Study ITCC 020 & I-BFM Relapsed AML 2009/02
Protocol Version Date: 05-11-2011

Incorporates substantial amendment no 1, 05-11-2011, administrative changes, and specific notification for France

A Phase I/II study of clofarabine in combination with cytarabine and liposomal daunorubicin in children with relapsed/refractory pediatric AML

A collaborative ITCC and I-BFM study.

Sponsor:
Erasmus MC
Dr Molewaterplein 60
3015 GJ Rotterdam
The Netherlands

EUDRA-CT number: 2009-009457-13
Principal Investigators:

CM Zwaan, MD, PhD  
Assoc. Prof. of Pediatric Oncology  
Erasmus MC/Sophia Children’s Hospital  
Dr Molewaterplein 60  
3015 GJ Rotterdam, the Netherlands  
Tel: +31-10-703.6691/6130  
Fax: +31-10-703.1134  
Email: c.m.zwaan@erasmusmc.nl

D Reinhardt, MD, PhD  
Prof. of Pediatric Oncology  
Medizinische Hochschule Hannover  
Carl-Neuberg Strasse 1  
30625 Hannover, Germany  
Tel: +49 511 532 6720  
Fax: +49 511 532 9029  
Email: reinhardt.dirk@mh-hannover.de

Co-Investigators:

Y. Bertrand, MD, PhD  
CHU Lyon  
Institut Hematologie et Oncologie Pediatrique  
1 Place Joseph Renaut  
69008 Lyon  
France  
Tel: +33-469166588  
Fax: +33-469162703  
Email: yves.bertrand@chu-lyon.fr

M. Dworzak, MD, PhD  
CCRI and St Anna Children’s Hospital  
Kinderspitalgasse 6  
A-1090 Vienna  
Austria  
Tel. +43-1-40170 377  
Fax +43-1-40170 752  
E-mail: michael.dworzak@ccri.at

J. Stary, MD, PhD  
Dept Pediatr Hematol/Oncol  
Univ. Hosp. Motol,  
V úvalu 84  
150 06, Praha 5 - Motol
Pharmacokinetic studies:

Simon Joel
Cancer Pharmacology Group
Centre for Experimental Cancer Medicine
Institute of Cancer & Cancer Research UK Clinical Centre Barts and The London School of Medicine and Dentistry
Charterhouse Square
London EC1M 6BQ
Tel: +44-(0)207 882 3821
Fax: +44-(0)207 882 3891
E-mail: S.P.Joel@qmul.ac.uk
APPROVAL OF A STUDY PROTOCOL

Study Title: A Phase II study of clofarabine in combination with cytarabine and liposomal daunorubicin in relapsed/refractory pediatric AML

The following persons declare their consent with the study protocol:

Principal investigators:

Prof. Dr. D Reinhardt  __05-NOV-2011______ __________________
(date, dd/MON/yy)  (signature)

Dr. CM Zwaan, Assoc Prof.  __05-NOV-2011______ __________________
(date, dd/MON/yy)  (signature)

Sponsor: On behalf of Erasmus MC:

Dr. CM Zwaan, Assoc Prof.  ___05-NOV-2011____
__________________
(date, dd/MON/yy)  (signature)

Herewith I confirm that I read the study protocol carefully and declare my consent with it. I will treat and examine the patients in accordance with the study protocol, the national applicable laws, the international guidelines on good clinical practice (ICH-GCP) and the Declaration of Helsinki.

Investigator:

Prof Dr.____________________  ___________________ __________________
(name)    (date, dd/MON/yy)  (signature)

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1 SUMMARY AND STUDY OBJECTIVES

1.1 Summary of Background and Study Rationale

AML is a rare disease, and accounts for approximately 20% of pediatric leukemias. Treatment with intensive chemotherapy results in approximately 60-70% survival in newly diagnosed patients.

Prognosis at relapse is worse, and is in the 30-40% range. A couple of years ago an international relapse study was opened asking a randomized question in relapsed/refractory AML (study relapsed AML 2001/01). This randomization concerned the superiority of the FLAG regimen in combination with DaunoXome® (liposomal daunorubicine) versus FLAG alone, and is still ongoing. Overall results show that poor response to the 1st course of therapy (>20% of blasts in the BM shortly before the 2nd course), was seen in 23% of patients, and more often in early relapses (31%) than in late relapses (15%). Early death occurred in 6% of patients. Complete remission (CR) was achieved in 63% of patients after 2 courses, and these patients have a probability of survival at 3 years of 47%, compared to 33% for the total group, and only 8% for patients not achieving CR. Compared to early relapses, patients with late relapse had higher CR rates (76 vs. 51%), and higher 3-yr probability of survival of 42% vs. 23%. Clearly, nor the induction nor the consolidation chemotherapy is effective enough - and we need new and better treatment options for these children.

Cytarabine (Ara-C) and the anthracyclines are the back-bone of pediatric AML treatment. Cytarabine can be given in various different schedules, including higher-dose regimens such as 1-3 gr/m² IV every 12 hours for 3 consecutive days, but also as a lower dose continuous IV schedule (0.5 gr/m² CI over 24 hours x 4 days). Cytarabine has been used in combination with fludarabine and cladribine, with the aim to induce synergism by increasing Ara-CTP accumulation, which can be seen as a surrogate marker for cytarabine induced cell-kill. In the current European relapsed AML study, the FLAG (fludarabine 30 mg/m²/day x 5 days, Ara-C 2 gr/m² bolus IV in 3 hours, 4 hours following fludarabine, for 5 days; plus GCSF) regimen is used as standard re-induction therapy. Synergy with cytarabine can also be achieved with clofarabine, which is a potent inhibitor of ribonucleotide reductase, leading to a depletion of normal deoxynucleotides and subsequently to increased Ara-CTP levels. In the experimental arm of the European relapsed AML protocol liposomal daunorubicin is added. This drug was chosen for its favorable toxicity profile, in particular the expected lack of long-term cardiac toxicity, which is of serious concern for those who survive relapsed AML given the cumulative anthracycline dose these patients may have received during their treatment. The current dose we use is 60 mg/m² on days 1, 3 and 5 of the FLAG-regimen.

Clofarabine has been tested in phase I/II studies in children with AML. For clofarabine, the DLT was hepatic toxicity and rash, and the MTD for children was defined as 52 mg/m²/day x 5 days per block. Clofarabine is myelo-suppressive, and minimal neurotoxicity has also been reported. The response rate to clofarabine as a single agent in children with AML was relatively low, but this is probably related to the very heavy pre-treatment status of these
children, and patients were transplanted in aplasia before proper response determination could take place. Adult AML data do show that the drug is active in AML, even at dosages from 15-20 mg/m2 onwards. Clofarabine has also been studied in combination with cytarabine in adults.

Several pediatric studies are ongoing in the US, evaluating clofarabine in combination with 1) cyclophosphamide; 2) cyclophosphamide and etoposide and 3) cytarabine 1 gram/m2 x 5 days, either in ALL or AML. The combination studies with cyclophosphamide have caused some toxicity concerns, especially hepatotoxicity.

We aim at developing a combination of clofarabine, cytarabine and DaunoXome®, based on the FLAG regimen, which is a combination that may be taken forward in the new European relapsed AML protocol, or in other studies.

In the adult studies the combination of cytarabine and clofarabine has been safe, using cytarabine 1 gr/m2 for 5 consecutive days on day 1-5, and clofarabine at 40 mg/m2 for 5 days on day 2-6. This is the same schedule as the COG is currently testing in children. No data on combination studies with anthracyclines are available as yet, but there is extensive experience with the FLAG regimen in combination with anthracyclines such as idarubicin or DaunoXome®.

We propose to use the cytarabine dose as administered in the FLAG regimen (2 gram/m2 bolus IV) – which will also allow a fair comparison with the FLAG regimen in later studies. This regimen is currently being tested in a trial in the USA in adults with relapsed/refractory AML by Agura et al., with a dosing regimen of cytarabine 2 gr/m2/day IV over 3 hours with clofarabine 40 mg/m2/day over 2 hours, daily, for 5 consecutive days. Considering GCSF priming, there are only data available in adults, with various results. As we expect that priming will in the future be done with CXCR4 inhibitors, and given the lack of hard evidence that GCSF priming works, we decided against using GCSF priming in this study.

1.2 Objectives

1.2.1 Primary Objective

To establish the recommended dose of clofarabine in combination with cytarabine and liposomal daunorubicin (DaunoXome®) in children with relapsed/refractory AML, based on the FLAG regimen as used in the Relapsed AML 2001/01 study.

1.2.2 Secondary Objectives

- To determine the safety and tolerability of this combination
- To determine (preliminary) efficacy in terms of the hematological remission rate in these patients
- To describe the durability of response, including the number of patients that undergo stem-cell transplant after re-induction with this regimen
- To describe the pharmacokinetics of clofarabine in combination with cytarabine and liposomal daunorubicin
- To preliminary assess the CSF blast disappearance, and the CSF-levels of clofarabine

1.3 Summary of study design

Clofarabine is the investigational medicinal product in this study, and will be made available by the sponsor. Cytarabine and liposomal daunorubicin should be used from commercial stock according to the Summary of Product Characteristics (SPC).

We will use an adapted Faderl regimen to combine cytarabine given at day 1-5 with clofarabine given at day 1-5, as described by Agura et al. In addition, we will add DaunoXome in the same schedule as prescribed in the relapsed AML 2001/01 protocol, on day 1, day 3 and day 5.

The infusion schedule is as follows (see also the time table on the next page):

- Clofarabine infusion will be given over 2 hours IV (rather than one hour, to improve tolerability), daily on day 1-5.
- DaunoXome® will be infused in 60 minutes on day 1, 3 and 5 only, 30 minutes following end of the infusion of clofarabine. We will first combine 40 mg/m² of DaunoXome®, and later dose-escalate to 80 mg/m² (see below).
- Cytarabine will be given at 2 gram/m²/day, and infused in 3 hours IV, daily on day 1-5. Cytarabine infusion will start 3 hours after the end of the clofarabine infusion. There will be no dose-escalation of cytarabine.

Clofarabine and DaunoXome® will be dose-escalated according to the following table. Dose level 5 will be restricted to patients with first early relapse of AML and will only open when dose level 4 has been proven to be safe. The dose to be used will be provided by the Clinical Trial Bureau when enrolling the patient.
NOTE: Patients <1 year of age should be dosed based on mg/kg.

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**Study population**

We will include patients with

- 2nd relapse of AML,
- patients who are refractory after re-induction in first relapse,

and patients with first early relapse (defined as a relapse occurring within one year from initial diagnosis). However, given the outcome data of patients with late relapse of AML in the current relapse European AML 2001/01 study, these patients should be treated with the best arm of the current relapse protocol. Hence, patients with late relapse of AML will not be eligible for this study.

**Safety**

Safety analysis includes frequency, severity, and relatedness of all AEs, frequency and severity of all laboratory abnormalities, frequency of dose interruptions, dose reductions and treatment discontinuation for toxicity, and use of concomitant medications.

All AEs occurring after any administration of the study drug will be followed until resolution. The descriptions and grading scales found in the revised NCI CTCAE version 3.0 will be used for adverse event reporting. A copy of the CTCAE version 3.0 can be downloaded from the CTEP web site ([http://ctep.cancer.gov/reporting/ctc.html](http://ctep.cancer.gov/reporting/ctc.html)).
Dose-limiting toxicities (DLTs) are adverse events (AEs) considered at least possibly drug-related and occurring within the first course of study treatment

Non-hematologic DLT will be defined as:

- Any ≥ grade 3 study treatment related non-hematologic toxicity occurring in spite of appropriate medical management.
- Any non-hematologic laboratory abnormality of Grade 4, or Grade 3 lasting ≥ 7 days, and requiring treatment discontinuation or interruption or dose-reduction in subsequent courses.
- Any clinically-important toxicity of Grade ≥ 2 requiring treatment discontinuation or interruption ≥ 7 days or dose-reduction in subsequent courses.

The following will not be considered DLT: grade 3 nausea and/or vomiting that can be subsequently controlled, including by pre-medication (uncontrollable conditions will be considered DLT), alopecia, drug fever, anorexia, and transient grade 3 transaminase elevations that return to ≤ grade 1 (i.e. > upper limit of normal (ULN) - 2.5 x ULN) within 7 days.

For hematologic toxicity, it is anticipated that the study treatment regimen will result in severe myelosuppression and its associated complications. Therefore, myelosuppression /pancytopenia and grade 3 febrile neutropenia will not be considered DLTs. The exception will be prolonged myelosuppression, which will be considered a DLT, and is defined as grade 3 or 4 myelosuppression, which represents a worsening from baseline, lasting more than 42 days with evidence of a hypocellular marrow (marrow cellularity less than 5% without evidence of recurrent leukemia). DLTs may result in delay of subsequent treatment cycles, or dose adjustments.

Safety will be assessed using a classical 3x3 design. A particular dose level will be expanded to 6 patients if one patient develops a grade 3 or 4 non-hematological toxicity out of 3 patients treated at that particular dose-level. Once this occurs, further dose-escalations needs to be halted until the dose has proven to be safe in the expanded cohort. If 2 patients in a cohort of no more than 6 develop dose-limiting toxicities (DLT) the maximal tolerated dose (MTD) has been reached. No further dose-escalation is allowed, and the next lower dose level will be expanded to 6 patients. If 6 patients have been treated already, we will further expand this dose-level to 10 patients in total at what will be the recommended phase II dose.

The number of patients will depend on the number of dose-escalation steps. A maximum number of 24 patients is expected to test 4 dose-levels with 6 patients at each dose-level. Additional patients will be accrued to expand the cohort treated at the recommended dose to a total number of 10 patients.

Amendment (number 1, 05 November 2011)

- Revision of the eligibility criteria due to observed DLTs (pulmonary fungal infection) in the current study:
All patients will be screened for subclinical fungal infections by HR-CT and a serum Aspergillus test (galactomannan) and excluded if positive.

To consider whether these DLTs can be avoided the revised exclusion criteria will be applied to another 3 patients at dose level 3. Normal dose escalation rules will be applied.

- Revision dose escalation schedule
  An additional dose level (dose level 5) will be added for patients with an early first relapse exclusively. Dose-level 5 can only be opened when dose-level 4 was assessed as safe. Dose-level 5 will then be considered as a separate cohort, given the restricted patient population. We will apply the 3x3 design, as defined in the protocol. When there are no DLTs, we will also expand this cohort to 10 patients, applying the restricted inclusion criteria. These patients may also not have been transplanted in CR1

Including cohort 5 the total number of patients for the entire study will be 39 patients (10 patients in dose level 5)

**Efficacy**

Efficacy is a secondary objective, and although this regimen is expected to induce responses in some patients, the results will be descriptive, and no statistical modeling is done considering response rates.

Results will be described as the percentage of patients achieving overall response, which includes CR, CRi, NEL and PR. For responding patients, survival estimates will be computed using the method of Kaplan and Meier.

**Pharmacokinetics (PK)**

The aim is to determine clofarabine PK on day 1 and 5 of treatment to investigate the effects of daunorubicin and Ara-C on clofarabine PK. Moreover, we will investigate CSF clofarabine levels together with the lumbar puncture on day 6. Resulting plasma concentration data will be modeled with previous data from patients receiving clofarabine alone (our data and Bonate data).

Considering samples: we need 3 samples on days 1 and 5 of treatment, collected at time 0 (pre infusion), time 2 hours (end of clofarabine infusion), time 5 hours (pre Ara-C infusion), as well as a CSF sample and blood sample will be collected on day 6, preferably ~24 hours after the last clofarabine infusion.

**Duration of therapy**

Courses may be repeated in the absence of significant safety concerns or progressive disease.
2 BACKGROUND AND STUDY RATIONALE

2.1 Relapsed/refractory AML

AML is a rare disease, and accounts for approximately 20% of pediatric leukemias. Treatment with intensive chemotherapy, including a stem cell transplant in selected poor-risk patients, results in approximately 60-70% survival in newly diagnosed patients.\(^1\)-\(^4\) Prognosis at relapse is worse, and is in the 30-40% range.\(^5\)-\(^8\) Outcome at relapse is mainly dependent on cytogenetics and on the time to relapse.\(^5\)

In 2001, the International Pediatric AML Working Group of the I-BFM AML Study Group opened a study asking a randomized question in relapsed/refractory AML (study relapsed AML 2001/01).\(^9\) This randomization concerned the superiority of the FLAG regimen in combination with liposomal daunorubicine (DaunoXome\(^®\)) versus FLAG alone, and is still ongoing. Overall results show that poor response to the 1st course of therapy (\(>20\%\) of blasts in the BM shortly before the 2nd course), was seen in 23% of patients, and more often in early relapses (31%) than in late relapses (15%). Early death occurred in 6% of patients. Complete remission (CR) was achieved in 63% of patients after 2 courses, and these patients have a probability of survival at 3 years of 47%, compared to 33% for the total group, and only 8% for patients not achieving CR. Compared to early relapses, patients with late relapse had higher CR rates (76 vs. 51%), and higher 3-yr probability of survival, i.e. 42% vs. 23%. Clearly, nor the induction, nor the consolidation chemotherapy is effective enough - and we need new and better treatment options for these children.

2.2 Overview of Clofarabine

Clofarabine (2-chloro-9-[2’-deoxy-2’-fluoro-β-D-arabinofuranosyl]adenine or Cl-F-ara A; CAFdA) is a rationally designed, second generation purine nucleoside analogue. Clofarabine was designed as a hybrid molecule to overcome the limitations and incorporate the best qualities of both fludarabine (F-ara-A) and cladribine (2-CdA, CdA) both of which are currently approved by various regulatory authorities for treatment of hematologic malignancies. Because clofarabine has a chloro- group at the 2-position of adenine, its chemical structure is more closely related to 2-CdA than to F-ara-A. Halogenation at the 2-position of adenine renders this class of compounds resistant to intracellular degradation by the enzyme adenosine deaminase. Substitution of a fluorine at the C-2’-position of the arabinofuranosyl moiety of clofarabine increases its stability in gastric acid and decreases its susceptibility to phosphorolytic cleavage by the bacterial enzyme Escherichia coli purine nucleoside phosphorylase in the gastrointestinal tract, both of which may lead to enhanced oral bioavailability.\(^10\),\(^11\)

Clofarabine was approved in December 2004 by the United States Food and Drug Administration (US FDA) for the treatment of pediatric patients with relapsed or refractory acute lymphoblastic leukemia (ALL) after at least 2 prior regimens based on the induction of complete responses. In addition, it was approved by EMEA for the same indication in May 2006.
2.2.1 Mechanism of Action

The precise mechanism of action of clofarabine on dividing and non-dividing cells is unknown. Like other nucleoside analogues (cytarabine, cladribine, and fludarabine), clofarabine must be converted within cells to the 5'-triphosphate form by deoxycytidine kinase (dCK) and mono- and di-phosphokinases to be active. Clofarabine is more efficient as a substrate for purified recombinant dCK, exceeding cladribine and the natural substrate, deoxycytidine.\textsuperscript{12} Evidence suggests that the primary cytotoxic effect of clofarabine is due to its inhibition of DNA synthesis and repair. The triphosphate form of clofarabine is an inhibitor of both DNA polymerase $\alpha$ and $\varepsilon$ and ribonucleotide reductase.\textsuperscript{13} These inhibitory effects lead to depletion of intracellular deoxynucleotide triphosphate pools and inhibition of elongation of DNA strands during synthesis and DNA repair.\textsuperscript{14} With respect to inhibition of ribonucleotide reductase, clofarabine and cladribine are superior to fludarabine.\textsuperscript{12} With respect to inhibition of DNA polymerase $\alpha$, clofarabine and fludarabine are similar and both are superior to cladribine.\textsuperscript{12} Thus, in comparison to cladribine and fludarabine, clofarabine more completely inhibits both ribonucleotide reductase and DNA polymerase $\alpha$, versus one or the other.

Unlike fludarabine, clofarabine is active in vitro in non-dividing cells and in cells with a low proliferation rate. Clofarabine can induce the apoptotic pathway as part of its cytotoxic effect on cells.\textsuperscript{11} Clofarabine has been shown to disrupt the integrity of mitochondria in primary chronic lymphocytic leukemia (CLL) cells. The damage leads to release of pro-apoptotic mitochondrial factors.\textsuperscript{15} These effects are postulated to induce apoptosis in indolent, non-dividing CLL cells. This result was not seen with fludarabine and may explain, at least in part, the enhanced cytotoxicity of clofarabine,\textsuperscript{15} although the physiologic and clinical implications of these observations remain uncertain and under continued investigation.

