Clofarabine added to intensive treatment in adult patients with newly diagnosed ALL: the HOVON-100 trial

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Abstract:
Clofarabine (CLO) is a nucleoside analogue with efficacy in relapsed/refractory acute lymphoblastic leukemia (ALL). This randomized phase III study aimed to evaluate whether CLO added to induction and consolidation would improve outcome in adults with newly diagnosed ALL. Treatment for younger (18-40 years) patients consisted of a pediatric inspired protocol and for older patients (41-70 years) of a semi-intensive protocol was used. 340 patients were randomized. After a median follow up of 70 months, 5-year EFS was 50% and 53% for arm A and B (CLO arm). For patients (less than or equal to)40 years, EFS was 58% vs 65% in arm A vs B, while in patients >40 years EFS was 43% in both arms. CR rate was 89% in both arms and similar in younger and older patients. Minimal residual disease (MRD) was assessed in 200 patients (60%). Fifty-four of 76 evaluable patients (71%) were MRD negative after consolidation 1 in arm A vs 75/81 (93%) in arm B (p=0.001). Seventy (42%) patients proceeded to allogeneic hematopoietic stem cell transplantation in both arms. Five years OS was similar in both arms, 60% vs 61%. Among patients achieving CR, relapse rates were 28% and 24%, and non-relapse mortality was 16% vs 17% after CR. CLO treated patients experienced more serious adverse events, more infections, and more often went off-protocol. This was most pronounced in older patients. We conclude that, despite a higher rate of MRD-negativity, addition of CLO does not improve outcome in adults with ALL, which might be due to increased toxicity. The trial is registered at www.trialregister.nl as NTR2004.

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Short title: Phase III trial of clofarabine in ALL

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- Clofarabine added to standard treatment for adults with newly diagnosed ALL does not improve event-free and overall survival.
- Clofarabine is associated with more toxicity and more patients going off-protocol which might have blunted a better MRD-response.
Abstract

Clofarabine (CLO) is a nucleoside analogue with efficacy in relapsed/refractory acute lymphoblastic leukemia (ALL). This randomized phase III study aimed to evaluate whether CLO added to induction and consolidation would improve outcome in adults with newly diagnosed ALL. Treatment for younger (18-40 years) patients consisted of a pediatric inspired protocol and for older patients (41-70 years) of a semi-intensive protocol was used. 340 patients were randomized. After a median follow up of 70 months, 5-year EFS was 50% and 53% for arm A and B (CLO arm). For patients ≤40 years, EFS was 58% vs 65% in arm A vs B, while in patients >40 years EFS was 43% in both arms. CR rate was 89% in both arms and similar in younger and older patients. Minimal residual disease (MRD) was assessed in 200 patients (60%). Fifty-four of 76 evaluable patients (71%) were MRD negative after consolidation 1 in arm A vs 75/81 (93%) in arm B (p=0.001). Seventy (42%) patients proceeded to allogeneic hematopoietic stem cell transplantation in both arms. Five years OS was similar in both arms, 60% vs 61%. Among patients achieving CR, relapse rates were 28% and 24%, and non-relapse mortality was 16% vs 17% after CR. CLO treated patients experienced more serious adverse events, more infections, and more often went off-protocol. This was most pronounced in older patients. We conclude that, despite a higher rate of MRD-negativity, addition of CLO does not improve outcome in adults with ALL, which might be due to increased toxicity. The trial is registered at www.trialregister.nl as NTR2004.
**Introduction**

Outcome in adult patients with ALL has substantially improved to approximately 50% event-free survival (EFS) during the last decades\(^1\)-\(^3\). However, a substantial proportion of patients, especially above 40 years of age, will develop a relapse, despite efforts to intensify established treatment approaches, including allogeneic hematopoietic stem cell transplantation (alloHSCT). Outcome after relapse is still very unsatisfactory in adult patients\(^4\),\(^5\). Therefore, prevention of relapse is still the major goal of frontline treatment in ALL. Several clinical studies have shown that patients with ALL and MRD negative CR who are consolidated with chemotherapy or alloHSCT had better survival than patients with MRD positive CR\(^5\),\(^9\). Therefore, the need to achieve MRD negativity before proceeding with consolidation treatment is currently considered a major treatment goal.

