Published in final edited form as:

Br J Haematol. 2012 July ; 158(2): 198–207. doi:10.1111/j.1365-2141.2012.09142.x.

Phase 1 Dose- Escalation Trial of Clofarabine Followed by Escalating Dose of Fractionated Cyclophosphamide in Adults with Relapsed or Refractory Acute Leukaemias

Amer M Zeidan, Rebecca M Ricklis, Hetty E Carraway, Hyun D Yun, Jacqueline M Greer, B Douglas Smith, Mark J Levis, Michael A McDevitt, Keith W Pratz, Margaret M Showel, Douglas E Gladstone, Steven D Gore, and Judith E Karp

Division of Hematologic Malignancies, the Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Department of Oncology, Baltimore, Maryland 21287

Summary

The prognosis of patients with relapsed and refractory acute leukaemia (RRAL) is very poor. Forty patients with RRAL were enrolled (28 acute myeloid leukaemia [AML], 12 acute lymphoblastic leukaemia [ALL]) in this Phase 1 dose-escalation trial of daily-infused clofarabine (CLO) followed by cyclophosphamide (CY) for 4 consecutive days (CLO-CYx4). The median age was 48.5 years. The median number of prior regimens was 2 (range 1–5), and 6/40 patients (15%) had prior allogeneic haematopoietic stem cell transplant. 28/40 patients (70%) had adverse genetic features. 6/40 patients (15%) died within 60 days of induction (2 infections, 4 progressive disease). The average time to neutrophil recovery (absolute neutrophil count $0.5 \times 10^9/l$ was 34 days, (range, 17–78). The overall response rate (ORR) was 33% (13/40), with 7 complete remissions (18%), 4 complete remissions with incomplete recovery of blood counts (10%), and 2 partial remissions (5%). ORR was 25% (7/28), and 50% (6/12), for AML and ALL, respectively. Notably, the clinical responses were independent of dose level. 7/17 patients (41%) exhibited CLO-mediated enhancement of CY-induced DNA, which was associated with, but not necessary for, improved clinical outcomes. In summary, the CLO-CYx4 regimen was well tolerated and had activity in patients with RRAL, especially relapsed ALL. Therefore, CLO-CYx4 can be considered a salvage therapy for adults with RRALs, and warrants further investigations.

Keywords

refractory leukaemia; clofarabine; cyclophosphamide; acute lymphoblastic leukaemia; acute myeloid leukaemia

Introduction

Refractory and relapsed acute leukaemias (RRALs) are characterized by poor response to salvage therapy and dismal outcomes. Clearly, development of more effective therapeutic modalities is vital to improve the outcomes of these patients. Clofarabine (CLO) is a second-

Corresponding Author: Judith E Karp, Division of Hematologic Malignancies, 1650 Orleans Street, Room 2M44, CRB1, Baltimore, Maryland 21231. Jkarp2@jhmi.edu. Phone: 410-502-7726. Fax: 410-614-1005.

This study was presented in part at the 53rd Annual American Society of Hematology meeting in San Diego, December 10–13, 2011.

A.M.Z. analysed the data and wrote the manuscript. J.E.K. and R.M.R. designed and performed research, analysed data, and helped with writing the paper. H.E.C. and H.D.Y. analysed the data and revised the manuscript. J.M.G. performed research, collected data, and assisted with data analysis. B.D.S., M.J.L., S.D.G., M.A.M., K.W.P., M.M.S., and D.E.G., performed research and reviewed the manuscript.

generation purine nucleoside analog that was rationally designed to overcome the limitations and incorporate the best qualities of the first-generation deoxyadenosine analogs fludarabine and cladribine (Carson *et al*, 1992). CLO has activity as a single agent and in combination with other drugs against refractory and relapsed acute lymphoblastic leukaemia (rrALL) (Jeha *et al*, 2006, McGregor *et al*, 2009, Advani *et al*, 2010). Similarly, CLO is active, as a single agent or in combination, in refractory and relapsed acute myeloid leukaemia (rrAML) and in poor-risk newly diagnosed AML (Faderl *et al*, 2005, Faderl *et al*, 2008b, Faderl *et al*, 2008a, Krawczyk *et al*, 2010, Agura *et al*, 2011, Tse *et al*, 2011). The cytotoxic effects of CLO are mediated by multiple mechanisms of action, with the major effects being inhibition of ribonucleotide reductase and DNA polymerases following the incorporation of CLO into DNA, leading to inhibition of DNA synthesis and repair (Parker *et al*, 1991 Xie & Plunkett, 1996). CLO depletes cellular deoxynucleotide triphosphate pools and enhances the incorporation of CLO 5'-triphosphate (CLO-TP) into DNA, which leads to termination of DNA chain prolongation (Parker *et al*, 1991, Lotfi *et al*, 1999). Cyclophosphamide (CY) is an alkylating agent that primarily induces DNA inter-strand cross-links, but these cross-links can be quickly repaired in malignant cells (Yamauchi *et al*, 2001). Phosphorylation of histone variant H2AX occurs as an early event in response to DNA double-strand breaks (Banath & Olive, 2003). Therefore, the degree of accumulation of phosphorylated H2AX (H2AX) has been used as a proxy for persistently-damaged and unrepaired DNA (Byrd *et al*, 2002). We have previously shown that administration of CLO prior to CY in a timed-sequential fashion enhanced the levels of DNA double-strand breaks induced by CY and resulted in enhanced blast cytotoxicity and apoptosis in RRAL, leading to objective responses in 67% and 25% of patients with rrALL and rrAML, respectively (Karp *et al*, 2007).

Unfortunately, the timed-sequential CLO-CY regimen was associated with significant haematological toxicity independent of CLO dosing. This observation was consistent with the possibility of stem cell toxicity related to recruitment and resulting in increased sensitivity to CLO-CY mediated cytotoxicity. Therefore, we hypothesized that a conventional dosing regimen of CLO followed by CY on a daily basis for 4 days (CLO-CYx4) would reduce haematological toxicity while maintaining clinical effectiveness. In this paper, we report the results of the 40 patients enrolled prospectively on this regimen.

