Published in final edited form as:
Clin Lymphoma Myeloma Leuk. 2014 June ; 14(3): 231–238. doi:10.1016/j.clml.2013.12.001.

Phase 1 and Extension Study of Clofarabine plus Cyclophosphamide in Patients with Relapsed/Refractory Acute Lymphoblastic Leukemia (ALL)

S Faderl, MD[1], **K Balakrishnan, PhD**[2], **DA Thomas, MD**[2], **F Ravandi, MD**[2], **G Borthakur, MD**[2], **J Burger, MD**[2], **A Ferrajoli, MD**[2], **J Cortes, MD**[2], **S O'Brien, MD**[2], **T Kadia, MD**[2], **J Feliu, RN**[2], **W Plunkett, PhD**[2], **V Gandhi, PhD**[2], and **HM Kantarjian, MD**[2]

[1]Division of Leukemia, Hackensack University Medical Center, Hackensack, New Jersey

[2]Department of Leukemia and Experimental Therapeutics, The University of Texas M.D. Anderson Cancer Center, Houston, Texas

Abstract

Background—Clofarabine is a nucleoside analog with activity in children with ALL. Based on the hypothesis that clofarabine inhibits DNA repair following exposure to DNA damaging agents, we designed a phase 1 and extension study to evaluate the combination of clofarabine with cyclophosphamide in adult patients with relapsed/refractory ALL.

Methods—The continual reassessment method (CRM) was used to define the maximum tolerated dose (MTD).

Results—Fifty patients with a median age of 30 years (range 21–72 years) were enrolled of whom 30 patients were part of the phase 1 group. Clofarabine 40 mg/m^2 iv daily x 3 days and cyclophosphamide 200 mg/m^2 iv q 12 hours x 3 days were established as the MTD. Dose limiting toxicities were diarrhea, transaminase elevations, and skin rashes. The response rate of the whole study group was 14% including 10% of patients who achieved complete remission (CR) or CR without platelet recovery. Three responses occurred in patients with primary refractory disease. Early mortality (< 30 days) was 6%. The median response duration was 69 days (range 5–315 days). Median overall survival was about 3 months. Compared to day 1 (cyclophosphamide alone), H2AX phosphorylation was increased on day 2 when clofarabine and cyclophosphamide were administered as a couplet (n = 8).

Conclusions—The combination of clofarabine plus cyclophosphamide at the doses used in this study and in a group of heavily pretreated patients with ALL is only moderately effective. Other

Corresponding author: Stefan Faderl, MD, Division of Leukemia, John Theurer Cancer Center at Hackensack University Medical Center, 92 Second Street, Hackensack, NJ 07601, Tel. 551-996-5481, Fax 551-996-0574, SFaderl@hackensackumc.org.

Conflict of interest

Dr. Faderl received research support from Genzyme (Sanofi). All other authors state that they have no conflicts of interest.

doses, alternative schedules, or a more favorable patient population may achieve better results. (Word count: 248)

Keywords

clofarabine; ALL; salvage chemotherapy

Introduction

Outcome of patients with relapsed and/or refractory ALL remains poor with response rates of less than 30% depending on prior therapy and duration of first remission. Median disease free survival is in the range of 2 to 7.5 months and long-term survival remains exceptional[1]. No effective salvage strategies save stem cell transplant (SCT) exist. Clofarabine, a second generation deoxyadenosine analog, is one of the most recently approved drugs for children with ALL relapse[2]. In a study of 61 children (median age 12 years, range 1–20) with a median number of 3 prior therapies (range 2–6), the overall response rate was 30% including 20% of children who achieved either complete remission (CR) or CR with incomplete platelet recovery (CRp)[3]. Median response duration was 29 weeks (range 1–48) and nine children were able to proceed with a stem cell transplant.

The role of clofarabine in adult patients with ALL is less well defined. Limited experience from single agent phase 2 studies indicates less activity than in children[4]. Combination therapies may help to improve the activity of clofarabine in adults with ALL. Clinical and laboratory observations suggested synergistic activity between clofarabine and cyclophosphamide[5]. Cyclophosphamide causes DNA interstrand crosslinks, which are rapidly repaired limiting its activity[6]. We hypothesized that in addition to its intrinsic anti-ALL activity, pretreatment with clofarabine inhibits repair of cyclophosphamide DNA strand breaks thus augmenting the activity of cyclophosphamide. In a phase 1 clinical and laboratory study of clofarabine followed by cyclophosphamide Karp et al. reported responses in 4 of 6 (67%) patients with refractory ALL using a timed-sequential approach where treatment is delivered on days 1–3 and again on days 8–10, albeit at the cost of significant toxicity[7]. We designed a daily up to times 5 schedule of both drugs in a phase 1 study for patients with relapsed and refractory ALL followed by an expansion cohort to assess activity of the combination further.

