPB Division:	Division of Pharmaceutical Evaluation I (HFD-860)
OND Division:	Division of Oncology Drug Products (HFD-150)
Submission Dates:	29-Mar-2004, 2-Aug-2004, 5-Aug-2004, Oct-3-2003
Primary Reviewer:	Roshni Ramchandani, Ph.D.
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TABLE OF CONTENTS

I. Executive Summary

Clofarabine is a purine nucleoside analog. The applicant has conducted studies evaluating the use of clofarabine in the treatment of acute lymphoblastic leukemia (ALL) and acute myelogenous leukemia (AML) in pediatric patients. The applicant has conducted 3 clinical studies that form the basis for the NDA application and include a Phase 1 study and 2 phase 2 studies. Study # ID99-383 was a phase 1 open-label, non-randomized, dose escalation study for pediatric patients with hematological malignancies (ALL and AML) who have failed standard therapy or for whom no such therapy existed (n=25). Patients received doses of clofarabine as 1-3 hr IV infusion daily × 5 days, every 2 to 6 weeks for a maximum of 12 cycles. The doses evaluated were 11.25, 15, 30, 40, 52 and 70 mg/m^2/day. The objective of this study was to establish the maximum tolerated dose and obtain pharmacokinetic data in this population. Study # CLO-212 was a phase 2 open-label, non-randomized study in pediatric patients (1-20 yrs) with refractory or relapsed acute lymphoblastic leukemia (ALL) (n=49). Patients received 52 mg/m^2/day of clofarabine as a 2-hr IV infusion daily × 5 days, every 2 to 6 weeks for a maximum of 12 cycles. The objective of this study was to examine the effectiveness of clofarabine in this population as well as to obtain data on the pharmacokinetics (PK) of clofarabine in the pediatric population. Study # CLO-222 was a phase 2 open-label, non-randomized study in pediatric patients (1-20 yrs) with refractory or relapsed acute myelogenous leukemia (AML) (n=35). Patients received 52 mg/m^2/day of clofarabine as a 2-hr IV infusion daily × 5 days, every 2 to 6 weeks for a maximum of 12 cycles. The objective of this study was to examine the effectiveness of clofarabine in this population as well as to obtain data on the PK of clofarabine in the pediatric population.

The population pharmacokinetics of clofarabine were studied in 40 pediatric patients, aged 2 to 19 years (21 males/19 females), from the above studies. Clofarabine pharmacokinetics were best described by a 2-compartment model with first order elimination. Body weight was a significant predictor for all model parameters (CL, Q, V1 and V2). BSA-normalized doses of 52 mg/m^2 produced equivalent exposure across a wide range of BSAs. Based on a non-compartmental analysis, systemic clearance and volume of distribution at steady-state were estimated to be 28.8 L/h/m^2 and 172 L/m^2, respectively. The terminal half-life was estimated to be 5.2 hours. The baseline White Blood Cell (WBC) count was found to be a significant predictor of the central compartment volume V1 by the applicant. However, the Agency's analysis determined that WBC counts were not correlated with the central volume estimates and inclusion of WBC in the parameter model did not reduce the population variance for the central volume. Renal excretion of unchanged clofarabine (over a 24-hour interval) accounted for 49-60% of the total clearance. In vitro studies using isolated hepatocytes indicate very limited hepatic metabolism, thus the pathways of non-renal elimination are unknown. No major pharmacokinetic differences were found between ALL and AML patients or between male and female patients. Intra-cellular concentrations of the active metabolite clofarabine triphosphate were also measured in some patients in the phase 1 study, however the data were too sparse for any meaningful evaluation. The inhibition and induction potential of clofarabine for cytochrome p450 enzymes has not been studied. The pharmacokinetics of clofarabine have not been evaluated in patients with renal or hepatic dysfunction, and use of the drug in these patients should be undertaken with caution.

No significant relationships were found between measures of clofarabine exposure and measures of clofarabine response or toxicity in this population. This may be because the majority of the patients received the 52 mg/m^2 dose and this did not provide an adequate range of exposures to effectively evaluate the exposure-response relationship for clofarabine.

A. Recommendations

The Office of Clinical Pharmacology and Biopharmaceutics (OCPB) has reviewed the Clinical Pharmacology section of NDA 21-673 and finds it to be acceptable, with some revisions to the applicant's proposed label.

FDA Proposed labeling

1. The following should be inserted under the Human Pharmacokinetics section, under CLINICAL PHARMACOLOGY

The population pharmacokinetics of CLOLAR™ were studied in 40 pediatric patients aged 2 to 19 years (21 males/19 females) with relapsed or refractory ALL or AML. At the given 52 mg/m^2 dose, similar concentrations were obtained over a wide range of BSAs. Clofarabine was 47% bound to plasma proteins, predominantly to albumin. Based on non-compartmental analysis, systemic clearance and volume of distribution at steady-state were estimated to be 28.8 L/h/m^2 and 172 L/m^2, respectively. The terminal half-life was estimated to be 5.2 hours. No apparent difference in pharmacokinetics was observed between patients with ALL and AML or between males and females.

No relationship between clofarabine or clofarabine triphosphate exposure and toxicity or response was found in this population.

Based on 24-hour urine collections in the pediatric studies, 49-60% of the dose is excreted in the urine unchanged. *In vitro* studies using isolated human hepatocytes indicate very limited metabolism (0.2%), therefore the pathways of non-renal elimination remain unknown.

Although no clinical drug-drug interaction studies have been conducted to date, on the basis of the *in vitro* studies, cytochrome p450 inhibitors and inducers are unlikely to affect the metabolism of clofarabine. The effect of clofarabine on the metabolism of cytochrome p450

substrates has not been studied. The pharmacokinetics of clofarabine have not been evaluated in patients with renal or hepatic dysfunction.

2. The following should be inserted under the Drug Interactions section under PRECAUTIONS

Although no clinical drug-drug interaction studies have been conducted to date, on the basis of the *in vitro* studies, cytochrome p450 inhibitors and inducers are unlikely to affect the metabolism of clofarabine. The effect of clofarabine on the metabolism of cytochrome p450 substrates has not been studied.

4. The following should be inserted under the Hepatic and Renal Impairment under WARNING and under DOSAGE AND ADMINISTRATION section

CLOLAR™ has not been studied in patients with hepatic or renal dysfunction. Its use in such patients should be undertaken only with the greatest caution.

Additional Recommendations

1. We recommend that you evaluate the pharmacokinetics of the active metabolite clofarabine triphosphate, both in future studies in adult and pediatric patients to better understand the exposure-response relationship for this drug and to help optimize dosing regimens in the future studies.

2. We recommend that you examine the effect of renal impairment on the safety and pharmacokinetics of clofarabine in patients in future studies.

3. Your studies have shown that clofarabine is not hepatically metabolized and that ~60% is renally excreted unchanged. The fate of the remaining 40% is not known. We suggest that you try to explore the fate of the fraction of the clofarabine that is not eliminated by renal or hepatic routes.

B. Phase IV Commitments

None.

C. Summary of Important Clinical Pharmacology and Biopharmaceutics Findings

Clofarabine pharmacokinetics were determined in 40 pediatric patients, ages 2 to 19 years, from 3 studies: a phase 1 dose escalation study and two phase 2 studies in ALL and AML patients. A population pharmacokinetic model was fit to the data from these studies. Clofarabine pharmacokinetics was best described by a 2-compartment model with first order elimination. Body weight was the best predictor in parameter models for all model parameters (CL, Q, V1 and V2). The applicant's model included baseline WBC count as a predictor of the central compartment volume V1. The Agency's analysis determined that WBC counts were not correlated with the central volume estimates and inclusion of WBC in the parameter model did not reduce the population variance for the central volume. Based on a non-compartmental analysis, systemic clearance and volume of distribution at steady-state were estimated to be 28.8 L/h/m^2 and 172 L/m^2, respectively. The terminal half-life was estimated to be 5.2 hours. No major pharmacokinetic differences were found between ALL and AML patients or between male and female patients. Intra-cellular concentrations of the active metabolite clofarabine triphosphate were also measured in some patients in the phase 1 study, however the data were too sparse for any meaningful evaluation.

Renal excretion of unchanged clofarabine, measured over a 24-hour period, accounts for 49-60% of the total clearance. *In vitro* studies using isolated hepatocytes indicate very limited hepatic metabolism, thus the pathways of non-renal elimination are unknown. The inhibition and induction potential of clofarabine for cytochrome p450 enzymes has not been studied. The pharmacokinetics of clofarabine has not been evaluated in patients with renal or hepatic dysfunction.

No significant relationships were found between measures of clofarabine exposure and measures of clofarabine response or toxicity. The applicant's analysis only included those patients who had PK measurements. The Agency's re-analysis of this data included estimation of the exposure (AUC) of clofarabine in all the patients in the studies, based on the parameter model for clearance which was a function of body weight. However this did not change the outcome, and there were still no significant associations between AUC and measures of toxicity or response. This may be partly because the majority of the patients received the 52 mg/m^2 dose, which did not provide an adequate range of exposures to effectively evaluate the exposure-response relationship for clofarabine.

<u>OCPB Briefing was held on December 14, 2004:</u>

Attendees: L. Lesko, S-M. Huang, C. Sahajwalla, A. Selen, J. Hunt, J. Collins, M. Cohen, M. Mehta, A. Rahman, S. Abraham, A. Men, C. Sung.

II. Question Based Review

A. General Attributes of the Drug

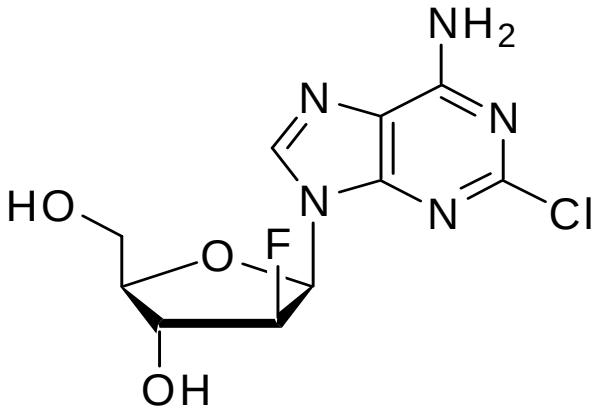
A1. What pertinent background or history contributes to the current assessment of the clinical pharmacology and biopharmaceutics of this drug?

Purine nucleoside analogues, including fludarabine and cladribine, have received FDA approval for the treatment of chronic and acute leukemias. These analogs have shown activity against lymphoproliferative disorders and this has led to the synthesis of a series of 2-halo-s'-halo-2' deoxyarabinofuranosyl adenine analogs that have also shown activity against a variety of preclinical tumor models, including tumor cell lines and xenografts. Clofarabine is one of these new analogs and was designed to be resistant to adenine deaminase and has greater gastric stability for improved oral administration.

This application for the use of clofarabine for the treatment of pediatric AML and ALL is the first submission for the use of clofarabine in any population.

A2. What are the highlights of the chemistry and physical-chemical properties of the drug substance, and the formulation of the drug product as they relate to clinical pharmacology and biopharmaceutics review?

Clofarabine is a second-generation purine nucleoside anti-metabolite from a family of compounds that includes marketed drugs like fludarabine, cladribine, and gemcitabine. The chemical name for clofarabine is 2-chloro-9- (2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)-9H-purine-6-amine. The molecular formula of clofarabine is C10H11ClFN5O3 with a molecular weight of 303.68.



Clofarabine

Clofarabine (1 mg/ml) is supplied in a 20 ml single-use vial. The 20 ml vial contains 20 mg of drug formulated in 20 ml of unbuffered normal saline (comprised of Water for Injection, United

States Pharmacopoeia [USP], and Sodium Chloride, USP). The pH range of the solution is 4.5 to 7.5. The solution is clear and practically colorless, and free from foreign matter.

A3. What are the proposed mechanism(s) of action and therapeutic indication(s)?

Clofarabine is sequentially metabolized intracellularly to the 5'-monophosphate metabolite by deoxycytidine kinase and mono- and di-phosphokinases to the active 5' triphosphate metabolite. Conversion of the monophosphate to the diphosphate is the rate-limiting step resulting in cellular accumulation of both clofarabine mono- and tri-phosphate. Clofarabine has high affinity for the activating phosphorylating enzyme, deoxycytidine kinase, equal to or greater than that of the natural substrate, deoxycytidine. Clofarabine inhibits DNA synthesis by decreasing cellular deoxynucleotide triphosphate (dNTP) pools through an inhibitory action on ribonucleotide reductase, and by terminating DNA chain elongation and inhibiting repair through incorporation into the DNA chain by competitive inhibition of DNA polymerases. The affinity of clofarabine triphosphate for these enzymes is similar to or greater than that of deoxyadenosine triphosphate. In preclinical models, clofarabine has demonstrated the ability to inhibit DNA repair by incorporation into the DNA chain during the repair process. Clofarabine 5'-triphosphate also disrupts the integrity of mitochondrial membrane, leading to the release of the pro-apoptotic mitochondrial proteins, cytochrome C and apoptosis inducing factor, leading to programmed cell death.
In summary, the anticancer activity of clofarabine is believed to be due to 3 mechanisms:

- DNA polymerase A inhibition resulting in termination of DNA elongation and/or DNA synthesis repair
- Ribonucleotide reductase inhibition with reduction of dNTP pools
- Induction of apoptosis through direct and indirect action on mitochondria by releasing cytochrome C and other proapoptotic factors

Experiments in pharmacology models have demonstrated that clofarabine is cytotoxic to a variety of rapidly proliferating and quiescent cancer cell types in vitro. Clofarabine causes tumor growth delay in human xenograft tumor models including leukemia. Studies in in vivo solid tumor models show that clofarabine causes tumor regression.

A4. What are the proposed dosage(s) and route(s) of administration?

The recommended pediatric dose and schedule is 52 mg/m^2 administered by intravenous infusion over 2 hours daily for 5 consecutive days. Treatment cycles are repeated every 2 to 6 weeks, following recovery or return to baseline organ function in case of toxicity.
The available formulation should be diluted with 5% dextrose injection, USP or EP or 0.9% sodium chloride injection, USP or EP prior to infusion. The dosage is based on the patient's body surface area (BSA), calculated using the actual height and weight before the start of each cycle.

B. Clinical Pharmacology

General attributes

B1. What are the design features of the clnical pharmacology and clinical studies used to support dosing or claims?

The applicant has conducted 3 clinical studies of clofarabine in pediatric populations. These studies form the basis for the NDA application and include:

- ID99-383 – A phase 1 open-label, non-randomized, dose escalation study for pediatric patients with hematological malignancies (ALL and AML) who have failed standard therapy or for whom no such therapy existed (n=25). Patients received doses of clofarabine as 1-3 hr IV infusion daily × 5 days, every 2 to 6 weeks for a maximum of 12 cycles. The doses evaluated were 11.25, 15, 30, 40, 52 and 70 mg/m^2/day. The objective of this study was to establish the maximum tolerated dose and obtain PK data in this population.

- CLO-212 – a phase 2 open-label, non-randomized study in pediatric patients (1-20 yrs) with refractory or relapsed acute lymphoblastic leukemia (ALL) (n=49). Patients received 52 mg/m^2/day of clofarabine as a 2-hr IV infusion daily × 5 days, every 2 to 6 weeks for a maximum of 12 cycles. The objective of this study was to examine the effectiveness of clofarabine in this population as well as to obtain data on the PK of clofarabine in the pediatric population.

- CLO-222 – a phase 2 open-label, non-randomized study in pediatric patients (1-20 yrs) with refractory or relapsed acute myelogenous leukemia (AML) (n=35). Patients received 52 mg/m^2/day of clofarabine as a 2-hr IV infusion daily × 5 days, every 2 to 6 weeks for a maximum of 12 cycles. The objective of this study was to examine the effectiveness of clofarabine in this population as well as to obtain data on the PK of clofarabine in the pediatric population.

Table I summarizes the design elements of the 3 pediatric studies. In addition, the applicant has conducted a phase I study of clofarabine in adult patients with solid and hematological malignancies (DM93-036) and a phase 2 study in adult patients with AML (CLO-221).

Table I: Summary of pediatric studies with clofarabine.

Study Number	**ID99-383**	**CLO-212**	**CLO-222**
Title	Phase I Study of CL-F-Ara-A (Clofarabine) in Pediatric Patients with Hematologic Malignancies	Study CLO-212: A Phase II, Open- Label Study of Clofarabine in Pediatric Patients With Refractory or Relapsed Acute Lymphoblastic Leukemia	A Phase II, Open- Label Study of Clofarabine in Pediatric Patients With Refractory or Relapsed Acute Myelogenous Leukemia
Patient population	Pediatrics with hematologic malignancies	Pediatrics with ALL	Pediatrics with AML
Number of patients Number in whom PK was done	N=25 12	N=49 14	N=35 14
Age	3 – 17 yrs	2 – 17 yrs	3 – 19 yrs
Gender	6 M / 6 F	7 M / 7 F	8 M /6 F
Doses (mg/m2) Schedule	11.25, 15, 30, 40, 52, 70 1-3 hr infusion daily x5 q2-6wks	52 2 hr infusion daily x5 q2-6wks	52 2 hr infusion daily x5 q2-6wks
PK sampling	Sparse data (0, end-of-infusion (EOI), EOI+1 hr, 24 h) Plasma only	Rich data Plasma and urine Day 1 and Day 5 Plasma: 0, 2 (EOI), 3, 4, 5, 10, 24 hrs Urine: 0-8, 8-24 hrs	Rich data Plasma and urine Day 1 and Day 5 Plasma: 0, 2 (EOI), 3, 4, 5, 10, 24 hrs Urine: 0-8, 8-24 hrs
Analytes	Plasma clofarabine Intracellular clofarabine triphosphate extracted from PMNC	Plasma clofarabine Intracellular clofarabine triphosphate extracted from PMNC*	Plasma clofarabine Intracellular clofarabine triphosphate extracted from PMNC*
Assay	Clofarabine: LC-MS/MS Clofarabine triphosphate: HPLC-UV	Clofarabine: LC-MS/MS Clofarabine triphosphate: HPLC-UV	Clofarabine: LC-MS/MS Clofarabine triphosphate: HPLC-UV
PK analysis, parameters	Data too sparse for PK analysis	Non-compartmental: Cmax, AUC, CL, Vss, CLr, %Ae	Non-compartmental: Cmax, AUC, CL, Vss, CLr, %Ae

*: intracellular clofarabine triphosphate measured in 2 patients in each of the phase 2 studies.

B2. What is the basis for selecting the response endpoints (i.e., clinical or surrogate endpoints) or biomarkers (collectively called pharmacodynamics (PD)) and how are they measured in clinical pharmacology and clinical studies?

The primary endpoint in the phase 2 studies was the objective response rate, defined as a complete remission (CR), complete remission in the absence of platelet recovery (CRp) or partial remission (PR), according to COG criteria (table II). These objective responses have been considered as evidence of therapeutic benefit of the drug in this population.

Table II: Objective response definitions according to Children's Oncology Group (COG) criteria.

Response Category	Response Criteria
CR	No evidence of circulating blasts or extramedullary disease M1 bone marrow (≤5% blasts) Recovery of peripheral counts (platelets ≥100 × 10^9/L and ANC ≥1.0 × 10^9/L)
CRp	Meets all of the criteria for a CR except platelet recovery to ≥100 × 10^9/L
PR	Complete disappearance of circulating blasts M2 bone marrow (>5% and ≤25% blasts) and appearance of normal progenitor cells M1 marrow that does not qualify for CR or CRp
Treatment failures	All other responses

B3. Are the active moieties in the plasma (or other biological fluid) appropriately identified and measured to assess pharmacokinetic parameters and exposure response relationships?

Clofarabine is a pro-drug that is taken up into WBCs where it is converted to its active triphosphate form. Plasma levels of clofarabine were measured in all three studies in pediatric populations. However, levels of intracellular clofarabine triphosphate were only measured in a few patients in the phase I study (ID99-383). Of the 70 patients in the two phase 2 studies, 4 patients had samples collected for measurement of intracellular clofarabine triphosphate levels, however the results have not been included in this application.