Materials and methods

Patient eligibility and selection

This study was a single-institution, modified “3+3” dose-escalation Phase 1 trial of daily CLO followed by fractionated CY in a 4-day regimen (CLO-CYx4) in adults ≥ 18 years of age with RRAL. The original study and its subsequent amendments were approved by the Johns Hopkins Medical Institutional Review Board (IRB). All patients provided informed consent, and the study was conducted in accordance with regulations of the local IRB and the Declaration of Helsinki. The study was registered with the National Cancer Institute at <http://clinicaltrials.gov> with identifier NCT00293410. The eligibility and exclusion criteria have been described previously (Karp *et al*, 2007). Patients with prior allogeneic haematopoietic stem cell transplantation (allo-HSCT) were eligible if they exhibited no evidence of graft-versus-host disease. There were no upper limits on age or on the number of prior relapses.

Treatment plan

This study was designed to determine the maximum-tolerated dose (MTD) and assess the toxicity of CLO-CYx4 regimen in patients with RRAL. Four cohorts of patients received escalating doses of CLO followed by fractionated CY at 4 dose levels (DLs). The 4 DLs

were chosen based on our previous results (Karp *et al*, 2007), and each DL enrolled 6 to 7 patients. If dose-limiting toxicities (DLTs) were noted in < 2 patients in any DL, the next DL would enroll patients. Intra-patient dose escalation was not permitted. An expansion cohort was planned to enroll an additional 14 patients at the optimal DL selected for further exploration. The treatment plan and the dose-escalation schema are shown in Table 1. On days 1 to 4, CLO was administered intravenously (IV) over 2 h. Two hours after finishing the CLO infusion, CY was infused IV over 2 h. The first dose of CY was split in half and given on day 0 and day 1 of cycle 1 to allow for comparison in the correlative studies between CY alone and the CLO-CY sequence.

Each cycle was defined as 28 days. A second cycle at the same DL was planned for patients who achieved an objective response without experiencing unacceptable toxicity.

End points and statistical considerations

Toxicity assessment—Using the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0 (NCI-CTCAE 3.0; http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcae3.pdf), all adverse events were reported and graded at the discretion of the treating physician. DLTs were assessed during the first cycle of therapy and were defined as the following: 1) Any grade 4 drug-related non-haematological toxicity, 2) Any grade 3 drug-related non-haematological toxicity that did not resolve to grade 2 within 24 h with the following exceptions: a) grade 3 elevations of one or more hepatic enzymes were considered a DLT only if resolution to grade 2 required > 7 days, b) grade 3 diarrhoea or oral mucositis were considered DLT only if resolution to grade 2 required > 48 h, and c) grade 3 neurological toxicity of any duration. If 2 patients in any DL developed DLTs, then the MTD would be defined as one DL below which the DLTs occurred.

Haematological toxicity was not considered in evaluating toxicity except where bone marrow (BM) aplasia lasted for 42 days with an overall BM cellularity 5% or lower with no evidence of leukaemia. Dose delays or dose reductions of CLO for haematological toxicity were not allowed. Myelosuppression-related DLT occurred when the absolute neutrophil count (ANC) did not return to $0.5 \times 10^9/l$ or the platelet count did not return to $75 \times 10^9/l$ or 20% of baseline values (whichever was less) by day 42 of therapy, in the absence of leukaemia in BM and in setting of BM cellularity of 5%.

Efficacy assessment—Responses were evaluated following cycle 1 according to the International Working Group (IWG) 2006 criteria (Cheson *et al*, 2006) for complete remission (CR), CR with incomplete blood count recovery (CRi), and partial remission (PR).

Statistical considerations—Demographics and baseline patient characteristics were summarized descriptively. The non-haematological toxicities were recorded according to NCI-CTCAE 3.0 and summarized by DLs. The haematological recovery was described by the median and range of duration to recovery of ANC and platelet count in responding patients. The overall response rate (ORR) was defined as the combination of CR, CRi, and PR. The duration of CR was calculated from the first documentation of CR. Survival was calculated from the first day of salvage chemotherapy to death from any cause or to time of last follow-up.

Laboratory correlates

Measurement of DNA damage—Samples from peripheral blood (PB) were obtained on day 0 before the infusion of CY (D0 Pre-Tx), day 0 at the end of the infusion of CY (D0

End-CY), day 0 at 2 h after the end of the infusion of CY (D0 Post-CY), day 1 before the infusion of CLO (D1 Pre-CLO), and on day 1 at 2 h after the end of the infusion of CY (D1 Post-CY). When feasible, BM samples at D0 Pre-Tx, D0 Post-CY, and D1 Post-CY were obtained. PB and BM blasts were isolated and prepared for flow cytometry as described previously (Karp *et al*, 2007). The standard 1×10^4 events were acquired from each sample during flow cytometry. Cellular debris was gated out of both the non-specific staining control and the experimental sample. The debris-corrected, non-specific staining, control histogram was subtracted from the debris-corrected histogram of the experimental sample, yielding the histogram of cells stained specifically for γ H2AX. The event count represented by this histogram of cells stained specifically for γ H2AX was divided by the event count represented by the debris-corrected histogram of the experimental sample, which included the total of cells stained specifically and non-specifically for γ H2AX. This value was multiplied by 100 to give the percentage of blasts with detected DNA-damage.

Immune reconstitution analysis—Previous generations of adenine-based nucleoside analogs were associated with T-cell depletion and immunosuppression. To evaluate the potential effects of CLO on immune function, in particular T-cell mediated immunity, we monitored absolute lymphocyte count (ALC), and CD4+ T-cell lymphocyte subset count at baseline, day 30 (D30) $\pm$ 3 days, and weekly thereafter until day 60 (D60), or until recovery to ALC of $0.2 \times 10^9/l$. Quantitative immunoglobulin levels (IgA, IgG, and IgM) were monitored at baseline, D30 $\pm$ 7 days, and D60 $\pm$ 7 days.

Results

Demographics and patient characteristics

Between December 2007 and March 2009, 26 adults with histologically confirmed RRAL were enrolled across the 4 dosing cohorts. Subsequently, 14 more patients were enrolled between April 2009 and March 2010 in the dose expansion cohort at the optimal DL determined by the Phase 1 trial (DL1). Baseline characteristics of the patients are summarized in Table 2. All 40 patients received at least one cycle of planned therapy, and 5 patients received a second cycle at the same DL. Thirty patients (75%) had refractory leukaemia, while 10 (25%) had relapsed disease. Of 10 patients with relapsed disease, 4 had first complete remission (CR1) duration of ≥ 12 months..