Patients, Materials, and Methods

Study Group

Patients aged 21 years and older with a diagnosis of previously treated acute lymphoblastic leukemia (ALL, including Burkitt leukemia/lymphoma and lymphoblastic lymphoma) whose disease has either relapsed or who have been refractory to induction therapy were eligible for the study. The study was later amended so that first remission duration of patients who were in first relapse had to be shorter than 12 months. Patients were required to be off previous therapy for at least 2 weeks by the time of study enrollment. Concurrent treatment for relapse in the central nervous system (CNS) or CNS prophylaxis with intrathecal chemotherapy was permitted. Other eligibility criteria included 1) performance

status of at least 3 (Eastern Cooperative Oncology Group [ECOG] scale); 2) adequate organ function (serum total bilirubin ≤ 2.5 mg/dL, alanine aminotransferase (ALT) or aspartate aminotransferase (AST) ≤ 3 x the upper limit of normal, and glomerular filtration rate ≥ 60 mL/min); 3) absence of active heart disease (≥ class 3 NYHA) based on history and physical examination; and 4) a cardiac ejection fraction that was at least 45% (based on multigated acquisition scan [MUGA] or echocardiogram). Informed consent was obtained by every participant according to institutional guidelines. Approval for the study was granted from the institutional review board (IRB) of The University of Texas M.D. Anderson Cancer Center. The study was conducted in accordance with the basic principles of the Declaration of Helsinki.

Treatment Design

Four dose levels were defined. A DLT was defined as any ≥ grade 3 drug-related non-hematologic toxicity, which occurred within the first 14 days after start of treatment. Patients in dose levels one and two received clofarabine 40 mg/m^2 as a 1-hour intravenous infusion daily for 3 consecutive days starting on day 2 (Figure 1). Cyclophosphamide 200 mg/m^2 (dose level 1) and 300 mg/m^2 (dose level 2), respectively, was given as a 3-hour intravenous infusion every 12 hours for 6 doses on days 1 to 4. The first dose of cyclophosphamide was given by itself (without accompanying clofarabine) and then continued following about 2 hours after the start of the clofarabine infusions with subsequent doses. For dose levels three and four, the number of days was extended to 4 and 5, translating into 4 and 5 doses of clofarabine and 8 and 10 doses of cyclophosphamide, respectively. Patients could receive up to two induction cycles and a maximum of 6 consolidation cycles depending on leukemia response and resolution of toxicities. To continue on study, achievement of at least a partial response was required. Consolidation cycles were to be administered at a 25% lower dose than the induction cycle and could be repeated every 3 to 7 weeks. Patients who developed CNS leukemia while on study were removed unless their response to the study drug combination was judged beneficial enough to continue.

The pretreatment evaluation included history, physical examination, complete blood count (CBC) with differential and platelet count, a chemistry survey including at least creatinine, bilirubin, ALT or AST, and uric acid levels, and marrow evaluation. A MUGA scan or echocardiogram was required within thirty days of treatment start. Follow up studies included physical examination weekly during the induction phase and then prior to each subsequent course, CBC with differential and platelet count once to three times weekly during the induction and at least weekly during the consolidation. A chemistry profile consisting of creatinine, bilirubin, ALT or AST was required weekly during induction and at least once every two weeks during consolidation. The first repeat marrow was performed on day 14 (+/− 3 days) and then every 1 to 2 weeks until the response to induction could be assessed. Further marrow examinations were scheduled as indicated by the clinical and laboratory picture. MUGA or echocardiogram testing was repeated up to 8 weeks following the last dose of therapy.

Response Criteria

A complete remission (CR) required normalization of the marrow (≤ 5% blasts) and peripheral counts with no circulating blast cells, a neutrophil count of ≥ 1×10^9/L and a platelet count of ≥ 100×10^9/L. A CRp required criteria similar to a CR, but with platelet counts between ≥ 20×10^9/L and < 100×10^9/L. A partial response (PR) consisted of a blood count recovery as for CR, but with persistence of 5% to 25% marrow blasts. All other responses were counted as treatment failures.

Collection and Isolation of Peripheral Blood Mononuclear Cells (PBMCs)

Whole blood was collected in heparinized tubes during therapy at indicated time points. The specimen was diluted 1:3 with cold PBS (0.135 M NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4, 8 mM Na_2HPO_4 [pH 7.4]) and layered onto Ficoll-Hypaque (specific gravity, 1.086; Life Technologies, Grand Island, NY). The mononuclear cells were isolated as described before[8]. Cells were washed twice with cold PBS. A Coulter channelyzer (Coulter Electronics, Hialeah, FL) was used to determine cell number and the mean cell volume. These cells were used to measure H2AX phosphorylation and clofarabine triphosphate levels.