2.2.2 Pharmacokinetics and Pharmacology

Pharmacokinetics of clofarabine in Adult Patients with AML

Pharmacokinetic data were collected from 13 adult patients with refractory or relapsed AML in an open-label study in which they were treated with clofarabine 40 mg/m²/day IV infusion over 1 hour for 5 consecutive days. (Clinical Report – Protocol CLO-221: A Phase II, open-label study of clofarabine in adult patients with refractory or relapsed acute myelogenous leukemia. San Antonio, TX: ILEX™ Products, Inc. Final report dated 13 February 2004). Stationary pharmacokinetics were observed between Days 1 and 5, and plasma concentrations declined rapidly thereafter and exhibited biphasic kinetics. The estimated terminal half-life was approximately 6 hours and ranged from 4.1 to 8.6 hours. Consistent with this short half-life, pre-dose concentrations on Day 2 were about 10% or less of maximal concentrations at the end of infusion. After 4 days of dosing, pre-dose concentrations averaged 13.8 ng/mL and ranged from 4.0 to 23.1 ng/mL. Because of the short half-life of clofarabine, there was little-to-negligible accumulation with once daily dosing of clofarabine at 40 mg/m² by 1-hour IV infusion.
Pharmacokinetics of Clofarabine in Pediatric Patients with ALL

The population pharmacokinetics of clofarabine was 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/m² dose, similar concentrations were obtained over a wide range of body surface areas (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/m² and 172 L/m², 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. (Clofarabine Package Insert, San Antonio, TX: Genzyme Corporation, final dated February 2008). Peripheral blood mononuclear cell (PBMC) clofarabine triphosphate concentrations were assessed in selected pediatric patients with relapsed or refractory ALL or AML in the pivotal phase 2 studies of clofarabine. Triphosphate concentrations were similar in magnitude to adults given the same or similar dose. The half-life of the triphosphate could not be adequately characterized but was estimated to be greater than 24 hours. No correlation between triphosphate concentrations and either dose or parent clofarabine concentration was found.

2.2.3 Toxicology

For detailed information on clofarabine toxicology refer to the Clofarabine Investigator’s Brochure.

2.2.4 Clinical Experience with Clofarabine

Clofarabine (marketed as Clolar® in the USA and as Evoltra® in Europe) is approved by the FDA and by EMEA for the treatment of pediatric patients 1 to 21 years old with relapsed or refractory ALL after at least 2 prior regimens based on the induction of complete responses (Clofarabine Package Insert. San Antonio, TX: Genzyme Corporation. Final dated February 2008). The maximum tolerated dose (MTD) of clofarabine IV for pediatric patients has been determined to be 52 mg/m²/day for 5 consecutive days and for adult patients 40 mg/m²/day for 5 consecutive days.
**Pediatric Leukemia**

A pediatric program was initiated in 2000. In the Phase I study in pediatric patients with hematologic malignancies, 25 patients were treated in cohorts of escalating doses up to 70 mg/m^2, a dose at which 1 patient had grade 4 hyperbilirubinemia and grade 3 elevated transaminases, and 1 had a grade 3 skin rash. The MTD was determined to be 52 mg/m^2.16

Of the 13 patients treated at 52 mg/m^2, grade 2 to grade 3 increases in bilirubin and liver transaminases were observed. A total of 5 patients achieved a CR and 3 achieved a PR for an overall response rate of 32%. Clofarabine plasma concentrations were generally lower in the pediatric population than the adult population when administered the same dose, but there did not appear to be much difference in intracellular clofarabine triphosphate concentrations. Thus, the MTD and the recommended phase 2 dose was determined to be 52 mg/m^2 and the recommended Phase II dose.

The Phase I studies in pediatric patients with hematologic malignancies led to the initiation in 2002 of 2 parallel Phase II trials in patients with either relapsed or refractory ALL or relapsed or refractory AML.17,18 Both studies evaluated clofarabine 52 mg/m^2.

In the 61 patients enrolled in the relapsed or refractory ALL study (61% males: 39% females, 1 to 20 years old), the overall remission rate (CR + CRp) was 20%; 30% (18/61) of patients showed a response (7CR, 5CRp, 6PR). Responses were noted in 15 of 50 (30%) patients with B-lineage ALL, 2 of 6 (33%) with T-cell ALL. Responders received a median of 3 prior induction regimens; 50% (9/18) had prior HSCT and 50% (9/18) were refractory to the preceding induction regimen. Response rate in refractory patients was 26% (9/35). After clofarabine treatment, 10 patients proceeded to transplant (including 8 responders). Six of 10 patients who received a transplant were alive at last follow up (survival range: 30.1+ - 145.1+ wks). Response duration in 6 patients with CR or CRp who did not receive a transplant ranged from 4.3 to 58.6 weeks; 2 patients maintained CR for 47.9 and 58.6 weeks after clofarabine therapy. Median overall survival for the patients who achieved at least a PR was 66.6 weeks compared to 12.9 weeks for all patients.

In 42 pediatric patients with relapsed or refractory AML, the response rate was 26% (1 CRp, 10 PR). Responders had received a median of 2 prior induction regimens, 36% (4/11) had prior HSCT and 55% (6/11) patients were refractory to the preceding induction regimen. Response rate in refractory patients was 21% (6/28). One patient who had received 5 prior induction regimens achieved CRp. 13 (31%) patients (1CRp, 6PR, 3NE, 3TF) underwent HSCT after completing clofarabine therapy, 5 of whom were alive at last follow-up (survival range: 62.7+ - 160.1+ wks). Many patients proceeded to HSCT as soon as a donor was identified without waiting for the patient to go into remission, making remission difficult to assess. Median overall survival for patients who achieved at least a PR was 32.1 weeks compared to 23.4 weeks for all patients.

Among the 113 pediatric patients with ALL and AML, the most frequently reported drug-related AEs were vomiting (66% ALL and 65% AML) and nausea (58% ALL and 70% AML) (Integrated Summary of Safety submitted to NDA 21-673 as A6 on 02 August 2004). Other drug-related AEs reported by at least 10% of the pediatric patients overall included febrile neutropenia (31% ALL and 28% AML), pyrexia (21% ALL and 26% AML),
pruritus NOS (24% ALL and 20% AML), dermatitis NOS (24% ALL and 17% AML), headache NOS (18% ALL and 35% AML), diarrhea NOS (21% ALL and 22% AML), anxiety NEC (16% ALL and 7% AML), fatigue (15% ALL and 13% AML), mucosal inflammation NOS (16% ALL and 15% AML), and flushing (12% ALL and 11% AML). Anorexia occurred in 12% ALL and 9% AML and palmar-plantar erythrodysesthesia syndrome in 12% ALL and 9% AML.

Currently, Genzyme is conducting a Phase I/II study to assess concomitant use of clofarabine, etoposide and cyclophosphamide in pediatric patients with acute leukemias. The study, “A Study of Clofarabine in Combination with Etoposide and Cyclophosphamide in Children with Acute Leukemias,” (CLO-218) aims to assess the safety and efficacy of clofarabine in combination with etoposide and cyclophosphamide in pediatric patients with relapsed or refractory acute lymphoblastic or myelogenous leukemia (ALL or AML). Refractory or relapsed acute leukemia patients between the ages of 1 and 21 years with adequate liver, renal, pancreatic and cardiac function were eligible for enrollment.

Summarized below are the preliminary findings of the Phase I/II study that were presented at the 2007 American Society of Hematology Annual Meeting and Exposition.19

**Phase I combination chemotherapy with clofarabine in pediatric leukemia**

The objectives of the Phase I portion of the combination chemotherapy study were to determine the maximum tolerated dose (MTD), the dose-limiting toxicities (DLTs), and the recommended Phase II dose of clofarabine when used in combination with etoposide and cyclophosphamide.

This part of the study enrolled 25 patients (20 ALL; 5 AML) with a mean age of 9.1 years (range: 2-21 years) and a mean of 2 prior induction regimens. Patients entered 1 of the 5 dosing cohorts and received clofarabine, etoposide and cyclophosphamide via intravenous (IV) infusion daily for 5 days (induction regimen) as outlined in Table 2-1. Patients could receive up to 2 induction cycles, followed by a 4-day consolidation cycle (up to a maximum of 8 cycles, including induction). A prior hematopoietic stem cell transplant (HSCT) was noted in 4 patients (1 ALL; 3 AML).

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Daily Dose mg/m²</th>
<th>Etoposide</th>
<th>Cyclophosphamide</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>75</td>
<td>340</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>75</td>
<td>440</td>
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<tr>
<td>3</td>
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<td>440</td>
</tr>
<tr>
<td>4</td>
<td>30</td>
<td>100</td>
<td>440</td>
</tr>
<tr>
<td>5</td>
<td>40</td>
<td>100</td>
<td>440</td>
</tr>
</tbody>
</table>

Complete remission (CR) was achieved in 10 patients (9 ALL; 1 AML) while an additional 6 patients (2 ALL; 4 AML) achieved a complete remission in the absence of total platelet recovery (CRp) for an overall response rate (ORR) of 64% (ALL: 55%; AML: 100%). After treatment, 4 patients proceeded to HSCT.
Febrile neutropenia, pyrexia and neutropenia were the most commonly reported serious adverse events and were reported in 64%, 20%, and 16% of patients, respectively. There were 10 patient deaths in the trial (malignant disease, n=5; adverse events, n=2: infection/micrococcus meningitis, CNS hemorrhage; and other causes, n=3: intracranial hemorrhage, multi-organ failure, unknown etiology). Dose-limiting toxicities included a grade 3 elevation of lipase, abdominal pain and possible veno-occlusive disease (VOD) that resolved (cohort 3) and 1 case of prolonged bone marrow aplasia (cohort 5).

The MTD, a Phase I objective, was not reached. Upon review of the data, an independent data monitoring committee recommended the following Phase II daily dosing regimen: clofarabine 40 mg/m²; etoposide 100 mg/m²; and cyclophosphamide 440 mg/m².

**Phase II combination chemotherapy with clofarabine in pediatric leukemia**

The objective of the Phase II portion of the study is to estimate the overall remission rate (CR + CRp) of clofarabine when used in combination with cyclophosphamide and etoposide. While the Phase II portion of the study has not yet been completed, 4 of the 8 patients enrolled reported serious adverse events involving signs and symptoms of hepatotoxicity causing patient enrollment to have been temporarily suspended.

Three of the 4 patients reported veno-occlusive disease (VOD) symptoms including hyperbilirubinemia, hepatomegaly, right upper quadrant pain, ascites and/or weight gain. VOD-like signs and symptoms initially presented within 5 to 12 days of the first dose of clofarabine in these patients. Two of the patients with VOD symptomatology had a history of HSCT and total body irradiation (TBI) within the previous 4 to 7 months. All 3 patients with VOD-like symptoms had ongoing severe infections and/or capillary leak syndrome preceding the occurrence of hepatotoxicity. The fourth patient reported grade 4 hyperbilirubinemia and had a history of HSCT and TBI greater than 1 year prior to study entry.

As a result of these safety findings, the updated protocol now states that patients with a history of prior HSCT, elevated conjugated serum bilirubin at study entry, uncontrolled systemic fungal, bacterial, or other infection, a history of hepatitis B or C infection or a history of cirrhosis are to be excluded from study participation. The warnings and precautions section of the clofarabine package insert has also been revised to reflect these recent safety findings (Clofarabine Package Insert. San Antonio, TX: Genzyme Corporation. Final dated February 2008).

**Salvage Single Agent Therapy in Adult AML**

Phase I trials were initiated in 1999 and the first study was a traditional dose-escalation study where the objective was to establish the MTD in adult patients with solid tumors or hematologic malignancies. The starting dose was 15 mg/m² IV administered daily for 5 days based on an animal study in which this dose was safe with no observable toxicities. However, 2 of the first few patients on study experienced myelosuppression and required dose de-escalations before the MTD of 2 mg/m² was identified in patients with solid tumors. Dose escalation in patients with hematologic malignancies increased to 55 mg/m², at which point patients experienced dose-limiting toxicities (DLTs) of reversible hepatotoxicity, and the MTD was determined to be 40 mg/m²/day. Among the 32 patients diagnosed with acute
leukemia, 2 patients achieved a CR and 3 achieved CRp for an overall response rate of 16%. Clofarabine pharmacokinetics were dose proportional across all the doses studied, but intracellular clofarabine triphosphate (which had large interpatient variability) began to show saturation at doses greater than about 20 mg/m²/day.

In a Phase II study reported by Kantarjian et al., 62 adult patients with relapsed or refractory acute leukemia received clofarabine 40 mg/m² IV once daily for 5 days every 3 to 6 weeks. Twenty (20) patients achieved a CR, 9 achieved a CRp, and 1 achieved a partial response for a total response rate of 48%. The predominant toxicities were reversible liver dysfunction (as indicated by elevated aminotransferases [alanine aminotransferase (ALT) and aspartate aminotransferase (AST)] and hyperbilirubinemia), skin rashes, palmar-plantar erythrodysesthesia, and mucositis. No correlation was observed between plasma clofarabine concentrations and intracellular clofarabine triphosphate concentrations, although it was observed that responders showed an accumulation of intracellular clofarabine triphosphate on Day 2 compared to nonresponders.

Another multi-center Phase II trial (CLO221) was initiated in 2002, in adult patients with relapsed or refractory acute myelogenous leukemia who received clofarabine 40 mg/m² once daily for 5 days every 28 days for 2 cycles, with subsequent cycles being dosed with 30 mg/m². Only 1/40 (3%) patients achieved a CR of 20.4 weeks duration. Nausea, vomiting, headache, diarrhea, anorexia, dermatitis, and stomatitis were the most frequently drug-related reported AEs. Drug-related renal toxicities were reported for 10% of the patients; however, these patients either had a concurrent clinical condition associated with renal toxicity or at least 1 concomitant medication known to increase the potential for renal toxicity. Pharmacokinetic data available for 33% of the patients indicate clofarabine had a high tissue distribution with minimal accumulation and rapid elimination (primarily as unchanged drug) in the urine. These seemingly discrepant results must be considered in the context of the patient populations treated. In the CLO-221 experience approximately 25% of patients were refractory to prior therapy, whereas no subgroup of patients with primary refractory disease was reported in the prior experience reported by Kantarjian and colleagues. In addition, the CLO 221 population included 14/29 patients with a duration of first remission <6 months and 8/29 patients with a duration of first remission between 6 – 12 months.

Previously Untreated Single Agent Therapy in Adult AML

In a Phase 2 study (UWCM-001) conducted by Burnett et al. clofarabine was evaluated as first line treatment in 30 older patients (median age 72, range 61-82 years) with AML who were considered unfit for intensive chemotherapy. Patients received clofarabine 30 mg/m² for 5 days, which could be repeated for up to 4 cycles at a minimum of 28 days between cycles. Overall response rate was 56% (43% CR, 13% CRp). Toxicities greater than grade 3 included increases in SGPT and bilirubin, hand foot syndrome, skin rash, and nausea and vomiting. There were 4 early deaths.

Burnett at al. also evaluated clofarabine in 66 previously untreated AML patients, aged >65 years who were considered unsuitable for standard intensive chemotherapy based on age, comorbidity, or performance status. This open-label, multi-center, non-randomized, Phase 2
study is known as BIOV-121. Patients received clofarabine at 30 mg/m²/day for 5 consecutive days repeated every 28-42 days, with a trial amendment to reduce to 20 mg/m² for subsequent cycles. Overall response rate (ORR) was 44% (21% CR, 23% CRi) for the whole population; 47% ORR in patients with high risk or intermediate cytogenetics, 50% ORR in de novo AML, and 31% ORR in secondary AML. Overall survival (OS) at 21 months was 25%, with 32% OS for patients with CR or PR. Median time to neutrophil recovery \((1.0 \times 10^9/L)\) was 24 days (16-30 days), and median platelet recovery was 38 days (25-46 days). The most common drug related serious adverse events were neutropenic sepsis (19.7%), renal failure (10.6%), renal insufficiency (7.6%), sepsis (6.1%), febrile neutropenia, atrial fibrillation, diarrhea, vomiting and rash (all 3%).

Thirty-nine percent of patients (26 out of 66) experienced renal adverse events of any grade. Fifty-six percent of patients with reduced estimated GFR (<60 ml/min/1.73 m² at study entry) reported renal adverse events, and only 29% of normal renal function patients reported a renal adverse event. Most patients who developed renal impairment or acute renal failure had co morbid conditions, sepsis and/or used nephrotoxic drugs during the study. Fourteen patients (21%) died within 30 days of clofarabine administration with 6 of these 14 deaths thought to be possibly related to clofarabine therapy.²⁴

Burnett et al.²⁵ also presented information on a subset of untreated adult AML patients derived from 2 separate studies (BIOV-121 and UWCM-001). This group consisted of 26 elderly AML patients with confirmed adverse cytogenetics who received 30 mg/m² of clofarabine daily for 5 days with a median follow-up of 10 months (range 5-17 months). This subset was compared to similar groups of elderly patients derived from other studies who received either non-intensive therapy (hydroxycarbamide or low dose 20 mg cytarabine, schedule not provided) or a daunorubicin/cytarabine-based intensive chemotherapy. Twelve out of 26 clofarabine patients (46%) achieved a CR or CRi which was comparable to the 42% remission rate observed after intensive chemotherapy. No complete responses were observed in patients in either of the low intensity treatment groups (cytarabine or hydroxycarbamide). The 1-year survival rate for clofarabine-treated patients was 21%, which was significantly better than the survival rates noted in the low intensity groups and comparable to the 23% survival rate noted in the intensive chemotherapy group.

In a Phase 2, single-arm, open-label study conducted by Erba et al.²⁶, the safety and efficacy of single agent clofarabine was evaluated in previously untreated older adult patients with AML who were unlikely to benefit from standard induction chemotherapy. The primary endpoint of the study was overall remission rate (ORR = CR + CRp). The study, known as CLASSIC II (CLO-243), enrolled 116 patients who were 60 years of age or older (median age 71 years, range 60-88) with Eastern Cooperative Oncology Group [ECOG] performance status [PS] of 0-2 and with at least one adverse prognostic factor (70 years of age or older, antecedent hematologic disorder [AHD], ECOG PS of 2, or intermediate or unfavorable karyotype). Patients received 30 mg/m²/day of clofarabine as a 1-hour intravenous (IV) infusion on days 1-5 for induction and 20 mg/m²/day on days 1-5 for re-induction or consolidation therapy (maximum of 6 total cycles). Subsequent cycles were begun after a minimum of 28 days from the start of the previous cycle.
An preliminary analysis of efficacy and safety data (investigator assessment as of 11 April 2008) for the 115 patients evaluated was presented at the 2008 ASCO Annual Meeting. The ORR was 45% (40% CR, 5% CRp), ORR by adverse prognostic factor is summarized in Table 2-2. Thirty-five patients achieved remission after cycle 1 and 17 patients achieved remission after cycle 2. Treatment failure occurred in 53% of patients (n=61).

Table 2-2: Overall Response Rate by Adverse Prognostic Factor

<table>
<thead>
<tr>
<th>Prognostic Factor</th>
<th>Patients in Subset</th>
<th>ORR (CR+CRp), n, (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age ≥70 years</td>
<td>70</td>
<td>28 (40%)</td>
</tr>
<tr>
<td>ECOG PS 2</td>
<td>26</td>
<td>10 (38%)</td>
</tr>
<tr>
<td>AHD</td>
<td>42</td>
<td>21 (50%)</td>
</tr>
<tr>
<td>Unfavorable karyotype</td>
<td>56</td>
<td>24 (43%)</td>
</tr>
<tr>
<td>Intermediate karyotype</td>
<td>46</td>
<td>24 (52%)</td>
</tr>
<tr>
<td>Number of Risk Factors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>24 (48%)</td>
</tr>
<tr>
<td>3</td>
<td>38</td>
<td>16 (42%)</td>
</tr>
</tbody>
</table>

The median time to hematopoietic recovery in responders for cycle 1 for neutrophils [ANC] (≥1 × 10⁹/L) and platelets (≥100 × 10⁹/L) were 31 and 28 days respectively.

Additionally the authors reported that patients received a median of 2 cycles of clofarabine (range 1-6). Of the sixty-nine patients whom were initiated a second cycle, 58% (n=40) were given clofarabine as re-induction and the remaining 29 patients as consolidation. The median duration between the first 2 cycles was 41 days.