Toxicity

Non-haematological toxicity—Table 3 summarizes the non-haematological, non-infectious, grade ≥ 2 toxicities according to the dosing cohort. During the dose-escalation phase, grade ≥ 3 non-haematological, non-infectious toxicities occurred in 0%, 0%, 14%, and 67%, of patients in DL1, DL2, DL3, DL4, respectively. After cohort expansion at DL1, grade ≥ 3 non-haematological, non-infectious toxicities were noted in 29% of all patients treated at DL1, and in 28% of the entire 40-patient cohort. The 30-day induction mortality was 2.5% (1 death due to polymicrobial sepsis). No DLTs were observed at DL1 or DL2, one patient had DLT at DL3, and 2 patients had DLTs at DL4. At DL4, one patient had DLT with grade 3 pericarditis leading to atrial fibrillation with pericardial and pleural effusions, while another patient developed a DLT related to a grade 3 non-oliguric acute renal failure (not requiring dialysis), and hand-foot syndrome. Although the MTD was determined to be DL3, it was decided to proceed with DL1 as the optimal dose to be used in the expansion cohort. The reasons included the higher response rates in DL1 (ORR 71%, compared to 17% and 14%, at DL2 and DL3, respectively), the increased incidence of toxicities at DL3 compared to DL1, and the lack of evidence of increased DNA damage in relation to increasing DLs (see below). A notable toxicity was pericarditis or pleuritis with or without

associated effusions or arrhythmias in 5 patients (12.5%). This toxicity was seen at all 4 DLs, and was of grade 2 in 4 patients, and grade 3 in 1 patient (a DLT at DL4).

Infectious toxicity—Neutropenic fevers developed in 68% of patients, and were more common in DL4 compared to DL1 (100% vs. 62%). Documented bacteraemias occurred in 9 patients (22.5%), while fungaemias (4 patients) and definitive or probable fungal pneumonias (5 patients) were seen in 19%, 17%, 14%, and 50% of patients in DL1, DL2, DL3, and DL4, respectively. There was no increased incidence of infections with *pneumocystis carinii* or viral infections.

Haematological toxicity—The prolonged aplasia that was previously reported in 7/18 patients (39%) with the timed-sequential CLO-CY regimen was not seen with the CLO-CYx4 regimen. The median duration of recovery of ANC $0.1 \times 10^9/l$ and $0.5 \times 10^9/l$ for the 13 patients who achieved clinical responses was 25 days (range, 14–59), and 34 days (range, 17–78), respectively. Of the 2/40 (5%) patients that needed >42 days to recover to ANC $0.5 \times 10^9/l$, one was treated at DL1 with baseline ANC $0.04 \times 10^9/l$, and the other had a prior allo-HSCT treated at DL4 with baseline ANC $0.04 \times 10^9/l$. The median time of recovery to an untransfused platelet count of $20 \times 10^9/l$, $50 \times 10^9/l$, and $100 \times 10^9/l$ for patients who achieved clinical responses was 29 (range 15–67), 39 (range 16–67), and 48 days (range 19–99), respectively.

Efficacy

Objective clinical responses—The objective clinical responses are summarized in Table 4. The ORR was 33% (13/40 patients). Seven patients had CR (18%), 4 patients had CRi (10%) and 2 had PR (5%). For AML, the ORR was 25% (7/28), with 3 CR (11%), 2 CRi (7%), and 2 PR (7%). For ALL, the ORR was 50% (6/12), including 4 CR (33%), and 2 CRi (17%). The clinical responses were not dependent on the DL. The ORR for DL1, DL2, DL3, and DL4 was 48%, 17%, 14%, and 17% respectively. The median duration of CR was 6 months (range, 2–28). All patients who had responses less than CR (CRi or PR) relapsed within 3 months.

Of the 11 patients with CR or CRi, 5 had adverse genetics (including Philadelphia-chromosome [Ph]-positive ALL and *FLT3*-internal tandem duplication-positive AML). Responses were seen across a wide age range (median 52 years, range 23–73). The median number of prior regimens in responders was 2, but responses were seen in patients who had up to 4 prior regimens (range 1–4). Seven of the 10 patients (70%) with relapsed disease had objective responses (2 AML, 5 ALL). Among patients with relapsed ALL, 5/6 (83%) responded. In contrast, for patients with refractory disease, 6/30 (20%) had clinical responses (5 AML, 1 ALL). One of the 6 patients who had prior allo-HSCT achieved CR and subsequently received donor lymphocyte infusion (DLI). In addition, 3 of the refractory patients who achieved CR were able to proceed to allo-HSCT after CLO-CYx4. Six patients (15%) died within the first 60 days of therapy, 2 from infections and 4 from progressive disease. The median survival for the entire 40-patient cohort was 3.8 months (range, 0.9–43.7). Among the 13 patients with clinical responses, the median survival was 6.4 months (range, 2.1–43.7). Three patients survived >12 months from induction date, and one remains alive after 43.7 months of follow-up.

Correlative studies

DNA Damage—Samples from PB were available for analysis of DNA damage in 17 patients (42.5%), 6 of whom had eventual clinical responses (Table 5). Seven of the 17 assayed patients (41%) exhibited greater increases in γ H2AX in PB blasts when pretreated with CLO (D1) than when treated with CY alone (D0), with a median D1 net γ H2AX

accumulation 18% (range 5 – 45%) greater than D0 net γ H2AX accumulation. Four of 6 clinical responders (67%) exhibited greater increases in γ H2AX when pretreated with CLO (D1) than when treated with CY alone (D0), compared to 3/11 non-responders (27%). The patients who demonstrated CLO-mediated enhancement of CY-induced DNA damage had an ORR of 57% (4/7) compared with the subpopulation in whom CLO-mediated enhancement of DNA damage was not observed (20%=2/10). Neither the presence nor the magnitude of increased net DNA damage in PB blasts was dependent on the CLO or CY dose (Table 5a). Serial BM samples for measurement of γ H2AX were available from 6 patients (15%) (Table 5b), of whom 1 achieved an eventual clinical response. Interestingly, this responder had a 3-fold increase in CY-induced DNA damage in BM blasts with CLO pretreatment.