Measurement of H2AX Phosphorylation During Therapy

The PBMCs obtained during therapy were washed with PBS and fixed in 6~8 mL ice-cold ethanol (70%) and stored at −20 degree until analysis for H2AX phosphorylation. For this assay, the cells were fixed with fresh 4% PFA/PBS (pH 7.4) at RT for 10 min; After 2 washes with 1% BSA/PBS, the cells were blocked by gently shaking with PBS containing 5% GS/1% BSA at RT for 1 hr. This is followed by the incubation with primary antibody anti-gamma-H2AX (Ser139) [Upstate, Clone JBW301] mAb for 2 hr (gentle shake) and secondary antibody FITC-anti-mouse IgG [Jackson Laboratory] for 1hr RT (gentle shake); The labeled cells were washed twice with cold PBS, and were resuspended in 1 ml of PBS containing the counter-stain PI (15 μg/ml) and RNAase (Roche) (2.5 μg/ml) and incubated in the dark for 5 min at 37°C before analysis using FACScalibur (Becton Dickinson). Data were expressed as percent H2AX phosphorylation[7].

Intracellular Clofarabine Triphosphate Quantification

Nucleotides were extracted using 0·4 N perchloric acid method and were separated on an analytical ion-exchange column (Partisil-10 SAX, 4·6 × 250 mm; Whatman, Maidstone, England) and quantified using external authentic standards[8]. The compositions of the mobile phases were 5 mmol/l $NH_4H_2PO_4$, pH 2·8 (mobile phase A) and 0·75 mol/l $NH_4H_2PO_4$, pH 3·7 (mobile phase B). The analytes were eluted at 1·5 ml/min using the following gradient conditions: 0 min 50% B, 0–15 min linear increase to 55% B, 15–25 min linear increase to 100% B, and 25–35 min 100% B. The intracellular concentrations of nucleotides were calculated using total cell count and the median cell volume.

Statistical Analysis

The primary objective of this phase 1 study was to determine the dose-limiting toxicity (DLT) and maximum tolerated dose (MTD) of the combination of clofarabine and cyclophosphamide in patients with previously treated relapsed/refractory ALL. We

employed the continuous reassessment method (CRM) to assess the MTD from 4 possible dose levels[9]. Patients were enrolled in cohorts of 2 patients. Operating characteristics were defined so that patients would not continue at the current dose level (possibility of dose escalation) or be allowed to enter the next higher dose level if the probability of toxicities exceeded 30%. Accrual would be stopped if there was a greater than 95% chance that the probability of does limiting toxicity at the current dose level was more than 0.30 and the dose level below would be defined as MTD. Once MTD was established, further patients were enrolled in an expansion cohort until at most 25 patients were treated at the MTD. The method of Thall and Simon was employed for interim safety monitoring of the expansion cohort[10]. Kaplan-Meier methodology is used to describe time-to-event outcomes.

Results

Study Population

Fifty patients were enrolled and treated, 30 in the phase 1 and 20 patients in the phase 2 part. Their characteristics are summarized in Table 1. The median age of the patients was 29 years and 31 years, respectively. The majority of patients had precursor B cell ALL followed in frequency by patients with T cell and mixed lineage ALL. The latter group of patients all had a diagnosis previously established as ALL and were treated with ALL regimens in the past. The median first remission duration of all patients was about 11 months. Patients received a median of 2 (phase 1) and 3 (phase 2) prior regimen. Overall, patients were extensively pretreated with most patients entering the study as their second or higher salvage regimen. Only one patient received a prior hematopoietic stem cell transplant. Various karyotype abnormalities were identified in 35% to 43% of patients. Three patients carried a Philadelphia translocation.

Definition of DLT and MTD (Phase I)

The first two patients were assigned to cohort 1 (clofarabine 40 mg/m^2 × 3 and cyclophosphamide 200 mg/m^2 × 6 doses). Patient 1 experienced a grade 3 elevation of total bilirubin and grade 3 diarrhea. No further ≥ grade 3 toxicities occurred in the second patient. Patients 3 to 8 were then enrolled on dose level 2 (clofarabine 40 mg/m^2 × 3 and cyclophosphamide 300 mg/m^2 × 6 doses). Three of the six patients demonstrated DLTs including diarrhea, skin rash, and elevation of transaminases. Whereas patients 9, 15, and 16 were treated at the first dose cohort, all other patients remained assigned to cohort 2. Only among the latter occurred further ≥ grade 3 toxicities such as nausea/vomiting, diarrhea, transaminase elevelations, and in one patient a grade 4 increase of lipase in the absence of the clinical manifestations of pancreatitis. Study drug-related toxicities by dose cohort are summarized in Table 2. As dose level 2 was considered too toxic (based on operating characteristics of CRM design), the doses applied for cohort 1 were established as the MTD and no escalation beyond dose level 2 was pursued.