Clofarabin (CLO) is a second generation, halogenated, nucleoside analogue that combines the positive activities of the two first generation purine nucleotides fludarabine and cladribine, but with less toxic and less neurological side effects\(^10\)-\(^12\). It was approved in 2004 for relapsed or refractory ALL in patients 1-21 years old. CLO proved to be well tolerated and effective as an antileukemic drug when used as monotherapy or in combination with other DNA damaging drugs\(^13\)-\(^16\). In a phase II study reported by Kantarjian et al 62 adult patients with relapsed or refractory acute leukemia received CLO for 5 days with an overall response rate of 48%, while in ALL this was only 2/12 (17%)\(^15\). Similar low response rates were shown in studies where CLO was combined with cytarabine\(^17\). CLO combined with cyclophosphamide was more promising in adults, especially at first salvage\(^18\)-\(^22\). The combination of CLO with cyclophosphamide and etoposide in relapsed or refractory ALL showed remarkable remission rates, but at the expense of substantial toxicities\(^16\),\(^19\),\(^23\). In addition, toxicity appeared also considerable in acute myeloid leukemia (AML), as was observed by the HOVON-SAKK study group, that performed a phase III study demonstrating that CLO integrated in standard treatment regimens did reduce relapse rate, but without improving survival\(^24\). So far, the efficacy and toxicity of CLO in upfront treatment of ALL in combination with induction and consolidation chemotherapy has not been addressed in adults. Here we report a large randomized phase III trial by the Dutch-Belgian HOVON
study group in adult patients with ALL, comparing induction and consolidation therapy with versus without CLO added to prephase and as an extra consolidation course.
Methods

Patients

This study was conducted in 29 centers in the Netherlands, Belgium and France from October 23, 2009 till November 7, 2016. Eligible patients were 18-70 years old and had a diagnosis of previously untreated precursor B- or T-ALL, mixed phenotype acute leukemia or T-lymphoblastic lymphoma. Patients with mature B-cell ALL and acute undifferentiated leukemia were not eligible. Adequate renal and hepatic function were required. The study protocol was approved by independent ethics committees at each participating center, and the study was conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent. The trial was supported by the Dutch Cancer Foundation (grant EMCR 2008-4300) and Sanofi Genzyme and was registered at www.trialregister.nl as NTR2004.

Study design and treatment

Patients were randomly assigned to receive standard treatment without or with CLO 30 mg/m\(^2\) for 5 days given as monotherapy during prephase and after consolidation 1. All patients proceeded at day 8 after pre-phase with induction chemotherapy (± CLO), irrespective of hematological toxicity. The study was started as a randomized phase II feasibility study and continued as a randomized phase III study with CLO 30 mg/m\(^2\) after 60 patients had been randomized and feasibility of this dose was evaluated and approved by the Data Safety and Monitoring Board (DSMB). We here report the results of the final analysis of the phase III part of the study. Randomization was stratified for age and immunophenotype (B- vs T-ALL). Treatment for younger (18-40 years) patients consisted of consecutive chemotherapy courses based upon a pediatric inspired schedule as reported before and older patients (41-70 years) were treated with a semi-intensive schedule\(^{25,26}\) (detailed in supplementary Tables 1 and 2). Central nervous system (CNS) prophylaxis was delivered intrathecally (IT) 12-18 times throughout the protocol. Before alloHSCT patients received at least 8 times IT prophylaxis. No prophylaxis was given after alloHSCT. Cranial irradiation was only given in case of CNS localization provided the patient did not proceed to alloHSCT, as these patients received their irradiation as part of
the conditioning regimen. All patients were given daily low dose trimethoprim/sulfamethoxazole for *Pneumocystis jirovecii* prophylaxis and valaciclovir for viral prophylaxis. Prevention of bacterial and fungal infections in patients with mucositis and neutropenia was recommended by use of penicillin, ciprofloxacin and fluconazole. Granulocyte colony-stimulating factor was strongly recommended to all patients with neutropenia until recovery and was mandatory for patients ≤ 40 years old during remission induction course 1. The protocol was amended to include low molecular weight heparin-prophylaxis (nadroparin 5700IU once daily) subcutaneously from start of prephase until 14 days after pegylated asparaginase for younger patients and until start of consolidation in older patients to reduce thrombo-embolic events in first course. Patients with t(9;22) positive-ALL received the same regimen with addition of a tyrosine kinase inhibitor (TKI) therapy, preferably imatinib, in conjunction with chemotherapy. AlloHSCT with an HLA identical sibling or HLA-identical (10/10) unrelated donor was offered to all CR1 patients after intensification 1 in patient ≤ 40 years and after consolidation 2 in patients > 40 years of age; only in high risk patients an alternative donor (cord blood, mismatched unrelated donor, or haploidentical related donor) was recommended in case of lack of a matched related or matched unrelated donor (10/10). AlloHSCT was not performed when there was no suitable donor or when the patient was not eligible for transplantation. Conditioning regimen before alloHSCT consisted of myeloablative conditioning (MAB) up to the age of 40. The regimens used were: busulfan/cyclophosphamide or cyclophosphamide/Total Body Irradiation (TBI). In patients > 60 years old a reduced intensity conditioning (RIC) regimen was applied. The regimens used were: fludarabin/TBI, fludarabin/melfalan/busulfan/fludarabin or cyclophosphamide/fludarabin/TBI. In patients between 40 and 60 years old the intensity of conditioning depended on physical fitness and centers policy.