The 30-day all-cause mortality was 9.6% (n=11). Three deaths were considered drug-related by the investigator (1 respiratory failure secondary to pneumonia, 1 gram negative sepsis and 1 acute respiratory distress). Clofarabine related adverse events, as determined by the investigator, were reported in a majority of patients (94%). Infection and febrile neutropenia were noted in 58% and 49% of patients respectively regardless of causality to clofarabine. The most common adverse events reported in ≥20% of patients were nausea (45%), febrile neutropenia (34%) vomiting (31%), diarrhea (26%) and rash (22%). The incidence of Grade 4 neutropenia and thrombocytopenia based on lab shift tables was 81% and 89%, respectively.²⁶

Salvage Combination Therapy in Adult AML

A Phase 1-2 study evaluated clofarabine plus intermediate dose cytarabine in 32 adult patients aged 18 years to 84 years (median 59 years) with relapsed acute leukemia (25 AML, 2 ALL), high-risk myelodysplastic syndromes (MDS; n=4), and blast phase chronic myelogenous leukemia (CML; n=1).²⁷ Clofarabine 40 mg/m²/day was given as a 1 hour intravenous (IV) infusion for 5 days on days 2-6 followed 4 hours later by cytarabine 1 g/m²/day as a 2 hour IV infusion for 5 days on days 1-5. Doses were reduced by 25% in subsequent cycles for grade 2 extramedullary toxicities and by 50% for grade 3 or higher extramedullary toxicities including life-threatening infections. Seven AML patients (28%)

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Footnotes:
²⁶: The incidence of Grade 4 neutropenia and thrombocytopenia based on lab shift tables was 81% and 89%, respectively.
²⁷: Clofarabine 40 mg/m²/day was given as a 1 hour intravenous (IV) infusion for 5 days on days 2-6 followed 4 hours later by cytarabine 1 g/m²/day as a 2 hour IV infusion for 5 days on days 1-5. Doses were reduced by 25% in subsequent cycles for grade 2 extramedullary toxicities and by 50% for grade 3 or higher extramedullary toxicities including life-threatening infections. Seven AML patients (28%)

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achieved a CR and 3 AML patients (12%) achieved a CRp, for an overall response rate of 40%. The response rate in 23 AML and high-risk MDS patients, who were either primary refractory or had a first remission duration <1 year, was 35% (22% CR and 13% CRp). Adverse events included myelosuppression, infection, transient liver test abnormalities, nausea/vomiting, diarrhea, skin rashes, mucositis and palmpoplantar erythrodysesthesias. Median time to platelet and neutrophil recovery in complete responders was 42 days and 33 days, respectively.

Faderl et al. conducted a dose-finding Phase 1 study in 44 adult patients with relapsed or refractory AML and high-grade MDS that compared 2 study arms using clofarabine in combination with idarubicin (CI group, n=23) and clofarabine/idarubicin plus cytarabine (CIA group, n=21). The CI group (median age 58 years; range 24-71 years), with 9 primary refractory and 15 abnormal cytogenetics patients completed five different dose levels. At the first dosing level, clofarabine 22.5 mg/m² for 5 days and idarubicin at 12 mg/m² for 3 days (n=6), 3 patients experienced grade 3 or higher toxicities (diarrhea, rash, and mucositis) necessitating a dose de-escalation. The subsequent dose levels were: clofarabine 15 mg/m² for 5 days plus idarubicin 8 mg/m² for 3 days (6 patients), clofarabine 18 mg/m² for 5 days plus idarubicin 10 mg/m² for 3 days (3 patients), clofarabine 22.5 mg/m² for 5 days plus idarubicin 10 mg/m² for 3 days (3 patients), and clofarabine 30 mg/m² for 5 days plus idarubicin 10 mg/m² for 3 days (5 patients). Dose limiting toxicities (DLT) at this level were increased bilirubin, increased SGPT and headaches. The maximum tolerated dose (MTD) was determined to be clofarabine 22.5 mg/m² and idarubicin 10 mg/m². Five patients responded in the CI treatment arm (22%) resulting in 3 CRs and 2 CRps. The CIA arm (median age 56 years; range 23-78 years) contained 8 primary refractory and 12 abnormal cytogenetics patients and evaluated 4 dose levels. In the first dosing level, clofarabine 22.5 mg/m² for 5 days plus idarubicin 8 mg/m² for 3 days plus cytarabine 1 g/m² for 5 days (3 patients), all 3 patients experienced grade 3 or higher toxicities (diarrhea, acute renal failure, and increased bilirubin) necessitating dose de-escalation. The subsequent doses included clofarabine 15 mg/m² for 5 days plus idarubicin 6 mg/m² for 3 days plus cytarabine 0.75 g/m² for 5 days (6 patients), clofarabine 22.5 mg/m² for 5 days plus idarubicin 6 mg/m² for 3 days plus cytarabine 0.75 g/m² for 5 days (6 patients), and clofarabine 30 mg/m² for 5 days plus idarubicin 6 mg/m² for 3 days plus cytarabine 0.75 g/m² for 5 days (6 patients). DLTs seen at the highest dose level were increased bilirubin, diarrhea, mucositis, and decreased left ventricular ejection fraction. The MTD was determined to be clofarabine 22.5 mg/m² plus idarubicin 6 mg/m² plus cytarabine 0.75 g/m². The CR rate in the CIA arm was 48%.

In another Phase 1 dose finding study, Karp et al. evaluated escalating doses of clofarabine followed by cyclophosphamide (CY) in 18 adults (median age 51 years) with refractory leukemia (12 AML and 6 ALL). One group received 10 mg/m²/day of clofarabine (n=12; 9 AML, 3 ALL) while the other was treated with 20 mg/m²/day of clofarabine (n=6; 3 AML, 3 ALL), and both clofarabine doses were followed with 400 mg/m²/day of CY on days 1-3 and days 8-10. This resulted in total dosages of either 60 mg/m² or 120 mg/m² of clofarabine and 2400 mg/m² of CY. In order to assess the effect of clofarabine on CY-induced cellular toxicity, the first CY dose was divided between days 0 and 1, and peripheral blood blasts were obtained prior to and after infusion of CY on days 0 and 1. In addition, bone marrows
were obtained at day 14. Both apoptosis and DNA damage were assessed on the peripheral blood blast and bone marrow samples. The use of clofarabine prior to CY appeared to augment both CY-induced DNA damage (by phosphorylated H2AX) and apoptosis (by sub-2N DNA). Two of the AML patients achieved a CR (10 mg/m² group) and 1 additional AML patient achieved a PR (20 mg/m² group). Four patients (22%; 2 from each dosing cohort) expired due to multi-organ failure (n=2), fungal pneumonia (n=1), and prolonged aplasia (n=1). The dose limiting toxicity in the 20 mg/m² group was prolonged marrow aplasia (>60 days). Since the increases in CY-induced cellular toxicity did not appear to be closely related to clofarabine dose, the authors intend to restudy this drug combination using lower total dosages of clofarabine and similar or slightly higher total dosages of CY.

Previously Untreated Combination Therapy in Adult AML

Faderl et al.\textsuperscript{30} conducted a Phase 2 study to evaluate the use of clofarabine plus cytarabine in 60 patients aged 50 years or older with newly diagnosed, untreated AML. The study was conducted in 2 study groups, the first group were 30 patients with diploid cytogenetics and the second group consisted of 30 patients with abnormal cytogenetics, [excluding inv(16) and translocation t(8;21) or t(15;17)]. Clofarabine 40 mg/m²/day was given as a 1 hour IV infusion for 5 days on days 2-6 followed 4 hours later by cytarabine 1 g/m²/day as a 2 hour IV infusion for 5 days on days 1-5. Cycles were repeated every 4-6 weeks depending on response. Patients were allowed to receive a maximum of 3 induction cycles with the second and third induction course administering both clofarabine and cytarabine on days 1-5. Responding patients could receive up to 6 additional consolidation courses of clofarabine 40 mg/m²/day and cytarabine 1 g/m²/day for 3 days (both days 1-3).

Thirty-one patients overall (52%) achieved CR and 5 (8%) achieved CRp, for an overall response rate (ORR) of 60%. Eighteen of the 30 patients (60%) with diploid cytogenetics achieved CR and 2 patients (7%) achieved CRp for an ORR of 67%. In patients with abnormal karyotypes, 13 out of 30 patients (43%) achieved CR, and 3 out of 30 patients (10%) achieved CRp resulting in an ORR of 53%. Median time to CR was 29 days (range 20-98 days) for all patients. Median time to CRp was 34 days (range 24-75 days) for all patients. Median follow up was 18.2 months (range 10.2-26.5 months), and median remission duration for all patients achieving a CR was 8.1 months (range 0.5-25.4+ months). Median overall survival of all patients achieving CR was 23.5 months (range 1.2-26.3+ months). The most common side effects were diarrhea, nausea, vomiting, headaches, skin rashes (including palmar-plantar erythrodysesthesia), facial flushing, and liver abnormalities including hyperbilirubinemia and elevations of ALT and/or AST. The most common grade 3 or higher adverse events were rash (including one patient with Stevens-Johnson syndrome), hyperbilirubinemia, and ALT/AST elevations. There were a total of 9 (16%) deaths: 4 (7%) patients died during the first induction course; 4 (7%) died in the second induction course; and 1 patient died on day 12 of the first consolidation course. Most of the deaths were attributed to sepsis-related complications.

A Phase I/II study combining clofarabine (30 mg/m²/day for 5 days) and cytarabine (100 mg/m²/day administered as a continuous infusion for 7 days) was conducted by Foran et al.\textsuperscript{31} in 4 patients (age range 61-77) with newly diagnosed AML. Two patients achieved a CR (50%), and 2 patients died of infectious complications. Due to the 2 deaths,
the protocol has been amended to decrease the dose of clofarabine by 25% (22.5 mg/m²/day for 5 days).

Faderl et al. studied the efficacy of clofarabine plus low-dose, subcutaneous cytarabine (LDAC) vs clofarabine in patients ≥60 years (median age 71, range 60-83) with untreated AML and high-risk MDS. Patients were randomized to an induction cycle (maximum of 2 cycles) of either 30 mg/m² of clofarabine for 5 days or 30 mg/m² of clofarabine for 5 days with 20 mg/m² of LDAC for 14 days. Consolidation courses (maximum of 12) could be given every 3 weeks to 6 weeks with the same daily dosages given for fewer days (clofarabine for 3 days and LDAC for 7 days). Most patients (54%) had an antecedent hematologic disorder (MDS, chronic myelomonocytic leukemia, non-Hodgkin’s lymphoma) or other malignancy, and 49% of patients had abnormal cytogenetics. Of the 67 evaluable patients, 43 patients achieved a CR and 2 a CRp for an overall response rate of 60%. The difference between the response rates between the 2 treatment arms was statistically significant (P≤0.05) in favor of the clofarabine with LDAC group. Most adverse events were less than grade 2. The following adverse events were grade 3 or higher: hyperbilirubinemia (26%), elevated SGPT (15%), skin rash (13%), elevated SGOT (9%), acute renal failure (8%), diarrhea (5%), elevated creatinine (5%), atrial fibrillation (4%), and elevated alkaline phosphatase (3%). Fifteen patients (20%) died during induction. Myelosuppression was universal and myelosuppression-related infectious complications were common.

A Phase 1, non-randomized, dose escalation study involving 37 elderly AML patients (median age 67 years ) in 5 different combination dosing cohorts was conducted by Burnett et al. All patients received daunorubicin 50 mg/m² on days 1, 3, and 5 and either 15, 20, 25, or 30 mg/m² of clofarabine on days 1-5. Dose limiting toxicities at clofarabine 30 mg/m² plus daunorubicin 50 mg/m² were oral toxicity, renal toxicity, diarrhea, cardiac toxicity, and increased bilirubin. Once the MTD for the two dose combination was determined, gemtuzumab was added, and the MTD for the three drug combination was determined to be clofarabine 20 mg/m² days 1-5 plus daunorubicin 50 mg/m² days 1,3, and 5 plus gemtuzumab 3 mg/m² day 1. The overall response rate for all dose levels combined was 65% (CR or CRi), with 3 out of 5 CRs at the MTD for the 3 drug combination.

Previously Untreated and Salvage Combination Therapy in Adult AML

Agura et al. conducted a single center, Phase 2 study of combination therapy with clofarabine and intermediate dose cytarabine in 30 treatment-naïve elderly patients with heart disease or relapsed adult AML patients at high risk of anthracycline toxicity. Eligible patients received 5 consecutive days of clofarabine 40 mg/m² over 1 hour followed by 1000 mg/m² of cytarabine four hours later. Patients also received IV fluids at 150 mg/m²/h, bumetanide 2-4 mg per day as needed to maintain initial body weight (≤1 kg of the starting weight), and dexamethasone 10 mg intravenous push daily as part of their chemotherapy regimen. Retreatment with clofarabine was allowed up to a total of 4 treatment cycles.

Thirty patients enrolled with a mean age of 64 years (range 38-82 years). At baseline, 13 patients (43%) had a history of cardiovascular disease (previous myocardial infarction, bypass grafting and/or cardiomyopathy). Twenty-nine of the 30 patients received at least
1 cycle (1 patient died within 24 hours of therapy initiation due to disease progression); 5 patients received 2 clofarabine cycles.\textsuperscript{34}

A morphological response was seen in 68\% of evaluable patients (17/25; 5 patients were deemed inevaluable due to early death), including CR in 14 patients (56\%) and PR in 3 patients (12\%). Twenty-seven patients were found to have intermediate or unfavorable cytogenetics, and only 1 patient possessed favorable cytogenetics at baseline. Within the intermediate or unfavorable population, 13 patients achieved a complete cytogenetic remission. Adverse events reported in more than 10\% of patients included: grade 3 or 4 neutropenia (100\%), grade 3 or less edema (70\%), diarrhea (60\%), grade 3 or less rash (57\%), nausea (40\%), elevated transaminases (33\%), mucositis (20\%), headache (20\%), atrial fibrillation/flutter (17\%), and elevated bilirubin (10\%). There were no case reports of treatment-related cardiac toxicity.\textsuperscript{34}

\section*{Consolidation Therapy in Adult AML}

Lobe et al.\textsuperscript{35} conducted a pilot study to assess the feasibility and safety of an intensive consolidation regimen consisting of clofarabine and cytarabine (CLARA) compared to high dose cytarabine (HDAC) in adult AML patients ages 65 years or less who had achieved their first complete remission (CR1) after induction therapy. Patients in the CLARA group received cytarabine (Ara-C) 1000 mg/m\textsuperscript{2}/day as a 2-hour infusion on days 1-5 and clofarabine 40 mg/m\textsuperscript{2}/day as a 1-hour infusion on days 2-6 given 4 hours before the cytarabine as opposed to the patients in the HDAC group who received cytarabine 3000 mg/m\textsuperscript{2}/12h as a 2-hour infusion on days 1, 3, and 5. As outlined in the entry criteria, the CLARA regimen was only offered to patients with relatively high-risk AML.

Thirty-six AML-CR1 patients between the ages of 17-63 years (median 39 years) received a combined total of 100 cycles of consolidation therapy (CLARA: n=5 patients, 15 cycles; HDAC: n=31 patients, 85 cycles). Although myelosuppression appeared to be more rapid and intense in the CLARA group compared to the HDAC group, the length of myelosuppression was similar. Median time to polymorphonuclear neutrophils (PMN) levels <0.5 × 10\textsuperscript{9}/L was 8 days for CLARA-treated patients vs. 11.5 days for HDAC-treated patients (p<0.001), while PMN recovery to >0.5 × 10\textsuperscript{9}/L was 25 days in both groups (p=0.33). All patients treated with the CLARA regimen required platelet transfusions compared to 35\% of patients treated with HDAC (p<0.001). Overall blood count recovery before day 30 was achieved by 100\% of the CLARA-treated patients as compared to 83\% of the HDAC-treated patients (p=0.08). Grade 3 and 4 infections were reported in 60\% (n=9) of the CLARA treatment cycles and in 19\% (n=16) of the HDAC treatment cycles (p=0.002). Septicemia and mucositis were also more frequently reported during or after CLARA treatment than HDAC treatment (47\% vs. 9\% and 73\% vs. 5\%, respectively). No treatment-related death occurred in either treatment arm. The anti-leukemic efficacy of treatment was not evaluated in this pilot study.

\subsection*{2.3 Study and Dose Rationale}

Cytarabine (Ara-C) and the anthracyclines are the most effective drugs in pediatric AML treatment.\textsuperscript{2,36} Cytarabine can be administered in many different schedules, including higher-dose regimens such as 1-3 gr/m\textsuperscript{2} IV every 12 hours for 3 consecutive days, but also as a
lower dose continuous IV schedule (0.5 gr/m² CI over 24 hours x 4 days), or a lower dose bolus infusion schedule (100 mg/m² IV, twice daily, for 10 consecutive days). Cytarabine has been used in combination with fludarabine and cladribine, with the aim to induce synergism by increasing Ara-CTP accumulation, which can be seen as a surrogate marker for cytarabine induced cell-kill. In the current European relapsed AML study, the FLAG (fludarabine 30 mg/m²/day IV x 5 days; Ara-C 2 gr/m² bolus IV in 3 hours, 4 hours following fludarabine, for 5 days; plus GCSF) regimen is used as standard re-induction therapy. Synergy with cytarabine can also be achieved with clofarabine, which is a potent inhibitor of ribonucleotide reductase, leading to a depletion of normal deoxynucleotides and subsequently to increased Ara-CTP levels.37

In the experimental arm of the European relapsed AML protocol liposomal daunorubicin was added.7 This drug was chosen for its favorable toxicity profile, in particular the expected lack of long-term cardiac toxicity, which is of serious concern for those who survive relapsed AML, given the cumulative anthracycline dose these patients may have received during their treatment.38 The current dose we use is 60 mg/m² on days 1, 3 and 5 of the FLAG-regimen. This combination appeared safe in terms of the overall safety, and there was no evidence of severe acute cardiac toxicity in the AML 2001/01 study. However, there are no data as yet on long-term cardiac follow-up after using liposomal daunorubicin. In newly diagnosed patients (in the AML-BFM 2004 study) the current dose of liposomal daunorubicin is 80 mg/m² on days 1, 3 and 5 of a combination regimen (Creutzig et al, ASH 2010).

Clofarabine has been tested in phase I/II studies in children with AML.16,39-41 For clofarabine, the DLT was hepatic toxicity and rash, and the MTD for children was defined as 52 mg/m²/day IV x 5 days. Clofarabine is myelo-suppressive, and minimal neurotoxicity has also been reported. The response rate to clofarabine as a single agent in children with AML was relatively low,16 but this is probably related to the very heavy pre-treatment status of these children. Moreover, several patients participating in these studies were transplanted in aplasia before proper response determination could take place. Adult AML data do show that the drug is active in AML, even at dosages from 15-20 mg/m² onwards.21,23 Clofarabine has also been studied in combination with cytarabine in adults.30,37

Several pediatric studies are ongoing in the US, evaluating clofarabine in combination with cyclophosphamid e, cyclophosphamide and etoposide, and cytarabine. The combination studies with cyclophosphamide have caused toxicity concerns, especially hepatotoxicity in patients treated with prior SCT. Therefore the studies now use very stringent entry criteria, excluding patients with pre-existing liver disease or prior SCT. Whether these modifications are successful needs to be awaited.

We aim at developing an alternative for the FLAG/liposomal daunorubicin combination. Hence, we will study the safety and preliminary efficacy of a combination of clofarabine, cytarabine and liposomal daunorubicin. GCSF priming will not be pursued, as there is limited evidence for its use, and there are more potent agents for priming being developed,
such as CXCR4 inhibitors.\textsuperscript{42-45} When successful, this combination may be taken forward in the new European relapsed AML protocol, or in other AML studies of national groups.

In the adult studies the combination of cytarabine and clofarabine has been demonstrated to be well tolerablated, using cytarabine 1 gr/m\textsuperscript{2} IV for 5 consecutive days on day 1-5, and clofarabine at 40 mg/m\textsuperscript{2} IV for 5 days on day 2-6.\textsuperscript{30,37} This is the same schedule as the COG is currently testing in children. No data on combination studies with anthracyclines are available as yet, but there is extensive experience with the FLAG regimen in combination with anthracyclines such as idarubicin or DaunoXome\textsuperscript{®}.