Efficacy

Seven patients responded (14%). Five patients (10%) achieved CR and one patient CRp and a marrow CR, respectively (Table 3). Except for one patient with CR all the remainder of the responses occurred in dose cohort 2. Three of the responses (2CR, 1 CRp) occurred in

patients with primary refractory disease. All of these 3 patients were refractory to intensive combination therapy (including HCVAD in two). Among the relapsed patients, the median first remission duration was 8.6 months (range 5.2 to 13.3 months). Six patients achieved the response following once cycle of therapy. The median number of days to response was 50 (range 29 to 89 days). One patient required 2 cycles after the first induction cycle resulted in a partial response. The median response duration was 69 days (range 5 to 315 days). The longest response duration of 315 days occurred in one of the patients with primary refractory disease (no response to HCVAD induction).

Three patients died (days 2, 5, and 16) during the induction course. All of these patients were part of cohort 2 of the phase 1 portion of the trial. No further early deaths occurred at the phase 2 doses in the expansion cohort. Two patients died in remission. All deaths were due to myelosuppression and associated infectious complications. As myelosuppression was worsened on chemotherapy, myelosuppression-related toxicities are indirectly related to the study drug combination. This should, however, be viewed in the context of patients with minimal marrow reserve based on their leukemia status. Median overall survivals of the phase 1 and 2 groups are shown in Figure 2. There were no significant differences between the groups.

Laboratory Endpoints

The protocol was designed to interrogate the effect of clofarabine on cyclophosphamide-induced DNA damage response which was measured by H2AX phosphorylation. Among eight patients' samples analyzed for H2AX phosphorylation, prior to treatment H2AX values were median 1.4% (range 0.3 to 4.6%, n=8). After first infusion of cyclophosphamide, the values increased on first day, the peak values were median 5.4 % (range 0,2 to 16.9%). The peaks occurred at different times (Figure 3). On the second day when cyclophosphamide was preceded by clofarabine, the median value was 25% (range 0.3 to 45.9%). Percentages of H2AX–positive of one representative patient samples (UPN 2) are shown in Figure 4. Clofarabine triphosphate concentrations in circulating ALL PBMCs ranged between 5 and 19 μM. None of these patients responded to therapy.

Discussion and Conclusion

We established clofarabine 40 mg/m^2 iv daily x 3 doses plus cyclophosphamide 200 mg/m^2 x 6 doses as the recommended phase 2 dose for this combination. It should, however be noted that the activity at was generally low and it is questionable whether this particular combination at this particular dose should be evaluated further in a larger study. DLTs included gastro-intestinal, hepatic, and skin-related adverse events. Neutropenia in the context of myelosuppression and the expected infectious complications were frequent. The latter were rather related to the far-advanced disease process and its attendant poor marrow reserve and compromised immune function. We describe responses in 14% of our patients including 5 patients (10%) who have achieved a complete remission. However, responses were short-lived with a median of 69 days and the median survival of the whole group of patients in the range of 3 months. Surprisingly on the other hand was that the longest-lasting response (315 days) occurred in one patient with ALL which was primary refractory to a

standard HCVAD induction indicating the potential for activity of this combination in subsets of patients whose characteristics remain undefined.

The activity of the combination of clofarabine with cyclophosphamide was lower in our study than what we were hoping to see. Clofarabine has single agent activity in children with ALL and there remains a compelling pre-clinical rationale for this combination. Clofarabine impairs DNA damage repair that follows the generation of DNA strand breaks from exposure to cyclophosphamide.

This concept was also explored by Karp et al in a phase I study in adult patients with refractory acute leukemias[7]. Included were 6 patients with ALL (in 3 patients disease recurred while on maintenance whereas 2 patients relapsed after an allogeneic stem cell transplant). Four of the patients responded including 3 patients who achieved a complete response. The investigators used a more intensive induction where the couplet of clofarabine and cyclophosphamide was administered on days 0 to 3 and again on days 8 through 10. Except for days 0 and 1 where the dose of cyclophosphamide was 200 mg/m^2, it was administered at 400 mg/m^2/dose. The starting dose of clofarabine was 20 mg/m^2/day, but required reduction to 10 mg/m^2/day because of prolonged aplasia which led to a number of adverse events. Similar to this investigation, there was an increase in H2AX phosphorylation after first infusion of cyclophosphamide. This was further induced after the second dose of cyclophosphamide which was preceded by clofarabine. As reported before, the clofarabine triphosphate value was heterogeneous[8]. Induction of H2AX phosphorylation is possibly caused by clofarabine incorporation into nucleotide excision repair of cyclophosphamide adducts[11], which could be increased by inhibition of ribonucleotide reductase by clofarabine triphosphate[6,11,12]. Alternatively, this may be indicative of double strand breaks generated secondary to failed repair of cyclophosphamide interstrand crosslinks[13]. While these cells are a low cycling population with low ribonucleotide reductase, increase in ribonucleotide reductase protein levels have been reported in quiescent lymphocytes by chlorambucil [14] and cyclophosphamide [15].