**Diagnostics**

Baseline evaluation included evaluation of bone marrow morphology and more than 20% leukemic cells were necessary for a diagnosis of ALL. Also, immunophenotyping was done and these diagnostics were centrally reviewed. Cytogenetic analysis and molecular assessment for t(9;22) and
11q23 aberrations or BCR-ABL, including MLL-AF4 screening were done. To detect extramedullary disease, a CT scan was performed.

Criteria for response and endpoints

CR was defined by less than 5% leukemic blasts in a normocellular bone marrow without peripheral leukemic cells and without extramedullary manifestations. Patients were considered as “CR on protocol” if CR was reached after at least one of the treatment cycles as planned. Relapse was defined by reappearance of disease either as unequivocal blasts in the BM (> 5%), in the liquor, or at extramedullary sites after prior achievement of CR. Primary endpoint was event free survival (EFS) which refers to the interval from randomization to the date of failure to enter a CR, death or relapse whichever occurs first. Secondary endpoints included CR rate, central assessment of MRD by real-time quantitative PCR (RQ-PCR) of rearranged immunoglobulin (IG) or T-cell receptor (TR) genes in the bone marrow or by flow cytometry, disease free survival (DFS; time from CR to relapse or death, whichever occurs first), relapse, non-relapse mortality (NRM), overall survival (OS; time from randomization to death from any cause, patients still alive at last contact were censored) and adverse events.

Molecular minimal residual disease analyses

MRD levels were determined in bone marrow by RQ-PCR of leukemia-specific rearranged IG and TR genes with the use of clone-specific primers and a set of different germline TaqMan probes and germline primers. Quality control and standardized interpretation of RQ-PCR data were achieved following the guidelines of the European Study Group on MRD detection in ALL (EuroMRD). Patients with MRD <10^−4 were classified as MRD negative. MRD evaluation took place after induction 1 and after consolidation 1 only in patients who were in CR. BM samples were sent to central reference laboratories at Erasmus MC (Rotterdam), Sanquin (Amsterdam) and VUB (Brussels). Results were classified as “molecular CR” in case of MRD negativity (defined as < 10^−4) at the respective time point with at least one RQ-PCR assay with a quantitative range of ≤ 10^4. Molecular MRD was the method of choice and was done centrally. If no material or targets were available for
molecular analysis, local flowcytometric MRD data were used if available and if the applied assay allowed a sensitivity of at least 0.01%.

**Immunophenotyping MRD analyses**

Bone marrow samples were processed, bulk-lysed and subsequently stained using 6 or 8 color stainings according to locally used protocols. One to four million cells (if available) were acquired and MRD positivity was defined if at least 20 ALL cells could be detected. MRD negativity was defined as MRD <0.01% using an assay with a sensitivity of at least 0.01%.

**Risk classification**

Patients were classified as having high risk disease if they met one of the following criteria: white blood cell count at diagnosis greater than 30x10^9/L for B-ALL and greater than 100x10^9/L in T-ALL, no CR after induction or specific cytogenetic/molecular abnormalities (Ph chromosome or BCR-ABL, 11q23 aberrations, hypodiploidy or complex karyotype). All other patients were classified as intermediate risk disease.

**Statistical analysis**

The study was powered on the randomization of patients that could be randomized to the final dose level of CLO for the phase III part of the study. Main endpoint for the comparison of the two treatment arms was EFS from registration. In order to detect with 80% power (two-sided significance level $\alpha = 0.05$; 1:1 randomization) an improvement of EFS with hazard ratio (HR) = 0.65 - which corresponds to an improvement of the CR rate from 85% to 90%, and 2-year EFS from 40% to 55% - 174 events had to be observed. This would require 316 patients to be accrued in 3.5 to 4 years – with an expected accrual of about 90 patients per year – and 2 years of follow up after the last registered patient. In order to overcome possible dropout, 340 patients would be registered in the phase III part.