In this study we will use the cytarabine dose as administered in the FLAG regimen (2 gram/m\textsuperscript{2} bolus IV for 5 consecutive days) – which will also allow a fair comparison with the FLAG regimen in later studies. The combination of this dose of Ara-C and clofarabine is currently being tested in a trial in the USA in adults with relapsed/refractory AML (Ara-C 2 gr/m2/day in 3 hours IV, with clofarabine 40 mg/m2/day in 2 hours IV, daily, for 5 consecutive days). Considering the dose of liposomal daunorubicin: we will first establish a recommended dose of clofarabine in combination with 60 mg/m\textsuperscript{2} of liposomal daunorubicin on days 1, 3 and 5. Later, in a separate cohort with restricted inclusion criteria, we will attempt to further dose-escalate liposomal daunorubicin to the dose to 80 mg/m\textsuperscript{2} on days 1, 3 and 5. This will allow incorporation of this schedule in front-line therapy.
3 ELIGIBILITY CRITERIA

3.1 Revision of eligibility regarding prior fungal infections (amendment 1)

3.1.1 Apergillosis

Pulmonary aspergillosis is a frequent complication in AML patients treated with intensive chemotherapy. Approximately 25% of children with relapsed or refractory AML had a history of proven or probable pulmonary fungal infection in a recent BFM-survey of 104 patients with 1st relapse (unpublished data, Prof. Reinhardt, 01-10-2011).

It is unclear if the infection can really be considered related to clofarabine use, although we cannot rule out that it has worsened by its use (in combination with cytarabine and liposomal daunorubicin). However, any other intensive chemotherapy cycle in such patients may show a similar worsening.

In the current version of the protocol we excluded patients with active uncontrolled infection, and we asked for a chest X-ray at screening in all patients. Only in case of an abnormal chest X-ray (which we know has a very low sensitivity to detect fungal infections) a HR-CT was required. We therefore have to face the situation that patients with a subclinical fungal infection may be enrolled, resulting in the DLTs mentioned above.

However, apart from infectious DLTs there were no other dose-limiting toxicities/organ-toxicities so far. We therefore propose to amend the in- and exclusion criteria, actively excluding patients who may have subclinical fungal infection. We will, if ethics approval has been obtained, then re-open the study at dose-level 3 to consider whether the DLTs can be avoided by implementing this strategy. If this is the case we feel it is also justified to proceed dose-escalation.

Therefore, we now propose that all patients must undergo a HR-CT scan as part of the screening procedure, as well as a serum Aspergillus test (galactomannan, which is a component of the cell wall of the mold Aspergillus). The latter test has been validated in the pediatric population in 2 studies showing somewhat discordant results, as reviewed by Lehrnbecher. One showed a poor specificity in the 42 children that were a subset of a larger study of 728 patients, as the specificity in the pediatric population was only 47.6%. However, another study looking at this test in HSCT recipients showed a specificity of 97.5% in 64 children. However, given that safety is our major concern, and we do not want to diagnose but rather exclude prior fungal infections, the likelihood that patients with both a negative HR-CT and a negative galactomannan are subclinically infected with aspergillus is probably low. In case of positive findings on either of these tests the patient will be excluded and considered a screen failure.
The modified inclusion and exclusion criteria are described below, as well as the amended work-up procedure.

### 3.1.2 Inclusion Criteria (dose level 1-4)

**Initial work-up:**
- Complete initial work-up within 7 days prior to first treatment, including bone-marrow aspiration, lumbar puncture (without intrathecal therapy), and assessment of organ toxicity

**General conditions:**
- 2nd relapse of AML
- refractory AML in 1st relapse (defined as ≥ 20% blasts in the bone marrow after the 1st course of standard re-induction therapy)
- 1st early relapse (relapse within one year from initial diagnosis) of AML
- ≤ 18 years old at initial diagnosis
- Lansky play score ≥ 60; or Karnofsky performance status ≥ 60
- Life expectancy ≥ 6 weeks
- Calculated creatinine clearance ≥ 90 ml/min/1.73m2 as calculated by the Schwartz formula for estimated glomerular filtration rate (GFR) where GFR (ml/min/1.73 m2) = k*Height (cm)/serum creatinine (mg/dl). k is a proportionality constant which varies with age and is a function of urinary creatinine excretion per unit of body size; 0.45 up to 12 months of age; 0.55 children and adolescent girls; and 0.70 adolescent boys.
- Liver function:
  - Serum bilirubin ≤ 1.5 × upper limit of normal (ULN)
  - Aspartate transaminase (AST)/alanine transaminase (ALT) ≤ 2.5 × ULN
  - Alkaline phosphatase ≤ 2.5 × ULN

**Other:**
- Able to comply with scheduled follow-up and with management of toxicity.
- For female patients with childbearing potential, a negative test for pregnancy is to be considered before entry on study
- Male and female patients must use an effective contraceptive method during the study and for a minimum of 6 months after study treatment.
- Written informed consent from patients or from parents or legal guardians for minor patients, according to local law and regulations
3.1.3 Exclusion Criteria (dose level 1-4)

General conditions:
- Isolated extramedullary relapse, including isolated CNS-relapse
- Symptomatic CNS leukemia in case of combined relapse
- Relapsed/refractory acute promyelocytic leukemia (APL)
- Relapsed/refractory myeloid leukemia of Down Syndrome (ML DS)
- Other serious illnesses or medical conditions
- Current uncontrolled infection
- Evidence of fungal infection by:
  - Evidence of pulmonary infiltrates suggestive of a fungal infection at HR-CT (within 3 weeks prior to enrollment)
  - A positive Aspergillus serum test (galactomannan), according to local laboratory practice (within 3 weeks prior to enrollment)
- Evidence of cardiac dysfunction (shortening fraction below 28%)
- Pregnant or lactating patients

Prior or current history:
- Use of any anticancer therapy within 2 weeks before study entry. The patient must have recovered from all acute toxicities from any previous therapy (note: hematological toxicities do not need to be considered since the patient has overt leukemia).
- History of prior veno-occlusive disease (VOD)
- Hypersensitivity to cytarabine, clofarabine or liposomal daunorubicin

Concomitant treatments:
- Concomitant administration of any other experimental drug under investigation, or concurrent treatment with any other anti-cancer therapy other than specified in the protocol is not allowed.
- GCSF will not be used for priming and no routine GCSF support is allowed during the 1st course, except for life-threatening infections.
- In case of non-symptomatic CNS-involvement, intrathecal therapy is allowed according to investigator's discretion. It is not allowed to give intrathecal therapy prior to treatment with clofarabine, as we do not know if this can be done without safety concerns. Hence, this should be delayed to day +7 of treatment, which will allow us to assess the CSF penetration of clofarabine (clofarabine CSF levels and early response). In case of neurotoxicity experienced during the IV treatment, the intrathecal may need to be further delayed.
3.2 Eligibility criteria for Dose Level 5 only

The protocol was designed to determine a recommended dose of clofarabine in combination with cytarabine and liposomal daunorubicine. The aim was to develop a new block that might be randomized against FLAG-DNX in patients with AML in 1st relapse. At relapse, 60 mg/m² of DNX (at day 1, 3 and 5) was used in the prior Relapsed AML 2001/01 study.

However, there is considerable interest in developing this block for newly diagnosed AML as a new block for an upfront randomization. Yet, in upfront AML, 80mg/m² of DNX (at day 1, 3 and 5) is used, rather than 3x60 mg/m², as described in the results from the AML-BFM 2004 study (Creutzig et al, ASH meeting 2010)55.

Therefore, we here propose to add a dose-level 5, with 40 mg/m² of clofarabine (day 1-5), 80 mg/m² liposomal daunorubicin (day 1, 3 and 5) and 2 gram/m² cytarabine (day 1-5).

We feel this is justified, given that we are seeing responses (3 out of 7 patients in dose-level 2 or 3 have responded) in patients who have relapsed after or are refractory to FLAG-DNX (the standard treatment in case of relapse). This suggests that clofarabine may be more active than our standard treatment in this situation, which is also supported by the available literature. For instance, in the CLOUD study (clofarabine 30 mg/m2 or 40 mg/m2, in combination with liposomal DNR, n=13) performed in the UK, also 40% responses were seen in patients with early first relapse or 2nd relapse (Kearns et al, ASCO 2011).

However, we do not want to expose patients with 2nd relapse or refractory first relapse or a prior SCT to this dose-level, as we anticipate this may be intolerable due to their very heavy pre-treatment status (risk of long-lasting aplasia and infectious death). Besides they may be exposed to a cumulative dose of anthracyclines that is considered too high. Therefore we want to restrict inclusion to patients with first relapse in this part of the study. However, patients with a ‘late’ first relapse (>1 year from initial diagnosis) have a reasonable chance of cure with standard chemotherapy treatment (i.e. FLAG/DNX). Patients with an early first relapse (less than 1 year from initial diagnosis, approximately 45-50% of the patients with 1st relapse) however had a dismal outcome using this regimen: the 2nd Cr rate was only 50% and the OS is approximately 20%. Therefore, we want to limit this part of the study to patients in 1st early relapse of AML without a prior SCT in CR1.

Dose-level 5 may only be opened when dose level 1-4 have been finalized and when dose-level 4 is considered safe. Dose-level 5 will then be considered as a separate cohort, given the restricted patient population.
3.2.1 **Inclusion criteria for Dose Level 5**

**Initial work-up:**
- Complete initial work-up within 7 days prior to first treatment, including bone-marrow aspiration, lumbar puncture (without intrathecal therapy), and assessment of organ toxicity

**General conditions:**
- **Newly diagnosed 1st relapse of AML:** only patients with early relapses occurring within 1 year of initial diagnosis are eligible
- ≤18 years old at initial diagnosis
- Lansky play score ≥ 60; or Karnofsky performance status ≥ 60
- Life expectancy ≥ 6 weeks
- Calculated creatinine clearance ≥ 90 ml/min/1.73 m^2^ as calculated by the Schwartz formula for estimated glomerular filtration rate (GFR) where GFR (ml/min/1.73 m^2^) = k*Height (cm)/serum creatinine (mg/dl). k is a proportionality constant which varies with age and is a function of urinary creatinine excretion per unit of body size; 0.45 up to 12 months of age; 0.55 children and adolescent girls; and 0.70 adolescent boys.
- Liver function:
  - Serum bilirubin ≤ 1.5 × upper limit of normal (ULN)
  - Aspartate transaminase (AST)/alanine transaminase (ALT) ≤ 2.5 × ULN
  - Alkaline phosphatase ≤ 2.5 × ULN

**Other:**
- Able to comply with scheduled follow-up and with management of toxicity.
- For female patients with childbearing potential, a negative test for pregnancy is to be considered before entry on study
- Male and female patients must use an effective contraceptive method during the study and for a minimum of 6 months after study treatment.
- Written informed consent from patients or from parents or legal guardians for minor patients, according to local law and regulations

3.2.2 **Exclusion Criteria for Dose Level 5 only**

**General conditions:**
- Isolated extramedullary relapse, including isolated CNS-relapse
- Symptomatic CNS leukemia in case of combined relapse
- Relapsed/refractory acute promyelocytic leukemia (APL)
- Relapsed/refractory myeloid leukemia of Down Syndrome (ML DS)
- Other serious illnesses or medical conditions
- Current uncontrolled infection
- Evidence of fungal infection by:
- Evidence of pulmonary infiltrates suggestive of a fungal infection at HR-CT (within 3 weeks prior to enrollment)
- A positive aspergillus serum test (galactomannan), according to local laboratory practice (within 3 weeks prior to enrollment)
- Evidence of cardiac dysfunction (shortening fraction below 28%)
- Pregnant or lactating patients
- Prior stem-cell transplant in CR1
4 PRODUCT CHARACTERISTICS

Clofarabine is the investigational medicinal product in this study, and will be made available by the sponsor. Cytarabine and liposomal daunorubicin should be used from commercial stock, and be handled according to the Summary of Product Characteristics (SPC). The text below summarizes the product characteristics of the 3 drugs which will be evaluated in this study in a combination regimen.

4.1 Clofarabine drug information

4.1.1 Nomenclature

Chemical Name: 2-chloro-9-(2’-deoxy-2’-fluoro-β-D-arabinofuranosyl)-9H-purine-6-amine

Other names: CLOLAR, EVOLTRA, clofarabine; CAFdA; Cl-F-ara-A;
2-chloro-2’-fluoro-deoxy-9-β arabinofuranosyladenine
2-chloro-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)adenine (Cl-F-ara-A)
2-chloro-2’-arabino-fluoro-2’-deoxyadenosine
2-chloro-2’-ara-fluorodeoxyadenosine (CAFdA)
2-chloro-2’-fluorodeoxyadenosine (CAFdA)

4.1.2 Molecular Structure

**Figure 4-1**: Molecular Structure of clofarabine

(C₁₀H₁₁ClFN₅O₃)

![Molecular Structure of clofarabine](image)

4.1.3 Physical and Chemical Characteristics

Clofarabine is a white to off-white solid with a melting point of 228°C to 230°C and a molecular weight of 303.5. The drug substance is very stable in the dry state, and aqueous solutions are stable to heat treatment. Clofarabine is freely soluble in water (1.5 mg/mL) or buffered solutions at room temperature. Clofarabine is not less than 97% pure on a dried basis by high performance liquid chromatography (HPLC) analysis.

Clofarabine is formulated at a concentration of 1 mg/mL. Clofarabine is supplied in 1 vial size: a 20-mL clear, glass vial with gray stopper and blue flip off seal. The 20-mL vials
contain 20 mL (20 mg) of sterile solution. The pH range of the solution is 4.5 to 7.5. The solution is clear and practically colorless, preservative free, and free from foreign matter.

**4.1.4 Storage and Handling**

Vials containing undiluted clofarabine for injection should be stored at controlled room temperature (15-25°C). The current commercial expiry period for clofarabine is 24 months at room temperature. Ongoing stability studies will continue to confirm the appropriate quality of drug product used for clinical trials beyond 24 months.

Clofarabine for injection should be diluted with 0.9% sodium chloride injection USP or European Pharmacopeia (EP) normal saline (NS) or 5% dextrose injection (D5W) USP or EP prior to IV infusion. The final volume is at the clinician’s discretion depending upon several factors including total clofarabine dose (in mg) and patient age/size, clinical condition and hydration status. The majority of clinical study sites used a final volume of 100-200 mL (0.9% Sodium Chloride or 5% Dextrose). The resulting admixture may be stored at room temperature, but must be used within 24 hours of preparation. 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 and should not be adjusted downward to “ideal” weight. To prevent drug incompatibilities, no other medications should be infused concurrently through the same IV lines as clofarabine. Also, no blood products should be administered at the same time as clofarabine.

**4.1.5 Drug order procedure**

Clofarabine will be distributed free of charge to the sites on behalf of the sponsor after re-labeling and QP-release by Penn Pharmaceutical Services (Penn), which will be done in accordance with the ICH GCP, GMP, EU and National legislation guidelines. The first drug shipment to a site will be issued after release of a “Investigational Product Authority To Ship Form”, which authorizes Penn to ship drug to the site once the sponsor has agreed that all regulatory documents are in place. Requests for drug shipment can be made by the site using a ‘Shipment Request Form’. Further details of the drug ordering procedure are specified in the Procedure Manual.

**4.1.6 Drug accountability**

It is the responsibility of the Investigator to ensure that the investigational product (clofarabine) is only dispensed to study subjects. The investigational product must be dispensed only from official study sites by authorized personnel according to local regulations.

The lot numbers, dosing start dates and the number of vials for each dosage strength must be recorded on drug accountability pages of the Case Report Form.

It is the responsibility of the Investigator to ensure that a current record of investigational product disposition is maintained at each study site where investigational product is inventoried and disposed. Records or logs must comply with applicable regulations and guidelines, and should include:
• Amount received and placed in storage area.
• Amount currently in storage area.
• Label ID number or batch number and use date or expiry date.
• Dates and initials of person responsible for each investigational product inventory entry/movement.
• Amount dispensed to and returned for each subject, including unique subject identifiers.
• Amount transferred to another area/site for dispensing or storage.
• Non-study disposition (e.g., lost, wasted, broken).
• Amount returned to Sponsor.
• Amount destroyed at study site, if applicable.
• Retain samples sent to third party for bioavailability/bioequivalence, if applicable.

Used or partially used investigational products will be destroyed on site, as well as unused vials, according to hospital procedures. It is the Investigator’s responsibility to ensure that arrangements have been made for disposal and written authorization has been granted by the sponsor, and that procedures for proper disposal have been established according to applicable regulations, guidelines, and institutional procedures. Appropriate records of the disposal must be maintained.

4.1.7 Toxicity

Adult Patients

Drug-related AEs observed in at least 10% of adult patients treated with clofarabine in previous clinical trials include myelosuppression, nausea, vomiting, infections, fatigue, headache, diarrhea, rigors, dermatitis, anorexia, febrile neutropenia, myalgia, asthenia, petechiae, transient elevated liver enzymes, stomatitis, mucositis, pyrexia, flushing, constipation, edema, dehydration, nervousness, stomach pain, insomnia, depression, dry skin, back pain, and decreased weight (Clofarabine Package Insert. San Antonio, TX: Genzyme Corporation. Final dated February 2008).

Adverse events reported in <10% of adult patients include tumor lysis syndrome, capillary leak syndrome, palmar plantar erythrodysesthesia, pancreatitis, seizures, irregular heart beat, edema, pericardial effusion, multi-organ failure, and death.

Pediatric Patients

The most common side effects observed in previous clinical studies of pediatric patients treated with clofarabine 52 mg/m² include vomiting NOS, nausea, febrile neutropenia, diarrhea NOS, headache NOS, pruritus NOS, pyrexia, dermatitis NOS, fatigue, rigors, abdominal pain NOS, tachycardia NOS, anorexia, petechiae, epistaxis, pain in limb, hypotension NOS, anxiety NEC, cough, constipation, erythema NEC, mucosal inflammation NOS, pain NOS, flushing, edema NOS, and hematuria.

Pediatric patients have also experienced increased liver enzymes and increased creatinine. Moderate neurological changes have been reported in some patients. Infections were reported in almost half of the patients treated with clofarabine.
Other potential SAEs include pericardial effusion, LVSD, tumor lysis syndrome, SIRS, and capillary leak syndrome.

Genzyme is conducting a Phase I/II study to assess concomitant use of clofarabine, etoposide and cyclophosphamide in pediatric patients with acute leukemias. In the Phase I portion of the study, febrile neutropenia, pyrexia and neutropenia were the most commonly reported serious adverse events and were reported in 64%, 20%, and 16% of patients, respectively. There were 10 patient deaths in the trial (malignant disease, n=5; adverse events, n=2: infection/micrococcus meningitis, CNS hemorrhage; and other causes, n=3: intracranial hemorrhage, multi-organ failure, unknown etiology). Dose-limiting toxicities included a grade 3 elevation of lipase, abdominal pain and possible veno-occlusive disease (VOD) that resolved (cohort 3) and 1 case of prolonged bone marrow aplasia (cohort 5). In the Phase II portion 3 of the 4 first patients treated reported veno-occlusive disease (VOD) symptoms including hyperbilirubinemia, hepatomegaly, right upper quadrant pain, ascites and/or weight gain. VOD-like signs and symptoms initially presented within 5 to 12 days of the first dose of clofarabine in these patients. Two of the patients with VOD symptomatology had a history of HSCT and total body irradiation (TBI) within the previous 4 to 7 months. All 3 patients with VOD-like symptoms had ongoing severe infections and/or capillary leak syndrome preceding the occurrence of hepatotoxicity. The fourth patient reported grade 4 hyperbilirubinemia and had a history of HSCT and TBI greater than 1 year prior to study entry. As a result of these safety findings, the updated protocol now states that patients with a history of prior HSCT, elevated conjugated serum bilirubin at study entry, uncontrolled systemic fungal, bacterial, or other infection, a history of hepatitis B or C infection or a history of cirrhosis are to be excluded from study participation. The warnings and precautions section of the clofarabine package insert has also been revised to reflect these recent safety findings.

4.2 Cytarabine Drug Information

4.2.1 Physical and Chemical Characteristics

Cytarabine is a deoxycytidine analogue which is metabolized to Ara-CTP, a substance which inhibits DNA polymerase. It is S-phase specific, and thus affects DNA synthesis. It has an initial plasma half-life of about 15 minutes, with a secondary phase of about 2 hours. Rapidly catabolized by hepatic cytidine deaminases to AraU. Intrathecally administered doses are catabolized and eliminated more slowly with a half-life of 1-11 hours.

4.2.2 Storage and Handling

Cytarabine Injection, an antineoplastic, is a sterile solution of Cytarabine for intravenous, intrathecal or subcutaneous administration.

Cytarabine is an odorless, white to off-white crystalline powder which is freely soluble in water and slightly soluble in alcohol and in chloroform. Cytarabine is a freeze-dried powder available in 100mg, 500mg, 1g and 2g vials. The unreconstituted form of the drug is stable at room temperature for at least 2 years.