In a phase II study of 25 children (median age 14 years) with relapsed/refractory ALL, Hijiya et al. combined clofarabine and cyclophosphamide with etoposide[16]. All drugs were given at a daily x 5 schedule with clofarabine 40 mg/m^2/day, cyclophosphamide 440 mg/m^2/day, and etoposide 100 mg/m^2/day. Sixty percent of the patients had refractory disease and 84% had received at least 2 prior treatment regimens. Forty-four percent of the patients responded (28% complete responses) with a median duration of response of 67.3 weeks. Ten patients were able to proceed to secondary therapy in the form of a stem cell transplant.

More recently and also more relevant for adult patients with ALL, a GRAALL multicenter study evaluated clofarabine combinations in the context of intensive anti-ALL therapy[17]. The so-called “ENDEVOL” schedule explored clofarabine 30 mg/m^2 daily x 5 days plus cyclophosphamide 300 mg/m^2 daily x 3. This schedule included 18 patients with a median age of 55 years (18–78). Six percent were primary-refractory and 44% of the patients were at least in their second relapse; 44% had received a prior stem cell transplant. The complete remission rate was 50% including 3 of 8 patients who relapsed after transplant. No conclusions can be drawn with regard to response duration and overall survival as these

parameters were presented for all patients including those receiving the “VANDEVOL” regimen, which is a more intense multidrug regimen including clofarabine and which was primarily administered to younger adult patients.

The various studies have obvious differences in their design and patient populations. In the present study, up to one third of the patients were primary refractory and up to 80% received the study combination as their second or subsequent salvage attempt. Also, the dose and schedule of clofarabine in combination with cyclophosphamide as in the current study may not have been optimal and higher dose of clofarabine (over several more days) such as in the French ENDEVOL study could have resulted in better results. Despite the moderate response rates in the current study, based on the aggregate experience, there appears to be a benefit of clofarabine in the context of combination regimens in adult patients with ALL. It will remain difficult to accurately assess the contribution of each individual agent within complex regimens.

Acknowledgments

Authors are thankful to Mary Ayers, Yuling Chen, Min Du, Brett Ewald and Min Fu for participating in pharmacodynamic analyses.

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Clinical Practice Points

Clofarabine is a nucleoside analog with activity in children with relapsed and/or refractory ALL. There is fairly limited clinical experience of clofarabine in adults. Given the scant treatment choices and poor prognosis of adult patients in this situation, trying to find new therapies is paramount and clofarabine is an obvious candidate. In an effort to try and augment single agent activity, we designed a combination study of clofarabine with cyclophosphamide. The DLTs included diarrhea, elevated transaminases and skin rashes, which defined the initial dose cohort as the MTD. Given this dose and the schedule chosen, the response rate was 14%. Even though some patients with primary refractory disease following a hyper-CVAD induction were able to achieve responses, the congregate experience suggests only modest clinical activity. Whether different doses and/or schedules or combinations with other drugs may be more effective remains to be investigated in future clinical studies.