Randomization between standard treatment without or with CLO was done with a minimization procedure, stratified by age (18- 40 versus 41-70 years), precursor- B-ALL versus T-ALL immunophenotype and center, ensuring balance within each stratum and overall. The formal test for
the difference in EFS between the two treatment arms would be done with a multivariate Cox regression analysis with adjustment for the stratification factors age (18–40 vs 41–70 years) and immunophenotype (B-cell vs T-cell). As a sensitivity analysis, we also performed a non-modelling based stratified logrank test for difference in EFS between the two treatment arms.

All analyses would be according to the intention to treat (ITT) principle, i.e. patients would be analyzed according to the treatment arms they were assigned to. However, patients initially randomized but considered ineligible afterwards based on information that should have been available before randomization, would be excluded from all analyses (modified-ITT).

Secondary efficacy endpoints were response rate (hematological, as well as molecular), DFS from CR, OS from randomization, and DFS and OS from allogeneic transplantation and from start maintenance. Actuarial probabilities of EFS, DFS and OS at appropriate time points including 95% confidence intervals (CIs) were calculated using the actuarial method of Kaplan and Meier. Kaplan-Meier survival curves were constructed to illustrate survival. Response rates were compared between the two arms using logistic regression analysis or the Fisher exact test, whichever appropriate.

Adverse events and infections were scored according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 3.0. A serious adverse event (SAE) was defined as any untoward medical occurrence that results in death, was life-threatening, leads to (prolongation of) hospitalization, resulted in disability, a congenital anomaly/birth defect, or any other medically important condition. SAEs had to be reported from the first study-related procedure until 30 days following the last protocol treatment or until the start of subsequent therapy for the disease under study. SAEs occurring after 30 days also had to be reported if considered to be at least possibly related to CLO by the investigator. All analyses were performed using Stata (StataCorp. 2019. Stata: Release 16. Statistical Software. College Station, TX: StataCorp LLC).
Results

Patient characteristics

Three hundred and forty patients were randomized between standard treatment without or with CLO. One hundred and sixty-eight patients were randomized to standard treatment and 172 patients to the CLO arm. Six patients were ineligible, 5 patients due to misdiagnosis and one due to breast cancer within 5 years of diagnosis of ALL (CONSORT diagram, Figure 1 and subgroups in supplementary Figure 1, 2 and 3). Table 1 summarizes the main baseline characteristics of eligible patients. Both groups were balanced in terms of age, WHO performance status, immunophenotype, risk group and BCR-ABL positivity. Two hundred and ten patients were considered high-risk, with 102 (61%) and 108 (64%) in the standard and CLO-arm, respectively.

Treatment and response

Overall, 236/334 (71%) patients completed protocol treatment, i.e. received alloHSCT or started with maintenance treatment. Patients randomized for standard arm completed treatment in 74% and 67% of patients completed treatment in CLO arm. According to age category, 78% and 71% (younger patients ≤ 40 years) and 70% and 64% (older patients > 40 years) completed treatment in the standard and CLO arm, respectively. Since CLO might induce early bone marrow toxicity and therefore might prolong regeneration after induction I, the time between start of induction I and start of CLO-Consolidation I in both arms and both age groups was calculated. For patients 18-40 years old, median duration between start of induction I and start of CLO-Consolidation I was 111 days (interquartile range [IQR], 102-122) in arm A versus 108 (IQR, 100-119) days in arm B. For older patients, median duration was 67 days (IQR, 61-75) in arm A versus 65 (IQR, 54-74) days in arm B. So neither in the younger nor in the older patients, there was a statistically significant difference. In total, 12 patients (see Figure 1) did not receive CLO consolidation while still on protocol. In 10/12 (83%) cases this seemed due to toxicity during CLO in pre-phase. Two patients refused to proceed. More CLO treated patients who were in CR went off-protocol due to other reasons (mainly toxicities) than relapse, death
or normal completion, which was statistically significant in older patients (p=0.024, Figure 2). Overall, 89% of patients achieved a CR during protocol treatment, which proved similar in both study arms (Table 2). Quantification of minimal residual disease by RQ-PCR analysis of rearranged IG/TR genes (n=167) or by flowcytometry (n=33) was performed in 200 (60%) patients, with 99 and 101 in arm A and B, respectively. The main reason for not having a MRD status was no available material. Fifty-four out of 76 evaluated patients (71%) were MRD negative (defined as <10^{-4}) after the first consolidation course in the standard arm versus 75 out of 81 (93%) in arm B (p=0.001) (Table 2). The MRD-response after the first consolidation was 75% and 94% in younger patients for the standard versus the CLO-arm, respectively. For older patients these percentages were 68% and 91%, respectively. Overall, as presented in Table 2, 78 out of 297 CR patients developed a relapse (26%), which were evenly distributed among the study arms: 42 of 148 patients in the standard arm (28%) versus 36 out of 149 CLO-treated patients (24%). The relapse rate in younger patients was 32/142 (23%) with no significant difference between both arms (27% in arm A vs 18% in arm B). For older patients these data were 30% vs 29%, respectively. Patients who were MRD negative after the first consolidation course developed a relapse in 23 out of 126 patients (18%), while CR1 patients who were MRD-positive developed a relapse in 11/25 (44%) of cases (Table 2). Thus, MRD-negativity after consolidation was associated with a lower relapse rate (HR:0.35, 95% confidence interval (CI) 0.17-0.71).