Protect from light. Retain in carton until time of use.
Reconstitute with sterile water or Bacteriostatic Water to a recommended concentration of 20mg/ml up to 100mg/ml, except for IT administration. (See Guidelines below.) Reconstituted solution stable for 28 days at room temperature or refrigerated (concentration dependent). Further diluted solutions of 0.5 mg to 25 mg/mL are stable at least 7 days at room temperature. A solution of 40 to 80 mg/mL diluted with bacteriostatic water in polypropylene syringes is stable 15 days at room temperature. A solution of 1 mg/mL in selected portable pump reservoirs is stable for 15 days at 37°C. Discard solution if haze develops. Compatible with potassium chloride and sodium bicarbonate. (Trissel, 9th edition). 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. Patients <1 year of age should be dosed based on mg/kg. To prevent drug incompatibilities, no other medications should be administered through the same IV line. Cytarabine will be infused in 3 hours, starting 3 hours after the end of the clofarabine infusion.

**Intrathecal administration**

IT cytarabine should be reconstituted with physiologic buffered diluents (lactated Ringer's, 0.9% sodium chloride, Elliott’s B solution) or patient's own CSF. Do not use Bacteriostatic Water to reconstitute for IT administration, use only preservative free solutions.

**4.2.3 Toxicity**

Cytarabine is a potent bone marrow suppressant. Therapy should be started cautiously in patients with pre-existing drug-induced bone marrow suppression. Facilities should be available for management of complications, possibly fatal, of bone marrow suppression (infection resulting from granulocytopenia and other impaired body defenses, and hemorrhage secondary to thrombocytopenia).

One case of anaphylaxis that resulted in acute cardiopulmonary arrest, and required resuscitation has been reported. This occurred immediately after the intravenous administration of Cytarabine injection.

Severe and at times fatal CNS, GI and pulmonary toxicity (different from that seen with conventional therapy regimens of Cytarabine injection) has been reported following some experimental dose schedules for Cytarabine injection. These reactions include reversible corneal toxicity, and hemorrhagic conjunctivitis, which may be prevented or diminished by prophylaxis with a local corticosteroid eye drop; cerebral and cerebellar dysfunction, including personality changes, somnolence and coma, usually reversible; severe gastrointestinal ulceration, including pneumatosis cystoides intestinalis leading to peritonitis; sepsis and liver abscess; pulmonary edema, liver damage with increased hyperbilirubinemia; bowel necrosis; and necrotizing colitis. Rarely, severe skin rash, leading to desquamation has been reported. Complete alopecia is more commonly seen with experimental high dose therapy than with standard treatment programs using Cytarabine injection. If experimental high dose therapy is used, do not use a preparation containing benzyl alcohol.

Cases of cardiomyopathy with subsequent death have been reported following experimental high dose therapy with Cytarabine in combination with cyclophosphamide when used for bone marrow transplant preparation.
A syndrome of sudden respiratory distress, rapidly progressing to pulmonary edema and radiographically pronounced cardiomegaly has been reported following experimental high dose therapy with Cytarabine used for the treatment of relapsed leukemia from one institution in 16/72 patients. The outcome of this syndrome can be fatal.

Two patients with childhood acute myelogenous leukemia who received intrathecal and intravenous Cytarabine injection at conventional doses (in addition to a number of other concomitantly administered drugs) developed delayed progressive ascending paralysis resulting in death in one of the two patients.

When large intravenous doses are given too quickly, patients are frequently nauseated and may vomit for several hours post-injection. This problem tends to be less severe when the drug is infused.

The human liver apparently detoxifies a substantial fraction of an administered dose. In particular, patients with renal or hepatic function impairment may have a higher likelihood of CNS toxicity after high-dose Cytarabine injection treatment. Use the drug with caution and possibly at reduced dose in patients whose liver or kidney function is poor.

Periodic checks of bone marrow, liver and kidney functions should be performed in patients receiving Cytarabine injection.

Like other cytotoxic drugs, Cytarabine Injection may induce hyperuricemia secondary to rapid lysis of neoplastic cells. The clinician should monitor the patient’s blood uric acid level and be prepared to use such supportive and pharmacologic measures as might be necessary to control this problem.

Acute pancreatitis has been reported to occur in a patient receiving Cytarabine Injection by continuous infusion and in patients being treated with Cytarabine Injection who have had prior treatment with L-asparaginase.

A Cytarabine syndrome has been described by Castleberry. It is characterized by fever, myalgia, bone pain, occasionally chest pain, maculopapular rash, conjunctivitis and malaise. It usually occurs 6-12 hours following drug administration. Corticosteroids have been shown to be beneficial in treating or preventing this syndrome. If the symptoms of the syndrome are deemed treatable, corticosteroids should be contemplated as well as continuation of therapy with Cytarabine Injection.

Ten patients treated with experimental intermediate doses of Cytarabine (1 g/m2) with and without other chemotherapeutic agents (meta-AMSA, daunorubicin, etoposide) at various dose regimes developed a diffuse interstitial pneumonitis without clear cause that may have been related to the Cytarabine.
Table 4-1: Potential toxicities of IV cytarabine

<table>
<thead>
<tr>
<th></th>
<th>Common</th>
<th>Occasional</th>
<th>Rare</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Happens to 21-100 children out of every 100</td>
<td>Happens to 5-20 children out of every 100</td>
<td>Happens to &lt;5 children out of every 100</td>
</tr>
<tr>
<td>Immediate:</td>
<td>Nausea, vomiting, anorexia (L), conjunctivitis¹</td>
<td>Flu-like symptoms with fever (L)</td>
<td>Rash (L), encephalopathy¹ (L), cerebellar dysfunction¹ (L)</td>
</tr>
<tr>
<td>Prompt:</td>
<td>Myelosuppression, stomatitis, alopecia</td>
<td>Diarrhea</td>
<td>Hepatotoxicity (L)¹, veno-occlusive disease¹ (L), pulmonary capillary leak¹</td>
</tr>
<tr>
<td>Delayed:</td>
<td>Any time later during therapy, excluding the above conditions</td>
<td></td>
<td>Pneumonitis</td>
</tr>
<tr>
<td>Late:</td>
<td>Any time after completion of treatment</td>
<td></td>
<td>Gonadal dysfunction</td>
</tr>
<tr>
<td>Unknown Frequency and Timing:</td>
<td><strong>Fetal and teratogenic toxicities</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹ Rare with low doses. (L) Toxicity may also occur later.
**Fetal toxicities and teratogenic effects of cytarabine (alone or in combination with other antineoplastic agents) have been noted in humans. Toxicities include: congenital defects, chromosome abnormalities, pancytopenia, and low birth weight.

Potential Toxicities of Cytarabine used as Intrathecal Therapy

Cerebrospinal fluid levels of Cytarabine are low in comparison to plasma levels after single intravenous injection. However, in one patient in whom cerebrospinal fluid levels were examined after 2 hours of constant intravenous infusion, levels approached 40 percent of the steady state plasma level. With intrathecal administration, levels of Cytarabine in the cerebrospinal fluid declined with a first order half-life of about 2 hours. Because cerebrospinal fluid levels of deaminase are low, little conversion to ara-U was observed.

Cytarabine injection has been used intrathecally in acute leukemia in doses ranging from 5 mg/m2 to 75 mg/m2 of body surface area. The frequency of administration varied from once a day for 4 days to once every 4 days. The most frequently used dose was 30 mg/m2 every 4 days until cerebrospinal fluid findings were normal, followed by one additional treatment. The dosage schedule is usually governed by the type and severity of central nervous system manifestations and the response to previous therapy.

Cytarabine injection given intrathecally may cause systemic toxicity and careful monitoring of the hematopoietic system is indicated. Modification of other anti-leukemia therapy may be necessary. Major toxicity is rare. The most frequently reported reactions after intrathecal administration were nausea, vomiting and fever; these reactions are mild and self-limiting. Paraplegia has been reported. Necrotizing leukencephalopathy occurred in 5 children; these patients had also been treated with intrathecal methotrexate and hydrocortisone, as well as by central nervous system radiation. Isolated neurotoxicity has been reported. Blindness occurred in two patients in remission whose treatment had consisted of combination systemic
Chemotherapy, prophylactic central nervous system radiation and intrathecal Cytarabine injection.

When Cytarabine injection is administered both intrathecally and intravenously within a few days, there is an increased risk of spinal cord toxicity, however, in serious life-threatening disease, concurrent use of intravenous and intrathecal Cytarabine Injection is left to the discretion of the treating physician.

Focal leukemic involvement of the central nervous system may not respond to intrathecal Cytarabine injection and may better be treated with radiotherapy.

**Table 4-2: potential toxicities of cytarabine used as intrathecal therapy (in a triple therapy combination):**

<table>
<thead>
<tr>
<th></th>
<th>Common</th>
<th>Occasional</th>
<th>Rare</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Immediate:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Within 1-2 days of</td>
<td>Nausea, vomiting</td>
<td>Headache, pleocytosis, fever</td>
</tr>
<tr>
<td></td>
<td>receiving drug</td>
<td></td>
<td>Rash, somnolence (L),</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>meningismus, convulsions, paresis</td>
</tr>
<tr>
<td><strong>Prompt:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Within 2-3 weeks, prior</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>to the next course</td>
<td></td>
<td>Myelosuppression, ataxia</td>
</tr>
<tr>
<td><strong>Delayed:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Any time later during</td>
<td>Learning disability</td>
<td></td>
</tr>
<tr>
<td></td>
<td>therapy, excluding the</td>
<td></td>
<td>Leukoencephalopathy (L)</td>
</tr>
<tr>
<td></td>
<td>above condition</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Late:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Any time after the</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>completion of treatment</td>
<td></td>
<td>Progressive CNS deterioration</td>
</tr>
</tbody>
</table>

(L) Toxicity may also occur later.

**Table 4-3: potential toxicities of cytarabine used as intrathecal therapy (as single agent):**

<table>
<thead>
<tr>
<th></th>
<th>Common</th>
<th>Occasional</th>
<th>Rare</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Immediate:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Within 1-2 days of</td>
<td>Nausea, vomiting</td>
<td>Headache, pleocytosis</td>
</tr>
<tr>
<td></td>
<td>receiving drug</td>
<td></td>
<td>Rash, fever, somnolence (L),</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>meningismus, convulsions (L),</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>paresis</td>
</tr>
<tr>
<td><strong>Prompt:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Within 2-3 weeks, prior</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>to the next course</td>
<td></td>
<td>Myelosuppression, ataxia</td>
</tr>
<tr>
<td><strong>Delayed:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Any time later during</td>
<td>Learning disability</td>
<td></td>
</tr>
<tr>
<td></td>
<td>therapy, excluding the</td>
<td></td>
<td>Leukoencephalopathy (L)</td>
</tr>
<tr>
<td></td>
<td>above condition</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Late:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Any time after the</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>completion of treatment</td>
<td></td>
<td>Learning disability (L)</td>
</tr>
</tbody>
</table>


4.3 Liposomal daunorubicin Drug Information

4.3.1 Physical and Chemical Characteristics

DaunoXome contains an aqueous solution of the citrate salt of daunorubicin encapsulated within lipid vesicles (liposomes) composed of a lipid bilayer of distearoylphosphatidylcholine and cholesterol (2:1 molar ratio), with a mean diameter of about 45 nm. The lipid to drug weight ratio is 18.7:1 (total lipid:daunorubicin base), equivalent to a 10:5:1 molar ratio of distearoylphosphatidylcholine:cholesterol:daunorubicin.

Daunorubicin is an anthracycline antibiotic with antineoplastic activity, originally obtained from Streptomyces peucetius. Daunorubicin has a 4-ring anthracycline moiety linked by a glycosidic bond to daunosamine, an amino sugar. Daunorubicin may also be isolated from Streptomyces coeruleorubidus and has the following chemical name: (8S - cis) - 8 - acetyl - 10 - [(3 - amino - 2,3,6 - trideoxy - α - L - lyxo - hexopyranosyl)oxy] - 7,8,9,10 - tetrahydro - 6,8,11 - trihydroxy - 1 - methoxy - 5,12 - naphthacenedione hydrochloride.

4.3.2 Storage and Handling

Store DaunoXome in a refrigerator, 2°–8°C (36°–46°F). Do not freeze. Protect from light. DaunoXome (daunorubicin citrate liposome injection) is a sterile, pyrogen-free, preservative-free product in a single use vial for intravenous infusion.

Each vial contains daunorubicin citrate equivalent to 50 mg of daunorubicin base, encapsulated in liposomes consisting of 704 mg distearoylphosphatidylcholine and 168 mg cholesterol. The liposomes encapsulating daunorubicin are dispersed in an aqueous medium containing 2,125 mg sucrose, 94 mg glycine, and 7 mg calcium chloride dihydrate in a total volume of 25 mL/vial. The pH of the dispersion is between 4.9 and 6.0. The liposome dispersion should appear red and translucent.

DaunoXome should be diluted 1:1 with 5% Dextrose Injection (D5W) before administration. Each vial of DaunoXome contains daunorubicin citrate equivalent to 50 mg daunorubicin base, at a concentration of 2 mg/mL. The recommended concentration after dilution is 1 mg daunorubicin/mL of solution. Aseptic technique must be strictly observed in all handling, since no preservative or bacteriostatic agent is present in DaunoXome or in the materials recommended for dilution.

Withdraw the calculated volume of DaunoXome from the vial into a sterile syringe, and transfer it into a sterile infusion bag containing an equivalent amount of D5W. Administer diluted DaunoXome immediately. If not used immediately, diluted DaunoXome should be refrigerated at 2°– 8 °C (36°– 46°F) for a maximum of 6 hours.

Caution: The only fluid which may be mixed with DaunoXome is D5W; DaunoXome must not be mixed with saline, bacteriostatic agents such as benzyl alcohol, or any other solution.

Do not use an in-line filter for the intravenous infusion of DaunoXome.
DaunoXome should be administered intravenously over a 60 minute period.

All parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. DaunoXome is a translucent dispersion of liposomes that scatters light to some degree. Do not use DaunoXome if it appears opaque, or has precipitate or foreign matter present.

4.3.3. Toxicity

The primary toxicity of DaunoXome is myelosuppression, especially of the granulocytic series, which may be severe, and associated with fever and may result in infection. Effects on the platelets and erythroid series are much less marked. Careful hematologic monitoring is required and since patients are immunocompromised, patients must be observed carefully for evidence of intercurrent or opportunistic infections.

Special attention must be given to the potential cardiac toxicity of DaunoXome. Although there is no reliable means of predicting congestive heart failure, cardiomyopathy induced by anthracyclines is usually associated with a decrease of the left ventricular ejection fraction (LVEF). Cardiac function should be evaluated in each patient by means of a history and physical examination before each course of DaunoXome. Patients who have received prior therapy with anthracyclines, have pre-existing cardiac disease, or have received previous radiotherapy encompassing the heart may be less "cardiac" tolerant to treatment with DaunoXome. In patients with Kaposi's sarcoma, congestive heart failure has been reported in one patient at a cumulative dose of 340 mg/m² of DaunoXome. In eight Kaposi's sarcoma patients, LVEF decreases were reported at cumulative doses ranging from 200 mg/m² to 2100 mg/m² (median dose 320 mg/m²) of DaunoXome. In clinical studies in malignancies other than Kaposi's sarcoma and treated with doses of DaunoXome greater than the recommended dose of 40 mg/m², congestive heart failure has been reported at a cumulative dose as low as 200 mg/m² of DaunoXome; seven patients have been reported with LVEF decreases. The proportion of patients at risk for cardiotoxicity is unknown because the denominator is uncertain since there were several instances of missing repeat cardiac evaluations.

A triad of back pain, flushing, and chest tightness has been reported in 13.8% of the patients (16/116) treated with DaunoXome in the randomized clinical trial and in 2.7% of treatment cycles (27/994). This triad generally occurs during the first five minutes of the infusion, subsides with interruption of the infusion, and generally does not recur if the infusion is then resumed at a slower rate. This combination of symptoms appears to be related to the lipid component of DaunoXome, as a similar set of signs and symptoms has been observed with other liposomal products not containing daunorubicin.

Daunorubicin has been associated with local tissue necrosis at the site of drug extravasation. Although no such local tissue necrosis has been observed with DaunoXome, care should be taken to ensure that there is no extravasation of drug when DaunoXome is administered.
Dosage should be reduced in patients with impaired hepatic function. Therefore, based on experience with daunorubicin, it is recommended that the dosage of DaunoXome be reduced if the bilirubin or creatinine is elevated as follows: serum bilirubin 1.2 to 3 mg/dL (= 20.5-51.3 μmol/l), give 25% dose reduction; serum bilirubin or creatinine > 3 mg/dL (= bili >51.3 μmol or creat > 265 μmol/l), give 50% dose reduction.
5 STUDY DESIGN AND TREATMENT PLAN

5.1 Primary Objectives
To establish the recommended dose of clofarabine in combination with cytarabine and liposomal daunorubicin (DaunoXome®) in children with relapsed/refractory AML.

5.2 Secondary Objectives
- To determine the safety and tolerability of this combination
- To determine (preliminary) efficacy in terms of the hematological remission rate in these patients
- To describe the durability of response, including the number of patients that undergo stem-cell transplant after re-induction with this regimen
- To describe the pharmacokinetics of clofarabine in combination with cytarabine and liposomal daunorubicin
- To preliminary assess the CSF blast disappearance, and the CSF-levels of clofarabine

5.3 Dose-escalation
We will use an adapted Faderl regimen to combine cytarabine given at day 1-5 with clofarabine given at day 1-5, as described by Agura et al.\textsuperscript{30,34,37}. In addition, we will use cytarabine and DaunoXome in the same schedule as prescribed in the relapsed AML 2001/01 protocol, with cytarabine 2 gram/m\textsuperscript{2} on day 1-5, and DaunoXome 60 mg/m\textsuperscript{2} on day 1, 3 and 5.

Clofarabine and DaunoXome\textsuperscript{®} will be dose-escalated according to table 5-1. Dose-escalation to a following dose-level can only take place once a prior dose-levels is considered safe. Cytarabine will be given at a fixed dose of 2 gram/m\textsuperscript{2} once daily, on day 1-5. The dose to be used will be provided by the Erasmus MC Clinical Trial Bureau when enrolling the patient.

The infusion schedule is as follows (see table 5-2):
- Clofarabine infusion will be given over 2 hours IV (rather than one hour, to improve tolerability), once daily, on day 1-5.
- DaunoXome\textsuperscript{®} will be infused in 60 minutes on day 1, 3 and 5 only, once daily, 30 minutes after the end of infusion of clofarabine. We will first combine 40 mg/m\textsuperscript{2} of DaunoXome\textsuperscript{®}, and later dose-escalate to 80 mg/m\textsuperscript{2} (see below).
- Cytarabine will be given at 2 gram/m\textsuperscript{2}/day, and infused in 3 hours IV, once daily, on day 1-5. Cytarabine infusion will start 3 hours after the end of the clofarabine infusion. There will be no dose-escalation of cytarabine.
5.3.1 Revision dose escalation schedule (Amendment 1, 05 November 2011)

Rationale

The protocol is designed to determine a recommended dose of clofarabine in combination with cytarabine and liposomal daunorubicine. This was initially considered a new block that might be randomized against FLAG-DNX in patients with AML in 1st relapse. At relapse, 60 mg/m² of DNX (at day 1, 3 and 5) was used in the prior Relapsed AML 2001/01 study.

However, there is considerable interest in developing this block for newly diagnosed AML as a new block for an upfront randomization. Yet, in upfront AML, 80mg/m² of DNX (at day 1, 3 and 5) is used, rather than 3x60 mg/m², as described in the results from the AML-BFM 2004 study (Creutzig et al, ASH meeting 2010). Therefore, we here propose to add a dose-level 5, with 40 mg/m² of clofarabine (day 1-5), 80 mg/m² liposomal daunorubicin (day 1, 3 and 5) and 2 gram/m² cytarabine (day 1-5).

We feel this is justified, given that we are seeing responses (3 out of 7 patients in dose-level 2 or 3 have responded) in patients who have relapsed after or are refractory to FLAG-DNX (the standard treatment in case of relapse). This suggests that clofarabine may be more active than our standard treatment in this situation, which is also supported by the available literature. For instance, in the CLOUD study (clofarabine 30 mg/m² or 40 mg/m², in combination with liposomal DNR, n=13) performed in the UK, also 40% responses were seen in patients with early first relapse or 2nd relapse (Kearns et al, ASCO 2011).