Exposure-response

B4. Is there a relationship between clofarabine exposure and effectiveness (response rates) in ALL and AML pediatric patients?

The applicant has examined the relationship between various measures of clofarabine exposure (Dose, Cmax and AUC) and response rates in the data, i.e., incidence of CR, CRp and PR. Their analysis, which only included those patients in whom PK data was available (n=32), did not reveal any significant relationships between exposure and response rates. However, this analysis is limited in that it totally excludes the patients in whom PK was not evaluated.

The Agency has repeated the analysis of exposure-response relationship for the entire dataset. The population PK model developed for clofarabine allowed the estimation of CL and therefore AUC for all the patients in the study, based on the body weight of the patients. This model-based estimate of AUC was used in logistic regression models to examine the exposure-response relationship for clofarabine in this population. The results, however, did not reveal any significant relationship between AUC and response rates in this population (see Pharmacometrics Review, Appendix C). This may be because the majority of the patients received the 52 mg/m^2 dose and this did not provide an adequate range of exposures to effectively evaluate the exposure-response relationship for clofarabine.

B5. Is there a relationship between clofarabine exposure and incidence of adverse events including hypotension, nausea+vomiting, neutropenia, anxiety, sepsis, dyspnea and hepatotoxicity (elevation in bilirubin and AST/ALT levels) in ALL and AML pediatric patients?

The applicant has examined the relationship between various measures of clofarabine exposure (Dose, Cmax and AUC) and incidence of adverse events in the data. The AEs evaluated included hypotension, nausea+vomiting, neutropenia, anxiety, sepsis, dyspnea and hepato-biliary toxicity. Their analysis, which only included those patients in whom PK data was available (n=32), did not reveal any significant relationships between exposure and incidences of the individual AEs. However, these analyses are limited in that they totally exclude the patients in whom PK was not evaluated.

The Agency has repeated the analysis of exposure-toxicity relationships in the entire dataset, using estimates of AUC obtained from the clearance that was imputed for each patient based on their body weight. The results of logistic regression of the adverse event listed above vs. AUC did not reveal any significant relationship.

Additional analysis was conducted on data from the lab tests for bilirubin and hepatic transaminase enzymes (SGPT and SGOT). The frequency of elevations in bilirubin, SGPT and SGOT were determined among the study patients, and logistic regressions were performed to examine if the frequency of elevations in any of these hepatic markers was associated with clofarabine AUC, which was estimated for each individual based on the population PK model for clearance. Results indicated that there was no significant relationship between clofarabine AUC and incidence of elevated bilirubin, SGPT or SGOT in the patients. Again, this might be related to the small sample size and limited range of clofarabine exposures seen in these studies.

B6. Does this drug prolong the QT or QTc interval?

QT/QTc prolongations were not seen in any of the pediatric patients in the phase 1 or phase 2 studies.

B7. Is the dose and dosing regimen selected by the applicant consistent with the known relationship between dose-concentration-response, and are there any unresolved dosing or administration issues?

The dose and dosing regimen selected by the applicant for the phase 2 studies were based on the MTD of clofarabine in the initial phase 1 study. The concentration-response relationship for clofarabine in this patient population has not been established.

At doses less than 52 mg/m^2, breakthrough DNA synthesis was observed in WBCs extracted from patients in the phase 1 study.
The rationale for the daily x 5 regimen is based on pre-clinical studies wherein optimal activity was obtained when the drug was given as multiple doses for several days.

Pharmacokinetics

B8. What are the PK characteristics of clofarabine and its active metabolite clofarabine triphosphate?

Clofarabine Figures 1A and B show the plasma clofarabine concentration-time profiles for patients in the two phase 2 studies.

Figure 1A: Plasma clofarabine concentration vs. time profiles for ALL patients (study CLO-212).

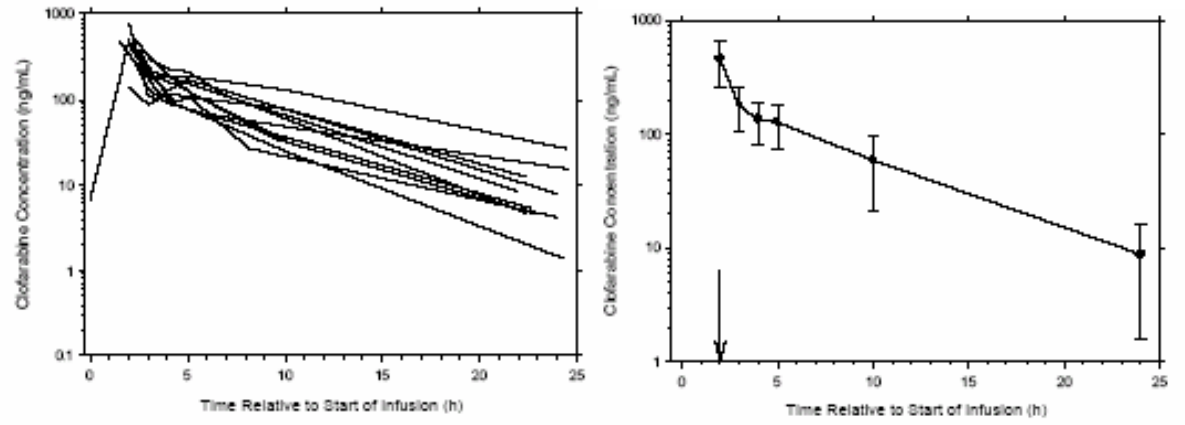
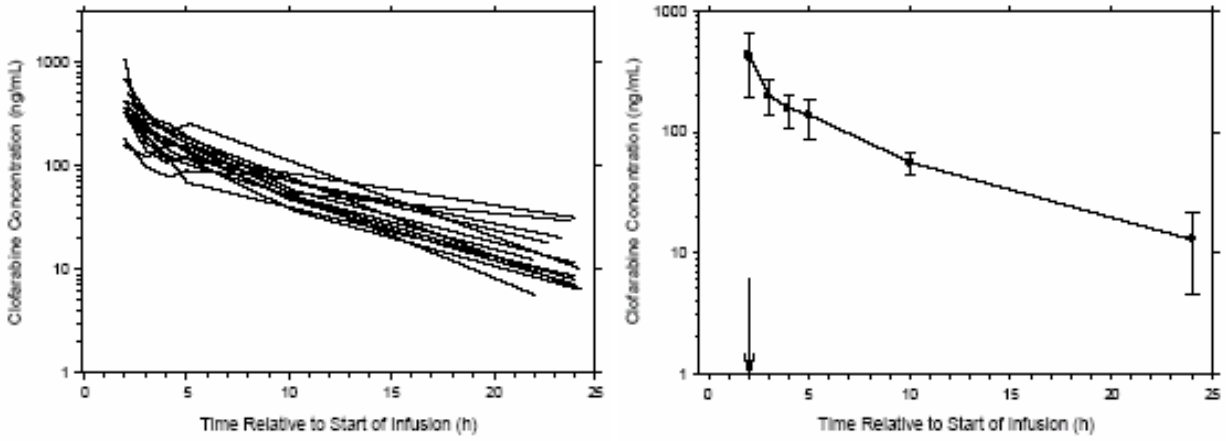


Figure 1B: Plasma clofarabine concentration vs. time profiles for AML patients (study CLO-222).



The population pharmacokinetics of clofarabine were studied in 40 pediatric patients ages 2 to 19 years old (21 males/19 females) with relapsed or refractory ALL or AML given multiple doses. Clofarabine pharmacokinetic parameters were dependent on body metrics (body weight, body surface area, with weight providing the best predictor in parameter models of clearances and volumes. BSA-normalized clofarabine doses of 52 mg/m^2 produced equivalent exposure across a wide range of weights. Clofarabine pharmacokinetics were best described by a 2-compartment model with first order elimination. Body weight was a significant predictor in parameter models for all model parameters (CL, Q, V1, V2), and WBC count was a significant predictor of the central compartment volume V1, although the applicant did not provide a clear rationale or

interpretation for including it as a covariate in the population analysis (also see Q.B13). Table III lists the PK parameters obtained from the best-fitting population PK model for clofarabine, for a typical 40 kg patient (WBC count = $10 \times 10^3/\mu L$).

Table III: Applicant's analysis: PK parameters for clofarabine (based on population PK analysis), for a typical 40 kg patient (WBC count = $10 \times 10^3/\mu L$).

Parameter	Typical Value [40 kg patient]	Between-subject variability
Clearance (CL)	32.8 L/hr	27%
Volume of distribution at steady-state (Vss)	210 L	72%
α-half-life	1.2 hr	-
β-half-life	6.4 hr	-

Simulations based on the applicant's PK model indicated that as WBC count was depleted (as a result of the cytotoxic effect of the drug), clofarabine AUC decreased and Cmax increased, although the change would likely not be clinically significant. No apparent difference in pharmacokinetics was observed between patients with ALL or AML or between males and females.

Based on the Agency's analysis, the inclusion of WBC could not be justified in the model for volume of distribution (see Pharmacometrics Review, Appendix C). The final model, according to the Agency's analysis, did not include WBC as a covariate in the analysis and resulted in the PK parameter estimates shown in table IV.

Table IV: Agency's analysis: *PK parameters for clofarabine (based on population PK analysis), for a typical 40 kg patient (n=30).*

	Model	Typical Value [40 kg patient]	Between-subject variability
CL	$32.5 \cdot (Wt/40)^{0.75}$	32.5 L/hr	20%
V1	$76.3 \cdot (Wt/40)^{1.0}$	76.3 L	57%
Q2	$25.3 \cdot (Wt/40)^{0.75}$	25.3 L/hr	15%
V2	$103 \cdot (Wt/40)^{1.0}$	103 L	37%

Clofarabine triphosphate

Clofarabine triphosphate concentrations extracted from peripheral mononuclear cells in the phase 1 study were assayed using a non-validated HPLC method. A limited sampling design (3 samples per subject) was used and the dataset included 10 patients. Values ranged from BLQ to (b) (4) ng/ml across all cycles, doses and times, and were much higher than concentrations of clofarabine in plasma on a per ml basis (median observed concentration=13.2 μM). No obvious triphosphate outliers were observed in the data. Clofarabine triphosphate concentrations appeared to increase with increasing dose and remained quantifiable 24 hours after the start of infusion.

Clofarabine triphosphate concentrations were found to be essentially constant over the sampling period with an estimated average concentration of 11.6 ± 0.262 μM. The in vivo half- life of clofarabine triphosphate in peripheral mononuclear cells could not be definitively established but was estimated to be greater than 24 hours.

A modest Pearson correlation of 0.42 (p = 0.0261) was observed between plasma clofarabine concentration and intracellular clofarabine triphosphate concentrations collected at the same time.

B9. What are the single dose and multiple dose PK parameters?

Single dose and multiple dose PK parameters were estimated from subjects in both phase 2 studies using non-compartmental methods. Table V lists the single dose PK parameters and table VI lists the multiple dose PK parameters obtained from the studies.

Table V: Single dose PK parameters (non-compartmental) for clofarabine in pediatric patients. Clofarabine dose = 52 mg/m^2.

	CLO 212 – ALL patients			CLO 222 – AML Patients		
Parameter	**N**	**Mean**	**Standard Deviation**	**N**	**Mean**	**Standard Deviation**
AUC(0-∞) (ng.h/mL)	11	1725.5	586.4	10	2044.2	480.3
Cmax (ng/mL)	11	403.1	171.4	13	417.5	228.9
CL (L/h)	11	43.2	18.8	10	38.9	15.9
CL (L/h/m^2)	11	33.1	10.3	10	26.8	6.4
Half-life (h)	11	4.7	1.8	10	5.7	2.0
Vdss (L)	11	251.0	142.4	10	274.4	156.3
Vdss (L/m^2)	11	187.1	75.1	10	182.1	67.2
Renal CL (L/h)	7	28.9	27.5	10	26.6	20.6
Renal CL (L/h/m^2)	7	18.3	16.8	10	17.7	13.4
% dose excreted in urine	7	48.5	20.6	11	60.6	36.8

Table VI: Multiple dose PK parameters (non-compartmental) for clofarabine in pediatric patients.

	CLO 212 – ALL patients (n=12)		CLO 222 – AML Patients (n=12)	
Parameter	**Mean**	**Range**	**Mean**	**Range**
Cmax (ng/mL)	558.9	(214.2 – 1389.8)	471	(249 – 748)
Renal CL (L/h)	28.9	(1.1 – 86.0)	34.7	(3.9 – 68.9)
% dose excreted in urine	48.5	(7.8 – 82.2)	69.4	(26.4 – 113.4)
Accumulation Ratio (based on AUC(0-8)	1.25	(0.51 – 1.72)	1.40	(0.82-1.99)

B10. How does the PK of clofarabine in healthy volunteers compare to that in patients?

Clofarabine has not been given to healthy volunteers and so the PK of clofarabine in that population has not been characterized.

B11. What are the PK characteristics of clofarabine in patients with cancers other than AML and/or ALL?

The PK of clofarabine in pediatric or adult patients has not been examined in cancers other than AML and ALL.
Comparison of PK parameters obtained in pediatric AML and ALL patients indicate no differences in the PK of clofarabine in these leukemia types (also see Q.C2).

B12. What are the characteristics of drug absorption?

Clofarabine is administered intravenously; therefore drug absorption is not an issue.

B13. What are the characteristics of drug distribution?

Clofarabine was 47% bound to plasma proteins, predominantly to albumin.
Clofarabine has a large volume of distribution (254-274 L), suggesting extensive tissue distribution.

Clofarabine does distribute extensively into almost all tissues, both normal and cancerous. Uptake of clofarabine into cells occurs by active transport via specific nucleoside transporters, hENT1, hENT2 and hCNT2. The hENTs are bidirectional transporters driven solely by the concentration gradient of the nucleoside permeant between the inside and outside of the cell membrane. The hCNT transporters inwardly transport purine nucleosides against their concentration gradient. The abundance of nucleoside transporters on a tumor or normal cell appears to be a major determinant of the amount of nucleoside or nucleoside analogue that is taken into a particular tissue type. These transporters are broadly expressed in most mammalian tissues and may explain the large volume of distribution of the drug. Uptake into WBCs, both normal and cancerous, is also driven by the abundance of these transporters. Once inside the cells, clofarabine is converted into its active form, i.e., clofarabine triphosphate.

Transporter	Predominant Substrate	Tissue Distribution
Equilibrative-type		
hENT1	Purine and pyrimidine nucleosides	Most mammalian cell types, including, erythrocytes, placenta, brain, heart, liver, lung, and colon
hENT2	Purine and pyrimidine nucleosides, hypoxanthine	Most mammalian cell types, especially high in skeletal muscle
Concentrative-type		
hCNT2	Purine nucleosides, Uridine	Liver, kidney, small intestine, brain, spleen, lung, heart, skeletal muscle, pancreas, macrophages, monocytes; lymphoma, and leukemia cells.

The population PK model indicated that WBC count was a significant predictor of the central compartment volume for clofarabine. The applicant did not provide a rationale for including the WBC count as a covariate in the modeling effort; neither did they provide an interpretation for the significance of the finding (also see Pharmacometrics Review, Appendix C). The Agency's analysis confirmed that WBC counts were not correlated with the central volume estimates and inclusion of WBC in the parameter model did not reduce the population variance for the central volume.

B14. Does the mass balance study suggest renal or hepatic as the major route of elimination?

Since this drug is intended for pediatric patients, a mass balance study was not expected in this population. Mass balance studies have not been conducted in adult patients. Measurement of clofarabine in 24-hr urine collections in the phase 2 studies indicates that 49 to 60% of the dose is excreted unchanged in the urine. The site or nature of the non-renal clearance of clofarabine is unknown. Typically, one assumes this to be hepatic clearance, a combination of hepatic metabolism and biliary clearance. In rats dosed with radioactive clofarabine, < 2% of the dose was recovered in the feces as unchanged drug and < 1% of the dose was recovered in bile using an isolated perfused rat liver model. Thus, metabolic clearance would be expected to be the other clearance component (see also Q.B15).

B15. What are the characteristics of drug metabolism?

There are four possible major metabolic routes that could be expected in humans based on preclinical studies and previous experience with nucleoside analogs: metabolism by cytochrome p450 enzymes; intracellular metabolism to the phosphate derivatives; metabolism to 2-chloroadenine; or metabolism to 6-ketoclofarabine.

- The metabolism of clofarabine was studied in vitro using rat, dogs and human cryopreserved hepatocytes. Results indicated that clofarabine metabolism was minimal to negligible in rat, dog, and human hepatocytes following 6 hours incubation with 10 mM clofarabine. Only one metabolite (clofarabine sulfate) was observed in human hepatocytes and accounted for less than 0.2% of the radioactivity in the sample. These results suggest that clofarabine was not metabolized to any appreciable extent in any of the species studied by Phase I and II hepatic enzymes, including CYP 450. Thus, clofarabine PK would not be expected to be affected by other drugs that inhibit or induce the CYP 450 enzymes.

- Metabolism to the mono-, di-, and triphosphate metabolites occurs for some small part of the dose, however the applicant does not believe that up to 40% of the dose would be metabolized by this pathway.

- Metabolism by non-CYP 450 pathways, to 6-ketoclofarabine (the metabolite of largest concentration in rat urine and feces after dosing with radiolabeled clofarabine).

Unfortunately, 6-ketoclofarabine concentrations have not been monitored in any of the clinical studies. Given the close structural similarity of clofarabine to adenosine and 6-ketoclofarabine to deoxyadeosine, a likely possible enzyme for the conversion of clofarabine to 6-ketoclofarabine is adenosine deaminase, an enzyme of purine metabolism that catalyzes the irreversible deamination of adenosine and deoxyadenosine to inosine and deoxyinosine, respectively. This enzyme is ubiquitous, occurring in all tissues having DNA synthesis.

- Metabolism to 2-chloroadenine (the major metabolite of cladribine). Clofarabine does not appear to be metabolized to 2-chloroadenine in humans as all clinical samples analyzed for 2-cloroadenine (Study DM93-036 in adults) were below the detection limit of 1 ng/mL.

Thus, the non-renal clearance of clofarabine may represent a combination of hepatic and extrahepatic metabolism occurring throughout the body. The source of extrahepatic metabolism will remain unknown until a human mass balance study can be performed.

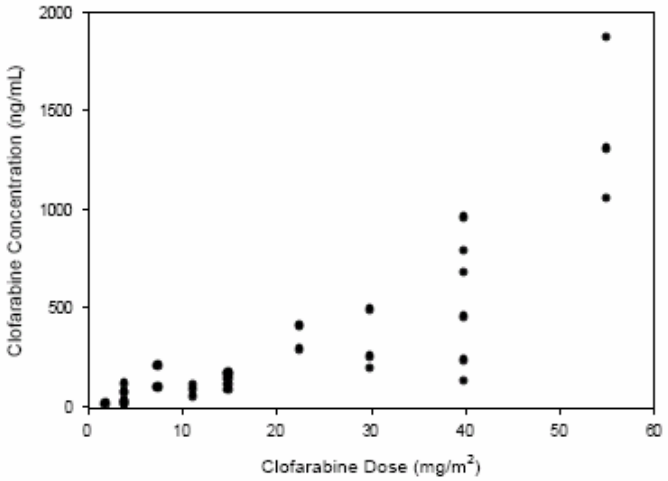
B16. What are the characteristics of drug excretion?

Based on the percent of clofarabine excreted in urine over a 24-hour period in the two phase 2 studies, 49 to 60% of the dose is excreted unchanged in the urine.

B17. Based on PK parameters, what is the degree of linearity or nonlinearity in the dose-concentration relationship?