Subgroup analysis

No convincing indication was found that subgroups (Ph+/Ph-, B-/T-ALL, T-ALL/T-LyLy, different age groups, intermediate/high risk group) would selectively benefit from addition of CLO (compared with the control treatment) (Supplementary Table 3+4). Also in Ph-negative patients, without a transplant, no advantage of CLO was found, but younger patients (Ph negative and positive) who were not consolidated by alloHSCT showed a lower relapse rate if they had received CLO during induction and consolidation (36% versus 14%, in standard versus CLO arm resp.) (Supplementary Table 4).
interest, 19 patients over 60 years of age showed similar CR and MRD negativity as the 68 patients of 41-60 years of age (Supplementary Table 3) in arm A. In arm B, CR and MRD rate were also similar between the two age groups, but MRD negativity rate was significantly higher in the CLO arm for both age groups than patients treated in standard arm. EFS after 5 years follow up was 47 versus 50% in arm A vs arm B in patients aged 41-60 years old and 26 vs 28% for patients over 61 years of age.

**Maintenance treatment and allogeneic hematopoietic stem cell transplantation.**

Among all patients who completed consolidation and intensification courses, 100 patients (41%) received maintenance treatment and 140 (58%) proceeded to alloHSCT, without significant differences between study-arms, nor in the time to maintenance or the time to alloHSCT, taking the extra time for the CLO consolidation cycle in arm B into account (CONSORT diagram, Figure 1). AlloHSCT was applied in 68 younger patients, 56 (82%) after MAB and 12 (18%) after RIC conditioning regimen and 72 older patients (17 (24%) MAB, 55 (76%) RIC). Donortype was a matched sibling in 55 patients, matched unrelated donors in 76 patients, mismatched unrelated donor in 1 patient, and 8 patients received cord blood stem cells. AlloHSCT was evaluated as a time dependent covariate to address the question whether alloHSCT was associated with better outcome. However, no such effect was observed. Results are shown in the supplementary Table 5, indicating that multivariable analysis with alloHSCT as a time-dependent covariate firmly showed that age 41-70 years was the predominant variable associated with DFS (HR: 2.13, 95% CI 1.47-3.09, p<0.001).

**Event free, overall and disease free survival**

The median follow-up of 203 patients alive was 70 months (interquartile range: 55 - 88 months).

Event free survival (EFS) at 5 years from randomization was 50% (95% CI, 42-57) in patients receiving standard treatment and 53% (95% CI, 45-60) for CLO (hazard ratio (HR)=0.93, 95% CI: 0.69-1.27, p=0.67; adjusted for age and phenotype). For patients ≤40 years, 5-year EFS was 58%
(95% CI, 47-69) versus 65% (95% CI, 53-74) in arm A and B, respectively, while in patients >40 years 5-year EFS was 43% (95% CI, 32-53) in both arms (Figure 3 A, B, C). In addition, overall survival (OS) was not significantly different. Five-year OS was 60% (95% CI, 52-68) in arm A versus 61% (95% CI, 53-68) in arm B (HR=0.98, 95% CI: 0.70-1.38, p=0.92). Patients ≤40 years old, showed an OS of 72% at 5 years versus 76% in arm A and B, respectively. Patients >40 years old, showed an OS of 50% and 47% in arm A versus B (Figure 3 D, E, F). Five year disease free survival from CR (DFS) was 56% in arm A versus 60% in arm B (HR=0.90, 95% CI: 0.64-1.28, p=0.57) and proved also similar in younger and older patients in either study-arm.