However, we do not want to expose patients with 2nd relapse or refractory first relapse or a prior SCT in CR1 to this dose-level, as we anticipate this may be intolerable due to their very heavy pre-treatment status (risk of long-lasting aplasia and infectious death). Besides they may be exposed to a cumulative dose of anthracyclines that is considered too high. Therefore we want to restrict inclusion to patients with first relapse in this part of the study. However, patients with a ‘late’ first relapse (>1 year from initial diagnosis) have a reasonable chance of cure with standard chemotherapy treatment (i.e. FLAG/DNX). Patients with an early first relapse (less than 1 year from initial diagnosis, approximately 45-50% of the patients with 1st relapse) however had a dismal outcome using this regimen: the 2nd Cr rate was only 50% and the OS is approximately 20%. Therefore, we want to limit this part of the study to patients in 1st early relapse of AML without prior SCT in CR1.
Dose level 5 will be added for patients with an early first relapse exclusively. Dose level 5 may only be opened when dose level 4 was assessed to be safe. Dose level 5 will then be considered as a separate cohort, given the restricted patient population. We will apply the 3x3 design, as defined in the protocol. When there are no DLTs, we will also expand this cohort to 10 patients, applying the restricted inclusion criteria.

**Table 5-1: Dose-escalation schedule (amendment 1)**

NOTE: Patients <1 year of age should be dosed based on mg/kg.

<table>
<thead>
<tr>
<th>Age ≥ 1 year</th>
<th>Clofarabine</th>
<th>DaunoXome®</th>
<th>Ara-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose-level -1</td>
<td>15 mg/m²/day x 5 d</td>
<td>40 mg/m²/d 1-3-5</td>
<td>2 gr/m²/day x 5d</td>
</tr>
<tr>
<td><strong>Dose-level 1 (starting dose)</strong></td>
<td><strong>20 mg/m²/day x 5 d</strong></td>
<td><strong>40 mg/m²/d 1-3-5</strong></td>
<td>2 gr/m²/day x 5d</td>
</tr>
<tr>
<td>Dose-level 2</td>
<td>30 mg/m²/day x 5 d</td>
<td>40 mg/m²/d 1-3-5</td>
<td>2 gr/m²/day x 5d</td>
</tr>
<tr>
<td>Dose-level 3</td>
<td>30 mg/m²/day x 5 d</td>
<td>60 mg/m²/d 1-3-5</td>
<td>2 gr/m²/day x 5d</td>
</tr>
<tr>
<td>Dose level 4</td>
<td>40 mg/m²/day x 5 d</td>
<td>60 mg/m²/d 1-3-5</td>
<td>2 gr/m²/day x 5d</td>
</tr>
<tr>
<td><strong>Dose level 5</strong></td>
<td><strong>40 mg/m²/day x 5 d</strong></td>
<td><strong>80 mg/m²/d 1-3-5</strong></td>
<td>2 gr/m²/day x 5d</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age &lt; 1 year</th>
<th>Clofarabine</th>
<th>DaunoXome®</th>
<th>Ara-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose-level -1</td>
<td>0.5 mg/kg/d x 5 d</td>
<td>1.3 mg/kg/d 1-3-5</td>
<td>70 mg/kg/d x 5d</td>
</tr>
<tr>
<td><strong>Dose-level 1 (starting dose)</strong></td>
<td><strong>0.7 mg/kg/d x 5 d</strong></td>
<td><strong>1.3 mg/kg/d 1-3-5</strong></td>
<td>70 mg/kg/d x 5d</td>
</tr>
<tr>
<td>Dose-level 2</td>
<td>1.0 mg/kg/d x 5 d</td>
<td>1.3 mg/kg/d 1-3-5</td>
<td>70 mg/kg/d x 5d</td>
</tr>
<tr>
<td>Dose-level 3</td>
<td>1.0 mg/kg/d x 5 d</td>
<td>2.0 mg/kg/d 1-3-5</td>
<td>70 mg/kg/d x 5d</td>
</tr>
<tr>
<td>Dose level 4</td>
<td>1.3 mg/kg/d x 5 d</td>
<td>2.0 mg/kg/d 1-3-5</td>
<td>70 mg/kg/d x 5d</td>
</tr>
<tr>
<td><strong>Dose level 5</strong></td>
<td><strong>1.3 mg/kg/d x 5 d</strong></td>
<td><strong>2.7 mg/kg/d 1-3-5</strong></td>
<td>70 mg/kg/d x 5d</td>
</tr>
</tbody>
</table>

Please also see the infusion time table (table 5-2) below.
Table 5-2: Time-table administration of Clofarabine/Ara-C/DaunoXome per course; dose-levels are given in table 5-1.

<table>
<thead>
<tr>
<th>Day</th>
<th>Drug</th>
<th>Infusion time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1#</td>
<td>Anti-emetic IV*</td>
<td>09.45 am</td>
</tr>
<tr>
<td></td>
<td>Clofarabine</td>
<td>10.00-12.00 am</td>
</tr>
<tr>
<td></td>
<td>DaunoXome</td>
<td>12.30-13.30 pm</td>
</tr>
<tr>
<td></td>
<td>Ara-C</td>
<td>15.00-18.00 pm</td>
</tr>
<tr>
<td></td>
<td>Anti-emetic IV*</td>
<td>22.00 pm</td>
</tr>
<tr>
<td>Day 2</td>
<td>Anti-emetic IV*</td>
<td>09.45 am</td>
</tr>
<tr>
<td></td>
<td>Clofarabine</td>
<td>10.00-12.00 am</td>
</tr>
<tr>
<td></td>
<td>Ara-C</td>
<td>15.00-18.00 pm</td>
</tr>
<tr>
<td></td>
<td>Anti-emetic IV*</td>
<td>22.00 pm</td>
</tr>
<tr>
<td>Day 3</td>
<td>Anti-emetic IV*</td>
<td>09.45 am</td>
</tr>
<tr>
<td></td>
<td>Clofarabine</td>
<td>10.00-12.00 am</td>
</tr>
<tr>
<td></td>
<td>DaunoXome</td>
<td>12.30-13.30 pm</td>
</tr>
<tr>
<td></td>
<td>Ara-C</td>
<td>15.00-18.00 pm</td>
</tr>
<tr>
<td></td>
<td>Anti-emetic IV*</td>
<td>22.00 pm</td>
</tr>
<tr>
<td>Day 4</td>
<td>Anti-emetic IV*</td>
<td>09.45 am</td>
</tr>
<tr>
<td></td>
<td>Clofarabine</td>
<td>10.00-12.00 am</td>
</tr>
<tr>
<td></td>
<td>Ara-C</td>
<td>15.00-18.00 pm</td>
</tr>
<tr>
<td></td>
<td>Anti-emetic IV*</td>
<td>22.00 pm</td>
</tr>
<tr>
<td>Day 5#</td>
<td>Anti-emetic IV*</td>
<td>09.45 am</td>
</tr>
<tr>
<td></td>
<td>Clofarabine</td>
<td>10.00-12.00 am</td>
</tr>
<tr>
<td></td>
<td>DaunoXome</td>
<td>12.30-13.30 pm</td>
</tr>
<tr>
<td></td>
<td>Ara-C</td>
<td>15.00-18.00 pm</td>
</tr>
<tr>
<td></td>
<td>Anti-emetic IV*</td>
<td>22.00 pm</td>
</tr>
<tr>
<td>Day 6#</td>
<td>Intrathecal therapy@</td>
<td>See section 5.5</td>
</tr>
</tbody>
</table>

* See text at section 5.4

# Day 1-5 and 6: PK sampling, see table 5-5

@ Intrathecal therapy is described in tables 5-3 and 5-4
5.4 Anti-emetics

Clofarabine is moderately emetogenic. Therefore, standard anti-emetic therapy (such as a 5HT3 antagonist) should be administered prior to therapy, per institutional protocol. Dexamethasone is not allowed due to concerns of inducing proliferation of AML cells, especially in acute monoblastic leukemia, as demonstrated in-vitro.46

5.5 Administration related supportive care measures

It is advised to vigorously hydrate the patient (2-5.3.0 liter/m²/day) with an IV solution starting at least 3 hours before administration of the clofarabine/Ara-C/DaunoXome course, and lasting until 24 hours post the last infusion of cytarabine.

In order to prevent tumor-lysis syndrome it is advised to hydrate the patient with a solution containing NaHCO₃ (for instance NaHCO₃ 4.2% at 4 ml/m²/hour, resulting in urine alkalinization which needs to be adjusted according to urine pH, aiming at a urine output with a pH between 7.0-8.0) and without potassium. For tumor-lysis prevention it is also advised to begin allopurinol 200 mg/m²/day, divided into 2 doses, before therapy begins. In case of high WBC (>50x10⁹/l), it is advised to use rasburicase instead of allopurinol. The recommended dose is 0.20 mg per kilogram body weight given as a daily infusion for up to seven days. The duration of treatment is adjusted depending on the patient’s uricaemia (blood levels of uric acid). The infusion should last 30 minutes. In case of rasburicase use, urine alkalinization is not needed.

Patients should have indwelling silastic catheters, preferably prior to the initiation of treatment. Therapy may be started with a temporary deep line until a permanent one can be placed.

Isotears should be used to prevent conjunctival and corneal pain. (2 gtt. OS/OD q 2 - 3 hours during AraC and for 24 - 48 hours after completion). Alternatively, centres may use steroid drops or saline per institutional guidelines.

5.6 Management of Capillary Leak Syndrome

In pediatric studies, during or shortly after IV clofarabine administration a few patients developed signs and symptoms consistent with capillary leak syndrome. In these heavily pretreated patients, it has been difficult to separate potential drug-related cases of capillary leak syndrome from concurrent medical conditions such as infection/sepsis, progressive disease, or other underlying problems resulting from prior antileukemic therapies.

For these reasons, during and after each dose of clofarabine investigators are to assess patients for the onset of the following signs or symptoms ≥ grade 2:

- Tachypnea or other evidence of respiratory distress;
- Unexplained hypotension; and/or
− Unexplained tachycardia.

If one or more of these signs or symptoms occurs during study drug infusion, clofarabine administration is to be interrupted or held as clinically indicated. It is recognized that the total infusion time for this clofarabine dose in this circumstance may exceed 1 hour. Thus, if the patient’s condition stabilizes or improves, clofarabine administration may resume. Pretreatment with steroids (e.g. hydrocortisone 100 mg/day or its equivalent or dexamethasone 20 mg/day as part of an anti-emetic regimen) is recommended for all subsequent doses during the remainder of that treatment cycle and for all subsequent treatment cycles.

5.7 Dose Modifications

All patients must have recovered from acute grade 3 or 4 side-effects from previous courses of chemotherapy before starting the 2nd or subsequent course, as specified in detail below:

Dose reduction for hematological toxicity

In case a 2nd or subsequent course cannot start before day 42 due to persisting aplasia, which is not caused by persistent leukemia (empty bone marrow), subsequent treatment courses should be given at the next lower dose-level. In case of persisting leukemia (without clear signs of progressive disease), no dose reductions apply.

Dose reduction for non-hematological toxicity

For grade 3 or 4 non-hematological toxicity, which is at least potentially related to study treatment, and that is not clinically manageable with regular supportive care/medical management, treatment needs to be interrupted until the toxicity decreases to ≤ grade 1 (or ≤ grade 2, if this is baseline). Subsequent courses may then be given at the next lower dose-level.

Alternatively, for clinically manageable toxicities which are at least potentially related to study treatment, and which are expected side-effects in case of intensive chemotherapy (febrile neutropenia, diarrhea, mucositis), no dose-reduction is needed, unless this toxicity was considered too severe per investigator’s discretion. When the toxicity was considered too severe by the investigator, subsequent courses may then be given at the next lower dose-level.

Clofarabine

Patients who experience grade 2 serum creatinine or serum total bilirubin toxicity during any clofarabine administration period, should have clofarabine held until recovery to baseline or grade <2 before resuming treatment.

Liposomal daunorubicin

It is recommended that the dosage of DaunoXome be reduced if the bilirubin or creatinine is elevated as follows: serum bilirubin 1.2 to 3 mg/dL (= 20.5-51.3 umol/l), give 25% dose reduction; serum bilirubin or creatinine > 3 mg/dL (= bili >51.3 umol or creat > 265 umol/l), give 50% dose reduction. In case the shortening fraction falls below 28% no further treatment with DaunoXome is allowed.
5.8 Supportive Care

5.8.1 Blood Products

Use of leukocyte-reduced and irradiated blood products is encouraged, according to each institution’s guidelines. Also, CMV- and ParvoB19 negative patients should receive CMV/ParvoB19 negative blood products, according to each institution’s guidelines.

Persistent bleeding may be attributable to thrombocytopenia and patients should receive platelet transfusions as quickly as possible. Platelet transfusions should also occur if the platelet count <10,000/ul and falling (relative indication). Splenomegaly, DIC, active bleeding, fever/sepsis, amphotericin B and platelet antibodies are reasons for poor recovery or survival of transfused platelets.

5.8.2 Infection Prophylaxis

The use of prophylactic antibacterial, antifungals, and antiviral agents is recommended according to each institution’s guidelines. Given the potential for liver toxicity by clofarabine it is recommended to at least interrupt prophylactic azoles during the days of clofarabine infusion, and, preferably delay the start of antifungal prophylaxis until 5 days after the clofarabine course, whenever possible. All azoles should be used with caution given that they may induce significant hepatotoxicity.

5.8.3 Treatment of Fever and Neutropenia

Patients with ANC of $\leq 500/\mu l$ (or $< 1,000$ and falling) and an oral temperature $> 38^\circ C$ twice in 12 hours, or $> 38.5^\circ C$ once, should have empiric systemic antibiotics started immediately. Broad-spectrum antibiotics should be initiated according to institutional guidelines, and should cover major gram-negative pathogens, as well as alpha hemolytic streptococcus and staphylococci.

Strep viridans is a bacterial organism responsible for much morbidity and mortality in children with febrile neutropenia and AML. Many strains of Strep viridans are resistant to penicillins and cephalosporins. Hence it is strongly recommend that 24 to 48 hours of vancomycin should be initiated for empiric gram-positive coverage at the time of fever. Vancomycin should be continued for all gram-positive organisms until results of culture and sensitivities are available. Use of penicillin prophylaxis or other prophylactic antibiotics to prevent Strep viridans should be in accordance with institutional guidelines.

The persistence of fever during broad spectrum antibiotic coverage or the emergence of a new fever in neutropenic patients with negative blood cultures warrants the initiation of IV antifungal treatment, unless other causes are apparent.

5.8.4 Colony Stimulating Factors

GCSF is not allowed during the administration of chemotherapy, and should be stopped 3 days prior to administration of clofarabine and cytarabine.
GCSF is allowed at investigator's discretion in case of febrile neutropenia or to shorten the duration of neutropenia, except that routine GCSF administration is NOT allowed after the 1st course to properly assess the duration of neutropenia after the 1st course. Only in case of life-threatening infections GCSF therapy can be initiated after consultation with the PIs of this study.

5.8.5 **Suppression of menstruation**

All menstruating females should receive oral contraceptives during the entire course of this protocol. Suppression of menses should be continued until the platelet count is 50,000/ul without transfusion support.

5.8.6 **Mucosal Evaluation and Care**

Mucositis may be severe on this regimen. Hence, liberal use of pain medications for this condition is encouraged. For patients with poor oral hygiene, consider consultation by Oral Surgery prior to initiating therapy since dental extractions/care may be necessary. Stomatitis and esophagitis due to Herpes virus may be confused with drug-induced mucositis. Hence, viral cultures and anti-viral treatment/prophylaxis should be considered according to institutional guidelines.

5.8.7 **Nutrition**

Active measures should be used to prevent weight loss of greater than 10% of pre-illness body weight. If possible, enteral feedings are preferred to parenteral. However, enteral feedings should be withheld at any time during therapy if gastrointestinal problems such as severe nausea, vomiting, or diarrhea, or signs and symptoms of illness (e.g. typhlitis) occur.

5.8.8 **Hyperleukocytosis and Metabolic Derangement**

Patients with high peripheral blast counts (> 100,000/ul) and significant organomegaly have increased problems related to metabolic abnormalities, bleeding, and hyperviscosity. Patients with high WBCs and/or evidence of disseminated intravascular coagulation (DIC) may be prone to more complications of general anesthesia. In these patients, platelets should be transfused in patients with platelet counts < 20,000. Hemoglobin levels should not be raised above 10 mg/dl ~ 6 mmol/l. The use of leukapheresis, exchange transfusion, or hemodialysis may be necessary with some patients.

5.9 **Concomitant Therapy**

No concomitant cytotoxic therapy or investigational therapy is allowed during the study other than prescribed in this protocol.

Clofarabine is excreted primarily by the kidneys. Therefore, drugs with known renal toxicity (e.g. vancomycin, amphotericin B, acyclovir, cyclosporin, methotrexate, tacrolimus, levaquin) should be administered cautiously and with close monitoring and should be avoided to the extent possible during the 5 days of clofarabine treatment.

Additionally, the liver is a known target organ for clofarabine toxicity. Therefore, concomitant use of medications known to induce hepatic toxicity (e.g. voriconazole,
cyclosporine, methotrexate, tacrolimus, levaquin) should be avoided to the extent possible or, if required, administered cautiously and with close monitoring during the 5 days of clofarabine treatment. Hepatic and renal function should be assessed prior to, during and after treatment with clofarabine and it is recommended that the patient’s fluid status and hepatic and renal function be carefully monitored during the drug administration period.

Use of alternative medications (eg, herbal or botanical) is not permitted during the entire study period.

For GCSF, see 5.8.4.

5.10 Duration of Therapy

There is no maximum to the number of cycles of study treatment, unless:

- there is evidence of disease progression (see chapter 7 for definitions)
- unacceptable toxicity occurs per investigator’s discretion
- general or specific changes in the patient’s condition render the patient unacceptable for further treatment per the investigator’s judgment
- the patient chooses to withdraw from the study
- the patient becomes pregnant or fails to use adequate birth control if able to conceive
- the patient is not able to comply with the protocol requirements
- the sponsor decides to terminate the study for significant risk-benefit concerns
- death or lost to follow-up

Subsequent stem cell transplantation

A stem cell transplantation is not part of the study protocol. Both the decision to transplant, plus the transplant procedure and conditioning regimen are therefore left to the discretion of the treating physician. We will however capture data on the SCT-procedure as well as follow-up data.

Once patients achieve a response following re-induction with this regimen some may go on to be transplanted per investigator discretion. However, in order to be evaluable for the study, patients need to have regenerated their counts before a SCT is scheduled after the 1st course. Hence, taking the patient off study after the 1st course when the patients is still in aplasia and not evaluable for response is considered a protocol violation.

However, it is allowed to proceed to SCT after the 2nd or subsequent course without awaiting full count recovery.

5.11 Intrathecal therapy

5.11.1 Prophylaxis

Cytarabine may be administered as central nervous system prophylaxis with each course. It needs to be administered intrathecally at age-adjusted doses (Table 5-3) via a lumbar puncture (LP). Because the combination of cytarabine and clofarabine may induce neurotoxicity, intrathecal prophylaxis should always be delayed to day 6, and only given when there are no concerns regarding neuro-toxicity that may potentially worsen by administration of intrathecal therapy. This delay to day 6 will also allow us to determine any
potential efficacy of the study treatment in CNS-2 patients. Whether any CNS-prophylaxis is given in subsequent courses is left to the discretion of the investigator. In case of neurotoxicity intrathecal prophylaxis should be delayed further, which is left to the discretion of the investigator.

**Table 5-3. Intrathecal medication (LP) in case of CNS prophylaxis**

<table>
<thead>
<tr>
<th>Age</th>
<th>Cytarabine, single dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1 year</td>
<td>20 mg</td>
</tr>
<tr>
<td>1&lt;2 year</td>
<td>26 mg</td>
</tr>
<tr>
<td>2&lt;3 year</td>
<td>34 mg</td>
</tr>
<tr>
<td>≥ 3 year</td>
<td>40 mg</td>
</tr>
</tbody>
</table>

**5.11.2 Treatment of CNS leukemia**

Patients with CNS3 status should be excluded when they are symptomatic or when they present with isolated CNS localization of disease.

Either single agent cytarabine (see under prophylaxis) or triple intrathecal medication at age-adjusted doses (Table 5-4) are allowed, by lumbar puncture. The first injection should be delayed until day 6, as it is not allowed to combine IV study treatment with intrathecal medication. The second and subsequent doses should be given every 7 days until 1 week after complete clearance of the CSF of leukemic blasts. Then, 2 more dosages must be given, if possible one at least 1 day before the beginning of the 2nd course, and the other after the 2nd course on day 6.

**Table 5-4. CNS leukemia: Intrathecal medication to be administered via lumbar puncture.**

Triple therapy or single agent cytarabine (see under prophylaxis for dosages) are allowed per investigator’s discretion.

