UNIVERSITES PARIS SUD XI - AIX-MARSEILLE II - LYON I - NANCY I - PARIS V - PARIS VI - PARIS VII et TOULOUSE III

DIPLOME INTER-UNIVERSITAIRE D'ONCOLOGIE PEDIATRIQUE

Année Universitaire 2010 - 2011

ETUDE RETROSPECTIVE DU DEVENIR DES PATIENTS AGES DE MOINS DE 21 ANS, TRAITES POUR UNE LEUCEMIE AIGUE LYMPHOBLASTIQUE AVEC LA CLOFARABINE (EVOLTRA®) OU UNE ASSOCIATION MEDICAMENTEUSE INCLUANT LA CLOFARABINE, DEPUIS SON AMM.

Mémoire présenté le 21 Octobre 2011

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Remerciements à :

Monsieur le Professeur André Baruchel,
Pour les conseils, l'aide et le temps qu'il a bien voulu me consacrer tout au long de la réalisation de ce mémoire.

Madame le Professeur Sylvie Chevret,
Pour sa disponibilité et son aide précieuse dans l'analyse statistique des données.

Mademoiselle Kristell Desseaux, biostatisticienne,
Pour sa disponibilité, sa réactivité et son aide dans l'analyse statistique.

Tous les médecins, membres de la SFCE, qui ont participé à cette étude,
Madame le Docteur Brigitte Nelken à Lille,
Monsieur le Professeur Gérard Michel à Marseille,
Madame le Docteur Isabelle Pellier à Angers,
Monsieur le Professeur Guy Leverger à Paris (Hôpital Trousseau),
Monsieur le Professeur Yves Bertrand à Lyon,
Monsieur le Professeur Pierre Rohrlich à Besançon,
Madame le Docteur Claudine Schmitt et Madame le Docteur Audrey Contet à Nancy,
Monsieur le Docteur Nicolas Sirvent et Madame le Docteur Anne Deville à Nice,
Monsieur le Docteur Patrick Boutard à Caen,
Madame le Docteur Geneviève Margueritte à Montpellier,
Madame le Docteur Brigitte Pautard à Amiens,
Monsieur le Professeur Yves Perel et Madame le Docteur Nathalie Aladjidi à Bordeaux,
Monsieur le Professeur Dominique Plantaz à Grenoble,
Monsieur le Docteur Alain Robert à Toulouse,
Madame le Docteur Caroline Thomas à Nantes,
Madame le Docteur Claire Berger à Saint-Etienne,
Madame le Docteur Virginie Gandemer à Rennes,
Monsieur le Docteur Patrick Lutz à Strasbourg,
Monsieur le Docteur Christophe Piguet et Madame le Docteur Caroline Oudot à Limoges.

Mesdames les Attachées de Recherche Clinique des différents centres,

Monsieur le Professeur Philippe Labrune,
Pour son soutien et sa confiance.

A toute l'équipe de l'hôpital de jour de l'hôpital Antoine Béclère,
Pour son soutien et sa compréhension.

A mon mari et à mes enfants,
Pour leur aide, leur soutien et leur patience.

Table of contents

French “real life” experience of clofarabine in children

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Summary

Clofarabine alone or in combination with cyclophosphamide and etoposide has shown, in previous studies, a good efficacy and a tolerable toxicity profile in children with relapsed or refractory leukaemia. This report describes a retrospective study of 38 French patients who received clofarabine as a monotherapy or in combination for an acute lymphoblastic leukaemia (ALL), outside of clinical trials, after the French Marketing Authorization. Thirty patients received clofarabine for a bone marrow relapse of ALL. Most of the patients presented with an advanced disease. An overall remission rate (ORR) of 37% was obtained in this population, which seems lower than the ones found in previous similar studies. Nevertheless, transplantation rate and survival for these patients were similar to those in published studies. Eight patients were treated in remission for a high level of minimal residual disease (MRD). A moderate improvement of MRD (one log or less) was documented in 4 patients. However, clofarabine treatment is associated with a high risk of infection and hepatotoxicity. Four deaths related to treatment have been observed in our study. Prospective studies using clofarabine during earlier phases of the disease should help to define the place of this new drug in childhood and adolescent ALL.

Introduction

Acute lymphoblastic leukaemia (ALL) is the most common form of cancer in children, comprising approximately 30 percent of all childhood malignancies. Despite dramatic improvements in survival for children with ALL that have occurred over the last 3 to 4 decades, 20 to 25% of children suffer a relapse (Gaynon *et al*, 2005; Pui *et al*, 2006). While a substantial proportion of children with relapse ALL achieve a second remission, the overall final outcome remains unsatisfactory with long-term overall survival rates range from 15 to 50% (Raetz *et al*, 2008). Predictive factors of survival include the site of the relapse and the length of the first complete remission (Malempati *et al*, 2007; Nguyen *et al*, 2008; Ko *et al*, 2010). In general, bone marrow and early relapse (< 36 months from initial diagnosis) have a worse prognosis than isolated extra-medullary or late relapse (≥ 36 months from initial diagnosis). Treatment for relapsed ALL primarily involves many of the same traditional chemotherapy agents initially used as well as hematopoietic stem cell transplantation (HSCT) (Malempati *et al*, 2007; Nguyen *et al*, 2008; Ko *et al*, 2010). In light of the very poor prognosis of these patients with a conventional therapy, innovative treatment strategies based on the use of novels anti-leukemic agents are needed.