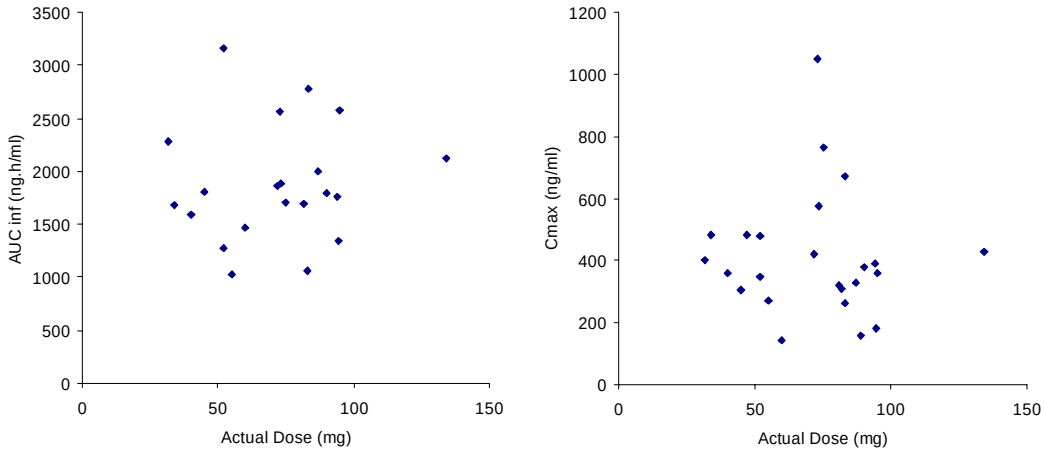
The phase I study (ID99-383) in pediatric leukemia patients included doses of clofarabine ranging from 11.25 to 70 mg/m^2. However, PK samples were not collected in any systematic manner, and therefore the dose-proportionality of the pharmacokinetics in pediatric patients could not be evaluated.
Dose-proportionality was assessed in the phase 1 study in adult patients (DM93-036) who received doses ranging from 2 to 55 mg/m2 of clofarabine. The peak clofarabine levels were found to be linearly related to dose.

Figure 2: Clofarabine concentration vs. dose in adults (study DM93-036).



Scatter-plots of the clofarabine AUC vs. dose and Cmax vs. dose following doses of 52 mg/m^2 in the patients in the phase 2 studies are shown in figure 3. As the figure shows, the actual doses (in mg) varied almost 2-fold, however the Cmax and AUC were fairly consistent across patients, providing further evidence of the dependence of the Vd and CL on body size.

Figure 3: Scatter-plots of AUC (0-8h) and Cmax vs. clofarabine dose in patients in the phase 2 studies. The AUC and Cmax do not appear to be related to the actual dose administered to the patients.



B18. How do the PK parameters change with time following chronic dosing?

There has not been any systematic evaluation of changes in PK parameters following chronic dosing with clofarabine. PK samples of clofarabine were collected on days 1 and 5 of the first 5-day cycle of treatment. Table VI (above) lists the summary statistics for Cmax and AUC(0-8h) on day 1 and day 5 for patients in the two phase 2 studies (n=24). Table VII indicates average accumulation ratios of 1.6 and 1.3 based on Cmax and AUC(0-8h) respectively.

Table VII: Cmax and AUC on days 1 and 5 of cycle 1 in patients in the phase 2 studies.

	Day 1	Day 5
Mean (CV%) Cmax (ng/ml)	410.9 (49%)	516.7 (45%)
Mean (CV%) AUC(0-8h) (ng.hr/ml)	1296 (30%)	1685 (47%)
Mean (Range) Accumulation ratio based on Cmax	-	1.6 (0.3 – 3.2)
Mean (Range) Accumulation ratio based on AUC(0-8)	-	1.3 (0.5 – 2.0)

B19. What is the inter- and intra-subject variability of PK parameters in volunteers and patients, and what are the major causes of variability?

Table VII indicates that clofarabine exposure (Cmax, AUC) showed moderate variability across patients receiving the 52 mg/m^2 dose in the phase 2 studies (CV% of 49% and 30% respectively).
The population PK model for clofarabine provided estimates of inter-individual variability in the PK parameters as well as an estimate of the residual variability. Based on the best fitting model, inter-individual variability was 27%, 56%, 27% and 39% for CL, V1, Q2 and V2 respectively. The residual variance was estimated to be 27%.
The major source of inter-individual variability was body size, with body weight proving to be the best predictor of the PK parameters. Age had no effect on the PK parameters after accounting for body weight. The only other significant covariate was the WBC count, which was significant in the model for V1. Models incorporating other covariates including gender, disease type (ALL or AML), serum creatinine, bilirubin did not result in improvement in fits or reduction in variance.

C. Intrinsic Factors

C1. What is the influence of age and body-size on the PK of clofarabine?

Figures 4 and 5 shows scatter plots of clofarabine clearance and volume of distribution (non-compartmental analysis) vs. BSA and body weight for the two phase 2 studies. There is a moderate correlation of clearance as well as volume with both BSA and weight.
Additionally, the population PK model incorporating weight as a covariate provided a better fit (lower OFV and lower variance of parameter estimates) than the model incorporating BSA as the covariate.

There was also a modest correlation between CL and age as well as Vdss with age in the two studies. However, BSA-normalized CL and Vdss did not show any relationship with age within the pediatric population (figure 6).

Figure 4: Total clearance vs. BSA (left panel) and vs. body weight (right panel) in patients from the phase 2 studies.

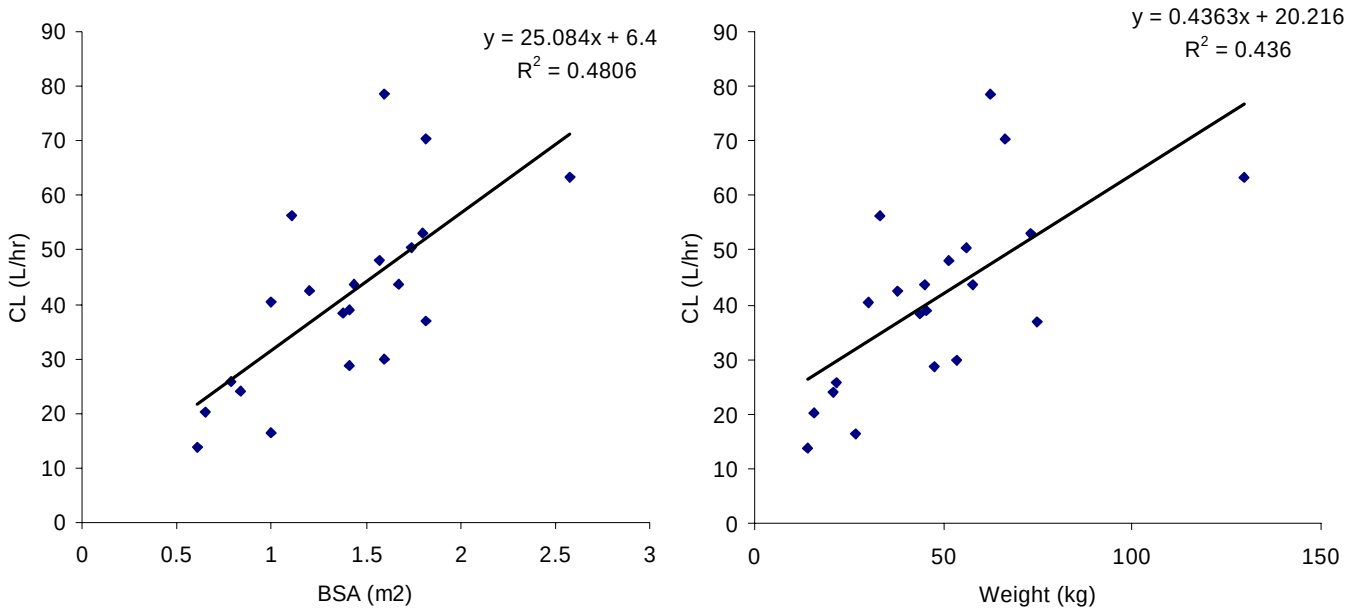


Figure 5: Volume of distribution vs. BSA (left panel) and vs. body weight (right panel) in patients from the phase 2 studies.

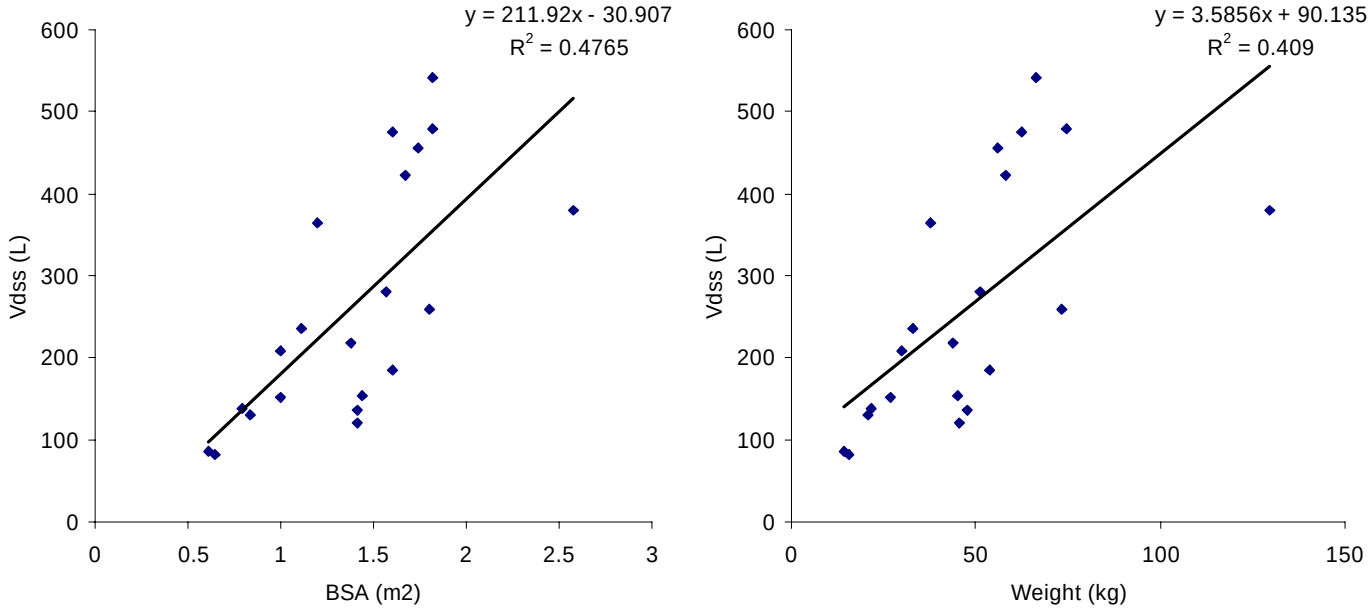
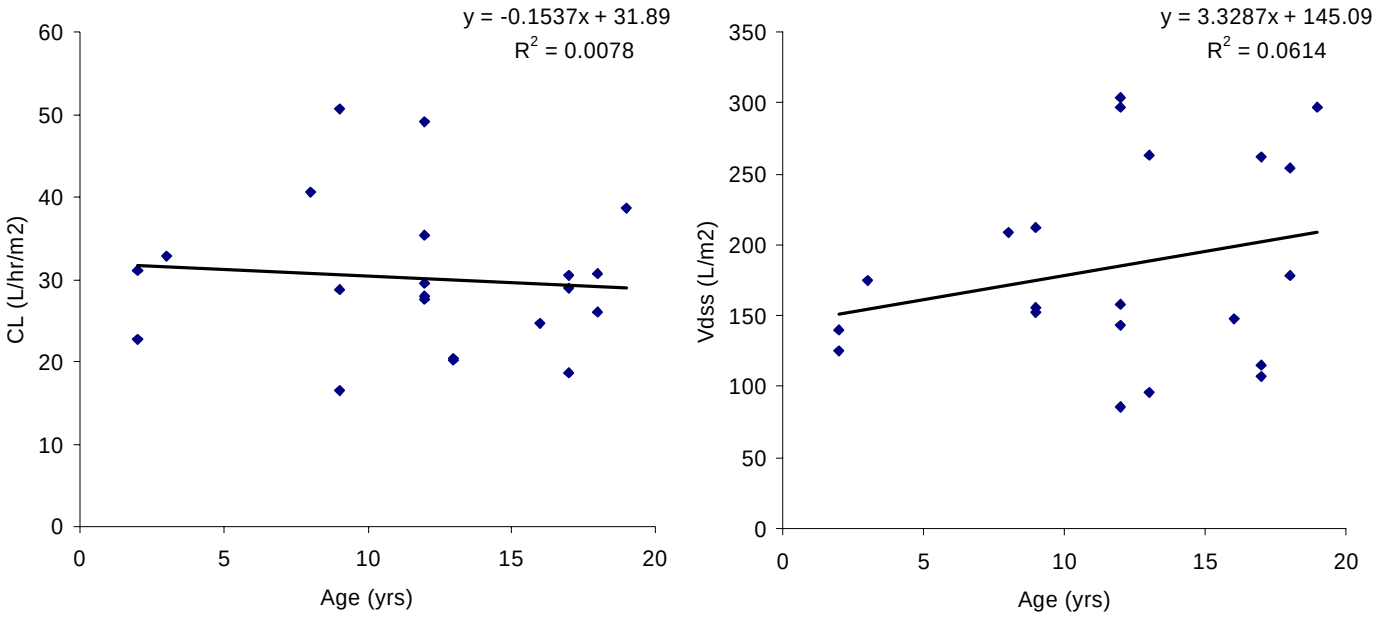


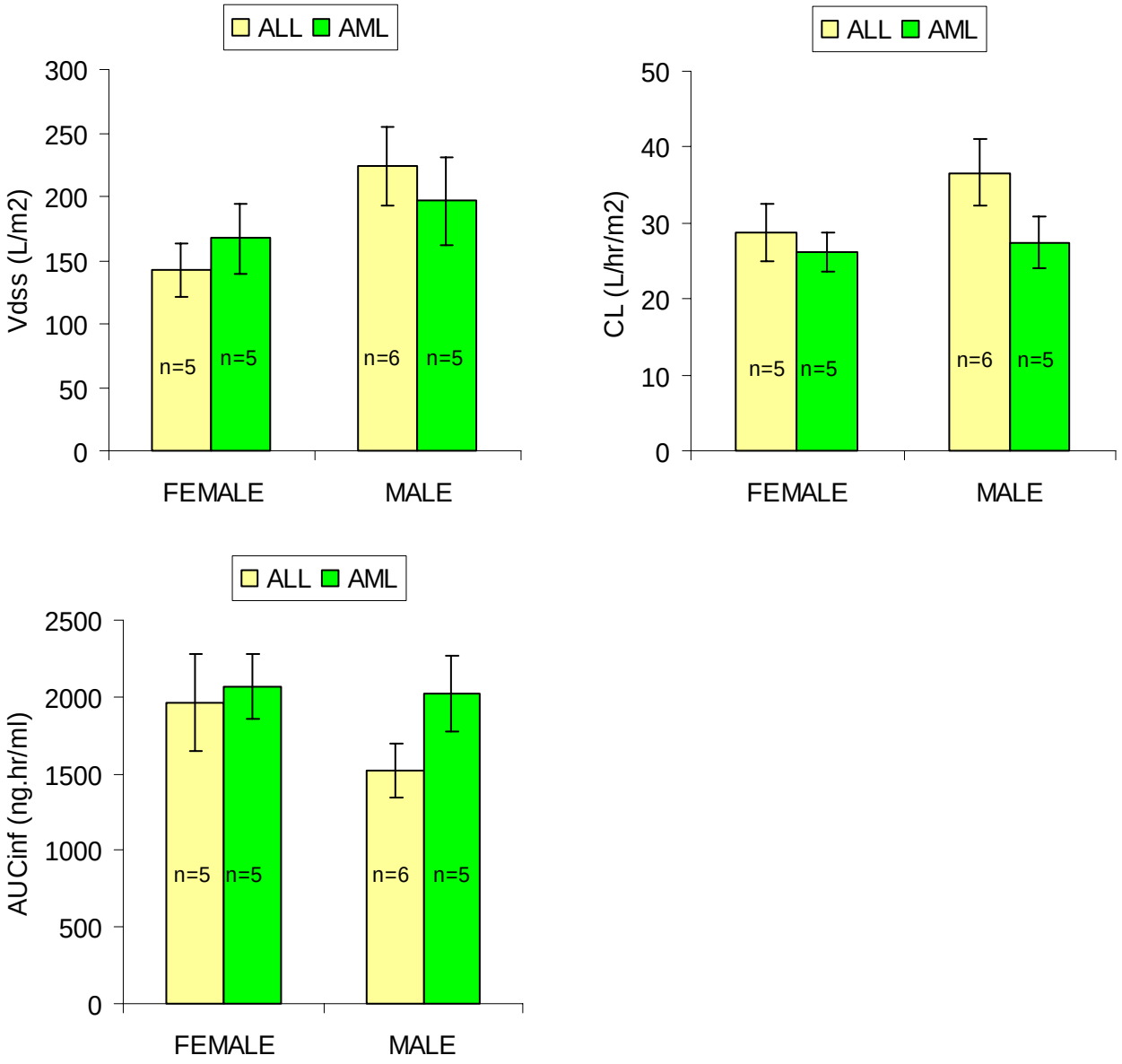
Figure 6: BSA-normalized CL vs. age (left panel) and BSA-normalized Vdss vs. age (right panel) in patients from the phase 2 studies.



C2. What is the influence of gender and disease type (AML vs. ALL) on the PK of clofarabine?

Regression analysis of the effect of gender and disease type (ALL or AML) on the non-compartmental parameters (CL, Vdss, AUCinf) did not show any significant associations. Figure 7 shows the CL, Vdss and AUC obtained in the two phase 2 studies separately for the males and females. There does not appear to be any difference in exposure between males and females or between ALL and AML patients.

Figure 7: PK parameters for clofarabine by gender and disease type. No significant differences were found by gender or disease type.



C3. How do the PK characteristics of clofarabine compare between pediatric and adult patient populations?

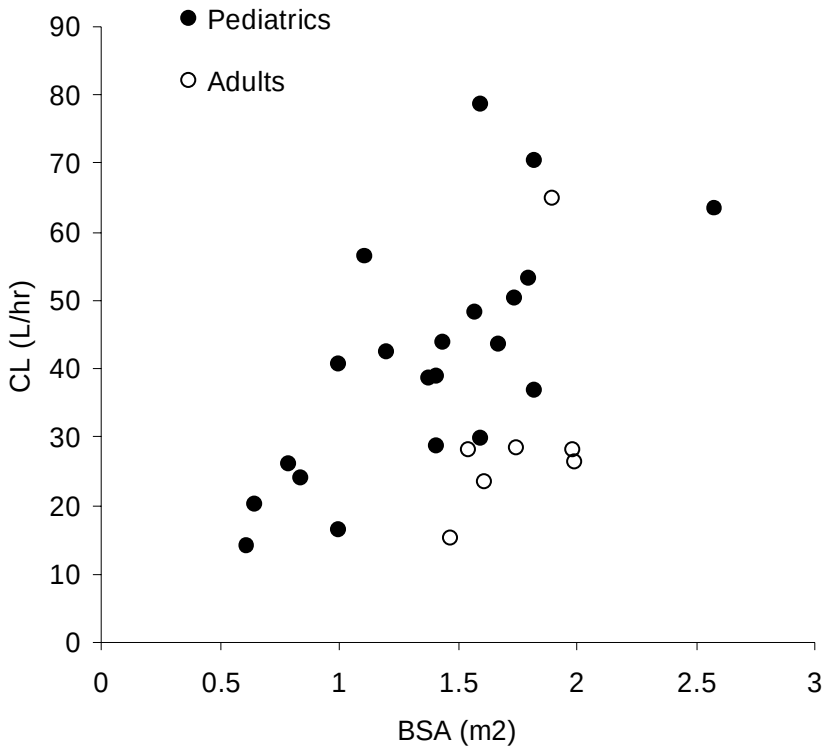
The PK of clofarabine was determined in adult AML patients in the phase 2 study CLO-221. In this study, patients received 40mg/m^2 of clofarabine for 5 days, every 3 weeks. Blood samples for PK were obtained, using a rich sampling scheme similar to that in the pediatric phase 2 studies. Table VIII lists the non-compartmental PK parameters obtained in the adult patients.