<table>
<thead>
<tr>
<th>Age</th>
<th>Cytarabine mg/dose</th>
<th>Methotrexate mg/dose</th>
<th>Prednisolone mg/dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1 year</td>
<td>16</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>1&lt;2 year</td>
<td>20</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>2&lt;3 year</td>
<td>26</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>≥ 3 years</td>
<td>30</td>
<td>12</td>
<td>10</td>
</tr>
</tbody>
</table>

**5.12 Pharmacokinetics (PK)**

The aim is to determine clofarabine PK on day 1 and 5 of treatment to investigate the effects of daunorubicin and Ara-C on clofarabine PK.

Moreover, we will investigate CSF clofarabine levels, which should be sampled at the lumbar puncture on day 6. After 10 patients with available CSF levels we will determine
whether this part of the study is successful. If we cannot measure clofarabine CSF levels this part of the study will not be continued.

Resulting plasma concentration data will be modeled with previous data from patients receiving clofarabine alone. PK-sampling will only be done during the 1st course of treatment, and in all patients.

Considering blood samples (see table 5-5): we need 3 samples on days 1 and 5 of treatment, collected at time 0 (pre infusion), time 2 hours (end of clofarabine infusion), time 5 hours (pre Ara-C infusion), as well as a CSF sample and blood sample will be collected on day 6, preferably ~24 hours after the end of the last clofarabine infusion.

For sample processing, PK-kits and shipment of PK-samples: see the Procedure Manual.

**Table 5-5**: Clofarabine PK sampling schedule

<table>
<thead>
<tr>
<th>Day</th>
<th>Pre-dose</th>
<th>Hours Post-dose</th>
<th>Hours Post-dose</th>
<th>Hours Post-dose</th>
<th>CSF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>5</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Clofarabine 1</td>
<td>X</td>
<td>X*</td>
<td>X*</td>
<td>X*</td>
<td></td>
</tr>
<tr>
<td>Clofarabine 5</td>
<td>X</td>
<td>X*</td>
<td>X*</td>
<td>X*</td>
<td></td>
</tr>
<tr>
<td>Clofarabine 6</td>
<td>X</td>
<td>X*</td>
<td>X*</td>
<td>X*</td>
<td></td>
</tr>
</tbody>
</table>

* End of clofarabine infusion

# Just prior to cytarabine

@ One CSF and plasma sample, approximately 24 hours after the last clofarabine infusion, which finishes at noon on day 5 (see table 5-2)

The PK analysis will be done under supervision of Simon Joel, Cancer Pharmacology Group, Centre for Experimental Cancer Medicine, Institute of Cancer and the CR-UK Clinical Centre Barts and The London Queen, Mary's School of Medicine and Dentistry, London, UK.
6 STUDY PROCEDURES

6.1 Pre-treatment Evaluations

Complete initial work-up within 7 days prior to first treatment, including bone-marrow aspiration, lumbar puncture, and assessment of organ toxicity.

Note: Signed written informed consent must be completed prior to performing any study-related procedures.

6.2 Screening Evaluations/Procedures

These evaluations should be carefully reviewed to determine the patient’s eligibility for the study.

1. Signed, written informed consent
2. Complete physical examination, including vital parameters (temperature, pulse, blood pressure) height, weight and body surface area.
3. Medical history: Detailed documentation of disease and treatment history with outcomes.
4. Lansky or Karnofski performance status (Appendix B).
5. Concurrent medical conditions.
6. Adverse event assessment from time of signing informed consent.
8. Peripheral blood and bone marrow to assess morphology (smears)
   Note: in case of a dry tap a trephine biopsy is also acceptable
9. Peripheral blood and bone marrow for flowcytometry (immunological classification)
   Note: in case of a dry tap a trephine biopsy is also acceptable
10. Peripheral blood and bone marrow for cytogenetic analysis
11. Send peripheral blood, bone marrow and CSF cytospin slides for central review as described in the procedure manual (note: only morphology will be centrally reviewed; there is no centralized flowcytometry, minimal residual disease detection, radiology review or cytogenetics).
12. Serum chemistries: Electrolytes (sodium, potassium, calcium, magnesium, phosphate, chloride, and bicarbonate), blood urea nitrogen (BUN), creatinine, CRP, glucose, and liver function tests (aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, lactate dehydrogenase (LDH), uric acid.
13. Calculate creatinine clearance (see inclusion criteria)
14. Lumbar puncture for WBC, erythrocytes and cytospins for morphology
15. Extramedullary leukemia needs to be documented by sonography, CT- or MRI scan, including abdominal organomegaly
16. HRCT of the lungs (available HRCT within 3 weeks prior to enrollment are admitted)
17. Aspergillus serum test (galactomannan) (available test results within 3 weeks prior to enrollment are admitted)
18. Echocardiography (shortening fraction)
19. Pregnancy test
6.3 **On Study Evaluations 1st course of treatment**

1. Hematology: CBC with differential and platelet count and peripheral blood smear: twice weekly during the chemotherapy administration, after that at least weekly, but may be needed more frequently depending on clinical circumstances.

2. Serum chemistries: Electrolytes (sodium, potassium, calcium, phosphate, chloride, and bicarbonate), uric acid, BUN, creatinine, and liver function tests (AST, ALT, ALP, total bilirubin, LDH): every other day during chemotherapy administration, or more frequently when clinically indicated. After that once weekly until the next course, but may be needed more frequently depending on clinical circumstances.

3. Adverse events evaluated and recorded using the NCI CTCAE, version 3.0

4. Lumbar punctation day +6: repeat WBC, erythrocytes and smears to evaluate CNS-status. Note: there will be central morphology review of day 6 CSF morphology as described in the procedure manual.

5. PK sampling according to table 5-5.

6.4 **Response evaluation after 1st course**

1. Response evaluation may be performed as early as day +21 after the study medication was started. However, in case of persisting aplasia, response determination may be delayed to day +28.

2. Response evaluation should consist of the following evaluations:
   a. Bone marrow and peripheral blood examination using morphology and immunological classification
   b. Lumbar puncture: WBC, erythrocytes and cytospins for morphology
   c. All manifestations of extramedullary leukemia need to be re-evaluated using the same technique as done at initial diagnosis
   d. Send peripheral blood, bone marrow and CSF cytospin slides for central review as described in the procedure manual

3. If the patient is still in aplasia at day +28, a bone marrow aspirate needs to be done to rule out persisting leukemia.
   a. In case of persisting leukemia the investigator may decide to perform a full evaluation as mentioned above and carry on with a 2nd course of treatment, unless there are signs of disease progression, in which case the patient will come of study. For definitions see chapter 7)
b. In case of persisting aplasia without evidence of leukemia at day +28, the next evaluation may be delayed to day +42, to neutrophil recovery (ANC at least 1000x10^6/l), or to progressive leukemia, whichever occurs first.

6.5 Criteria to start subsequent courses of treatment

1. In case of CR: peripheral blood recovery to ANC at least 1000x10^6/l and platelets at least 80x10^9/l. In all other cases these criteria do not apply.

2. Must have recovered from the acute side-effects of previous courses, especially infectious complications, diarrhea and mucositis. See also dose-reduction guidelines.

3. No evidence of progressive disease

4. Ecocardiography should be performed before each course. In case of a SF below 28% additional courses may be given, but without liposomal daunorubicin.

6.6 On study evaluation subsequent courses of treatment

1. Complete physical examination, including vital parameters (temperature, pulse, blood pressure) height, weight and body surface area.

2. Lansky or Karnofski performance status (Appendix B).

3. Hematology: CBC with differential and platelet count and peripheral blood smear: twice weekly during the chemotherapy administration, after that at least weekly, but may be needed more frequently depending on clinical circumstances

4. Serum chemistries: Electrolytes (sodium, potassium, calcium, phosphate, chloride, and bicarbonate), CRP, BUN, creatinine, and liver function tests (AST, ALT, ALP, total bilirubin, LDH): every other day during chemotherapy administration, or more frequently when clinically indicated. After that once weekly until the next course, but may be needed more frequently depending on clinical circumstances.

5. Adverse events evaluated and recorded using the NCI CTCAE, version 3.0

6. Lumbar punction day +6 (per investigator’s discretion only): repeat WBC, erythrocytes and smears to evaluate CNS-status.

6.7 Response evaluation after subsequent courses of treatment

1. Response evaluation must be performed as mentioned in paragraph 6.4.

2. Response evaluation should consist of the following evaluations:
   a. Bone marrow and peripheral blood examination using morphology and immunological classification
   b. Lumbar puncture: WBC, erythrocytes and cytospins for morphology
   c. All remaining manifestations of extramedullary leukemia need to be re-evaluated using the same technique as done at initial diagnosis. Once in CR they do not need to be re-evaluated using CT or MRI, unless relapse is suspected.
d. Send peripheral blood, bone marrow and CSF cytospin slides for central review until CR has been documented (see procedure manual)

6.8 Off Study Evaluations

1. Complete physical examination, including vital parameters (temperature, pulse, blood pressure).
2. Lansky or Karnofski performance status (Appendix B).
3. Adverse event assessment
5. Peripheral blood and bone marrow to assess morphology (smears)
   Note: in case of a dry tap a trephine biopsy is also acceptable
6. Peripheral blood and bone marrow for immunological classification of leukemia
   Note 1: in case of a dry tap a trephine biopsy is also acceptable
   Note 2: in case of relapse which is demonstrable in peripheral blood by morphology and confirmed with flowcytometry, a bone marrow aspirate is NOT needed.
7. Serum chemistries: Electrolytes (sodium, potassium, calcium, magnesium, phosphate, chloride, and bicarbonate), blood urea nitrogen (BUN), creatinine, CRP, glucose, and liver function tests (aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, lactate dehydrogenase (LDH), uric acid.
8. Echocardiography (shortening fraction)

6.9 Follow up visits

All subjects will be followed q.3 months after end-of-treatment for date of progression (only the first occurrence of disease progression after study entry) and date of death.

If drug-related AEs are present at end-of-treatment, early follow-up visits are required at a maximum interval of 4 weeks until all such AEs resolve to baseline or CTC Grade $\leq 1$, or are deemed irreversible.

6.10 Study Calendar

See Appendix A.
7 DEFINITIONS AND CRITERIA FOR EVALUATION

7.1 Safety and toxicity

Safety and tolerability of study treatment will be reported for all treated subjects. Safety assessments will be performed during screening, prior to treatment, on Days 1-6, Day 14, and Day 21 (and weekly thereafter until recovery from aplasia or persistent leukemia is diagnosed) during each treatment cycle, and at the End of Study visit.

Safety assessments will include physical examinations, vital signs (systolic/diastolic blood pressure, pulse rate, and body temperature), clinical laboratory tests (hematology, serum chemistry), and reported or observed adverse events.

Treatment interruptions and discontinuations, and dose reductions for toxicity will be analyzed by dose level. Moreover, the frequency and severity of all laboratory abnormalities will be tabulated.

All laboratory test values captured as part of the study should be recorded on the appropriate laboratory test results pages of the CRF. In addition, in order for the principal investigators to collect additional information about clinically-important laboratory abnormalities, at a minimum, the following laboratory abnormalities recorded on an AE or SAE form as appropriate:
- Any laboratory test result that meets the criteria for a Serious Adverse Event
- Any laboratory abnormality that requires the subject to have investigational product discontinued or interrupted

It is expected that wherever possible, the clinical, rather than the laboratory term would be used by the reporting Investigator (e.g., fatigue rather than low hemoglobin value or tumor lysis syndrome, versus reporting individual chemistry values).

7.2 Adverse events

Adverse events (AEs) are defined as any untoward medical occurrence in a patients or a clinical investigation subject administered a pharmaceutical product, and which does not necessarily have a causal relationship with the treatment. An AE can therefore be any unfavorable and unintended sign, symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related. Pre-existing conditions which worsen during a study are to be reported as AEs. AEs will be assessed continuously and graded according to NCI Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 (a copy can be downloaded from the CTEP web site [http://ctep.cancer.gov/reporting/ctc.html]). Frequency and severity, and outcomes of AEs will be determined.
The causality relationship of the three drug combination and in particular of clofarabine to the AEs will be assessed by the investigator as either:

1. **Certain**: There is a reasonable causal relationship between the combined drug treatment and the AE. The event responds to withdrawal of study treatment (dechallenge), and recurs with rechallenge when clinically feasible.
2. **Probable**: There is a reasonable causal relationship between the treatment and the AE. The event responds to dechallenge. Rechallenge is not required.
3. **Possible**: There is reasonable causal relationship between the treatment and the AE. Dechallenge information is lacking or unclear.
4. **Not likely**: There is a temporal relationship to treatment administration, but there is not a reasonable causal relationship between the treatment and the AE.
5. **Not related**: There is not a temporal relationship to treatment administration (too early, or late, or study drug not taken), or there is a reasonable causal relationship between another drug, concurrent disease, or circumstance and the AE.

AEs should be followed to resolution or stabilization, and reported as SAEs as they become serious. AEs occurring in the first course will be tabulated separately from the 2nd or subsequent courses.

### 7.3 Serious adverse events

A serious adverse event (SAE) is any adverse drug experience that occurs at any dose and results in any of the following outcomes:

1. Death.
2. Life-threatening adverse drug experience.
3. Requires inpatient hospitalization or prolongation of existing hospitalization.
4. Persistent or significant disability/incapacity.
5. A congenital anomaly/birth defect.
6. Requires medical or surgical intervention to prevent one of the outcomes listed above.

**NOTE 1:**
- Pregnancy: Incidence of pregnancy is not considered a SAE; pregnancy must, however, be reported immediately by E-mail to the principal investigators of this study;
- Overdose: All cases of overdose must be reported immediately by E-mail to the principal investigators of this study.

**NOTE 2:**
Criteria for hospitalizations not reported as SAEs include admissions for:
- Planned as per protocol medical/surgical procedure
- Routine health assessment requiring admission for baseline/trending of health status documentation
- Medical/surgical admission for purpose other than remedying ill health state (planned prior to entry into study trial; appropriate documentation required)
- Admission encountered for other life circumstance that carries no bearing on health status and requires no medical/surgical intervention (e.g. lack of housing, economic inadequacy, care-giver respite, family circumstances, administrative)
- Admissions for protocol-scheduled procedures or blood product transfusions will not be considered SAEs.

An SAE report should be completed for any event where doubt exists regarding its status of seriousness.

7.4 Response Rate

The proportion of subjects with CR, CRi, NEL and PR (see 7.4.1. for definitions) will be determined after the first and second course of study treatment. However, we decided to lower the threshold for platelet regeneration to \(80 \times 10^9/l\), based on the recommendations of Creutzig et al. The response rates may be determined by dose-level, and by early versus late relapse.

7.4.1 Response definitions

Morphologic leukemia-free state (no evidence of leukemia or NEL): This designation requires less than 5% blasts in an aspirate sample with marrow spicules and with a count of at least 200 nucleated cells. There should be no blasts with Auer rods or persistence of extramedullary disease. The presence of a unique phenotype (by flow cytometry) identical to what was found in the pretreatment specimen (e.g., CD34, CD7 coexpression) should be viewed as persistence of leukemia if this occurs in >5% of cells.

Morphologic complete remission with incomplete blood count recovery (CRi): After chemotherapy, some patients fulfill all of the criteria for CR except for residual neutropenia (<1,000/µL) or thrombocytopenia (<80,000/µL). Although this category of response indicates activity, it should not be included with CR.

Morphologic complete remission (CR) requires that the patient achieve the morphologic leukemia-free state, and have an absolute neutrophil count of \(\geq 1,000/\mu\)L and platelets of \(\geq 80,000/\mu\)L. Hemoglobin concentration or hematocrit has no bearing on remission status, although the patient must be independent of transfusions. There should be no residual evidence of extramedullary leukemia.

Partial remission (PR). This designation requires all of the hematologic values for a CR but with a decrease of at least 50% in the percentage of blasts to 5-25% in the bone marrow aspirate. Thus, if the pretreatment bone marrow blast percentage was 50% to 100%, the percentage of blasts must decrease to a value between 5% and 25%; if the pretreatment blast percentage was 20% to less than 50%, they must decrease by at least half to a value of more than 5%.

Stable disease (SD). This includes all patients who do not qualify as PR or PD.

Progressive disease (PD). This designation requires an increase of at least 50% in the percentage of blasts in the bone marrow aspirate or in peripheral blood, or the occurrence of new disease localizations.
Treatment failure. Treatment failure includes those patients for whom treatment has failed to achieve less than a PR.

7.4.2 Time to and Duration of Responses

Time to response is defined as the time from first dose of study treatment until the first day the response criteria are met, computed only for subjects whose best response is CR, Cri, NEL or PR.

Duration of response will be computed from the first day all criteria are met for response until the date progressive disease (PD) is reported or death.

Overall Survival

Overall survival (OS) is defined as time from first dose of study treatment to date of death. All subjects will be followed until death or (if study drug is stopped) until 2 years after end-of-treatment. Subjects lost to follow-up will be censored on the last date the subject was known to be alive. Probabilities of survival will be estimated by the method of Kaplan and Meier and were compared using the log-rank test.

Event-free survival

Event-free survival (EFS) will be defined as the time between diagnosis and first event (relapse, death of any cause, failure to achieve remission or second malignancy) or date of last follow-up. Patients who were classified as treatment failures will be considered as failures at time zero. Probabilities of survival will be estimated by the method of Kaplan and Meier and were compared using the log-rank test.

7.5 Other Definitions

- CNS disease: $\geq 5$ white blood cells/mm$^3$ (ie, $\geq 5/\mu l$, or $\geq 15/3\mu l$) and unequivocal evidence of blasts on cytospin examination, and/or clinical (seizures, cranial nerve palsy and symptoms of increased cranial pressure or other signs/symptoms not readily explained by another disease) and/or radiological evidence of leukemic infiltration in the central nervous system. In all symptomatic cases, an MRI should be made.
- Early Death: Death during the first 3 weeks of treatment (ie, before the time that complete remission could have been documented). The cause of death (disease, therapy or both) should be recorded as well as the last known percentage of blasts in the BM.
- Relapse: After a documented CR/Cri/NEL, this designation is defined as a reappearance of leukemic blasts in the peripheral blood, or $\geq 5\%$ blasts in the bone marrow not attributable to any other cause (eg, bone marrow regeneration after consolidation therapy). In case of leukemic cells demonstrable in the peripheral blood, and confirmed with flowcytometry, a bone marrow aspirate is not needed to diagnose relapse. In case of CSF with $<$5 cells/mm$^3$, but with blasts on the cytospin examination, a second lumbar puncture should be performed at least 7 days later to confirm relapse. In the setting of recent treatment, if there are no circulating blasts and the bone marrow contains 5% to 20% blasts, a repeat bone marrow performed at
least a week later is necessary to distinguish relapse from bone marrow regeneration, unless there is confirmation of the leukemic origin with flow cytometry. In such instances the date of recurrence is defined as the first date that more than 5% blasts were observed in the marrow. The reappearance or development of cytologically proven extramedullary disease also indicates relapse.

- Early Relapse: Relapse within 12 months from initial/1st relapse diagnosis
- Late Relapse: Relapse later than 12 months from initial/1st relapse diagnosis
8 REGISTRATION OF PATIENTS

Subjects will be recruited from a population of children and adolescents treated at or referred to the investigational centers.

The site will enroll subjects into the study at the time of eligibility screening by contacting the Study Coordinating Center after informed consent is obtained.

Patient registration will be done by faxing the enrollment forms to:

**Clinical Trial Bureau Pediatric Oncology Erasmus MC**
Fax: +31-10-703.6681 (in case of fax problems the back-up number is +31-10-703.1134)
E-mail: research-kocr@erasusmc.nl

In case any enrollment issues need to be discussed prior to faxing the enrollment forms, please send an e-mail to both PIs simultaneously for discussion: c.m.zwaan@erasusmc.nl and reinhardt.dirk@mh-hannover.de, and send a copy to research-kocr@erasusmc.nl

It is the responsibility of the Investigator to ensure that the subject is eligible for the study before enrolling the subject.

The following information will be required when the fax call is placed to the Study Coordinating Center:
1. Gender, Date of Birth
2. Leukemia Type and Phase; Date of Diagnosis
3. Review of Eligibility Criteria
4. Expected Date of Study Treatment Start.

A subject number will be assigned at this time. The Clinical Trial Bureau will inform the investigator of the subject number and the dose to be used.

The first dose of study therapy should be administered within 3 days. Therefore enrollment can only be started once the investigational medicinal product is available at the site.
9 REGULATORY AND REPORTING REQUIREMENTS

9.1 Adverse Event Monitoring and Reporting

The principal investigator is responsible for monitoring the safety of patients who enroll in the study. All AEs occurring after any administration of the three drug combination will be followed until resolution. The descriptions and grading scales found in the revised NCI CTCAE version 3.0 will be used for adverse event reporting. A copy of the CTCAE version 3.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/reporting/ctc.html).