**Safety and tolerability**

The two treatment arms were compared with respect to adverse events (AE), NRM in CR patients, and number of patients in each treatment arm stopping protocol treatment not due to completion, relapse or death. Percentages of grades 3-4 AE were 89% and 93% in arms A and B, respectively. CLO treated patients experienced more serious adverse events (SAEs), 70% versus 82% in younger patients (≤40 years of age), and 52% versus 75% in patients >40 years of age (Table 3). Grade 3 and 4 infections occurred in both groups but were significantly more present in the CLO treated patients (45% versus 66% in arm A and B respectively (p<0.001)), with respiratory tract infections, sepsis and abdominal infections being the most prevalent types of infection. In younger as well as in older patient this significant difference between arms was similar (≤40 years of age 53% vs 66% and in patients >40 years of age 37% vs 67% in arm A vs B resp (Table 3 and detailed in supplementary Table 6). Thrombo-embolic events were frequently reported, but no difference was seen between arms in both age groups (34% versus 25% in patients ≤40 years in arm A and B resp., and 26% in patients >40 years on both arms).

The rates of treatment discontinuations due to any adverse event were 6% (10/166) in the control group and 11% (22/168) in the CLO group (<40 years: 8% vs 11% and >40 years 5% vs 14% in arm A and B resp.) (Figure 1 and supplementary Figures 1, 2 and 3). Figure 2 shows that CLO treated
patients more often went off-protocol due to other reasons than completion, relapse or death while being in CR, especially in older patients (12% versus 27% in arm A and B). These other reasons consisted of toxicity (20), lost to follow-up (2) and alternative treatment (1), and therefore is a convenient parameter for cumulative toxicity (Figure 1 and supplementary Figures 1, 2 and 3). Fatal serious adverse events were reported in 2 versus 6 patients (3% versus 8%) in patients less than 40 years old, and in 11 versus 12 patients (13% versus 14%) of patients above 40 years of age. The most common reason for a fatal AE was infection. 5-year NRM in CR patients was 16% (standard error (SE) 3%) in arm A versus 17% (SE 3%) in arm B.
Discussion

CLO is an effective anti-neoplastic drug that proved efficacious in several phase II studies in younger and older patients with relapsed or refractory ALL\textsuperscript{12,13,15,30,31}. Furthermore, combining CLO with conventional chemotherapeutic drugs including alkylating drugs and anthracyclines was suggested to result in synergistic activity\textsuperscript{17,22,32}. Combining CLO with conventional chemotherapy in an upfront treatment setting of pediatric very high risk precursor B-ALL, resulted in unacceptable toxicity\textsuperscript{33}. Its additive value in adult patients in the context of intensified chemotherapy, however, was not studied before. This paper reports the first large phase III study with mature follow-up on the use of CLO as an integrated drug in intensified chemotherapeutic schedules for both younger (\(\leq 40\) years of age) and older adult patients (> 40 years of age) with ALL as part of first line treatment. The results of this study failed to reveal an improvement of event free survival or overall survival, both in the subgroups of younger and older patients. While also hematological response rates were similar in both study arms, the addition of CLO resulted in a significantly better MRD response. In a subset of patients (60\% of patients were evaluable) CLO was more effective than standard treatment in terms of eradication of residual disease. Nevertheless, the overall relapse rate appeared similar in either study arm as well as DFS and OS, which might be explained by a higher number of patients not completing full protocol treatment, due to prematurely going off-protocol for toxicity reasons.

The prognostic value of MRD early in the course of treatment has been shown in pediatric patients\textsuperscript{34-39} but also extensively in adult patients\textsuperscript{8,40-43}. Therefore, the question why the depth of the response did not translate into an improved outcome is relevant. The most likely explanation for the lack of efficacy is the finding that an increased rate of adverse events and possibly also death in CR patients may have counterbalanced an early advantage of CLO. However, while more adverse events were observed in CLO-treated patients, non-relapse mortality (NRM) appeared not significantly different between both study arms. Of interest, we did find a significantly higher proportion of CLO-treated patients going off-protocol, especially in elderly patients, while being in CR. Going off-protocol for other reasons than normal completion, relapse or death, most often implies a continuation with a less intensive course of succeeding chemotherapy or an earlier switch to maintenance chemotherapy. Thereby, an initial beneficial effect of CLO may have been blunted by succession of insufficient intensified
consolidation chemotherapy. That explanation would compare well to earlier findings in AML patients, in whom incorporation of CLO into intensified induction and consolidation chemotherapy also proved associated with a better MRD response, but no improved outcome\textsuperscript{24}. The current results also confirm earlier concerns about toxicity with an increase in SAE’s and infection rate related to CLO treatment. Since earlier studies exploring CLO in combination with cyclophosphamide for heavily treated and relapsing leukemia patients led to prohibitive toxicity\textsuperscript{19,21} arguing for CLO dose reductions\textsuperscript{20,22}. In addition, the Children’s Oncology group (COG) study group performed a randomized phase III study evaluating CLO upfront in children and adolescents with very high risk ALL. Intensification of chemotherapy with CLO in this subgroup did not improve survival and appeared associated with considerable toxicity\textsuperscript{33}.