Table VIII: Non-compartmental PK parameters for clofarabine in adult AML patients. Clofarabine dose = 40 mg/m^2.

Parameter	N	Mean	Standard Deviation
AUC(0-inf) (ng.h/mL)	7	2617.5	826.4
Cmax (ng/mL)	13	620.5	467.7
CL (L/h)	7	30.6	15.8
Half-life (h)	7	6.2	1.6
Vd,ss (L)	7	173.1	77.4
Renal CL (L/h)	11	17.7	12.4
% dose excreted in urine	11	58.3	32.6

Figure 8 shows the clearance for the adult and pediatric patients as a function of BSA. The adult patients appeared to show lower clearances compared to that seen in the pediatric patients. Among the adult patients, the lowest observed clearance was 15.8 L/hr, seen in a 63 year old female while the highest observed clearance of 65 L/hr was seen in a 32 year old male. Such differences in demographics might suggest that factors such as creatinine clearance (which is a function of age and gender) might also explain the variability in clearance. Unfortunately creatinine data were not submitted for the adults which limits our ability to interpret these apparent differences in clearance between the pediatrics and adults.

Figure 8: PK parameters (CL, Vdss) for clofarabine in pediatric and adult patients.



C4. What is the influence of hepatic or renal impairment on the PK of clofarabine?

The pharmacokinetics of clofarabine has not been studied in patients with any degree of renal or hepatic dysfunction. The applicant recommends that, given the percent of dose excreted in urine as unchanged clofarabine, clofarabine should not be administered to patients with severe hepatic or renal impairment and that caution should be exercised when administering clofarabine to patients with mild to moderate renal or hepatic impairment.

Given that 49–60% of clofarabine is excreted unchanged in urine, any degree of renal impairment would be expected to increase the exposure to clofarabine and the potential for toxicity. The phase 2 studies included 8 patients who had slightly elevated serum creatinine levels, which would put them in the category of mild renal impairment (creatinine clearance between 50 and 80 ml/min). Unfortunately, PK data was only collected in 2 of these patients, and the estimated CL in these individuals was not different from the average values for the entire group. Also, examination of the frequency of AEs in this group compared with the remaining patients did not indicate any major differences (table IX).

Table IX: Incidence of toxicity in patients with mild renal impairment and others with normal renal function.

AEs	Pts with CLcr = 60-80 ml/min (n=8)	Others (n=105)
Anxiety	3/8 (37.5%)	23/105 (21.9%)
Hypotension	4/8 (50%)	27/105 (25.7%)
Nausea, vomiting	7/8 (87.5%)	94/105 (89.5%)
Neutropenia	7/8 (87.5%)	68/105 (64.7%)
Sepsis	2/8 (25%)	27/105 (25.7%)
Dyspnea	1/8 (12.5%)	25/105 (23.8%)
Hepato-biliary	0/8 (0%)	5/105 (4.8%)

There were no patients with creatinine clearances that would put them in the moderately impaired category. In this group, the renal clearance of these patients would be markedly decreased, however the non-renal clearance would remain unaffected, so that the change in total clearance (CLtot) in patients with moderate renal impairment can be estimated as follows:

In pts with normal renal function: CLtot=30 L/hr/m^2 =60 L/hr for a normal patient (BSA=2 m^2).
In normals, CLr = 60% and CLnr = 40% of total, i.e., CLr = 36 L/hr and CLnr = 24 L/hr.
In moderate renal impairment (CLcr=30 ml/min at the lower limit), fraction of renal clearance remaining can be calculated as: CLr = 30/120 * 36 = 9 L/hr.
Therefore, CLtot = CLr + CLnr = 9 + 24 = 33 L/hr.

Thus, CLtot in moderate renal impairment is about half that in normals and AUC in moderate renal impairment would be approximately 2-fold that in normals. The impact of this increased exposure on the incidence of toxicity is unclear.

Thus, clofarabine should be contra-indicated in patients with moderate and severe renal impairment until the relationship between renal dysfunction and clofarabine exposure (and toxicity) has been evaluated.

The effect of hepatic impairment on clofarabine PK is more difficult to establish since the pathways of non-renal clearance of clofarabine have not yet been determined (see Q.B14 and Q.B15).

D. Extrinsic Factors

D2. Is there a significant pharmacokinetic interaction of clofarabine with other drugs administered concomitantly in this patient population?

The in vitro metabolism of clofarabine in rat, dog, and human hepatocytes was evaluated using 10 μM (=3030 μg/L or 3030 ng/ml) clofarabine. Positive and negative controls were used to confirm the viability of the hepatocytes. All samples and controls were stored at -20°C after termination of the incubation until analyzed by LC-MS for metabolite identification and assessed quantitatively using radioactivity standards.
Clofarabine metabolism was minimal to negligible in rat, dog, and human hepatocytes following 6 hours incubation with 10 mM clofarabine. Only one metabolite (clofarabine sulfate) was observed in human hepatocytes and accounted for less than 0.2% of the radioactivity in the sample. These results suggest that clofarabine was not metabolized to any appreciable extent in any of the species studied by Phase I and II hepatic enzymes, including cytochrome p450. The concentration of clofarabine used in these experiments was about 8-fold higher than the Cmax of ~400 ng/ml) following the 52 mg/m2 dose in pediatric ALL and AML patients.
Thus, no significant PK interactions would be expected with drugs that are CYP inhibitors or inducers.

The CYP inhibiton or induction potential of clofarabine has not been evaluated, therefore the influence of clofarabine on concomitant drugs that are CYP substrates is unknown.

<u>Concomitant Medications:</u>
The pediatric patients in the two phase 2 studies were receiving at least one concomitant medication at baseline, i.e., on entry into the study. There was no attempt to restrict concomitant medication use, and the profile of concomitant medication use during clofarabine treatment was quite similar to that at baseline, with the exceptions that the incidence of use generally increased following clofarabine. Table X lists several classes of medications with the highest frequency of use in the study population. Due to the large number of medications and the high frequency of use in this relatively small sample, a meaningful analysis of differences in exposure or toxicity based on concomitant medication use was not feasible.

Table X: Medication use during clofarabine treatment in phase 2 pediatric studies.

ILEXDRUG Classification	ALL/AML (N=111)	
	N	%
Antiinfectives	111	100.0
Gastrointestinal Tract Agents	111	100.0
Analgesics/NSAIDs	110	99.1
Antihistamines	108	97.3
Antifungals	107	96.4
Narcotic Analgesics	101	91.0
Vitamins/Minerals/Electrolytes	96	86.5
Benzodiazepines	95	85.6
Antiseptics & Topical Agents	87	78.4
Corticosteroids	86	77.5
Anesthetics	80	72.1
Hypouricemics	67	60.4
Antivirals	65	58.6
Diuretics	61	55.0
Nutritionals/Parenterals/Herbals	47	42.3
Anxiolytics	40	36.0
Colony Stimulating Factors	33	29.7
Hormones	33	29.7
Central Nervous System Agents	31	27.9
H2 Blockers	24	21.6
Thrombolytics	24	21.6
Anticonvulsants	20	18.0
Antidepressants	18	16.2
Anticoagulants	17	15.3
Antineoplastic Antimetabolites	17	15.3
Bronchodilators	17	15.3
Cardiovascular Agents	17	15.3
Immunoglobulins	17	15.3
Skeletal Muscle Relaxers	17	15.3
Antihypertensives	16	14.4
Cough and Cold Preparations	14	12.6
Anticholinergics	13	11.7
Antiinflammatory	12	10.8

D3. Based on the above (intrinsic and extrinsic factors), are there any recommendations for dosing adjustments for this population?

There are no recommendations at this time. Possible dosage adjustments in patients with renal dysfunction may be recommended after the renal impairment study is completed. Additionally, possible dosage recommendations may be made regarding the concomitant use of CYP substrates after inhibition and induction potential studies with clofarabine have been completed.

Other

D4. Are there any additional unresolved issues or omissions with regard to the evaluation of the PK and PD of clofarabine?

The relationship between clofarabine exposure and response has not been characterized in the pediatric patient population.

E. Analytical Section

E1. How are the active moieties identified and measured in the plasma in the clinical pharmacology and biopharmaceutics studies?

Clofarabine levels in plasma and urine of patients in the phase 1 and both phase 2 studies were measured by HPLC/MS/MS.

E2. Which metabolites have been selected for analysis and why?

No metabolites were measured in any of the studies in the pediatric patients. In the phase I study in adults (DM93-036), samples were assayed for the 2-cloroadenine metabolite, however the levels were below the limit of quantitation of 1 ng/ml. The other putative metabolite, 6-ketochofarabine was not measured in any of the studies, since the preclinical studies indicated it was formed at very low levels.

In a selected subset of samples, levels of intracellular clofarabine triphosphate were measured in WBCs obtained from patients following clofarabine. However, these samples were not collected in a consistent manner and were only obtained from patients at one study site (MD Anderson Cancer Center). Additionally, the assay method was not validated according to FDA guidelines.

E3. For all moieties measured, is free, bound or total measured? What is the basis for that decision, if any, and is it appropriate?

For all moieties measured, the total concentrations were measured as clofarabine is only 47% plasma protein bound.

E4. What is the bioanalytical method that isused to assess concentrations of clofarabine and its metabolites?

Clofarabine plasma and urine concentrations were measured using HPLC/MS/MS.
Briefly, 100 ml of plasma along with an internal standard, cladribine, was deproteinized with 1.0 ml of acetonitrile. The mixture was centrifuged and the supernatant was dried. The residue was reconstituted in (b) (4) and injected onto the HPLC system. For urine, 100 ml aliquot was

mixed with cladribine and then diluted with 6.0 ml of [redacted] (b) (4) Separation was conducted in a [redacted] (b) (4) phases consisting of formic acid, water and acetonitrile. The HPLC eluent was [redacted] (b) (4) /interface. The compounds were ionized by electrospray in the positive mode and the transition of m/z from 304 to 170 and 286 to 170 was monitored using a tandem [redacted] (b) (4) spectrometer for clofarabine and the internal standard, respectively. The peak height ratio of clofarabine and the internal standard was used in the construction of the calibration curve and calculation of the concentration in the samples.

E5. What are the figures of merit and performance characteristics for the methods used to assess concentrations of clofarabine?

The assays were validated according to the Guidance to Industry Bioanalytical Method Validation issued by FDA.

The applicant has submitted the following analytical reports:

MSN02018	Analytical method summary for clofarabine in human plasma
MC02017	Full validation of method in human plasma
MC02293	Partial validation for change in calibration range
MC03065	Partial validation for change in injection volume
MC02301	Partial validation for assay of clofarabine in presence of cytarabine
MC03171	Stability study: long term frozen stability in human plasma
MC03240	Stability study: long term processed sample stability in human plasma
MSN02075	Analytical method summary for clofarabine in human urine
MC02298	Full validation of method in human urine
MC03135	Partial validation for change in injection volume
MC03105	Stability study: long term frozen stability in human plasma
MC03240	Stability study: long term processed sample stability in human plasma
MSN02033	Analytical method summary for chloroadenine in human plasma
MC02299	Full validation of method for chloroadenine in human plasma

Appendix B includes summaries of each of the reports. Based on the full and partial validation methods, the following performance characteristics were established for the assays:

Clofarabine:

Plasma

- Assay calibration range: 1.0 to 500.0 ng/ml
- LLOQ = 1 ng/ml (%CV = 13.5%)
- Inter-day:
  - Accuracy: %DFN = 0.7 to 7.0
  - Precision: %CV = 4.6 to 8.8
- Intra-day:

    - Accuracy: %DFN = 4.4 to 10
    - Precision: %CV = 6.7 to 9.6
- Specificity: Determined in 6 lots of blank human plasma. No interference peaks were seen at the retention times of clofarabine or internal standard (typical chromatograms included in Appendix B).
- Recovery: Overall mean recovery for clofarabine: 92.4%
    - 95.4% at 30 ng/ml, 92.3% at 300 ng/ml, 89.4% at 4000 ng/ml
    - Overall mean recovery for internal standard: 94.6%
    - Recovery study was not repeated with lower calibration range of 1-500 ng/ml.
- Matrix effect: comparison of peak height of standards spiked into plasma blanks to peak heights spiked into reagent blanks: Overall matrix effect: 0.993
    - 0.98 at 30 ng/ml, 0.991 at 300 ng/ml, 1.01 at 4000 ng/ml.
    - Overall matrix effect for internal standard: 1.025
- Stability:
    - QC Sample storage frozen at -20^{o}C: 89 days (samples were ± 8% of nominal)
    - QC Sample storage thawed at room temperature: 26 hours
    - QC Sample storage stability (freeze/thaw): 3 cycles
    - Processed sample stability (refrigerated at 5^{o}C): 16 days
    - Stock standard solution stability (frozen at -20^{o}C): 195 days

Urine

- Assay calibration range: 1.0 to 500.0 ng/ml
- LLOQ = 1 ng/ml (%CV = 5.3%)
- Intra-day:
    - Accuracy: %DFN = 0.3 to 11.8
    - Precision: %CV = 3.4 to 5.8
- Specificity: Determined in 6 lots of blank human urine. No interference peaks were seen at the retention times of clofarabine or internal standard (typical chromatograms included in Appendix B).
- Recovery: Overall mean recovery for clofarabine: 99.8%
    - 99.7% at 3 ng/ml, 97.3% at 30 ng/ml, 102.5% at 400 ng/ml
    - Overall mean recovery for internal standard: 98.9%
- Matrix effect: comparison of peak height of standards spiked into plasma blanks to peak heights spiked into reagent blanks: Overall matrix effect: 1.130
    - 1.146 at 3 ng/ml, 1.132 at 30 ng/ml, 1.111 at 400 ng/ml.
    - Overall matrix effect for internal standard: 1.456
- Stability:
    - QC Sample storage frozen at -20^{o}C: 202 days (samples were ± 5.2% of nominal)
    - QC Sample storage thawed at room temperature: 24 hours
    - QC Sample storage stability (freeze/thaw): 3 cycles
    - Processed sample stability (refrigerated at 5^{o}C): 122 hours
    - Stock standard solution stability (frozen at -20^{o}C): 56 days

Chloroadenine:

Plasma

- Assay calibration range: 1.0 to 500.0 ng/ml

- LLOQ = 1 ng/ml (%CV = 7.0%)
- Inter-day:
    - Accuracy: %DFN = 0.7 to 2.3
    - Precision: %CV = 4.6 to 7.6
- Intra-day:
    - Accuracy: %DFN = 2.3 to 3.5
    - Precision: %CV = 6.1 to 12.1
- Specificity: Determined in 6 lots of blank human urine. No interference peaks were seen at the retention times of chloroadenine or internal standard (typical chromatograms included in Appendix B).
- Recovery: Overall mean recovery for chloroadenine: 101.8%
    - 106.2% at 3 ng/ml, 95.7% at 30 ng/ml, 103.4% at 400 ng/ml
- Matrix effect: comparison of peak height of standards spiked into plasma blanks to peak heights spiked into reagent blanks: Overall matrix effect: 0.743
    - 0.662 at 3 ng/ml, 0.743 at 30 ng/ml, 0.823 at 400 ng/ml.
- Stability:
    - QC Sample storage frozen at -20^{o}C: 33 days (samples were ± 7.3% of nominal)
    - QC Sample storage thawed at room temperature: 25 hours
    - QC Sample storage stability (freeze/thaw): 3 cycles
    - Processed sample stability (refrigerated at 5^{o}C): 91 hours

III. Detailed Labeling Recommendations

1. The following should be inserted under the Human Pharmacokinetics section, under CLINICAL PHARMACOLOGY

The population pharmacokinetics of CLOLAR™ were studied in 40 pediatric patients aged 2 to 19 years (21 males/19 females) with relapsed or refractory ALL or AML. At the given 52 mg/m^2 dose, similar concentrations were obtained over a wide range of BSAs. Clofarabine was 47% bound to plasma proteins, predominantly to albumin. Based on non-compartmental analysis, systemic clearance and volume of distribution at steady-state were estimated to be 28.8 L/h/m^2 and 172 L/m^2, respectively. The terminal half-life was estimated to be 5.2 hours. No apparent difference in pharmacokinetics was observed between patients with ALL and AML or between males and females.

No relationship between clofarabine or clofarabine triphosphate exposure and toxicity or response was found in this population.

Based on 24-hour urine collections in the pediatric studies, 49-60% of the dose is excreted in the urine unchanged. *In vitro* studies using isolated human hepatocytes indicate very limited metabolism (0.2%), therefore the pathways of non-renal elimination remain unknown.

Although no clinical drug-drug interaction studies have been conducted to date, on the basis of the *in vitro* studies, cytochrome p450 inhibitors and inducers are unlikely to affect the metabolism of clofarabine. The effect of clofarabine on the metabolism of cytochrome p450 substrates has not been studied. The pharmacokinetics of clofarabine have not been evaluated in patients with renal or hepatic dysfunction.

2. The following should be inserted under the Drug Interactions section under PRECAUTIONS

Although no clinical drug-drug interaction studies have been conducted to date, on the basis of the *in vitro* studies, cytochrome p450 inhibitors and inducers are unlikely to affect the

metabolism of clofarabine. The effect of clofarabine on the metabolism of cytochrome p450 substrates has not been studied.

4. The following should be inserted under the Hepatic and Renal Impairment under WARNING and under DOSAGE AND ADMINISTRATION section

CLOLAR™ has not been studied in patients with hepatic or renal dysfunction. Its use in such patients should be undertaken only with the greatest caution.

27 Page(s) of Draft Labeling have been Withheld in Full as b4 (CCI/TS) immediately following this page

B. Individual Study Reviews

I.

Study #:	**ID99-383**
Title:	**Phase I study of Cl-F-ara-A (Clofarabine) in Pediatric Patients with Hematologic Malignancies**

Clinical Study Summary:

Objectives:
Primary: To determine the maximum tolerated dose (MTD) and toxicity profile of clofarabine administered by IV infusion over 1 hour each day for 5 consecutive days in pediatric patients with hematologic malignancies.
Secondary: To characterize the pharmacologic profile, including metabolism of clofarabine in circulating leukemic cells and mononuclear cells during therapy; to quantitate the effects of treatment on deoxynucleotide levels, rate of DNA synthesis, and stability of DNA in these cells; and seek correlation between these parameters and clinical response.

Study Design: Phase I, open- label, dose-escalating study of clofarabine administered to pediatric patients with refractory leukemia who failed standard therapy or for whom no such therapy existed. Patients 21 years or younger and diagnosed with refractory leukemia or lymphoma and who were not candidates for treatment of higher efficacy or priority were eligible for enrollment. The study was conducted by the MD Anderson Cancer Center (Houston, TX).

Additionally, patients were not to have received chemotherapy, immunotherapy, or radiotherapy for 2 weeks before entering this study and were to have recovered from the toxic effects of that therapy, except for patients with leukemia who were allowed to start treatment with the study drug if life-threatening increases in leukemia cell burden occurred during the 2-week period. All patients were to have a Zubrod performance status no greater than 2 and must have had adequate liver function (bilirubin < 2 mg/dL) and renal function (creatinine < 1.5 mg/dL). Pregnant and lactating females were not eligible. Patients were to receive up to 2 cycles of therapy beyond the best response or a maximum of 12 cycles. Patients who failed to achieve a response after 2 cycles were to be discontinued from the study.

Results: The doses studied were 11.25, 15, 30, 40, 52, and 70 mg/m^2 administered over 1 hour once daily for 5 consecutive days every 2 to 4 weeks depending on toxicity and response. More than half the patients enrolled in the study received 52 mg/m^2. Complete or partial responses were observed in 8 of 25 (32%) patients with some hematologic improvement in another 7 of 25 (28%) patients. Specifically, 5 of 25 (20%) patients achieved a complete remission and 3 of 25 (12%) patients achieved a partial remission. Among the patients who achieved a complete response, 4 were diagnosed with ALL (1 received 30 mg/m^2, 2 received 40 mg/m^2, and 1 received 70 mg/m^2 decreased to 52 mg/m^2 at the time of response) and 1 was diagnosed with

AML (52 mg/m^2). Among those who obtained a partial response, 2 were diagnosed with AML (40 mg/m^2) and 1 was diagnosed with ALL (52 mg/m^2). The MTD was determined to be 52 mg/m^2 with the dose limiting toxicity observed at 70 mg/m^2 being hepatic toxicity (hyperbilirubinemia and elevated transaminase activity) and skin rash.