Immunological reconstitution—The median ALC before initiation of therapy for the 13 responders was $1.393 \times 10^9/l$ (range, $0.11\text{--}11.43 \times 10^9/l$), while the median absolute CD4+ lymphocyte count in this group was $0.352 \times 10^9/l$ (range, $0.039\text{--}1.023 \times 10^9/l$). The lowest ALC achieved after therapy was zero $\times 10^9/l$ for 10 of the 13 patients, and $0.01\text{--}0.1 \times 10^9/l$ for the other 3 patients, with a median percentage decline of 100% in ALC. The median duration of recovery of ALC $0.2 \times 10^9/l$ was 46 days (range, 15–75), with all 13 patients recovering to ALC of $0.2 \times 10^9/l$. Median ALC on D30 of therapy (± 3 days) for the group was $0.130 \times 10^9/l$ (range, $0\text{--}0.630 \times 10^9/l$), while the median absolute CD4+ lymphocyte subset count on D30 (± 3 days) was $0.01 \times 10^9/l$ (range, $0.003\text{--}0.156 \times 10^9/l$, n=10).

The quantitative immunoglobulin levels were evaluated pre-therapy, on D30 (± 7 days), and on D60 (± 7 days). For the patients with clinical responses, the median IgG levels pre-therapy, on D30, and on D60 were 10.04 g/l (range, 2.26–19.30), 9.19 g/l (range, 4.44–16.90), and 7.81 g/l (range, 2.84–16.40), respectively. The median IgM levels pre-therapy, on D30, and on D60 were 0.62 g/l (range, 0.1–1.91), 0.47 g/l (range, 0.09–0.98), and 0.38 g/l (range, 0.08–0.73), respectively. The median IgA levels pre-therapy, on D30, and on D60 were 1.5 g/l (range, $<0.07\text{--}3.08$), 0.74 g/l (range, $<0.07\text{--}3.82$), and 0.78 g/l (range, $<0.07\text{--}2.65$), respectively. The median percentage reduction in IgG levels from pre-therapy levels to D60 was 6% (range, –27 to 31%), for IgM 39% (range, –21 to 72%), and for IgA 32% (range, –18 to 83%).

Discussion

The outcomes of the majority of patients with RRAL with commonly used salvage chemotherapeutic regimens continue to be dismal, with limited clinical responses and poor long-term survival (Estey, 2000, Fielding *et al*, 2007, O'Brien *et al*, 2008). CLO has been studied extensively as a single agent and in combination with other drugs in RRAL in both adults and children. Table 6 lists published prospective trials that evaluated CLO-based combination regimens for salvage therapy of RRAL in adults. Most of these Phase I and II trials have combined CLO with cytarabine or with CY (Karp *et al*, 2007, Advani *et al*, 2010, Faderl *et al*, 2005, Faderl *et al*, 2008a, Agura *et al*, 2011, Tse *et al*, 2011, Becker *et al*, 2011). The ORR in these trials ranged from 17% to 61%, with CR in the range of 13% to 47%. The wide variability in results can be partly attributed to the different prognostic characteristics of patients enrolled in each study, such as the relative proportions of patients with refractory disease, multiple relapses, and short CR1 (Estey *et al*, 1996, Estey *et al*, 1997, Estey, 2000, Becker *et al*, 2011).

We have previously reported an ORR of 67% and 33% in rrALL and rrAML, respectively, using a timed-sequential CLO-CY regimen, but the haematological toxicity was significant, consistent with the notion of possible haematopoietic stem cell recruitment and enhanced chemotherapy susceptibility (Karp *et al*, 2007). In the current study, we used a more

conventional dosing regimen of CLO followed by CY on a daily basis for 4 days (CLO-CYx4) to decrease the putative stem cell recruitment and potential damage. This regimen was well tolerated, with a manageable side-effect profile, and no haematological DLT was seen. Notable non-haematological toxicities with the CLO-CYx4 regimen were pericarditis or pleuritis, which occurred in 12.5% of patients independent of DL, but were reversible in all cases. Out of 40 patients in this study, 75% had refractory leukaemia, 70% of patients had adverse cytogenetics or molecular features, and 15% had prior allo-HSCT. The use of CLO-CYx4 regimen in this heavily pretreated population resulted in an ORR of 33% (CR, 18%). The ORR for rrALL was 50% (CR, 33%), while for rrAML it was 25% (CR, 11%). While we observed more toxicity with increased DL, there was no clear correlation between the DL and clinical response rates. As a result of these findings, we used DL1 for the dose expansion group. In DL1 (CLO 10 mg/m²/day → CY 600 mg/m²/day × 4 days), the ORR was 48% (CR, 38%).

These findings confirm the clinical activity of CLO-CY combination in RRAL that we previously reported, especially for rrALL (ORR 50% in this trial, and 67% in previous trial) (Karp *et al*, 2007). In addition, our current findings confirm that CLO-pretreatment can be instrumental in augmenting CY-induced DNA damage in some patients, as measured by γ H2AX, albeit not CLO dose-dependent. The immune reconstitution analysis of patients with clinical responses showed a profound and protracted decline in ALC, including the CD4⁺ subset, with a median recovery duration of ALC of $0.2 \times 10^9/l$ of 46 days (range, 15–75). There were no significant changes in the level of IgG between pre-therapy and D60 values for most clinical responders, but there was a trend for reduction in IgA and especially for IgM levels. These findings suggested a more pronounced negative impact on early immunoglobulin responses (IgM level). Further immunological correlates should accompany clinical studies using CLO.

In summary, CLO-CYx4 regimen is well tolerated in patients with RRAL, and has a meaningful clinical activity, especially against relapsed ALL. In line with our results, Vitale et al (2009) reported meaningful clinical responses to a regimen of CLO followed by CY in 2 adult patients with relapsed/refractory Ph-positive ALL who were tyrosine-kinase inhibitor-resistant, one of whom had the highly-resistant *ABL1* T315I mutation. Therefore, CLO-CYx4 can be considered as a salvage therapy for adult patients with RRAL, especially for relapsed ALL, and should be considered for further investigations.