Adverse events, as well as serious adverse events (see 9.2), need to be reported until 30 days after the last administration of study medication, or until another treatment regimen is started, whichever occurs first. However, in the case of AEs occurring later than this deadline but which are considered related to the study medication by the investigator, such AEs still need to be reported.

9.2 Reporting Serious Adverse Events

All SAEs occurring during the study or within 30 days of the last administration of the combination chemotherapy treatment must be reported to the principal investigator within 24 hours of occurrence. Adverse events classified as "serious" must be recorded on the SERIOUS AE (SAE) page of the CRF and require expeditious handling and reporting (within 24 hours after occurrence) to The pediatric Oncology Trial Bureau to comply with regulatory requirements.

SAE reporting by TELEPHONE, FAX and E-MAIL:
CM Zwaan, M.D., PhD
Tel: +31-10-703.6568
Fax: +31-10-703.6801/1134
E-mail: c.m.zwaan@erasasmusmc.nl; and copy e-mail to: research-kocr@erasasmusmc.nl and reinhardt.dirk@mh-hannover.de.

Collection of complete information concerning SAEs is extremely important. If only limited information is initially available, follow-up reports are required. Thus, follow-up information which becomes available as the SAE evolves, as well as supporting documentation (e.g., hospital discharge summaries and autopsy reports), should be collected subsequently, if not available at the time of the initial report, and immediately sent using the same procedure as the initial SAE report. For ongoing SAEs a follow-up report should be sent at least once-monthly. The investigator is responsible for submitting these follow-up reports for all SAEs, until the SAE has resolved or until the patient’s condition stabilizes (in the case of persistent impairment), or the patient dies.

The principal investigators are responsible for reporting SAEs/SUSARS to the IRB or other applicable regulatory authority.
In accordance with local regulations, the trial bureau will notify Investigators of all AEs that are serious, unexpected, and certainly, probably, or possibly related to the investigational product. This notification will be in the form of a SUSAR report.

Upon receiving such notices, the Investigator must review and retain the SUSAR reports with the Investigator Brochure.

Where required by local regulations or when there is a central Institutional Review Board (IRB)/Independent Ethics Committee (IEC) for the study, the Sponsor will submit the SUSAR report to the appropriate IRB/IEC. The sponsor, together with the principal investigators, will determine if the informed consent requires revision.

9.3 Compliance with the protocol

The study shall be conducted as described in this approved protocol. All revisions to the protocol must be discussed with, and be prepared by the sponsor. The Investigator should not implement any deviation or change to the protocol without prior review and documented approval/favorable opinion from the IRB/IEC of an Amendment, except where necessary to eliminate an immediate hazard(s) to study subjects.

If a deviation or change to a protocol is implemented to eliminate an immediate hazard(s) prior to obtaining IRB/IEC approval/favorable opinion, as soon as possible the deviation or change will be submitted to:
- IRB/IEC for review and approval/favorable opinion;
- the sponsor;
- Regulatory Authority(ies), if required by local regulations.

Documentation of approval signed by the chairperson or designee of the IRB(s)/IEC(s) must be sent to the sponsor.

If the revision is a non-substantial amendment, investigators must inform their IRB(s)/IEC(s).

If an Amendment substantially alters the study design or increases the potential risk to the subject:
(1) the consent form must be revised and submitted to the IRB(s)/IEC(s) for review and approval/favorable opinion;
(2) the revised form must be used to obtain consent from subjects currently enrolled in the study if they are affected by the Amendment; and
(3) the new form must be used to obtain consent from new subjects prior to enrollment.

9.4 CRFs and Monitoring

The investigator will receive case report forms (CRF) for documentation. All relevant data collected during the study for all of the patients enrolled into the study have to be entered into these CRF’s by the responsible investigator or someone authorized by him in a timely manner so that they are clear and legible. The principal investigator will review all the CRF’s of each patient and confirm the completeness, medical correctness and plausibility of the
documented data by his signature on a special CRF page. The entries should be made with black ball-point pen.

Additions and corrections in the CRF will be dated and signed by the responsible physician or an authorised person. Reasons must be given for corrections that are not self-explanatory. Copies of the originals will be retained at the centre. If corrections and/or additions are needed after collection of the original CRF, a corresponding query has to be filled out on a Query Form and forwarded to the physician for his response. The Query Form is part of the CRF.

Monitors will ensure that the clinical trial is conducted, recorded, and reported in accordance with the protocol, ICH-GCP, and the applicable regulatory requirement(s). The sponsors may delegate monitoring activities to national co-sponsors in each of the participating countries, provided they are appropriately qualified and approved by the sponsor. The site of the sponsor will be monitored by one of the co-sponsors.

Representatives of the sponsor and/or co-sponsor must be allowed to visit all study site locations periodically to assess the data, quality and study integrity. On site they will review study records and directly compare them with source documents, and discuss the conduct of the study with the Investigator, and verify that the facilities remain acceptable. Source documents are defined as: patient files, letters, laboratory / histology records. The type and scope of monitoring will be defined in the Monitoring Manual and documented on study specific Source Data Verification Forms.

With his participation in the study the investigator is obligated to support the activities of the monitors, provide them with direct access to the files and give them the opportunity to inspect the laboratory facilities, storage of the investigational product, etc.

9.5 Quality assurance
Apart from the monitoring process described above, the sponsor may implement procedures to assure the quality of every aspect of the study. During the course of the study, external auditors contracted by the Sponsor may conduct an onsite audit visit.

In addition, the study may be evaluated by government inspectors who must be allowed access to CRFs, source documents and other study files. THE INVESTIGATOR MUST NOTIFY THE SPONSOR PROMPTLY OF ANY INSPECTIONS SCHEDULED BY REGULATORY AUTHORITIES, AND PROMPTLY FORWARD COPIES OF INSPECTION REPORTS TO THE SPONSOR.

9.6 Delegation of authority
The Investigator will maintain a Delegation of Authority Form in the Investigator File to document signatures and initials of all persons to provide a legal delegation of study specific principal responsibilities. Each member of study staff should sign to agree the acceptance of these, and each delegation should be signed by the Investigator.
9.7 Records retention

The confidentiality of records that could identify subjects must be protected, respecting the privacy and confidentiality rules in accordance with the applicable regulatory requirement(s).

The Investigator must retain investigational product disposition records, copies of CRFs and source documents for the maximum period required by applicable regulations and guidelines, or Institution procedures, or for the period specified by the Sponsor, whichever is longer. The Investigator must contact the sponsor prior to destroying any records associated with the study.

9.8 Institutional Review Board/Independent Ethics Committee (IRB/IEC)

Before study initiation, the Investigator must have written and dated approval/favorable opinion from the local IRB/IEC for the protocol, consent form, subject recruitment materials/process (e.g., advertisements), and any other written information to be provided to subjects. The Investigator or Sponsor should also provide the IRB/IEC with a copy of the Investigator Brochure or product labeling, information to be provided to subjects.

The Investigator or Sponsor should provide the IRB/IEC with reports, updates and other information (e.g., ESR, Amendments, Administrative Letters) according to regulatory requirements or Institution procedures.

Freely given written informed consent must be obtained from every subject or their legally acceptable representative prior to clinical trial participation, including informed consent for any screening procedures conducted to establish subject eligibility for the trial.

For minors, according to local legislation, one or both parents or legally acceptable representative must be informed of the study procedures and must sign the informed consent form approved for the study prior to clinical trial participation. The explicit wish of a minor who is capable of forming an opinion and assessing this information to refuse participation in, or to be withdrawn from, the clinical trial at any time should be considered by the Investigator.

9.9 Informed Consent Procedures

Preparation of the consent form is the responsibility of the Investigator and must include all elements required by ICH, GCP and applicable regulatory requirements, and must adhere to GCP and to the ethical principles that have their origin in the Declaration of Helsinki. The consent form must also include a statement that the sponsor and regulatory authorities have direct access to subject records.

Prior to the beginning of the study, the Investigator must have the IRB/IEC’s written approval/favorable opinion of the written informed consent form and any other information to be provided to the subjects.
The Investigator must provide the subject or legally acceptable representative with a copy of
the consent form and written information about the study in the language in which the subject
is most proficient. The language must be non-technical and easily understood. The
Investigator should allow time necessary for subject or subject's legally acceptable
representative to inquire about the details of the study, then informed consent must be signed
and personally dated by the subject or the subject's legally acceptable representative and by
the person who conducted the informed consent discussion. The subject or legally acceptable
representative should receive a copy of the signed informed consent and any other written
information provided to study subjects prior to subject's participation in the trial.

9.10 Update of Informed Consent

The informed consent and any other information provided to subjects or the subject's legally
acceptable representative, should be revised whenever important new information becomes
available that is relevant to the subject's consent, and should receive IRB/IEC
approval/favorable opinion prior to use. The Investigator, or a person designated by the
Investigator should fully inform the subject or the subject's legally acceptable representative
of all pertinent aspects of the study and of any new information relevant to the subject's
willingness to continue participation in the study. This communication should be
documented. During a subject's participation in the trial, any updates to the consent form and
any updates to the written information will be provided to the subject.
10  **STATISTICAL CONSIDERATIONS**

The intent of this study is to establish a recommended phase II dose of the combination of clofarabine, cytarabine and DaunoXome for children and adolescents with relapsed/refractory AML.

10.1  **Data set description**

The following data sets will be used in this study:

- **Enrolled subjects:** All subjects who signed the informed consent form. This dataset will be used for ineligibility tabulation.
- **Treated subjects:** All subjects who received at least one dose of study treatment. Demographic and baseline characteristics and safety analyses will be performed on all treated subjects.
- **Evaluable subjects:** All subjects who received at least one course of study treatment and who have had at least one on-treatment efficacy evaluation. Analyses of dosing and efficacy will be performed on the dataset of all efficacy-evaluable subjects.

10.2  **Safety**

Safety analysis includes frequency, severity, and relatedness of all AEs, frequency and severity of all laboratory abnormalities, frequency of dose interruptions, dose reductions and treatment discontinuation for toxicity, and use of concomitant medications.

All AEs occurring after any administration of the study drug will be followed until resolution. The descriptions and grading scales found in the revised NCI CTCAE version 3.0 will be used for adverse event reporting. A copy of the CTCAE version 3.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/reporting/ctc.html).

10.3  **Dose limiting toxicities**

Dose-limiting toxicities (DLTs) are adverse events (AEs) considered at least possibly drug-related and occurring within the first course of study treatment.

**Non-hematologic DLT** will be defined as:

- Any ≥ grade 3 study treatment related non-hematologic toxicity occurring in spite of appropriate medical management.
- Any non-hematologic laboratory abnormality of Grade 4, or Grade 3 lasting ≥ 7 days, and requiring treatment discontinuation or interruption or dose-reduction in subsequent courses.
- Any clinically-important toxicity of Grade ≥ 2 requiring treatment discontinuation or interruption ≥ 7 days or dose-reduction in subsequent courses.

The following will not be considered DLT: grade 3 nausea and/or vomiting that can be subsequently controlled, including by pre-medication (uncontrollable conditions will be considered DLT), alopecia, drug fever, anorexia, and transient grade 3 transaminase elevations that return to ≤ grade 1 within 7 days.
For hematologic toxicity, it is anticipated that the study treatment regimen will result in severe myelosuppression and its associated complications. Therefore, myelosuppression/pancytopenia and grade 3 febrile neutropenia will not be considered DLTs. However, prolonged myelosuppression will be considered a DLT, and is defined as grade 3 or 4 myelosuppression, which represents a worsening from baseline, lasting more than 42 days with evidence of a hypocellular marrow (marrow cellularity less than 5% without evidence of persisting or recurrent leukemia). DLTs may result in delay of subsequent treatment cycles, or dose adjustments.

10.4 Number of treated subjects

Safety will be assessed using a classical 3x3 design. A particular dose level will be expanded to 6 patients if one patient develops a grade 3 or 4 non-hematological toxicity out of 3 patients treated at that particular dose-level. Once this occurs, further dose-escalations needs to be halted until the dose has proven to be safe in the expanded cohort. If 2 patients in a cohort of no more than 6 develop dose-limiting toxicities (DLT) the maximal tolerated dose (MTD) has been reached. No further dose-escalation is allowed, and the next lower dose level will be expanded to 6 patients. If 6 patients have been treated already, we will further expand this dose-level to 10 patients in total at what will be the recommended phase II dose.

10.4.1 Revised statistical approach and sample size (amendment 1)

We first need to identify the recommended dose based on dose-level 1-4, as described in the original protocol. At the recommended dose we will expand to 10 patients to get a better assessment of toxicity.

So far we have recruited 13 patients into the study. The minimum number of patients we need to enroll to finalize this part of the study is 10 patients, and the maximum number of patients is 16. Therefore the total number of patients will vary between 23 and 29 patients.

Dose-level 5 can only be opened when dose level 1-4 has been finalized and when dose-level 4 was considered safe. Dose-level 5 will then be considered as a separate cohort, given the restricted patient population. We will apply the 3x3 design, as defined in the protocol. When there are no DLTs, we will also expand this cohort to 10 patients, applying the restricted inclusion criteria.

In cohort 5 the total number of patients for the entire study will then be 10 patients, and the maximum number will be 39 patients.

10.5 Preliminary Efficacy

Efficacy is a secondary objective, and although this regimen is expected to induce responses in some patients, the results will be descriptive, and no statistical modeling is done considering response rates.
Results will be described as the percentage of patients achieving overall response, which includes CR, CRi, NEL and PR. For responding patients, survival estimates will be computed using the method of Kaplan and Meier.
11 ETHICS

This study will be conducted in accordance with the ethical principles that have their origin in the current Declaration of Helsinki, and will be consistent with International Conference on Harmonization Good Clinical Practice (ICH GCP) and applicable regulatory requirements.

The study will be conducted in compliance with the protocol. The protocol and any Amendments and the subject informed consent will receive Institutional Review Board (IRB)/Independent Ethics Committee (IEC) approval/favorable opinion prior to initiation of the study.

The rights, safety and well-being of the trial subjects are the most important considerations and should prevail over interests of science and society.

Study personnel involved in conducting this trial will be qualified by education, training, and experience to perform their respective task(s), and will be authorized to perform study related procedures as laid down in the delegation of authority log.

Systems with procedures that assure the quality of every aspect of the study will be implemented.
12 END OF STUDY REPORT

The sponsor will notify the accredited METC and the competent authority of the end of the study within a period of 90 days. The end of the study is defined as the last patient’s last visit.

In case the study is ended prematurely, the sponsor will notify the accredited METC and the competent authority within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC and the Competent Authority.
13 PUBLICATION

The results of the clinical trial will be published after complete data collection and evaluation. Partial or preliminary results will not be published beforehand. Publications and/or presentations are to be initiated and/or authorized by the principal investigators, and will be prepared by the principal investigators as first and last author of the paper.

The following persons will be considered as co-authors:

- investigators who have recruited at least 1 patient into the trial. In case the journal we want to publish the paper in does not allow a large number of co-authors this may have to be restricted to investigators enrolling at least 2 patients.
- the co-investigators representing their country/national group as co-sponsor.

The final publication manuscript should be prepared within six months after last patient last visit. The co-authors must notify the main author in writing concerning their approval or proposed changes to the manuscript within four weeks after receiving the publication draft. Failing to do this, their approval will be assumed.

Any publication in the form of a lecture, poster or publication of data must basically be approved by the coordinating investigator. Such publication should generally not occur before the joint publication of the study group. Enquiries from the press and general public concerning study results may only be answered by the coordinating investigator of the clinical trial after consultation with the sponsor.
14 INVESTIGATOR SPONSOR AND CO-SPONSOR RESPONSIBILITIES AND PATIENT INSURANCE

Erasmus MC is the coordinating sponsor of the study. There is a co-sponsor for each country involved in the study.

The (co)-sponsor of each country will provide insurance or indemnity in accordance with the applicable regulatory requirements for all patients within that country.

Any investigator or co-investigator who signed this protocol agrees to carry out this research in accordance with the protocol approved by the ethic committee, GCP and regulatory requirements.

Study personnel involved in conducting this trial will be qualified by education, training, and experience to perform their respective task(s).

The investigator should provide the Sponsor with his CV.

The Sponsor has the right to prematurely discontinue the study for significant efficacy or safety problems and will notify the Investigator in writing, as well as the ethics committees and the competent authorities according to local law and regulations.
### APPENDIX A: STUDY CALENDAR

<table>
<thead>
<tr>
<th>Assessment</th>
<th>Screening Within 7 Days</th>
<th>Baseline</th>
<th>Treatment Phase</th>
<th>Additional courses</th>
<th>End of Treatment</th>
<th>Long Term Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Informed Consent</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Medical History/Concurrent Conditions</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height, Weight, BSA</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Physical examination/Vital Signs</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Performance Status</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Hematology</td>
<td>X</td>
<td>X</td>
<td>X (see 6.6)</td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Serum Chemistries(^a)</td>
<td>X</td>
<td>X</td>
<td>X (see 6.6)</td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Bone Marrow Aspirate(^**)</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>X'</td>
<td>X*</td>
</tr>
<tr>
<td>BM and PB for central review</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumbar puncture</td>
<td>X</td>
<td>X (day 6)</td>
<td>X (day 6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extramedullary leukemia(^*)</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>(sonography/CT/MRI)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>HR-CT lungs(^##)</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspergilus serum test (galactomannan)</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Echocardiography (shortening fraction)</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Pregnancy test</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Response Assessment/Confirmation of Continued Remission Status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Study Treatment Administration</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Concomitant Medications/Transfusions</td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Adverse Event Assessment</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
</tr>
</tbody>
</table>

\(^a\) Electrolytes (sodium, potassium, chloride, calcium, phosphate), BUN, creatinine, CRP, glucose, AST, ALT, alkaline phosphatase, total bilirubin, LDH and uric acid.

\(^*\) For morphology, flow-cytometry and cytogenetics; Trephine needed only in case of dry tap.

\(^\prime\) Only morphology and flow-cytometry

\(^\dagger\) PD may be diagnosed on PB when morphology is confirmed by flow-cytometry

\(^*\) Until documented CR

\(^##\) HR-CT: high resolution CT; within 3 weeks prior to enrollment. Obligatory in all patients.

\(^***\) According to local laboratory practice; within 3 weeks prior to enrollment. Obligatory in all patients.
APPENDIX B: PERFORMANCE STATUS CRITERIA

Please use the score which is most applicable for this particular child.

<table>
<thead>
<tr>
<th>Lansky score: for younger children</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fully active</td>
<td>100</td>
</tr>
<tr>
<td>Minor restriction in normal physical activity</td>
<td>90</td>
</tr>
<tr>
<td>Active, but tires more quickly</td>
<td>80</td>
</tr>
<tr>
<td>Both greater restriction and less time spent in active play</td>
<td>70</td>
</tr>
<tr>
<td>Minimal active play, busy with quieter activities</td>
<td>60</td>
</tr>
<tr>
<td>Gets dressed, but no active play, able to participate in all quiet play and activities</td>
<td>50</td>
</tr>
<tr>
<td>Mostly in bed, participates in quiet activities</td>
<td>40</td>
</tr>
<tr>
<td>In bed, needs assistance even for quiet play</td>
<td>30</td>
</tr>
<tr>
<td>Often sleeping, play limited to passive activity</td>
<td>20</td>
</tr>
<tr>
<td>No play, does not get out of bed</td>
<td>10</td>
</tr>
<tr>
<td>Unresponsive</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Karnofsky score: for older children</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal, no complaints, no evidence of disease</td>
<td>100</td>
</tr>
<tr>
<td>Able to carry on normal activities</td>
<td>90</td>
</tr>
<tr>
<td>Normal activity with effort</td>
<td>80</td>
</tr>
<tr>
<td>Cares for self, unable to carry on normal activity or to do active work</td>
<td>70</td>
</tr>
<tr>
<td>Requires occasional assistance, is able to care for most of own needs</td>
<td>60</td>
</tr>
<tr>
<td>Requires considerable assistance, frequent medical care</td>
<td>50</td>
</tr>
<tr>
<td>Disabled, requires special care/assistance</td>
<td>40</td>
</tr>
<tr>
<td>Severely disabled, hospitalization</td>
<td>30</td>
</tr>
<tr>
<td>Hospitalization, very sick, active treatment</td>
<td>20</td>
</tr>
<tr>
<td>Moribund, fatal processes in progression</td>
<td>10</td>
</tr>
<tr>
<td>Dead</td>
<td>0</td>
</tr>
</tbody>
</table>
15 REFERENCES