Pharmacokinetic Analysis Summary:

Objectives:
To evaluate the pharmacokinetics of clofarabine in patients enrolled in the phase I study.

Methods:
Pharmacokinetic data were available from 12 of 25 patients enrolled in the study. All total, 98 samples were collected and analyzed for plasma clofarabine concentrations while 40 samples were collected for intracellular clofarabine triphosphate analysis. Blood samples for pharmacokinetic analysis (plasma clofarabine and intracellular clofarabine triphosphate) were to be collected at pre-dose and at 1 hour post-infusion. In general, pharmacokinetic samples were collected at predose, at the end of infusion (1 hour), 1 hour post-infusion, and sometimes at random times thereafter.
Plasma and urine samples were assayed for clofarabine using a validated HPLC/MS/MS method. Intracellular clofarabine triphosphate levels were assayed using HPLC.

Results:
- There were insufficient samples per patient with unstructured sample collection times to perform non-compartmental analysis. Similarly, there were too few subjects to perform any type of population analysis on the data. The median number of samples per patient collected for clofarabine and clofarabine triphosphate analysis was 9 (range: 2 to 15) and 1 (range: 0 to 8), respectively, collected over multiple cycles and days.
- Plasma clofarabine concentrations increased with increasing dose and were still quantifiable 24 hours after the start of infusion, albeit about < 10% of their maximal end of infusion value. As expected, peak plasma concentrations were reached at the end of infusion and declined thereafter. Large inter-patient variability was observed with clofarabine concentrations within a dose level.
- Clofarabine triphosphate concentrations appeared to increase with increasing dose and remained quantifiable 24 hours after the start of infusion. Figure 3 presents a scatter plot of plasma clofarabine concentrations against matched intracellular clofarabine triphosphate concentrations. A modest Pearson correlation of 0.42 (p = 0.0261) was observed.

Figure B1: Clofarabine plasma concentration vs. time data across subjects and doses.

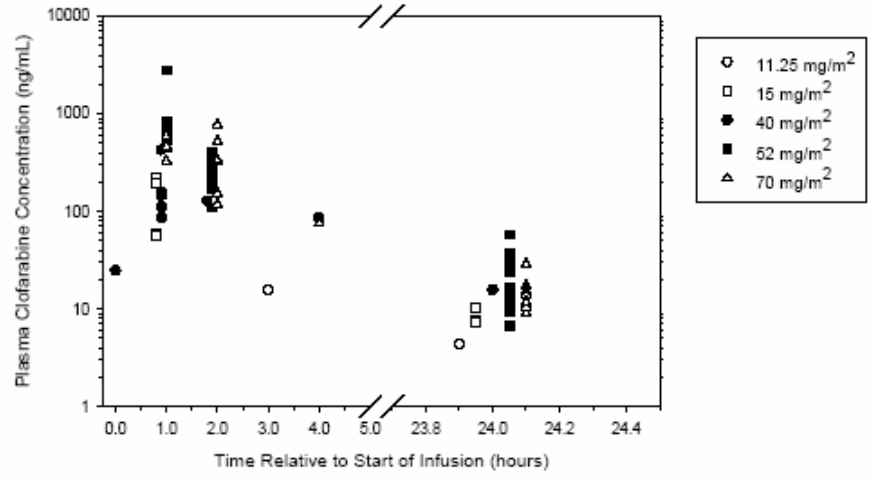


Figure B2: Clofarabine triphosphate concentration vs. time data across subjects and doses.

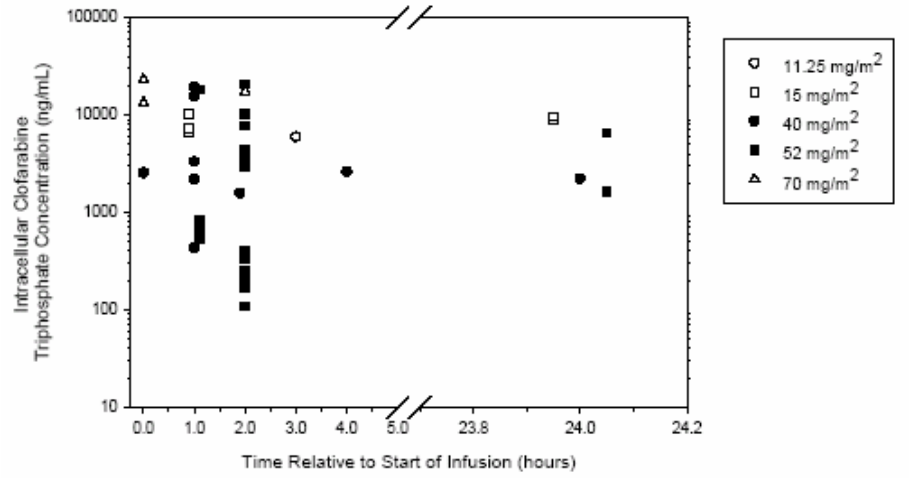
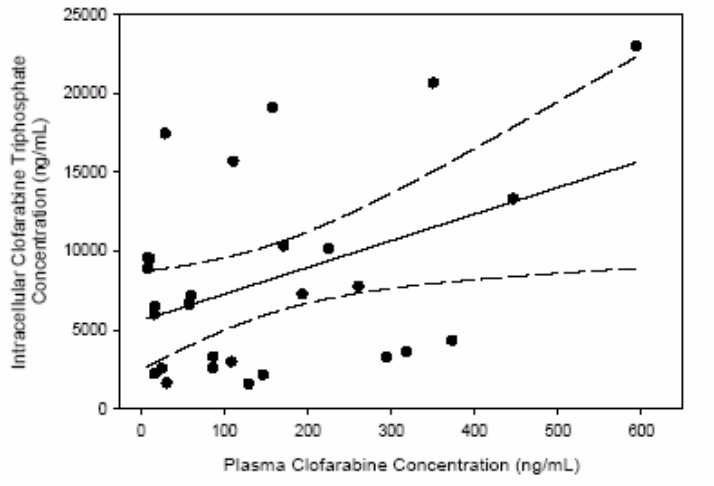


Figure B3: Correlation between plasma clofarabine and intracellular clofarabine triphosphate concentrations across time, subjects and doses.



II.

Study #: CLO-212

Title: A Phase II, Open- Label Study of Clofarabine in Pediatric Patients with Refractory or Relapsed Acute Lymphoblastic Leukemia

Clinical Study Summary:

Objectives:
Primary: The primary objective of this study was to determine the overall remission (OR) rate of clofarabine in pediatric patients with refractory or relapsed ALL.

Secondary:
Secondary objectives included documentation of CR, CRp, and PR rates, as well as duration of remission and overall survival (OS), and the safety profile and tolerability of clofarabine for this dosing regimen in this population.

Study Design:
Phase II, non-randomized, open-label, single-arm study of clofarabine in pediatric patients (21 years of age or less at the time of initial diagnosis) with ALL who were not eligible for therapy of higher curative potential, and who were in second or subsequent relapse and/or refractory. Patients with a Karnofsky Performance Status (KPS) ≥50 with adequate renal (serum creatinine <2 × upper limit of normal for age [ULN]) and liver function (serum bilirubin ≤1.5 × ULN; AST and ALT ≤5 × ULN) within 2 weeks before registration were eligible to enroll.

Patients in this study received clofarabine 52 mg/m2/day for 5 consecutive days as IVI over 2 hours for each cycle up to a maximum of 12 cycles. Treatment was to be continued until disease relapse or recovery of normal hematopoiesis (defined as an absolute neutrophil count [ANC] of ≥0.75 × 109/L). Each cycle was to be repeated every 2 to 6 weeks. Doses were to be reduced for nonhematologic or hematologic toxicities.
The OR rate was defined as the sum of the number of patients with either complete remission (CR) or complete remission in the absence of total platelet recovery (CRp) divided by the total number of eligible patients.

Results:
The OR rate (CR + CRp) was 20.4% (95% CI: 10% to 34.0%) according to the IRRP. Of note, 30.6% of the patients achieved at least a PR. The median duration of remission (CR + CRp) was 20.2 weeks (95% CI: 6.1 to 28.6 weeks). Furthermore, 8/49 (16.3%) patients went on to receive a HSCT after treatment with clofarabine, 5 of whom were alive at last follow up. Survival times for patients who went on to transplant ranged from 17.6+ to 63.1+ weeks. At last follow up, 11/49 (22%) patients were alive, including 7 of 10 patients who achieved an OR (CR + CRp); survival times for patients who had an OR ranged from 9.1+ to 63.1+ weeks. Of the 15 responding pediatric ALL patients, 6 had post-clofarabine bone marrow transplantation. In the 9 responding patients that were not transplanted, the response durations for CR were 43, 50, 82,

93+, and 160+ days; for CRp the response duration was 32 days; and for PR the response durations were 7, 16, and 21 days.

Pharmacokinetic Analysis Summary:

Objectives:
To characterize the pharmacokinetics in pediatric patients enrolled in this study.

Methods:
Blood samples for pharmacokinetic analysis were collected at 0 (immediately prior to infusion), end of infusion (2 hours), 3 hours, 4 hours, 5 hours, 10 hours (or just prior to leaving clinic), and 24 hours (pre-dose on Day 2) on Day 1 and at 0 (immediately prior to infusion), end of infusion (2 hours), 3 hours, 4 hours, 5 hours, 10 hours (or just prior to leaving clinic) on Day 5. Urine samples were to be collected prior to infusion on Day 1 and at 0 (pre- infusion) to 8 hours and 8 to 24 hours on Days 1 and 5. Plasma and urine samples were to be analyzed for clofarabine concentrations using a validated analytical LC-MS/ MS method.
Urine samples were to be collected at pre-dose (on Day 1), 0 to 8 hours post-dose, and 8 to 24 hours post-dose on Days 1 and 5 and analyzed for parent clofarabine concentrations using a validated analytical LC-MS/MS method.
Plasma clofarabine concentrations were obtained from 14 of 49 patients and analyzed using noncompartmental methods. Subsequently the data from all 3 studies in pediatric populations were combined for a population analysis.

Results:
Figure B4 presents spaghetti plots of individual concentration-time profiles on semi-log scale for Day 1 and 5, respectively. Figure B5 presents mean concentration-time profiles on semi-log scale on Day 1 and 5, respectively. Table B1 lists the demographics of the patients with PK data.

Figure B4: Spaghetti plots of individual concentration-time profiles on semi-log scale for Day 1 (left panel) and day 5 (right panel).

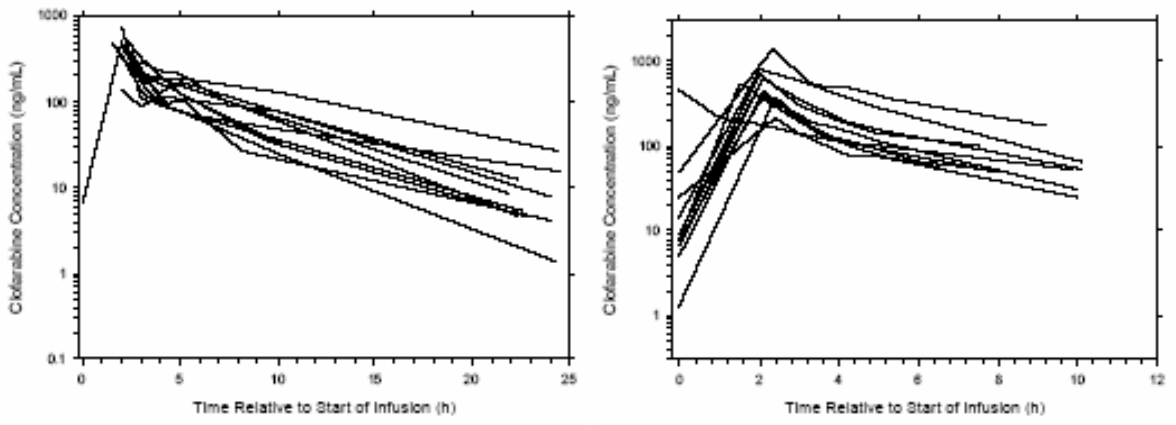


Figure B5: Spaghetti plots of mean concentration-time profiles on semi-log scale for Day 1 (left panel) and day 5 (right panel).

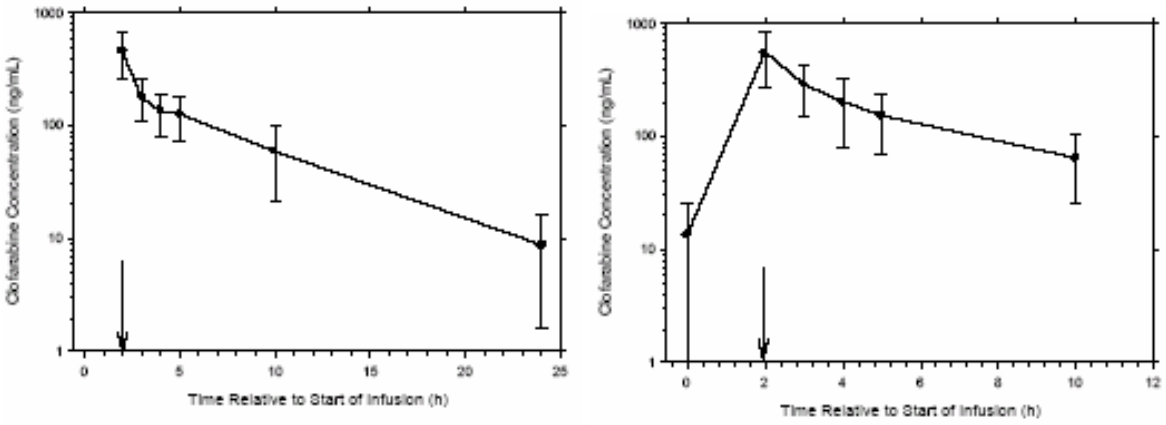


Table B1: Demographics of patients with PK data.

Patient ID	Sex	Age (years)	Race	BSA (m**2)	Weight (kg)	Height (cm)	Daily Dose (mg)	Dose (mg/m**2)
004-0005	Female	9	Hispanic	0.84	21.0	121.0	45.0	52
004-0031	Male	12	Hispanic	1.20	37.8	134.5	60.0	52
005-0037	Male	17	Caucasian	1.74	55.9	180.0	90.0	52
006-0003	Female	8	Caucasian	1.00	30.0	128.9	52.0	52
006-0004	Female	15	Other	1.25	35.9	154.3	65.0	52
009-0024	Male	2	Caucasian	0.65	15.6	100.0	34.0	52
010-0010	Female	12	Caucasian	1.41	45.6	153.6	73.3	52
010-0016	Male	8	Black	0.92	24.5	.	47.8	52
011-0035	Male	9	Black	1.11	33.0	134.3	55.0	52
012-0014	Male	12	Black	1.60	62.4	.	83.0	52
016-0008	Female	17	Caucasian	1.44	45.0	166.5	75.0	52
018-0036	Female	9	Caucasian	1.00	26.8	132.3	52.0	52
019-0011	Male	16	Black	2.58	129.8	184.7	134.0	52
019-0017	Female	13	Caucasian	1.71	67.2	157.0	88.0	52

Summary of PK findings (see table B2 for PK results):

- Clofarabine showed rapid elimination with most of the dose being eliminated in a 24 hour period, which is consistent with an observed half- life of about 5 hours. Little accumulation was observed with once-daily dosing of 52 mg/m2 administered as a 2 hour intravenous infusion as most pre-dose concentrations on Day 5 were less than 5% of maximal concentrations at the end of infusion.

- Clofarabine was eliminated largely unchanged in the urine with a renal clearance of about 14 L/h. Given an unbound fraction of 0.53 in human plasma, the unbound renal clearance of clofarabine was estimated at about 26 L/h, which was much greater than the glomerular filtration rate in humans (7.5 L/h) [3]. Hence, clofarabine showed evidence of filtration and tubular secretion as kidney elimination mechanisms.

- Given a systemic clearance of about 43 L/h and a renal clearance of about 14 L/h, nonrenal clearance was estimated as 29 L/h. Hence, clofarabine showed balanced clearance with

regards to renal and nonrenal elimination pathways – no single pathway dominated the clearance of clofarabine from plasma.

- Clofarabine had a volume of distribution at steady-state of 251 L and an unbound volume of distribution at steady-state of 474 L, indicating the clofarabine showed high tissue distribution.

- A relationship between weight and clearance and between weight and volume of distribution at steady-state was observed. However, when corrected for BSA the correlation was removed.

- Age was not observed to be correlated to pharmacokinetics to any significant extent, which may have been due to the small sample size. Since age and weight are often highly correlated it was expected that since weight was correlated with pharmacokinetics that age would be as well. But that was not observed. Since the youngest age in this study was 3 years old, it may be that by this time most renal and metabolic processes have reached maturation and there is no difference in this regard between a child and an adult.

Table B2: PK parameters in patients.

Patient ID	BSA (m**2)	Half-Life (hours)	Tmax (hours)	Cmax (ng/mL)	Total CL (L/h)	Total CL (L/h/m**2)	Vd,ss (L)	Vd,ss (L/m**2)
004-0005								(b) (4)
004-0031								
005-0037								
006-0003								
009-0024								
010-0010								
011-0035								
012-0014								
016-0008								
018-0036								
019-0011								
Mean	1.32	4.7	2.2	403.1	43.2	33.1	251.0	187.1
n	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0
Std Dev	0.53	1.8	0.4	171.4	18.8	10.3	142.4	75.1
CV(%)	40.0	38.3	16.9	42.5	43.5	31.1	56.7	40.1
Minimum	0.65	2.2	1.6	140.9	16.5	16.5	81.4	86.3
Median	1.20	4.3	2.1	380.0	42.5	30.4	208.6	156.0
Maximum	2.58	8.6	3.0	765.8	78.7	50.7	474.6	303.4

III.

Study #: CLO-222

Title: A Phase II, Open- Label Study of Clofarabine in Pediatric Patients with Refractory or Relapsed Acute Myelogenous Leukemia

Clinical Study Summary:

Objectives:
Primary: The primary objective of this study was to establish the efficacy of clofarabine in pediatric patients by determining the OR rate of clofarabine in pediatric patients with refractory or relapsed AML.
Secondary:
Secondary objectives included documenting the CR, CRp, and PR rates in this population, documenting duration of remission and overall survival, and documenting the safety profile and tolerability for this dosing regimen of clofarabine in this population.

Study Design:
A Phase II, non-randomized, open-label, single-arm study of clofarabine in pediatric patients (21 years of age or less at time of initial diagnosis) who were in first or subsequent relapse and/or refractory AML and were not eligible for therapy of higher curative potential. Refractory AML was defined as failure to achieve remission after 2 cycles of the same therapy or 2 different regimens. Patients with a KPS ≥50 with adequate renal (serum creatinine <2 × ULN for age) and liver function (serum bilirubin ≤1.5 × ULN; AST and ALT ≤5 × ULN) within 2 weeks before registration were eligible to enroll.

Patients in this study received clofarabine 52 mg/m2/day for 5 consecutive days as a 2-hour IVI for each cycle up to a maximum of 12 cycles. Treatment was to be continued until disease relapse or recovery of normal hematopoiesis (ANC ≥0.75 × 109/L). Each cycle was to be repeated every 2 to 6 weeks. Doses were to be reduced for nonhematologic or hematologic toxicities. The OR rate was defined as the sum of the number of patients with either CR or CRp divided by the total number of eligible patients. For patients who achieved a CR, an HSCT was an option. Patients who received an HSCT were discontinued from clofarabine therapy.