Acknowledgments

This study was supported in part by an investigator-initiated grant from Genzyme (JEK), NCI Cancer Center Support Grant 2P30 CA06973-47, and by philanthropic funds from Dr. Robert Fischell in memory of his wife Marian (JEK).

References

- Advani AS, Gundacker HM, Sala-Torra O, Radich JP, Lai R, Slovak ML, Lancet JE, Coutre SE, Stuart RK, Mims MP, Stiff PJ, Appelbaum FR. Southwest oncology group study S0530: A phase 2 trial of clofarabine and cytarabine for relapsed or refractory acute lymphocytic leukaemia. *British Journal of Haematology*. 2010; 151:430–434. [PubMed: 21113977]
- Agura E, Cooper B, Holmes H, Vance E, Berryman RB, Maisel C, Li S, Saracino G, Tadic-Ovcina M, Fay J. Report of a phase II study of clofarabine and cytarabine in de novo and relapsed and refractory AML patients and in selected elderly patients at high risk for anthracycline toxicity. *The Oncologist*. 2011; 16:197–206. [PubMed: 21273514]
- Banath JP, Olive PL. Expression of phosphorylated histone H2AX as a surrogate of cell killing by drugs that create DNA double-strand breaks. *Cancer Research*. 2003; 63:4347–4350. [PubMed: 12907603]

- Becker PS, Kantarjian HM, Appelbaum FR, Petersdorf SH, Storer B, Pierce S, Shan J, Hendrie PC, Pagel JM, Shustov AR, Stirewalt DL, Faderl S, Harrington E, Estey EH. Clofarabine with high dose cytarabine and granulocyte colony-stimulating factor (G-CSF) priming for relapsed and refractory acute myeloid leukaemia. *British Journal of Haematology*. 2011; 155:182–189. [PubMed: 21848522]
- Byrd JC, Mrozek K, Dodge RK, Carroll AJ, Edwards CG, Arthur DC, Pettenati MJ, Patil SR, Rao KW, Watson MS, Koduru PR, Moore JO, Stone RM, Mayer RJ, Feldman EJ, Davey FR, Schiffer CA, Larson RA, Bloomfield CD. Cancer Leukemia Group B (CALGB 8461). Pretreatment cytogenetic abnormalities are predictive of induction success, cumulative incidence of relapse, and overall survival in adult patients with de novo acute myeloid leukemia: Results from cancer and leukemia group B (CALGB 8461). *Blood*. 2002; 100:4325–4336. [PubMed: 12393746]
- Carson DA, Wasson DB, Esparza LM, Carrera CJ, Kipps TJ, Cottam HB. Oral antilymphocyte activity and induction of apoptosis by 2-chloro-2'-arabino-fluoro-2'-deoxyadenosine. *Proceedings of the National Academy of Sciences of the United States of America*. 1992; 89:2970–2974. [PubMed: 1348362]
- Cheson BD, Greenberg PL, Bennett JM, Lowenberg B, Wijermans PW, Nimer SD, Pinto A, Beran M, de Witte TM, Stone RM, Mittelman M, Sanz GF, Gore SD, Schiffer CA, Kantarjian H. Clinical application and proposal for modification of the international working group (IWG) response criteria in myelodysplasia. *Blood*. 2006; 108:419–425. [PubMed: 16609072]
- Estey EH. Treatment of relapsed and refractory acute myelogenous leukemia. *Leukemia*. 2000; 14:476–479. [PubMed: 10720145]
- Estey E, Kornblau S, Pierce S, Kantarjian H, Beran M, Keating M. A stratification system for evaluating and selecting therapies in patients with relapsed or primary refractory acute myelogenous leukemia. *Blood*. 1996; 88:756. [PubMed: 8695828]
- Estey E, Thall P, David C. Design and analysis of trials of salvage therapy in acute myelogenous leukemia. *Cancer Chemotherapy and Pharmacology*. 1997; 40(Suppl):S9–12. [PubMed: 9272127]
- Faderl S, Gandhi V, O'Brien S, Bonate P, Cortes J, Estey E, Beran M, Wierda W, Garcia-Manero G, Ferrajoli A, Estrov Z, Giles FJ, Du M, Kwari M, Keating M, Plunkett W, Kantarjian H. Results of a phase 1–2 study of clofarabine in combination with cytarabine (ara-C) in relapsed and refractory acute leukemias. *Blood*. 2005; 105:940–947. [PubMed: 15486072]
- Faderl S, Ferrajoli A, Wierda W, Huang X, Verstovsek S, Ravandi F, Estrov Z, Borthakur G, Kwari M, Kantarjian HM. Clofarabine combinations as acute myeloid leukemia salvage therapy. *Cancer*. 2008a; 113:2090–2096. [PubMed: 18756533]
- Faderl S, Ravandi F, Huang X, Garcia-Manero G, Ferrajoli A, Estrov Z, Borthakur G, Verstovsek S, Thomas DA, Kwari M, Kantarjian HM. A randomized study of clofarabine versus clofarabine plus low-dose cytarabine as front-line therapy for patients aged 60 years and older with acute myeloid leukemia and high-risk myelodysplastic syndrome. *Blood*. 2008b; 112:1638–1645. [PubMed: 18565853]
- Fielding AK, Richards SM, Chopra R, Lazarus HM, Litzow MR, Buck G, Durrant IJ, Luger SM, Marks DI, Franklin IM, McMillan AK, Tallman MS, Rowe JM, Goldstone AH. Medical Research Council of the United Kingdom Adult ALL Working Party and Eastern Cooperative Oncology Group. Outcome of 609 adults after relapse of acute lymphoblastic leukemia (ALL); an MRC UKALL12/ECOG 2993 study. *Blood*. 2007; 109:944–950. [PubMed: 17032921]
- Jeha S, Gaynon PS, Razzouk BI, Franklin J, Kadota R, Shen V, Luchtman-Jones L, Rytting M, Bomgaars LR, Rheingold S, Ritchey K, Albano E, Arceci RJ, Goldman S, Griffin T, Altman A, Gordon B, Steinherz L, Weitman S, Steinherz P. Phase II study of clofarabine in pediatric patients with refractory or relapsed acute lymphoblastic leukemia. *Journal of Clinical Oncology*. 2006; 24:1917–1923. [PubMed: 16622268]
- Karp JE, Ricklis RM, Balakrishnan K, Briel J, Greer J, Gore SD, Smith BD, McDevitt MA, Carraway H, Levis MJ, Gandhi V. A phase 1 clinical-laboratory study of clofarabine followed by cyclophosphamide for adults with refractory acute leukemias. *Blood*. 2007; 110:1762–1769. [PubMed: 17562873]
- Krawczyk J, Ansar N, Swords R, Murphy T, MacDonagh B, Meenaghan T, Hayden P, Hayad A, Murray M, O'Dwyer M. Clofarabine in the treatment of poor risk acute myeloid leukaemia. *Hematological Oncology*. 2010; 28:118–123. [PubMed: 19768694]