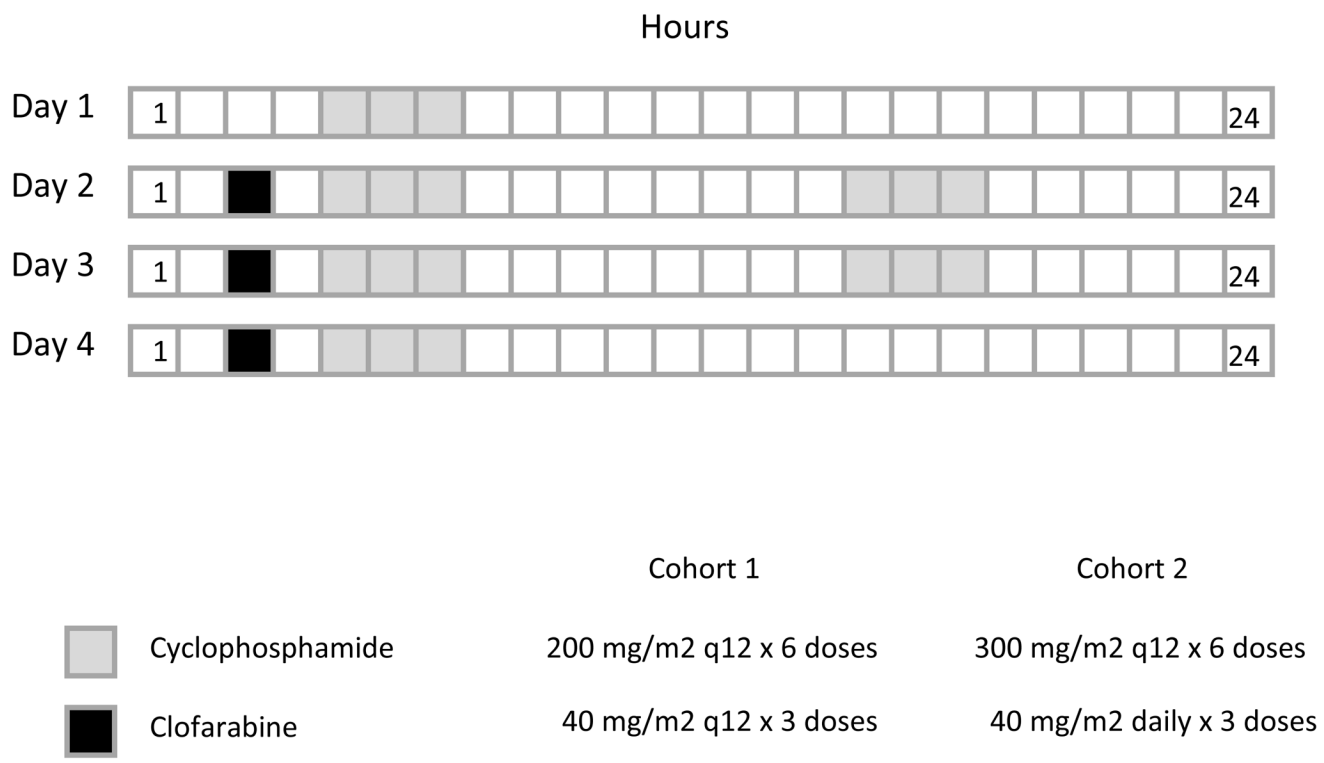

Figure 1.
Treatment design

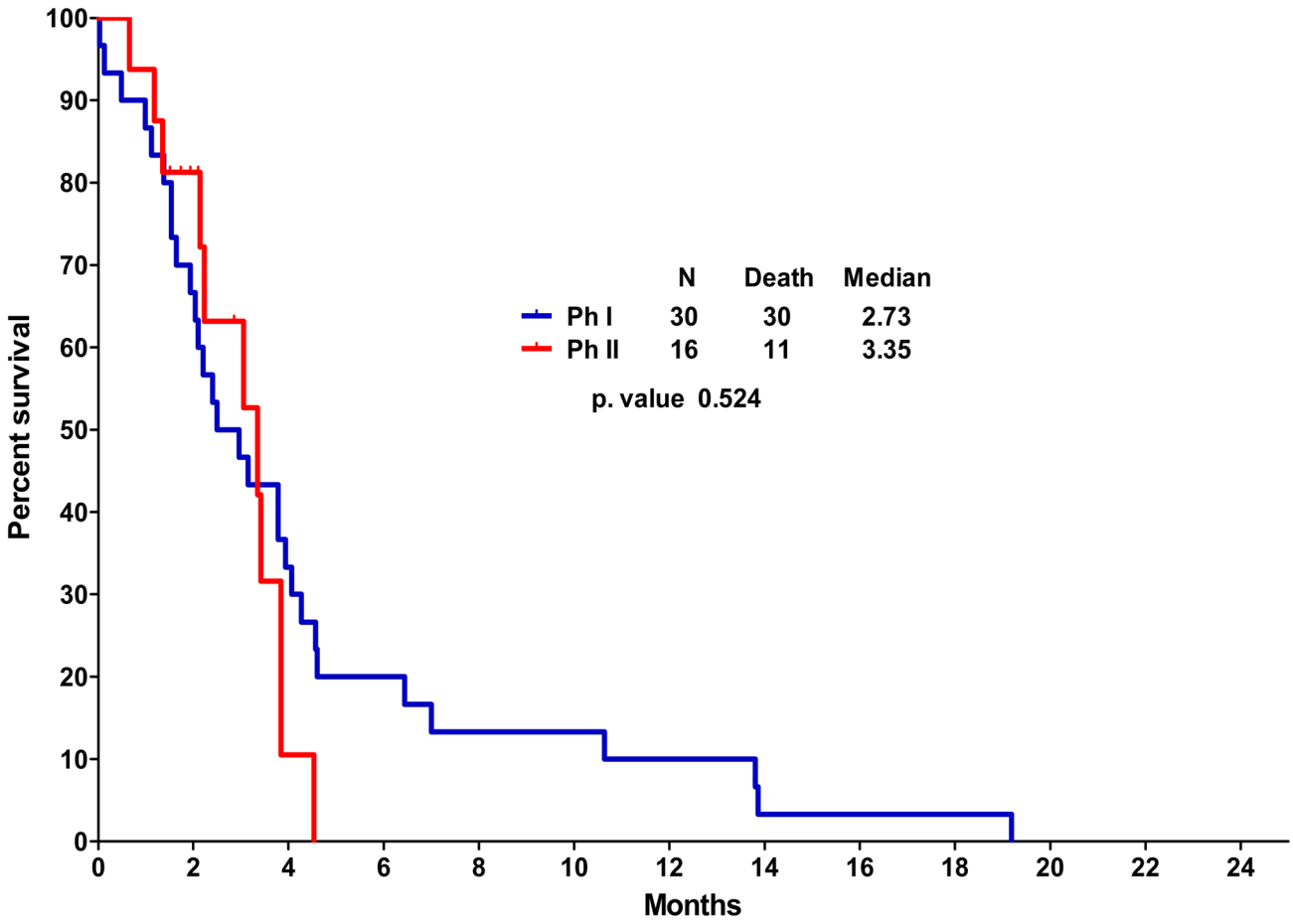

Figure 2.
Kaplan-Meier Curve for overall survival.