Results:
The OR (CR + CRp) was 3%, and 26% among the patients who achieved at least a PR. Of note, there were 24 patients with measurable peripheral absolute blast counts at baseline, and all of these patients showed a rapid decrease in blast counts during or after Cycle 1 of clofarabine administration.
The median duration of remission for ALL patients (CLO-212) refractory to the most recent multi-agent therapy who achieved an OR (CR + CRp) was 6.1 weeks. The median duration of remission in this subpopulation for patients achieving at least a PR was 4.6 weeks. The time to progression for the 1 AML patient (CLO-222) who achieved a CRp was 44.0+ weeks and for patients who achieved at least a PR was 20.2 weeks. The time for survival for the 1 AML patient

(CLO-222) who achieved a CRp was 93.6+ weeks and for patients who achieved at least a PR was 39.0 weeks.

Pharmacokinetic Analysis Summary:

Objectives:
To characterize the pharmacokinetics in pediatric patients enrolled in this study.

Methods:
Blood samples for pharmacokinetic analysis were collected at 0 (immediately prior to infusion), end of infusion (2 hours), 3 hours, 4 hours, 5 hours, 10 hours (or just prior to leaving clinic), and 24 hours (pre-dose on Day 2) on Day 1 and at 0 (immediately prior to infusion), end of infusion (2 hours), 3 hours, 4 hours, 5 hours, 10 hours (or just prior to leaving clinic) on Day 5. Urine samples were to be collected prior to infusion on Day 1 and at 0 (pre- infusion) to 8 hours and 8 to 24 hours on Days 1 and 5. Plasma and urine samples were to be analyzed for clofarabine concentrations using a validated analytical LC-MS/ MS method.
Urine samples were to be collected at pre-dose (on Day 1), 0 to 8 hours post-dose, and 8 to 24 hours post-dose on Days 1 and 5 and analyzed for parent clofarabine concentrations using a validated analytical LC-MS/MS method.
Plasma clofarabine concentrations were obtained from 14 of 49 patients and analyzed using noncompartmental methods. Subsequently the data from all 3 studies in pediatric populations were combined for a population analysis.

Results:
Figure B6 presents spaghetti plots of individual concentration-time profiles on semi-log scale for Day 1 and 5, respectively. Figure B7 presents mean concentration-time profiles on semi-log scale on Day 1 and 5, respectively. Table B3 lists the demographics of the patients with PK data.

Figure B6: Spaghetti plots of individual concentration-time profiles on semi-log scale for Day 1 (left panel) and day 5 (right panel).

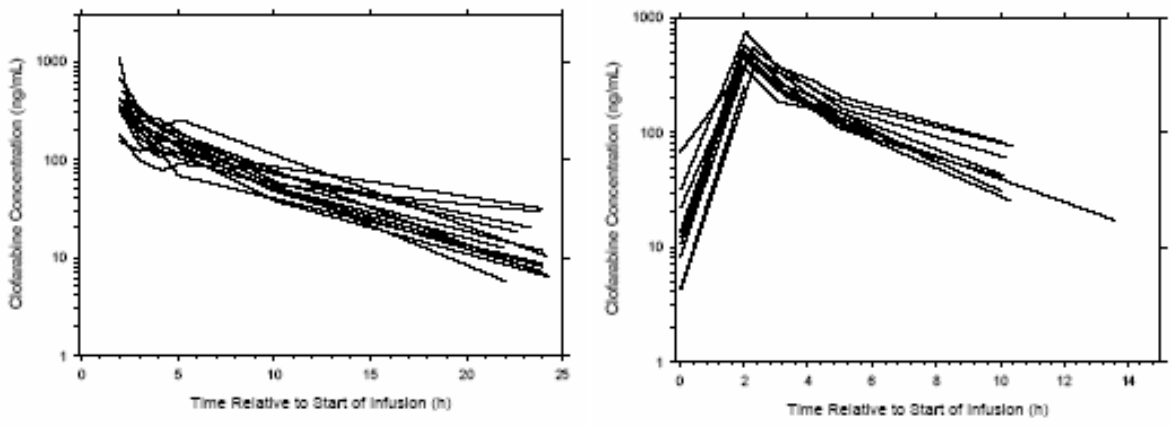


Figure B7: Spaghetti plots of mean concentration-time profiles on semi-log scale for Day 1 (left panel) and day 5 (right panel).

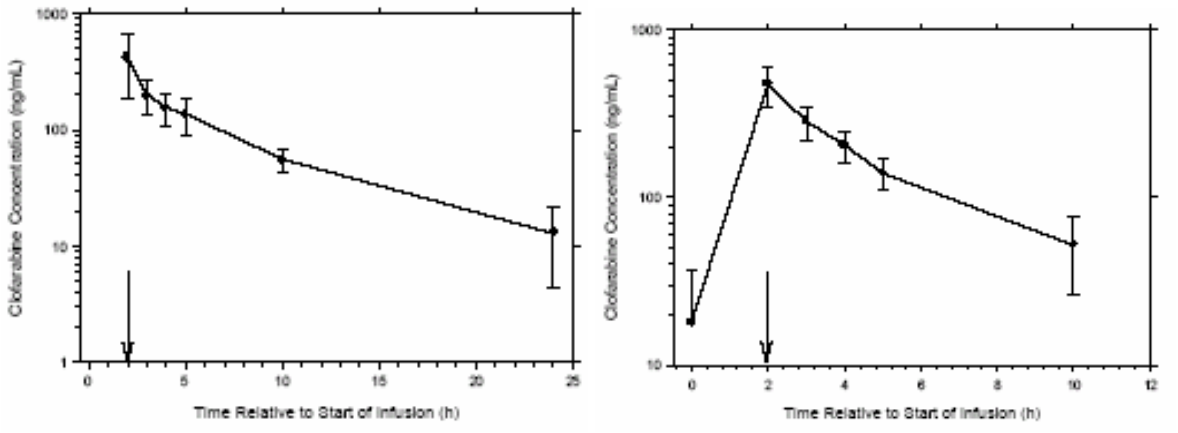


Table B3: Demographics of patients with PK data.

Patient ID	Sex	Age (years)	Race	BSA (m**2)	Weight (kg)	Height (cm)	Daily Dose (mg)	Dose (mg/m**2)
001-0021	Female	13	Hispanic	1.41	47.7	150.0	73.0	52
004-0024	Female	13	Other	1.82	74.7	158.4	95.0	52
006-0010	Male	18	Caucasian	1.67	58.0	172.0	87.0	52
006-0013	Male	10	Hispanic	1.00	30.7	133.2	52.0	52
009-0018	Female	12	Other	1.80	73.1	162.0	94.0	52
010-0015	Female	12	Black	1.38	43.7	155.6	71.8	52
010-0020	Male	18	Caucasian	1.57	51.3	173.7	81.6	52
010-0022	Male	17	Caucasian	1.60	53.6	166.3	83.2	52
010-0023	Male	2	Caucasian	0.61	14.1	88.0	31.7	52
010-0026	Male	19	Black	1.82	66.5	174.5	94.6	52
011-0008	Female	3	Asian	0.79	21.8	102.3	40.0	52
011-0028	Male	15	Other	1.72	59.0	180.0	89.0	52
012-0011	Female	9	Caucasian	0.88	22.0	.	47.0	52
015-0017	Male	11	Caucasian	1.55	61.0	149.0	81.0	52

Summary of PK findings (see table B4 for PK results):

- Clofarabine showed rapid elimination with most of the dose being eliminated in a 24 hour period, which is consistent with an observed half- life of about 6 hours. Little accumulation was observed with once-daily dosing of 52 mg/m2 administered as a 2 hour intravenous infusion as most pre-dose concentrations on Day 5 were less than 5% of maximal concentrations at the end of infusion.

- Clofarabine was eliminated largely unchanged in the urine with a renal clearance of about 18 L/h. Given an unbound fraction of 0.53 in human plasma, the unbound renal clearance of clofarabine was estimated at about 34 L/h, which was much greater than the glomerular

filtration rate in humans (7.5 L/h). Hence, clofarabine showed evidence of filtration and tubular secretion as kidney elimination mechanisms.

- Given a systemic clearance of about 39 L/h and a renal clearance of about 18 L/h, nonrenal clearance was estimated as 21 L/h. Hence, clofarabine showed balanced clearance with regards to renal and nonrenal elimination pathways – no single pathway dominated the clearance of clofarabine from plasma.

- Clofarabine had a volume of distribution at steady-state of 274 L and an unbound volume of distribution at steady-state of 517 L, indicating that clofarabine showed high tissue distribution.

- Total systemic clearance and volume of distribution at steady-state were both linearly related to body weight and BSA. As weight or BSA increased so did clearance and volume of distribution.

- A relationship between age and systemic clearance and volume of distribution at steady state was observed. However, since age and weight were confounded when these parameters were corrected for BSA the correlation dissipated, which would suggest that the age effect was really an effect of body size.

Table B4: PK parameters in patients.

Patient ID	BSA (m**2)	Half-life (hours)	Tmax (hours)	Cmax (ng/mL)	Total CL (L/h)	Total CL (L/h/m**2)	Vdss(L)	Vdss(L/m**2)
001-0021								(b) (4)
004-0024								
006-0010								
009-0018								
010-0015								
010-0020								
010-0022								
010-0023								
010-0026								
011-0008								
011-0028								
012-0011								
015-0017								
Mean	1.43	5.7	2.3	417.5	38.9	26.8	274.4	182.1
n	13.0	10.0	13.0	13.0	10.0	10.0	10.0	10.0
Std Dev	0.41	2.0	0.8	228.9	15.9	6.4	156.3	67.2
CV(%)	28.7	34.4	35.2	54.8	41.0	23.9	56.9	36.9
Minimum	0.61	3.6	2.0	156.9	13.9	18.7	85.3	96.2
Median	1.57	4.9	2.0	360.1	37.6	27.0	238.6	166.8
Maximum	1.82	10.3	5.0	1048.9	70.4	38.7	540.8	297.2

IV.

Title: Summary of Analytical Methods

A series of method development and validation studies were conducted to establish the analytical method for clofarabine in human plasma and urine. Brief summaries of these studies are presented below.

MSN02018	**Analytical method summary for clofarabine in human plasma** This report summarizes the method used for analysis of clofarabine in human plasma. Human plasma samples containing clofarabine, cladribine (as internal standard) and heparin were precipitated with acetonitrile, and the supernatant was evaporated and reconstituted in a water: acetonitrile:formic acid (b) (4) mixture. Samples were analyzed by (b) (4) Volume of matrix: 100.0 µl Type of matrix: heparin containing human plasma Standard concentrations: 1.00 to 500.0 ng/ml (9 standards) QC concentrations: 3.0, 30.0 and 400.0 ng/ml Chromatographic conditions: Column (b) (4) (b) (4) Flow rate: (b) (4) Temperature: 45°C Injection volume: (b) (4)
MC02017	**Full validation of method in human plasma** This report describes the validation experiments conducted for the HPLC method in human plasma. The following characteristics were evaluated as part of the validation process: interday variation, intraday variation, LLOQ precision and accuracy, partial volume verification, sample storage stability: freeze-thaw, thawed, and processed samples, recovery and matrix effect. Based on the results, the analytical method has been validated according to the applicant's criteria. The method has a range of linearity from 10 to 5000 ng/ml. the method is reproducible and robust, with no significant matrix effect.

MC02293	**Partial validation for change in calibration range** This report characterizes the change in the calibration range for the assay from 10-5000 ng/ml to 1-500 ng/ml. As part of the partial validation, intraday and interday variation was assessed and found to be acceptable for the new calibration range.
MC03065	**Partial validation for change in injection volume** This report characterizes a change in the injection volume from (b) (4). Intraday and interday variations were small. The calibration curve was within acceptable limits.
MC02301	**Partial validation for assay of clofarabine in presence of cytarabine** This report characterizes the effect of cytarabine on the measurement of clofarabine. The results indicated that the calibration curve for clofarabine in the presence of cytarabine was acceptable. The mean %deviation of QC samples spiked with various amounts of cytarabine was less than 15% from theoretical values, indicating that cytarabine does not interfere in the detection of clofarabine.
MC03171	**Stability study: long term frozen stability in human plasma** This report characterizes the long term stability of clofarabine in human plasma frozen for 89 days. Three replicates of each QC level (3.0, 30.0 and 400.0 ng/ml) were stored frozen at -20^{o}C for 89 days. Samples were analyzed by HPLC/MS/MS to determine the clofarabine concentrations following freezing. Results showed that the mean value of the QC samples remained within ± 8% from theoretical for all three levels indicating acceptable stability for at least 89 days at -20^{o}C.
MC03240	**Stability study: long term processed sample stability in human plasma and human urine** This objective of this study was to investigate the processed sample stability of clofarabine in human plasma and urine. One plasma and one urine run (standards and QC solutions) were extracted and stored for 16 and 20 days respectively, at 5^{o}C to determine the stability of clofarabine extracts under refrigerated conditions. Samples were assayed using HPLC/MS/MS. Results showed that the standards and QC solutions remained within 10% for plasma and within 15% for urine (with one exception: the lowest QC deviated by 22% from theoretical) after refrigeration for 16 and 20 days respectively.

MSN02075	**Analytical method summary for clofarabine in human urine** This report summarizes the method used for analysis of clofarabine in human urine. Human urine samples containing clofarabine and cladribine (as internal standard) were diluted with water: acetonitrile:formic acid (90:10:0.1 v/v/v) mixture. Samples were analyzed by (b) (4) HPLC using a (b) (4) column maintained at 45°C. The (b) (4) was nebulized using heated nitrogen in a Z-spray source/interface and the ionized compounds were detected using a tandem (b) (4) spectrometer. Volume of matrix: 100.0 µl Type of matrix: human urine Standard concentrations: 1.00 to 500.0 ng/ml (9 standards) QC concentrations: 3.0, 30.0 and 400.0 ng/ml Chromatographic conditions: Column (b) (4) (b) (4) Flow rate: (b) (4) Temperature: 45°C Injection volume: (b) (4)
MC02298	**Full validation of method in human urine** This report describes the validation experiments conducted for the HPLC method in human urine. The following characteristics were evaluated as part of the validation process: interday variation, intraday variation, LLOQ precision and accuracy, partial volume verification, sample storage stability: freeze-thaw, thawed, and processed samples, recovery and matrix effect. Based on the results, the analytical method has been validated according to the applicant's criteria. The method has a range of linearity from 10 to 5000 ng/ml. the method is reproducible and robust, with no significant matrix effect.
MC03135	**Partial validation for change in injection volume** This report characterizes a change in the injection volume from (b) (4). Intraday and interday variations were small. The calibration curve was within acceptable limits.
MC03105	**Stability study: long term frozen stability in human urine** This report characterizes the long term stability of clofarabine in frozen human urine. Three replicates of each QC level (3.0, 30.0 and 400.0 ng/ml) were stored frozen at -20°C for 202 days. Samples were analyzed by HPLC/MS/MS to

	determine the clofarabine concentrations following freezing. Results showed that the mean value of the QC samples remained within ± 5.2% from theoretical for all three levels indicating acceptable stability for at least 202 days at -20^{o}C.

Based on the above studies and reports, the following performance characteristics were developed for clofarabine in plasma and urine.

Clofarabine:

Plasma

- Assay calibration range: 1.0 to 500.0 ng/ml
- LLOQ = 1 ng/ml (%CV = 13.5%)
- Inter-day:
  - Accuracy: %DFN = 0.7 to 7.0
  - Precision: %CV = 4.6 to 8.8
- Intra-day:
  - Accuracy: %DFN = 4.4 to 10
  - Precision: %CV = 6.7 to 9.6
- Specificity: Determined in 6 lots of blank human plasma. No interference peaks were seen at the retention times of clofarabine or internal standard (typical chromatograms included in Appendix B).
- Recovery: Overall mean recovery for clofarabine: 92.4%
  - 95.4% at 30 ng/ml, 92.3% at 300 ng/ml, 89.4% at 4000 ng/ml
  - Overall mean recovery for internal standard: 94.6%
  - Recovery study was not repeated with lower calibration range of 1-500 ng/ml.
- Matrix effect: comparison of peak height of standards spiked into plasma blanks to peak heights spiked into reagent blanks: Overall matrix effect: 0.993
  - 0.98 at 30 ng/ml, 0.991 at 300 ng/ml, 1.01 at 4000 ng/ml.
  - Overall matrix effect for internal standard: 1.025
- Stability:
  - QC Sample storage frozen at -20^{o}C: 89 days (samples were ± 8% of nominal)
  - QC Sample storage thawed at room temperature: 26 hours
  - QC Sample storage stability (freeze/thaw): 3 cycles
  - Processed sample stability (refrigerated at 5^{o}C): 16 days
  - Stock standard solution stability (frozen at -20^{o}C): 195 days

Urine

- Assay calibration range: 1.0 to 500.0 ng/ml
- LLOQ = 1 ng/ml (%CV = 5.3%)
- Intra-day:
  - Accuracy: %DFN = 0.3 to 11.8
  - Precision: %CV = 3.4 to 5.8
- Specificity: Determined in 6 lots of blank human urine. No interference peaks were seen at the retention times of clofarabine or internal standard (typical chromatograms included in Appendix B).

- Recovery: Overall mean recovery for clofarabine: 99.8%
    - 99.7% at 3 ng/ml, 97.3% at 30 ng/ml, 102.5% at 400 ng/ml
    - Overall mean recovery for internal standard: 98.9%
- Matrix effect: comparison of peak height of standards spiked into plasma blanks to peak heights spiked into reagent blanks: Overall matrix effect: 1.130
    - 1.146 at 3 ng/ml, 1.132 at 30 ng/ml, 1.111 at 400 ng/ml.
    - Overall matrix effect for internal standard: 1.456
- Stability:
    - QC Sample storage frozen at -20^{o}C: 202 days (samples were ± 5.2% of nominal)
    - QC Sample storage thawed at room temperature: 24 hours
    - QC Sample storage stability (freeze/thaw): 3 cycles
    - Processed sample stability (refrigerated at 5^{o}C): 122 hours
    - Stock standard solution stability (frozen at -20^{o}C): 56 days

Assay for Chloroadenine:
An analytical method was developed for chloroadenine, which is a putative metabolite of clofarabine. Samples from a study done in adult patients receiving clofarabine were analyzed for chloroadenine. In all cases, samples showed levels below the LLOQ.
The analytical method was identical to the one used for clofarabine, i.e., HPLC with tandem mass spectrometry.

Chloroadenine:
Plasma
- Assay calibration range: 1.0 to 500.0 ng/ml
- LLOQ = 1 ng/ml (%CV = 7.0%)
- Inter-day:
    - Accuracy: %DFN = 0.7 to 2.3
    - Precision: %CV = 4.6 to 7.6
- Intra-day:
    - Accuracy: %DFN = 2.3 to 3.5
    - Precision: %CV = 6.1 to 12.1
- Specificity: Determined in 6 lots of blank human urine. No interference peaks were seen at the retention times of chloroadenine or internal standard (typical chromatograms included in Appendix B).
- Recovery: Overall mean recovery for chloroadenine: 101.8%
    - 106.2% at 3 ng/ml, 95.7% at 30 ng/ml, 103.4% at 400 ng/ml
- Matrix effect: comparison of peak height of standards spiked into plasma blanks to peak heights spiked into reagent blanks: Overall matrix effect: 0.743
    - 0.662 at 3 ng/ml, 0.743 at 30 ng/ml, 0.823 at 400 ng/ml.
- Stability:
    - QC Sample storage frozen at -20^{o}C: 33 days (samples were ± 7.3% of nominal)
    - QC Sample storage thawed at room temperature: 25 hours
    - QC Sample storage stability (freeze/thaw): 3 cycles
    - Processed sample stability (refrigerated at 5^{o}C): 91 hours

C. Pharmacometrics Review

SUMMARY

The purpose of this review is to evaluate the population PK and PK-PD analysis conducted by the applicant for clofarabine in pediatric patients and to address the following two questions regarding the analysis:
1) Is the inclusion of WBC as a covariate in the parameter model for central volume V1 appropriate?
2) Can the analysis of exposure-toxicity and exposure-response be improved by using data from all the patients in the database and does this approach result in any significant PK-PD relationships for clofarabine in this population?