- Lotfi K, Mansson E, Spasokoukotskaja T, Pettersson B, Liliemark J, Peterson C, Eriksson S, Albertioni F. Biochemical pharmacology and resistance to 2-chloro-2'-arabino-fluoro-2'-deoxyadenosine, a novel analogue of cladribine in human leukemic cells. *Clinical Cancer Research*. 1999; 5:2438–2444. [PubMed: 10499616]
- McGregor BA, Brown AW, Osswald MB, Savona MR. The use of higher dose clofarabine in adults with relapsed acute lymphoblastic leukemia. *American Journal of Hematology*. 2009; 84:228–230. [PubMed: 19260120]
- O'Brien S, Thomas D, Ravandi F, Faderl S, Cortes J, Borthakur G, Pierce S, Garcia-Manero G, Kantarjian HM. Outcome of adults with acute lymphocytic leukemia after second salvage therapy. *Cancer*. 2008; 113:3186–3191. [PubMed: 18846563]
- Parker WB, Shaddix SC, Chang CH, White EL, Rose LM, Brockman RW, Shortnacy AT, Montgomery JA, Secrist JA 3rd, Bennett LL Jr. Effects of 2-chloro-9-(2-deoxy-2-fluoro-beta-D-arabinofuranosyl)adenine on K562 cellular metabolism and the inhibition of human ribonucleotide reductase and DNA polymerases by its 5'-triphosphate. *Cancer Research*. 1991; 51:2386–2394. [PubMed: 1707752]
- Tse E, Leung AY, Sim J, Lee HK, Liu HS, Yip SF, Kwong YL. Clofarabine and high-dose cytosine arabinoside in the treatment of refractory or relapsed acute myeloid leukaemia. *Annals of Hematology*. 2011; 90:1277–1281. [PubMed: 21455604]
- Vitale A, Grammatico S, Capria S, Fiocchi C, Foa R, Meloni G. Advanced philadelphia chromosome positive acute lymphoblastic leukemia patients relapsed after treatment with tyrosine-kinase inhibitors: Successful response to clofarabine and cyclophosphamide. *Haematologica*. 2009; 94:1471–1473. [PubMed: 19608688]
- Xie KC, Plunkett W. Deoxynucleotide pool depletion and sustained inhibition of ribonucleotide reductase and DNA synthesis after treatment of human lymphoblastoid cells with 2-chloro-9-(2-deoxy-2-fluoro-beta-D-arabinofuranosyl) adenine. *Cancer Research*. 1996; 56:3030–3037. [PubMed: 8674058]
- Yamauchi T, Nowak BJ, Keating MJ, Plunkett W. DNA repair initiated in chronic lymphocytic leukemia lymphocytes by 4-hydroperoxycyclophosphamide is inhibited by fludarabine and clofarabine. *Clinical Cancer Research*. 2001; 7:3580–3589. [PubMed: 11705880]

Table 1
Treatment schedule and dose-escalation schema for the clofarabine/cyclophosphamide (CLO-CYx4) regimen

Table 1a. Treatment Schedule:				
Treatment	Day 0	Day 1	Days 2–4	
On day 0, CY 300 mg/m ² or 400 mg/m ² (depending on DL) administered by 2-h IVI. On day 1, CY 300 mg/m ² or 400 mg/m ² (depending on DL) administered by 2-h IVI started 2 h after the end of the CLO infusion.	X	X		
CLO 10 to 20 mg/m ² (depending on DL) administered by 2-h IVI.		X		X
CY 600 mg/m ² or 800 mg/m ² (depending on DL) administered by 2-h IVI				X

Table 1b. Dose-escalation schema:				
Dose-Escalation Schema				
DL	CLO		CY	
	Daily dose (mg/m ² /day)	Total dose (mg/m ²)	Days 0 and 1 of cycle 1 (mg/m ² /day)	Days 2–4 of cycle 2 (mg/m ² /day)
Level 1	10	40	300	600
Level 2	10	40	400	800
Level 3	15	60	400	800
Level 4	20	80	400	800
				Total dose (mg/m ²)
				2400
				3200
				3200
				3200

Treatment Schedule (Table 1a); dose-escalation schema (Table 1b). CLO: clofarabine, CY: cyclophosphamide, DL: dose level, IVI: intravenous infusion. Both CLO and CY were administered intravenously, each over 2 h. CY was infused 2 h after completion of CLO infusion on each of the 4 days (days 1–4). The CY dose of day 1 was divided in half between day 0 and day 1 to assess the impact of CLO on CY- induced DNA damage. No CLO was given on day 0.

Table 2
Demographic and biological characteristics of 40 patients with relapsed and refractory acute leukaemias enrolled in the study

	Dose escalation phase (n= 26)	Expansion cohort (n=14)	Total (n=40)
Sex			
Male	17	8	25
Female	9	6	15
Age			
Median (range), years	47 (20–72)	49 (22–73)	48.5 (20–73)
AML	21 (81%)	7 (50%)	28 (70%)
Relapsed	2	2	4
Primary refractory	5	0	5
Multi-refractory	14	5	19
ALL	5 (19%)	7 (50%)	12 (30%)
Relapsed	3	3	6
Refractory	2	4	6
Prior regimens Median (range)	2 (1–5)	2 (1–4)	2 (1–5)
Prior allo-HSCT	4	2	6
Adverse genetics *	18 (69%)	10 (71%)	28 (70%)
AML	15 (71%)	6 (86%)	21 (75%)
Single cytogenetics	3	1	4
Complex karyotype	6	3	9
<i>FLT3</i> mutation	6	2	8
ALL	3 (60%)	4 (57%)	7 (58%)
Ph-positive	1	2	3
Complex	2	2	4

AML: acute myeloid leukaemia; ALL: acute lymphoblastic leukaemia; allo-HSCT: Allogeneic haematopoietic stem cell transplantation; *FLT3*: Fms-like tyrosine kinase 3 gene; Ph: Philadelphia chromosome.