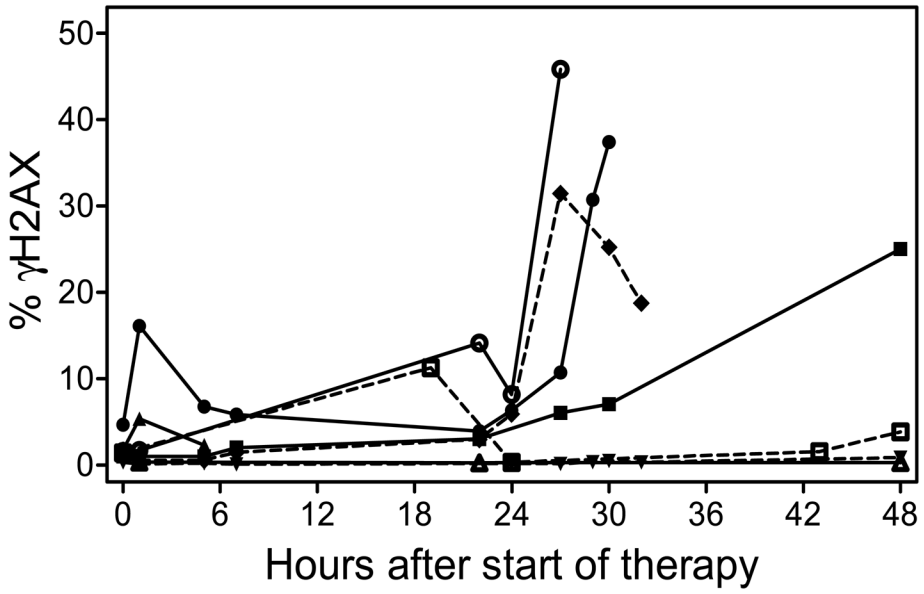

Figure 3.

Percent H2AX phosphorylation during the first 2 days of therapy. Data are summarized for all patients (n=8). On the first day at 0 hr, cyclophosphamide was infused alone. At 24 hours, cyclophosphamide was preceded by clofarabine. H2AX phosphorylation was measured as described under material and methods.