The applicant's analysis focused on the population pharmacokinetics of clofarabine studied in 40 pediatric patients, ages 2 to 19 years old (21 males/19 females) with relapsed or refractory acute lymphoblastic or acute myelogenous leukemia given multiple doses in 3 studies (a phase1 and two phase 2studies). Clofarabine pharmacokinetics were dependent on body size, with intravenous infusion of 52 mg/m^2 resulting in equivalent exposure across a wide range of weights. Clofarabine pharmacokinetics were best described by a 2-compartment model. The model developed by the applicant indicated that the WBC count was a significant covariate of the parameter model for the central compartment volume (V1). However, the Agency's analysis questioned the validity of including this covariate as there does not appear to be a physiological rationale for its inclusion and scatter-plots showed a lack of correlation between volume estimates and the WBC count. Moreover there was no decrease in the variance in the model for V1 when the WBC count is included in the model. No apparent difference in pharmacokinetics was observed between patients with ALL or AML or between males and females. Intracellular clofarabine triphosphate concentrations were essentially constant over the sampling period having an estimated concentration of 11.6 ± 0.262 μM. The *in vivo* half- life of clofarabine triphosphate in peripheral mononuclear cells could not be definitively established but was estimated to be at least 24 hours.

No relationship between exposure and toxicity or response was seen in this population. The applicant's analysis only included a subset of the sample, i.e., those in whom PK data was available. The Agency has repeated the analysis using the complete dataset, including data from all available patients by estimating individual exposures from the population model, however this analysis also did not result in any significant relationships between exposure (clofarabine AUC) and measures of toxicity or response.

OBJECTIVES

- To evaluate the population pharmacokinetic model developed by the applicant for clofarabine and intracellular pharmacokinetics of its active metabolite clofarabine triphosphate, in pediatric ALL and AML patients.
    - A specific aim is to examine the models for covariates of the PK parameters.

- To evaluate the pharmacokinetic-pharmacodynamic analysis conducted by the applicant for measures of response and toxicity of clofarabine.

METHODS

The applicant has conducted extensive analyses to develop a population PK model for clofarabine in pediatric ALL and AML patients.

Data:
Concentration and exposure data were pooled from three clinical studies:
ID99-383 was a Phase I, open label, dose escalation study in pediatric patients with refractory leukemia who failed standard therapy or for whom no such therapy existed.
CLO-212 was a Phase II, open-label, single-arm study of clofarabine administered to pediatric patients with refractory or relapsed acute lymphoblastic leukemia (ALL) who were not eligible for therapy of higher curative potential.
CLO-222 was a Phase II, open-label, single-arm study of clofarabine administered to pediatric patients with refractory or relapsed acute myelogenous leukemia (AML) who were not eligible for therapy of higher curative potential.
(Please see individual study reviews for details of study designs and PK sampling and analysis).
The full dataset consisted of 40 patients. The pharmacokinetic modeling was conducted on a dataset of 32 patients, since 8 patients had missing values for the covariates.

Pharmacokinetic Model:
Nonlinear mixed effects modeling using NONMEM was used to characterize the pharmacokinetics of plasma clofarabine and intracellular clofarabine triphosphate in the patient population. Traditional model building techniques were used with parent concentrations being modeled first followed by metabolite concentrations. Briefly, a structural model for clofarabine without patient covariates was first developed. Covariates were screened and then entered into the model using forward selection with a significance level of 0.05. The model was reduced using backwards selection with a significance level of 0.005. Model performance, model stability, and influence analysis were assessed using the nonparametric bootstrap and n-1 jackknife. Once the parent model was developed, a model using intracellular clofarabine triphosphate concentrations as the dependent variable was developed.

Pharmacodynamics:
A secondary objective was to characterize relationships between measures of exposure (clofarabine AUC, Cmax, total dose, clearance, and average clofarabine triphosphate concentration in patients with quantifiable concentrations) and response (neutropenia, dyspnea, nausea-vomiting, hypotension, sepsis, death, anxiety, and response rates). Exposure-response relationships were explored using the empirical Bayes estimates of individual measures of exposure using logistic regression models.
Simulations were performed to understand the relationship between any important covariates and pharmacokinetics-pharmacodynamic relationships.

Number of Patients with PK and PD data:

There were a total of 40 patients with PK data (male 21; female 19). There were 14 ALL patients, 14 AML patients, and 12 patients from the phase 1 study with concentration – time data. Of these 40 patients, eight patients had missing information on covariates, therefore the applicant's final dataset had 32 patients.

RESULTS

I. PK Model:

Various structural PK models, 1-compartment, 2-compartment, with linear and non-linear elimination, were evaluated. Weight was included as a covariate in the parameter models *a priori*. Goodness of fit and selection of models was based on precise standard error of the parameter estimates, unbiasedness of residual plots, and precise estimation of the variability associated with the random effects as well as a significant reduction in objective function value (OFV) and on the reduction in Akaike Information Criteria (AIC).

Comparison of models indicated that a 2-compartment model with zero-order input (infusion) and first-order output provided the best fit. To verify that another predictor such as BSA or age did not provide a better fit than body weight, the models were run with BSA and with age as covariates instead of weight (table PM1). The resulting fits had larger OFV, larger variance of parameter estimates and poorer goodness of fit statistics. Hence the final "base model" had weight as the covariate on the parameter models. Table PM2 provides a summary of the base model parameters and equations, as well as a comparison with a model minus weight as a covariate.

Table PM1: Comparison of models with weight, BSA and age as covariates.

	Covariate Model		
	Weight	BSA	Age
OFV	3378	3385	3519
Population Variance [%] for Parameter			
CL	20%	65%	49%
V1	57%	46%	31%
Q2	15%	158%	60%
V2	37%	70%	34%

Table PM2: Base model summary for plasma clofarabine with and without weight as a covariate in the model (applicant table 7.3-2)

Parameter	With Weight	Without Weight
Filename	Base	Basenowgt
Objective Function Value	3395.976	3455.501
Systemic Clearance (L/h)	$28.9\left(\frac{Weight}{40\ kg}\right)^{0.75}$	29.6
Central Volume (L)	$70.2\left(\frac{Weight}{40\ kg}\right)^{1.00}$	70.4
Intercompartmental Clearance (L/h)	$24.1\left(\frac{Weight}{40\ kg}\right)^{0.75}$	24.6
Peripheral Volume (L)	$99.9\left(\frac{Weight}{40\ kg}\right)^{1.00}$	109
Variance Component for Systemic Clearance [(Eq. 1)]	43%	71%
Variance Component for Central Volume [(Eq. 1)]	86%	102%
Variance Component for Intercompartmental Clearance [(Eq. 1)]	63%	77%
Variance Component for Peripheral Volume [(Eq. 1)]	Not estimable	Not estimable
Residual Variance (additive)	0.0719	0.0692
Residual Variance (proportional)	934	1060
Estimated α-half-life for a 40 kg child (hours)	0.79	0.78
Estimated β-half-life for a 40 kg child (hours)	6.2	6.5
Estimated volume of distribution at steady-state for a 40 kg child (L)	170	179

II. Selection of covariates:

Scatter plots of baseline covariates against the estimates of individual parameters from the base model were examined to determine potential covariates for further model development. Covariates of particular interest were sex, BSA, age, creatinine, disease type, and WBC counts. Figure PM1 shows the scatter plots for clearance and figure PM2 shows the scatter plots for central volume (V1). Additional covariates evaluated included bilirubin and serum transaminases (SGPT and SGOT) (scatter-plots not shown).

The correlations with age and BSA were not surprising as these covariates are correlated with weight, which was already included in the model. As determined from the non-compartmental analysis and verified in this analysis, substitution of weight in the model with age or BSA did not provide a better fit. The plots for WBC indicate the presence of one patient with an extremely high baseline WBC count. This patient's data was retained in the applicant's dataset.

The following covariates were identified using NONMEM screening with a significance level of 0.05 for further inclusion in the covariate sub-models: (1) Age on V1, (2) WBC count on CL, V1, Q2, and V2, (3) SGPT (ALT) on V1 and CL, and (4) Bilirubin on CL, V2, and Q2.

Covariate models incorporating the above covariates were evaluated, and the applicant concluded that WBC count was a significant covariate in the parameter model for V1. Other covariates evaluated for CL, Q2 and V2 did not result in significant reductions in OFV.

Table PM2 lists the final model developed by the applicant. The final model includes WBC as a covariate of V1, in addition to weight which was included as a covariate for all PK parameters.

Figure PM1: Scatter-plots of clearance vs. a selection of potential baseline covariates.

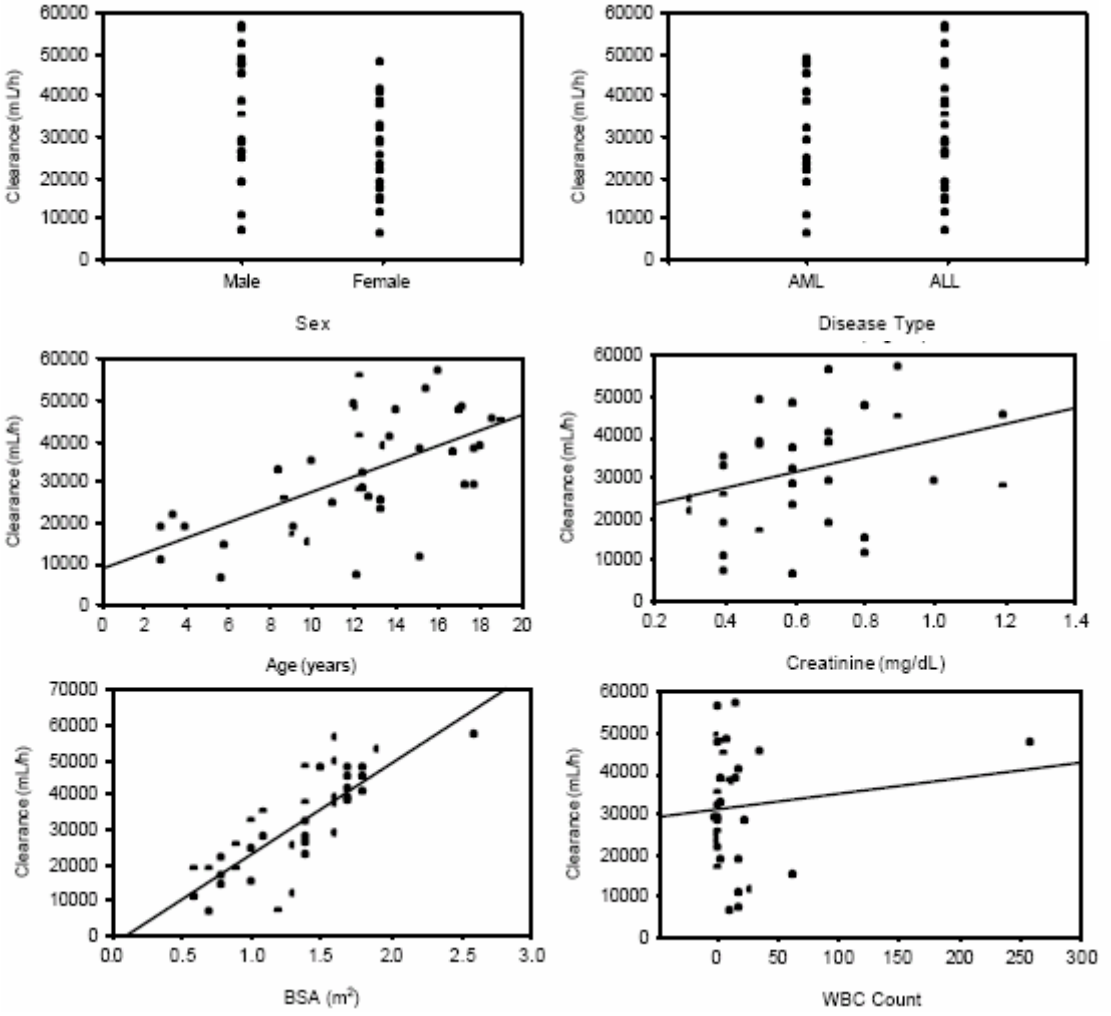


Figure PM2: Scatter-plots of central volume vs. a selection of potential baseline covariates.

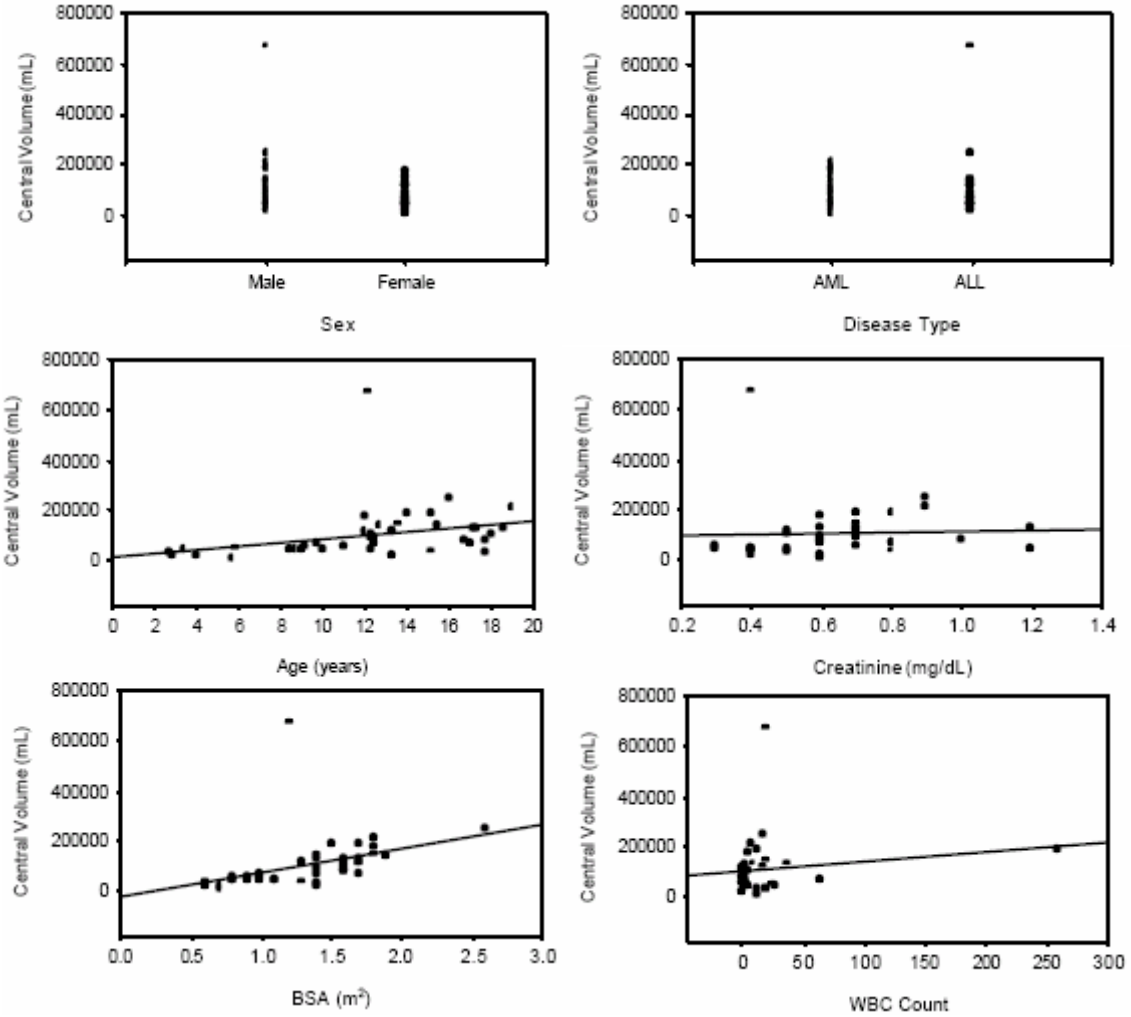


Table PM2: Final model summary for plasma clofarabine developed by the applicant.

Parameter	Model	Population Variance [(Eq. 1)]
Systemic Clearance (L/h)	$CL = 32.8 \pm 1.55 \left(\frac{Weight}{40\ \text{kg}} \right)^{0.75}$	27%
Central Volume (L)	$V1 = 115 \pm 19.6 \left(\frac{Weight}{40\ \text{kg}} \right)^{1.00} \left(\frac{WBCcount}{10 \times 10^3\ \text{per}\ \mu\text{L}} \right)^{0.128 \pm 0.0316}$	56%
Intercompartmental Clearance (L/h)	$Q2 = 20.5 \pm 3.39 \left(\frac{Weight}{40\ \text{kg}} \right)^{0.75}$	27%
Peripheral Volume (L)	$V2 = 94.5 \pm 9.80 \left(\frac{Weight}{40\ \text{kg}} \right)^{1.00}$	39%
Objective Function Value: 2420.819. Residual variance: Proportional, 0.0691 (27%); Additive, 0.00001 Fixed. Estimated α-half-life for a 40 kg child with WBC count of $10 \times 10^3/\mu L$: 1.2 hours Estimated β-half-life for a 40 kg child with WBC count of $10 \times 10^3/\mu L$: 6.4 hours Estimated volume of distribution at steady-state for a 40 kg person with a WBC count of $10 \times 10^3/\mu L$: 210 L (72% variability) Estimable model parameters are reported as estimate ± standard error of the estimate. Filename: FINAL		

III. Model Validation:

The applicant has evaluated the model performance and validation using internal methods, including (1) non-parametric bootstrap and (2) influence analysis using jackknife methods. Briefly, the nonparametric bootstrap involves the re-sampling of subjects from the final data set with replacement and fitting of the final model to the bootstrap data set using first-order conditional estimation with interaction. This process was repeated 1000 times. The distribution of the bootstrapped parameter estimates was then examined graphically for precision and bias from the model building dataset. Results showed that in all cases, the relative bias in the final model pharmacokinetic parameter estimates was less than ± 6%. The relative bias of the variance components was small, less than ± 5%, except for the bias associated with intercompartmental clearance, which was estimated at 65.8%.

The influence analysis was undertaken by creating n new data sets where n is the number of subjects in the final data set, with each new data set having one subject removed so that each data set has n-1 subjects. These new data sets are referred to as jackknifed data sets. Each jackknifed data set was used to fit the final model using first-order conditional estimation with interaction. The percent change in parameter estimates between the final model and the jackknife data set was calculated and graphically examined for any subject who had a consistent effect on parameter values (as defined as a percent change from the final model parameter estimate of more than ± 20%). Results showed that when individual subjects were removed from the data set, no subject showed a consistent effect on the parameter estimates. Therefore it was not necessary to exclude any individual subject's data from the analysis.

IV. PK model for intracellular clofarabine triphosphate

Once the model for plasma clofarabine concentrations was identified, attempts were made to develop a combined model to include intracellular clofarabine triphosphate concentrations. However, the available data for the intracellular clofarabine triphosphate was very limited: only 29 observations from 10 patients in the phase 1 study. Models with first-order and zero-order rates of formation and first-order rates of elimination were fir to the data, however, the models either did not converge or were unstable.

The available data seemed to indicate that the best model would be one where sustainable intracellular clofarabine triphosphate concentrations were rapidly achieved and maintained with no indication that triphosphate concentrations declined over the 24-hour interval over which data was collected time. For most patients, concentrations at 24 hours (day 2 pre-dose) were essentially the same as the end of infusion concentrations on day 1, indicating that the half-life is long (more than 24 hours)

Thus, the final model fit by the applicant to the clofarabine triphosphate concentrations was a random intercepts model without any covariates where both the random effects and residual error were modeled as log-normal in distribution. A multiplier factor was incorporated to account for the increase in clofarabine triphosphate levels following multiple cycles, although this was based on data from only 1 individual (levels ranged from 2.9 to 6.1 μM in the first cycle and 28.9 μM to 35.1 μM during cycle 3) and therefore the appropriateness of this approach is questionable. The average clofarabine triphosphate concentration (intercept) was 11.6 ± 2.62 μM, which was very near the median observed concentration of 13.2 μM, with a between-subject variability of 80%.