*
:or adverse molecular features.

Table 3
Non-haematological therapy-related toxicities grade 2 according to dose level

Dose level	1 (n=21 ⁺)	2* (n=6)	3* (n=7)	4 (n=6)
Toxicity grade	2	3	2	2
Cardiovascular				
arrhythmias			1	1
Reduction in LVEF		1		1
Gastrointestinal				
D/N/V	5	2	4	2
Hepatobiliary		3		1
Renal				
Acute renal impairment	1	1		1 [#]
Neurological				
Sedation/confusion	1	1		
Skin				
Rash	2	1	1	
Hand-foot syndrome				1
Inflammatory				
CLO cytokine release	2			
Pericarditis/pleuritis ± pericardial/pleural effusion	2	1	1	1

DL: dose level; G2: grade 2 toxicity; G 3: grade 3 or higher toxicity; n: number of patients; D/N/V: diarrhoea, nausea, vomiting; LVEF: left ventricular ejection fraction; CLO: clofarabine;

⁺: 7 patients in initial cohort, and 14 more patients in expansion cohort;

^{*}: no therapy-related, non-haematological G 3 grade seen at DL2 or DL3;

[#]: associated with tumour lysis syndrome.

Table 4
Clinical outcome for 40 adult patients with relapsed or refractory acute leukaemia according to CLO/CY dose level, disease status, and genetics

	CR+CRi	PR	NR	NE	ORR
Dose level (total dose, mg/m²)					
1. 40/2400 (n=21)	8	2	9	2	48%
AML (n=12)	4	2	5	1	50%
ALL (n=9)	4	0	4	1	44%
2. 40/3200 (n=6)	1 (AML)	0	5	0	17%
3. 60/3200 (n=7)	1 (ALL)	0	5	1	14%
4. 80/3200 (n=6)	1 (ALL)	0	5	0	17%
Stage of disease (n=40)					
Relapsed (n=10)	7	0	3	0	70%
CR1 12 months (n=4)	2	0	2	0	50%
CR1 >12 months (n=6)	5	0	1	0	83%
Refractory (n=30)	4	2	21	3	20%
Genetics					
Non-adverse (n=12)	5	0	6	1	42%
Adverse (n=28)	6	2	18	2	29%
Single (n=7)	1	1	4	1	29%
Complex (n=14)	4	1	8	1	36%
<i>FLT3</i> mutations (n=7)	1	0	6	0	14%
Subtotal					
AML (n=28)	5	2	19	2	25%
ALL (n=12)	6	0	5	1	50%
Total (n=40)	11	2	24	3	33%

CLO: clofarabine; CY: cyclophosphamide; n: number of patients; CR: complete remission; CRi: complete remission with incomplete recovery of blood counts; PR: partial remission; NR: no remission; NE: not evaluable; ORR: overall response rate; CR1: first complete remission.

Adverse karyotype, for acute myeloid leukaemia (AML): complex (3 or more chromosomal abnormalities), abnormalities of chromosome 5 or 7, and *FLT3*-internal tandem duplication positivity; for acute lymphoblastic leukaemia (ALL): Philadelphia positive chromosome-positive or complex karyotype.

Table 5
Percentage of blasts with detected DNA-damage, as measured by phosphorylated-H2AX
in relation to dose level and reported as the median (range) of percentage change relative
to D0 Pre-treatment values

5a. Peripheral blood blasts:				
Dose Level (n) CLO dose	D0 End-CY	D0 Post-CY	D1 Pre-CLO	D1 Post-CY
DL1+2 (7) 10 mg/m²	104 (48–216)	105 (78–139)	83 (45–253)	172 (64–327)
DL3 (6) 15 mg/m²	100 (92–161)	104 (94–197)	87 (52–241)	126 (30–195)
DL4 (3–4) 20 mg/m²	80 (65–237) (n=3)	104 (79–263) (n=3)	130 (115–254) (n=4)	158 (80–353) (n=4)

5b. Bone marrow blasts:		
Dose Level (n) CLO dose	D0 Post-CY	D1 Post-CY
DL1+2 (4) 10 mg/m²	93 (60–120)	150 (101–316)
DL3+4 (2) 15 mg/m²; 20 mg/m²	177 (116–238)	173 (99–247)

Peripheral blood blasts (Table 5a); bone marrow blasts (Table 5b). CY: cyclophosphamide; CLO: clofarabine; n: number of patients.

D0 Pre-treatment: Day 0 before infusion of CY; D0 End-CY: Day 0 at the end of infusion of CY; D0 Post-CY: Day 0 at 2 h after end of infusion of CY; D1 Pre-CLO: Day 1 before the infusion of CLO; D1 Post-CY: Day 1 at 2 h after end of infusion of CY.

Table 6
Published prospective trials evaluating clofarabine-based combinations in adult patients with relapsed and/or refractory acute myeloid leukaemia and acute lymphoblastic leukaemia