This study has some limitations that must be considered when interpreting the results. First, adult ALL consists of many subgroups nowadays and much larger numbers of patients would be needed to address the value of CLO in each subgroup. This study was powered to detect a difference in the entire, combined group of both younger and older and B-cell and T-cell ALL, in which a difference in favour of CLO was not found. We cannot exclude that CLO might be beneficial in a specific subset. Moreover, CLO was used at a lower dose (30 mg/m\textsuperscript{2}) than initially used in CLO combination studies (40 mg/m\textsuperscript{2}) with cytarabine, cyclophosphamide with or without etoposide\textsuperscript{18-22}. This might have affected efficacy, but it has to be taken into account that cumulative toxicity leading to more “off-protocol-treatment” (as described in this study) suggests that intensive chemotherapy administered in successive courses might not allow the introduction of a higher dose of CLO. Lastly, the results with respect to MRD appeared encouraging with a better MRD-response in CLO-treated patients. It however did not translate into better outcome, probably due to toxicity.

In conclusion, CLO added to induction and consolidation chemotherapy in adult patients with ALL does not improve EFS and OS, while CLO appeared associated with more toxicity and more patients going off-protocol not due to completion, relapse or death. While CLO appeared associated with a higher incidence of MRD-negativity in a subset of patients, relapse in either study arm appeared
similar, which might possibly be due to increased toxicity and more patients going off-protocol not due to completion, relapse or death.

**Data Sharing Statement:** To request data, please e-mail Anita Rijneveld at a.rijneveld@erasmusmc.nl.

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Table 1. Patient characteristics at baseline according to randomisation with or without CLO

|                                | Standard treatment (arm A) | CLO + standard treatment (arm B) |
|--------------------------------|---------------------------|----------------------------------|
| **Total, n**                   | 166                       | 168                              |
| **Age**                        |                           |                                  |
| Median (range), y              | 42 (18-70)                | 43 (18-70)                       |
| 18-40 y, n (%)                 | 79 (48)                   | 80 (48)                          |
| 41-70 y, n (%)                 | 87 (52)                   | 88 (52)                          |
| **Performance status, n (%)**  |                           |                                  |
| WHO 0                          | 69 (42)                   | 72 (43)                          |
| WHO 1                          | 69 (42)                   | 67 (40)                          |
| WHO 2                          | 12 (7)                    | 13 (8)                           |
| WHO 4                          | 1 (1)                     | -                                |
| unknown                        | 15 (9)                    | 16 (10)                          |
| **Sex, n (%)**                 |                           |                                  |
| Male                           | 96 (58)                   | 100 (60)                         |
| Female                         | 70 (42)                   | 68 (40)                          |
| **Immunophenotype, n (%)**     |                           |                                  |
| B-ALL                          | 118 (71)                  | 119 (71)                         |
| T-ALL                          | 29 (17)                   | 28 (17)                          |
| Mixed phenotype                | 5 (3)                     | 4 (2)                            |
| T-LBL                          | 14 (8)                    | 17 (10)                          |
| **WBC**                        |                           |                                  |
| Median x10^9/L, range          | 10.8 (0.5-524)            | 11.0 (0.5-540)                   |
| >30x10^9/L for B-lineage       | 33/120 (27)               | 30/123 (24)                      |
| >100x10^9/L for T-lineage      | 6/46 (13)                 | 2/45 (4)                         |
| **% Blast count in bone**      | 88 (0-100)                | 88 (0-100)                       |
|                      |                  |                  |
|----------------------|------------------|------------------|
| **marrow, median (range)** |                  |                  |
| **Cytogenetics and/or molecular analysis, n (%)** |                  |                  |
| Not done/failure      | 7 (4)            | 2 (1)            |
| t(9;22)/BCR-ABL       | 38/158 (24)      | 30/166 (18)      |
| 11q23 abnormality/MLL | 10/156 (6)       | 18/164 (11)      |
| fusion                |                  |                  |
| Hypodiploidy          | 16/151 (11)      | 15/156 (10)      |
| Complex karyotype     | 23/151 (15)      | 32/156 (21)      |
| **CNS involvement, n (%)** | 5 (3)            | 7 (4)            |
| **Risk group, n (%)** |                  |                  |
| High risk             | 102 (61)         | 108 (64)         |
| Standard risk         | 64 (39)          | 60 (36)          |