V. PK-PD Relationships:

A secondary objective of the analysis was to characterize relationships between measures of exposure and toxicity or response. Logistic regression was used to assess whether any measures of exposure (AUC, Cmax, total dose, clearance, and average clofarabine triphosphate concentration in patients with quantifiable concentrations) could predict either toxicity (neutropenia, dyspnea, nausea-vomiting, hypotension, sepsis, death, anxiety) or efficacy (responder or nonresponder).

The applicant's analysis only included those subjects in whom PK data was collected (n=32. The applicant's analysis showed that none of the measure of exposure was a significant predictor of either toxicity or efficacy.

AGENCY'S ANALYSIS

The Agency's analysis focused on addressing two primary questions:

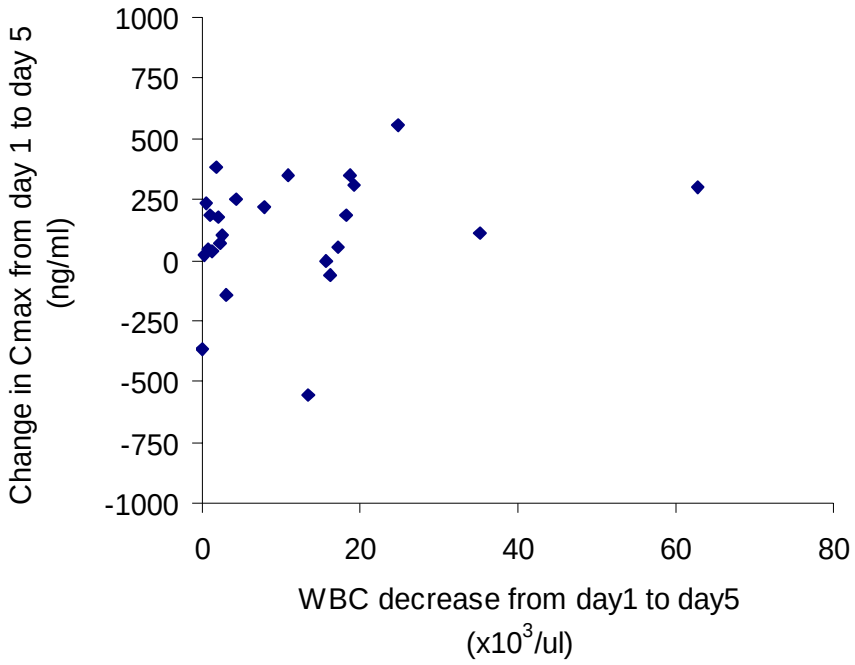
1) Is the inclusion of WBC as a covariate in the parameter model for central volume V1 appropriate?
2) Can the analysis of exposure-toxicity and exposure-response be improved by using data from all the patients in the database and does this approach result in any significant PK-PD relationships for clofarabine in this population?

I: WBC as a covariate of central compartment volume:
There is a concern about the inclusion of WBC as a covariate in the parameter model for central volume V1, for the following reasons:

1) There does not appear to be any clear physiological rationale for a relationship between V1 and the WBC count. Clofarabine does distribute into WBCs, this is facilitated by nucleoside transporters hCNT2 and hENT2, however these transporters are widely distributed across almost all body tissues, and there does not appear to be any evidence for a higher prevalence of these transporters on WBCs.

2) The applicant claims that, based on their final model and simulations, a decrease in WBC counts following treatment with clofarabine would result in a decrease in volume of distribution of clofarabine, and a related increase in the concentrations. To examine this, the Cmax and WBC counts from first and last day of the 5 day regimen were tabulated and the difference in Cmax from day 1 to day 5 (deltaCmax) was plotted against the difference in WBC from day 1 to day 5 (deltaWBC). The plot showed no consistent pattern or relationship between the two variables.

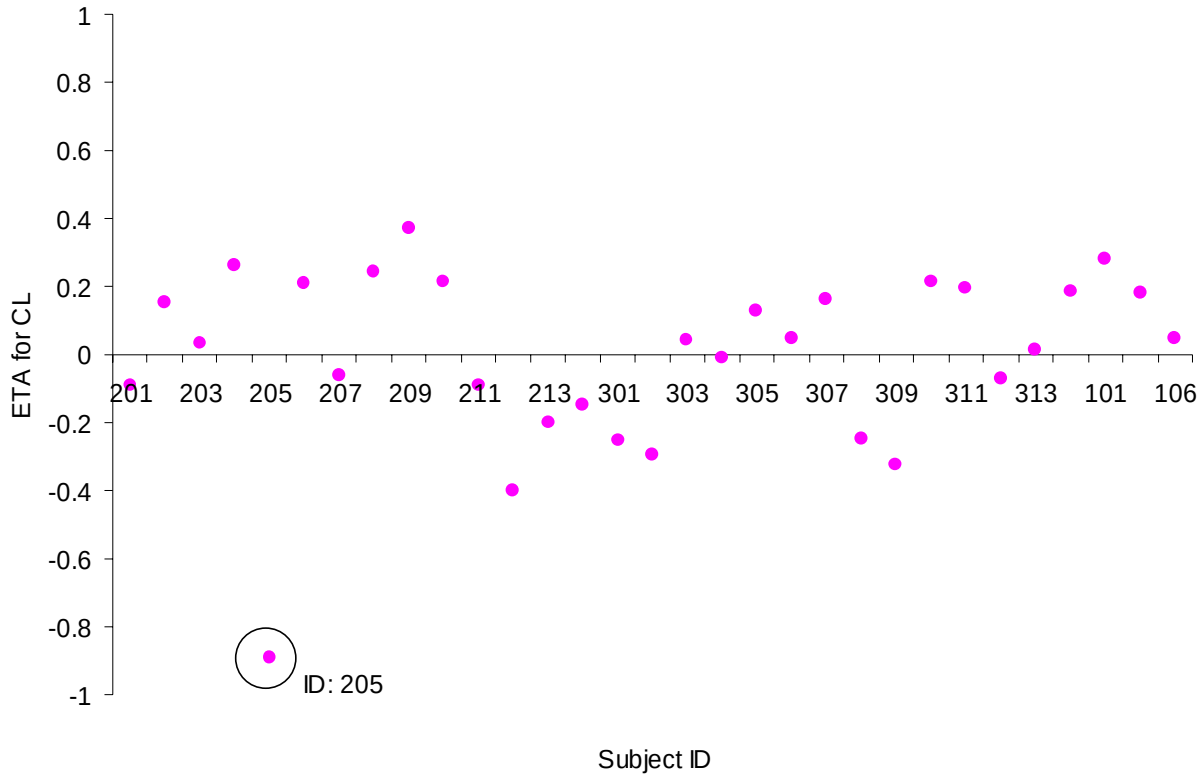
Figure PM3: Scatter-plot of change in Cmax from day 1 to day 5 (DeltaCmax) vs. change in WBC from day 1 to day 5 (DeltaWBC). The plot does not show any consistent pattern or correlation.



3) The scatter-plot of V1 estimated from the base model and baseline WBC counts is driven by the presence of one individual with a WBC count that is much higher than that in the remainder of the sample (see figure PM2). This individual's data was included in the applicant's final dataset and the subsequent significant findings might have been influenced by this outlier.

Dataset modification for Agency's analysis:
The applicant's dataset (n=32) was modified to exclude the data from the patient with the extremely high WBC count (#103). An additional subject (ID: 205) was identified as an outlier based on an extreme ETA(CL) value (see figure PM4). This patient had only one observed concentration following the first dose, which might have had an undue influence on the overall fit.

Figure PM4: ETA for CL vs. Subject ID. ID number 205 was identified as an outlier.



Software:
The following programs were used by the Agency for the analysis:
Database management and PK-PD analysis (logistic regression) – Microsoft Excel and SAS® (Ver 8.0).
Population PK analysis - NONMEM® (Compiler: Visual Fortran Ver 6.5).

Methods:
The Agency dataset (n=30) was used for running the final models again in NONMEM with and without the inclusion of WBC count as a covariate in the V1 parameter model.

Results:
Table PM3 lists the estimates from the model with and without WBC in the model. The population variance for the V1 model excluding WBC counts was not different from that of the model that included the WBC counts. This indicates that inclusion of WBC into the V1 model does not help reduce the variance. Estimates and variance of the parameters seem comparable

between the 2 models. Visual inspection of the goodness of fit suggests that both models resulted in comparable plots (figure PM4).

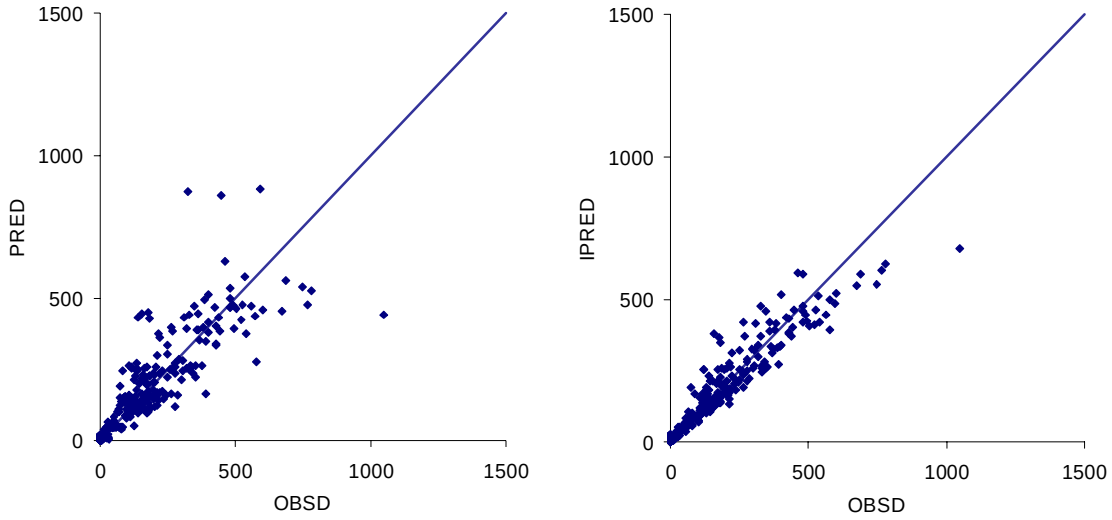
Thus, the inclusion of WBC does not appear to be justified, and the model incorporating WBC into the parameter model for V1 did not result in a lower variance than the model without the WBC (see table PM3).

Table PM3: Parameter estimates, including variance, for PK model with and without WBC counts (30 patients).

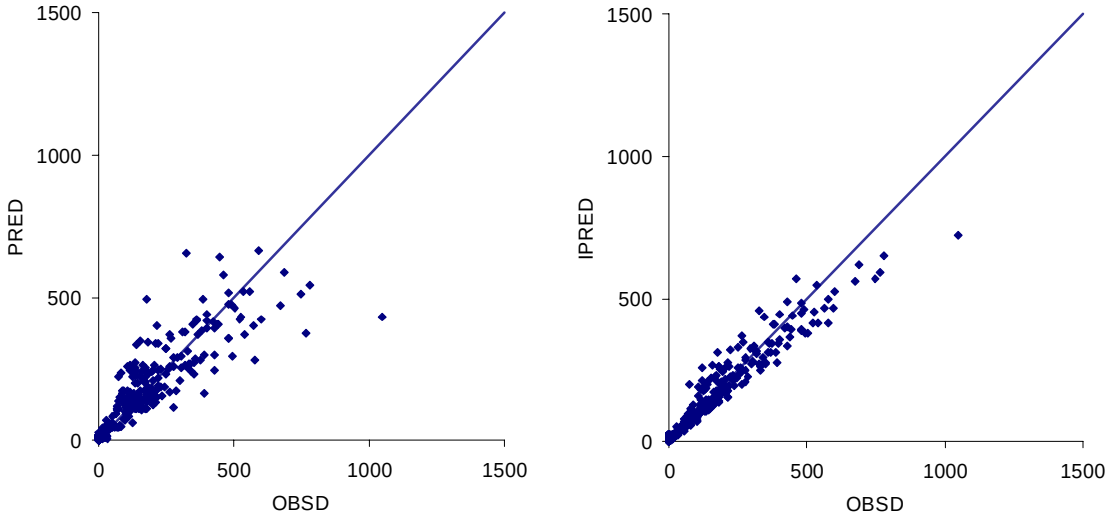
	Model including WBC		Model excluding WBC	
	Model	Population Variance [%]	Model	Population Variance [%]
CL	$33.3 \cdot (Wt/40)^{0.75}$	20%	$32.5 \cdot (Wt/40)^{0.75}$	20%
V1	$118 \cdot (Wt/40)^{1.0} \cdot (WBC/10^4)^{0\ 13}$	56%	$76.3 \cdot (Wt/40)^{1.0}$	57%
Q2	$20.8 \cdot (Wt/40)^{0.75}$	23%	$25.3 \cdot (Wt/40)^{0.75}$	15%
V2	$94.1 \cdot (Wt/40)^{1.0}$	36%	$103 \cdot (Wt/40)^{1.0}$	37%
ERR	6%		7%	-
OFV	2236.117		2254.079	-
AIC*	2256.117		2272.079	
*: AIC calculated as OFV+2p				

Figure PM4: Observed vs. predicted concentrations of clofarabine for the 2 models, including WBCs (upper panel) and excluding WBC (lower panel). Plots are shown for the population-predicted and individual-predicted concentrations.

Model including WBCs



Model excluding WBCs



II: PK-PD relationships:

The applicant's analysis used only those patients in whom PK data was available (n=32), which excluded about 60% of the study sample since toxicity and response data was available for all 95 subjects in the database.

The Agency has repeated the analysis using the complete dataset of patients. The AUC for each subject was calculated from the dose and the individual estimate of clearance. The logistic regression models were repeated for the measures of toxicity and response used by the applicant, neutropenia, dyspnea, nausea-vomiting, hypotension, sepsis, death, anxiety, and responder or nonresponder.

Inclusion of data from all the subjects did not change the results. None of the measures of toxicity or response was found to be related to the AUC of clofarabine. This may be due to the limited sample size and the limited range in exposures obtained in this study – the majority of the patients received only one dose level, i.e., 52 mg/m^2.

Additional analysis was conducted on data from the lab tests for bilirubin and hepatic transaminase enzymes (SGPT and SGOT). The frequency of elevations in bilirubin, SGPT and SGOT were determined among the study patients, and logistic regressions were performed to examine if the frequency of elevations in any of these hepatic markers was associated with clofarabine AUC, which was estimated for each individual based on the population PK model for clearance. Results indicated that there was no significant relationship between clofarabine AUC and incidence of elevated bilirubin, SGPT or SGOT in the patients. Again, this might be related to the small sample size and limited range of clofarabine exposures seen in these studies.

CONCLUSIONS

A population PK model was developed for clofarabine in pediatric leukemia patients by the applicant. The Agency conducted additional analysis to examine the appropriateness of including the baseline WBC count as a covariate in the parameter model for central compartment volume in the PK model. Based on the Agency's analysis, the inclusion of baseline WBC counts is not justified and the final model should not include the WBC count as a covariate in the model.

Additional analysis conducted by the Agency to extend the exposure-response analysis of the applicant by including all patients in the 2 studies did not change the lack of significant associations between clofarabine exposure (AUC) and measures of response and toxicity.

D. CPB Filing/Review Form

Office of Clinical Pharmacology and Biopharmaceutics

New Drug Application Filing and Review Form

General Information About the Submission

	Information		Information
NDA Number	21-673	**Brand Name**	CLOLAR
OCPB Division (I, II, III)	DPE-I	**Generic Name**	Clofarabine
Medical Division	HFD-150	**Drug Class**	Anti-cancer – anti-metabolite
OCPB Reviewer	Roshni Ramchandani	**Indication(s)**	Pediatric primary relapsed or refractory acute leukemia
OCPB Team Leader	Brian Booth	**Dosage Form**	IV solution (1mg/ml) for dilution
		Dosing Regimen	52 mg/m^2 as a 2 hr IV infusion daily x5 days every 2-6 weeks
Date of Submission	3/29/2004	**Route of Administration**	Intravenous
Estimated Due Date of OCPB Review	7/10/04	**Sponsor**	Ilex Products Inc.
PDUFA Due Date	9/29/04	**Priority Classification**	P
Division Due Date	8/29/04		

Clin. Pharm. and Biopharm. Information

	"X" if included at filing	Number of studies submitted	Number of studies reviewed	Critical Comments If any
STUDY TYPE				
Table of Contents present and sufficient to locate reports, tables, data, etc.	X			
Tabular Listing of All Human Studies	X			
HPK Summary	X			
Labeling	X			
Reference Bioanalytical and Analytical Methods	X	14		14 reports for assay of 4 analytical methods: clofarabine in plasma, clofarabine in urine, intracellular clofrabine triphosphase, 2-chloroadenine in plasma
I. Clinical Pharmacology				
Mass balance:				
Isozyme characterization:	X	2		- In vitro metabolism in rat, dog and human hepatocytes (study report) - In vitro metabolism by lymphoblastic cultured cells (publication)
Blood/plasma ratio:				
Plasma protein binding:	X	1		Publication
Pharmacokinetics (e.g., Phase I) -				
Healthy Volunteers-				
single dose:				
multiple dose:				
Patients-				
single dose:				
multiple dose:	X	1		- Phase 1 study of clofarabine in pediatrics with hematologic malignancies (ID99-383)
Dose proportionality -				
fasting / non-fasting single dose:				
fasting / non-fasting multiple dose:				
Drug-drug interaction studies -				
In-vivo effects on primary drug:				
In-vivo effects of primary drug:				
In-vitro:				
Subpopulation studies -				
ethnicity:				
gender:				

~~Pediatrics~~ adults:	X	2		- Phase 1 study of clofarabine in adults with solid and hematological malignancies (DM93-036) - Phase 2 study of clofarabine in adults with refractory or relapsed AML (CLO-221)
geriatrics:				
renal impairment:				
hepatic impairment:				
PD:				
Phase 2:	X	2		- Phase 2 study of clofarabine in adults with refractory or relapsed ALL (CLO-212). - Phase 2 study of clofarabine in adults with refractory or relapsed AML (CLO-222).
Phase 3:				
PK/PD:				
Phase 1 and/or 2, proof of concept:				
Phase 3 clinical trial:				
Population Analyses -				
Data rich:	X	1		- Report of Population PK analysis of pediatric phase2 study data (n=28)
Data sparse:				
II. Biopharmaceutics				
Absolute bioavailability:				
Relative bioavailability -				
solution as reference:				
alternate formulation as reference:				
Bioequivalence studies -				
traditional design; single / multi dose:				
replicate design; single / multi dose:				
Food-drug interaction studies:				
Dissolution:				
(IVIVC):				
Bio-wavier request based on BCS				
BCS class				
III. Other CPB Studies				
Genotype/phenotype studies:				
Chronopharmacokinetics				
Pediatric development plan				
Literature References				
Total Number of Studies		**23 reports**		

Filability and QBR comments

	"X" if yes	*Comments*
Application filable?	**X**	Reasons if the application is not filable (or an attachment if applicable) For example, is clinical formulation the same as the to-be-marketed one?
Comments sent to firm?		Comments have been sent to firm (or attachment included). FDA letter date if applicable.
QBR questions (key issues to be considered)	• PK parameters for clofarabine in pediatric populations.	
Other comments or information not included above	**This is a new drug application for a pediatric indication. Studies in adults are still ongoing.**	
Primary reviewer Signature and Date	Roshni Ramchandani	
Secondary reviewer Signature and Date	Brian Booth	

CC: NDA 21-673, HFD-850 (Electronic Entry), HFD-150 (Cottrell),
HFD-860 (Mehta, Rahman, Booth, Ramchandani), CDR (Biopharm).

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/s/

Roshni Ramchandani
12/22/04 03:33:10 PM
BIOPHARMACEUTICS

Atiqur Rahman
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Signing for Brian Booth

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