Study and patient population	Leukaemia characteristics	Treatment regimens and schema	Clinical responses and survival
Faderl <i>et al</i> (2005) Phase I/II 32 patients 18 years old Age (median 59, range 18–84 years)	<ul style="list-style-type: none"> AML (25), ALL (2), HR-MDS (4), CML-BC (1). Primary refractory (4), 1st relapse (24) 16 AML patients with CR1 12 months 44% of AML patients with adverse karyotype 	CLO (phase 2 dose 40 mg/m ² /day) daily × 5 days (D2→D6) → Ara-C (1 g/m ² /day) daily × 5 days (D1→D5)	<ul style="list-style-type: none"> ORR 38% (CR 22%, CRi 16%) No responses in ALL or CML-BC In AML patients, ORR 40%, CR 28% Median response duration 3.2 months (range 0.5–14 months) Induction death of 3% Median OS 5.5 months (range, 0.2–15.2 months) Median OS for responders 7.9 months (range, 1.7–8.3 months)
Faderl <i>et al</i> (2008a) Phase I 44 patients 18 years old Age (median 56 and 58 years in both arms, range 23–78 years)	<ul style="list-style-type: none"> AML (42), HR-MDS (2) Primary refractory (17), 1st relapse (27) 4 patients had prior BMT 8 of relapsed patients had CR1 12 months 	Two arms: <ul style="list-style-type: none"> Arm A (23 patients): CLO × 5 days → Ida 10 mg/m²/day × 3 days Arm B (21 patients): CLO × 5 days → Ida 6 mg/m²/day × 3 days → Ara-C 0.75 g/m²/day × 5 days 	<ul style="list-style-type: none"> MTD CLO both arms 22.5 mg/m²/day Arm A: ORR 22% (13% CR + 9% CRi), median response duration 4.4 months (range, 2.3–8.1 months) Arm B: 48% CR, median response duration 15.5 months (range, 3–27.8 months) 30% patients proceeded to BMT
Karp <i>et al</i> (2007) Phase I 18 patients 18 years old Age: median 51 years, range 27–67 years	<ul style="list-style-type: none"> AML (12), ALL (6) 10 AML patients had refractory disease, 2 had relapsed AML. 2 ALL patients relapsed after BMT 78% had adverse cytogenetics 3 patients had prior BMT 	2 dose levels: <ul style="list-style-type: none"> DL1 (6 patients): CLO (20 mg/m²/day (D1–D3, D8–D10) → CY (400 mg/m²/day, D1–D3, D8–D10) DL0 (12 patients): CLO (10 mg/m²/day (D1–D3, D8–D10) → CY (400 mg/m²/day, D1–D3, D8–D10) 	<ul style="list-style-type: none"> At DL1, ORR was 50% (CR 33%); for AML 33% (CR 0%), and for ALL 67% (CR 67%). At DL0, ORR 33% (CR 25%); for AML 22% (CR 22%), and for ALL 67% (CR 33%). In both dose levels, ORR in AML was 25%, and 67% in ALL.
Advani <i>et al</i> (2010) Phase II 36 patients 16 years old Age: median 41 years, range 20–68 years	<ul style="list-style-type: none"> All patients had ALL (36) Refractory (9), 1st relapse (20), Subsequent relapse (7) 2 patients had prior BMT 59% poor-risk cytogenetics 	CLO (40 mg/m ² /day) daily × 5 days (D1→D5) → Ara-C (1 g/m ² /day) daily × 5 days (D1→D5)	<ul style="list-style-type: none"> ORR 17% (3 patients had CR, 3 patients had CRi) CR in untreated first relapse was 15% (no difference in response between 1st relapse and refractory patients) Median survival 3 months, with 3 patients alive at 8–13 months 8 patients proceeded to BMT

Study and patient population	Leukaemia characteristics	Treatment regimens and schema	Clinical responses and survival
Tse <i>et al</i> (2011) Phase II 21 patients 18 years old Age: median 45 years, range 22–62 years	<ul style="list-style-type: none"> All patients had AML (21) Refractory (4), 1st relapse (11), 2nd relapse (6) 3 patients had prior BMT 39% had poor-risk cytogenetics or <i>FLT3</i>-mutation. 	CLO (40 mg/m ² /day) daily × 5 days (D1 → D5) → Ara-C (1 or 2 g/m ² /day) daily × 5 days (D1 → D5)	<ul style="list-style-type: none"> 9 patients (43%) achieved CR (all received Ara-C 2 g/m²/day) CR was not related to age, disease status, cytogenetic risk, or number of prior regimens 3 patients proceeded to BMT
Agura <i>et al</i> (2011) Phase II 30 patients 18 years old Age: median 67 years, range 38–82 years	<ul style="list-style-type: none"> All patients had AML (30) 40% were elderly with no prior therapy, 60% had prior therapy (5 patients with 1 prior regimen, 13 with 2 prior regimens) 47% poor risk cytogenetics, 4 patients with prior BMT 	CLO (40 mg/m ² /day) daily × 5 days (D1 → D5) → Ara-C (1 g/m ² /day) daily × 5 days (D1 → D5)	<ul style="list-style-type: none"> ORR 53%, with CR (47%), PR (7%). ORR for therapy-naïve patients was 75% (CR 67%), ORR for patients with 1 prior regimen was 39% (CR 33%) 30-day mortality 20% (for relapsed/refractory patients 28%) 7 patients proceeded to BMT (3 had relapsed or refractory disease, 4 had no prior therapy) Median DFS 9.5 months, median OS 6 months (range, 0–60 months)
Becker <i>et al</i> (2011) Phase I/II 50 patients 18 years old Age: median 53 years, range 19–69 years	<ul style="list-style-type: none"> All patients had AML (50) 64% had relapsed disease (median CR1 = 26 weeks), 34% had refractory disease 40% with adverse cytogenetics 5 patients with prior BMT 	<ul style="list-style-type: none"> Most patients treated at maximum dose of regimen (GCLAC); CLO 25 mg/m²/day × 5 days (D1 → D5) → Ara-C 2 g/m²/day (D1 → D5), G-CSF 5 µg/kg/day from D0 until neutrophil recovery 	<ul style="list-style-type: none"> ORR in 46 evaluable patients was 61% (CR 46%) Higher CR rates in relapsed patients with CR1 6 months No relationship between response rates and age, cytogenetics, disease status 2 deaths in induction Median survival 9 months (range, 5.2–13 months) 25 patients proceeded to BMT

AAML, acute myeloid leukaemia; ALL, acute lymphoblastic leukaemia; HR-MDS, high-risk myelodysplastic syndrome (10% blasts); CML-BC, chronic myelocytic leukaemia in blast crisis; CR1, first complete remission; BMT, bone marrow transplant; CLO, clofarabine; Ara-C, cytarabine; Ida, idarubicin; GCLAC, granulocyte colony-stimulating factor (G-CSF) priming, clofarabine and high dose cytarabine; D, day; DL, dose level; ORR, overall response rate; CR, complete remission; CRi, complete remission with incomplete recovery of blood counts; OS, overall survival. DFS, disease-free survival. MTD, maximum-tolerated dose. G-CSF, granulocyte colony-stimulating factor. DLi, donor lymphocyte infusion.