Abbreviations: N; number of patients. LBL; lymphoblastic lymphoma. WBC; white blood cell count at diagnosis.
### Table 2 Response according to randomisation for CLO

|                                    | Standard treatment (arm A) | CLO + standard treatment (arm B) |
|------------------------------------|-----------------------------|----------------------------------|
| **CR**                             |                             |                                  |
| After induction cycle 1, N (%)     | 131 (79)                    | 131 (78)                         |
| After consolidation, N (%)         | 143 (86)                    | 145 (86)                         |
| CR on protocol, N (%)              | 148 (89)                    | 149 (89)                         |
| **MRD negativity**                 |                             |                                  |
| After RI I                         | 45/83 (54)                  | 62/88 (70)                       |
| After consolidation I              | 54/76 (71)                  | 75/81 (93)                       |
| On protocol                        | 76/99 (77)                  | 88/101 (87)                      |
| **Relapsed disease**               |                             |                                  |
| Relapse after CR                   | 42/148 (28)                 | 36/149 (24)                      |
| Relapse in MRD negative patients   | 10/54 (19)                  | 13/72 (18)                       |
| after consolidation I              |                             |                                  |
| Relapse in MRD positive patients   | 8/20 (40)                   | 3/5 (60)                         |
| after consolidation I              |                             |                                  |
| **Non-relapse mortality in CR, n (%)** |                     |                                  |
| ≤40y                               | 5/71 (7)                    | 7/71 (10)                        |
| >40y                               | 19/77 (25)                  | 17/78 (22%)                      |

N; number of patients. CR; complete remission. MRD; minimal residual disease. RI; remission induction course. * Denominator indicates the number of patients for whom a sample was obtained
Table 3 Adverse events occurring during treatment (CTCAE grade ≥3)

|                            | ≤40y | >40y | ≤40y | >40y |
|---------------------------|------|------|------|------|
| N=79                      |      |      |      |      |
| N=87                      |      |      |      |      |
| Any AE grade ≥3, N (%)    | 73 (92) | 75 (86) | 74 (93) | 81 (92) |
| Infection                 | 42 (53) | 32 (37) | 52 (65) | 59 (67) |
| Gastro-intestinal         | 29 (37) | 25 (29) | 29 (36) | 29 (33) |
| Neurological event        | 12 (15) | 10 (11) | 15 (19) | 13 (15) |
| Thrombo-embolic events    | 27 (34) | 23 (26) | 20 (25) | 22 (25) |
| (CTCAE grade ≥ 2)         |      |      |      |      |
| Any serious adverse event, n (%) | 55 (70) | 45 (52) | 66 (82) | 66 (75) |
| Fatal serious adverse event, n (%) | 2 (3) | 11 (13) | 6 (8) | 12 (14) |

Toxicity is graded according to NCI Common Terminology Criteria for Adverse Events (CTCAE, version 3). N; number of patients. CLO; clofarabine.
Figure legends

Legend to figure 1

CONSORT diagram of study

Arm B included CLO in prephase. In this diagram the two age groups (younger and older than 40 years of age) are combined (for CONSORT diagrams in subgroups, see supplementary Figure 1, 2 and 3). Treatment protocols are detailed in supplementary Tables 1 and 2. In this figure induction includes induction course and consolidation A and B for patients ≤40 years, and remission induction 1 and consolidation 1 for patients > 40 years of age. Consolidation I consists of Intensification course 1A and 1B and remission induction course 2 for younger and older patients respectively and consolidation II contains interphase and intensification 2 for younger and consolidation 2 for older patients.

Legend to Figure 2

Cumulative incidence of going off-protocol not due to completion, relapse or death in CR patients in standard arm and CLO-arm

Cumulative incidence for going of off-protocol not due to completion, relapse or death is shown in patients ≤ 40 years of age (A), and patients > 40 years of age (B) in control (blue) versus CLO group (red).

Legend to Figure 3

Event-free survival and overall survival of patients treated according to standard arm versus additional CLO

Event-free survival (A, B, C) and overall survival (D, E, F) of patients treated according to standard arm versus additional CLO are shown. Figure A and D show survival of all age groups together. B and E are patients ≤ 40 years of age, C and F are patients > 40 years of age.
Figure 2

Cumulative percentage

(A: control) N 77 off
C: clof 30 78 21
P=0.024

(B)
Figure 3

(A) Cumulative percentage over months for different conditions. At risk numbers are shown for each condition:

- A:control: 166, N: 84
- C:clof 30: 168, N: 79

The graph illustrates the cumulative percentage decrease over time, with distinct lines for each condition.
Figure 3
Figure 3
Figure 3