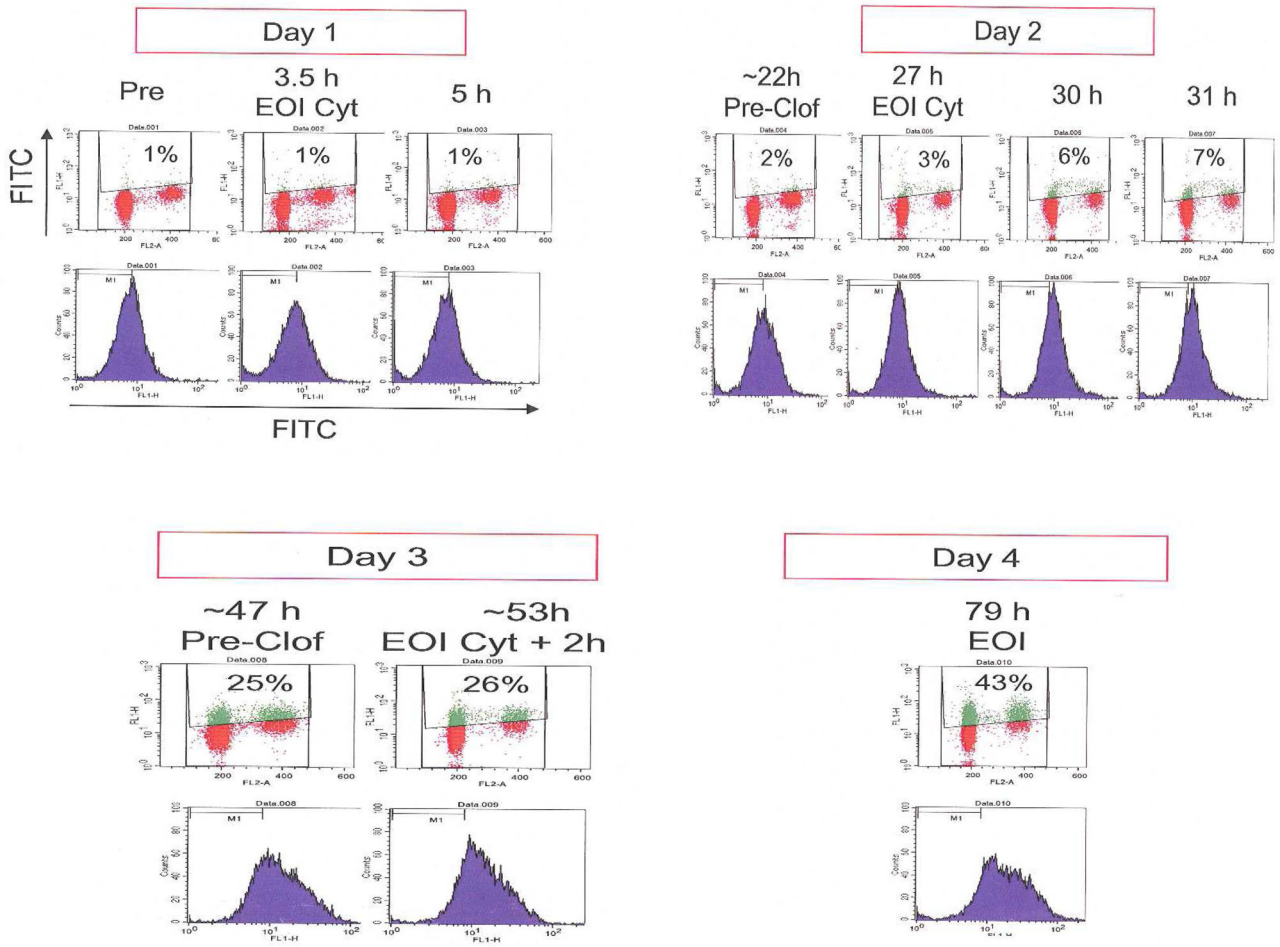

Figure 4.

Percentages of H2AX phosphorylation are shown for one representative patient sample (UPN 2).

Table 1

Patient Characteristics

	Numerical Value	
Characteristic	**Phase 1**	**Phase 2**
N	30	20
Median age, yrs (range)	29 (21–72)	31 (22–69)
Male/female	19/11	9/11
Diagnosis (%)		
pre-B	21 (70)	13 (65)
mature B	1 (3)	1 (5)
T	5 (17)	4 (20)
Mixed phenotype [1]	3 (10)	2 (10)
Extrameduallary disease [2] (%)	6 (20)	2 (10)
Disease status (%)		
Primary refractory	5 (17)	6 (30)
Salvage 1	6 (20)	1 (5)
Salvage 2+	24 (80)	19 (95)
Median CRD1, mos (range)	11.4 (1–39)	10.7 (4.3–46.4)
Median number of prior therapies (range)	2 (1–6)	3 (1–5)
Karyotype (%)		
Diploid	12 (39)	12 (60)
Abnormal	13 (43) [3]	7 (35)[4]
IM/not available	5 (16)	1 (5)

[1] with previous diagnosis of and treatment for ALL

[2] breast, mediastinum/retroperitoneal, testicular, skin, CNS, pleural fluid

[3] includes one patient with t(9;22)

[4] includes two patients with t(9;22)

CRD1, first complete remission duration; IM, insufficient metaphases

Table 2

Non-Hematologic Toxicities (Phase 1)

	Cohort 1 (N=5)		Cohort 2 (N=25)	
Toxicity	**≤ 2, N (%)**	**> 2, N (%)**	**≤ 2, N (%)**	**> 2, N (%)**
Nausea/vomiting	4 (80)	-	22 (88)	1 (4)
↑ transaminases	3 (60)	1 (20)	15 (60)	3 (12)
↑ bilirubin	1 (20)	-	11 (44)	4 (16)
Diarrhea	-	1 (20)	8 (32)	3 (12)
Mucositis	1 (20)	-	3 (12)	-
Rash	-	-	3 (12)	-
Hand-foot syndrome	1 (20)	-	-	-
Headache	1 (20)	-	3 (12)	-
↑ creatinine	-	-	1 (4)	1 (4)
↑ lipase	-	-	-	1 (4)
Neuropathy	-	-	1 (4)	-

Table 3

Characteristics of Responding Patients

UPN	2	17	19	21	34	42	46
Age/Gender	70/M	21/M	25/M	21/M	65/M	38/M	29/F
Diagnosis	Pre-B	Pre-B	Pre-B	Pre-B	Bi	Pre-B	Pre-T
Salvage #	2	3	1	2	3	3	4
CRD1 (m)	0	13.3	5.2	6.4	10.7	0	0
Karyotype	Complex	IM	Ph	Diploid	Diploid	Ph/-5/-7	Diploid
Cycles to response	2	1	1	1	1	1	1
Response	CR	CR	CR	BMCR	CR	CR	CRp
Response duration (d)	44	29	43	46	55	57	89

UPN, unidentified patient number; Bi, biphenotypic acute leukemia; CRD1, duration of first complete remission (prior to study entry); IM, insufficient metaphases; Ph, Philadelphia chromosome; BMCR, marrow CR

Clin Lymphoma Myeloma Leuk. Author manuscript; available in PMC 2015 June 01.