Antimetabolites are some of the most effective drugs against haematological malignancies. Fludarabine and cladribine are active in the treatment of relapsed acute leukaemia although their use is associated with an important dose-limiting neurotoxicity (Gandhi *et al*, 2001). Among new antimetabolic drugs, clofarabine (2-chloro-9(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)adenine) is a novel second-generation purine nucleoside analogue synthesized with the aim to overcome the limitations (neurotoxicity), but to retain the favourable properties of fludarabine and cladribine (Kline *et al*, 2005; Bonate *et al*, 2006; Kantarjian *et al*, 2007; Robak *et al*, 2009; Robak *et al*, 2011). Its antitumor activity is due to 3 mechanisms: inhibition of DNA polymerase α, inhibition of ribonucleotide reductase and disruption of mitochondrial membrane integrity with the release of proapoptotic factors leading to programmed cell death even in non-dividing lymphocytes. To exert its cytotoxic effects, clofarabine needs to be phosphorylated by deoxycytidine kinase (dCK) to its active triphosphate form.

Phases I and II studies with single-agent clofarabine were performed in paediatric and adult patients with multiple relapse or refractory leukaemia (Kantarjian *et al*, 2003a; Kantarjian *et al*, 2003b; Jeha *et al*, 2004; Jeha *et al*, 2006; Kearns *et al*, 2006). These studies have shown the safety and the efficacy

of clofarabine leading an overall remission rate of 20% in paediatric patients. The most frequently observed grade ≥ 3 adverse events were febrile neutropenia, hypokalemia, elevated aspartate or alanine transaminases, hyperbilirubinemia and neutropenia. Systemic inflammatory response-like or cytokine release-like events, skin rash and hand-foot syndrome were also observed.

In view of these results clofarabine has been approved by both the Food and Drug Administration in the United States and the European Medicinal Evaluation Agency for the treatment of ALL in paediatric patients (≤ 21 years old) who have relapsed or are refractory after receiving at least two prior regimens and where there is no other treatment option anticipated to result in a durable response (Jeha *et al*, 2006).

Clofarabine inhibits the repair of DNA damage. An increased cytotoxic effect could thus be expected using a combination with alkylating agents such as cyclophosphamide. A synergistic effect was first demonstrated by in-vitro studies (Yamauchi *et al*, 2001) and was confirmed in clinical trials (Karp *et al*, 2007; Hijiya *et al*, 2009; Locatelli *et al*, 2009). Phases I and II studies with the combination clofarabine, cyclophosphamide and etoposide were performed by Hijiya *et al* and Locatelli *et al* (Hijiya *et al*, 2009; Locatelli *et al*, 2009). The results were very encouraging with an overall remission rate (ORR) of 56% and 55% respectively for patients with relapse or refractory ALL. The most common adverse events were the same as clofarabine in single use. In some cases, however, severe and potentially life-threatening hepatotoxicity occurred with the use of clofarabine in combination. In the phase II study reported by Hijiya *et al*, four of the first eight patients enrolled developed a severe hepatotoxicity (three veno-occlusive diseases (VOD) and one hyperbilirubinemia) (Hijiya *et al*, 2009). But no case of VOD was reported by Locatelli *et al* with the same combination of drugs at slightly different dosages (Locatelli *et al*, 2009).

This report describes a retrospective study of 38 patients who received clofarabine as a monotherapy or in combination for a relapse or refractory ALL (30 cases) or in remission but with a high MRD (8 cases). These patients were treated in 17 French haematological centres after the obtention of the EMEA marketing authorization (2006).

Patients and methods

Study Group

This study has been proposed to 26 centres treating children and adolescents with leukaemia belonging to the French society for paediatric hemato-oncology (SFCE: Société Française de lutte contre les Cancers et les leucémies de l'Enfant et de l'adolescent). Five of them have not answered and four of them had not used this treatment. Finally, 17 centres provided information about 38 patients. Anonymized data were collected using a standardized Case Report Form.

All the patients who received at least one course of clofarabine for a relapse or refractory ALL (30 cases) or in remission but with a high MRD (8 cases), and were less than 21 years old at the moment of treatment have been included. No other eligibility criteria were required. We have collected, for all the patients, demographic data, leukaemia characteristics, previous treatments, modality of administration of clofarabine, adverse events and outcome after clofarabine therapy.

A validation step of data collected has been made by two of us (PT and AB) before database freezing and analysis. The cut-off date for this analysis was September 1st, 2010.

Treatment

Clofarabine was administered alone (9 patients) at a dosage of 52 mg/m^2 daily for five days or in combination. The two major combinations were clofarabine 40 mg/m^2/day, cyclophosphamide 440 mg/m^2/day and etoposide 100 mg/m^2/day as in Hijiya *et al* (2009) study (21 patients) or clofarabine 40 mg/m^2/day, cyclophosphamide 400 mg/m^2/day and etoposide 150 mg/m^2/day as in Locatelli *et al* (2009) study (5 patients). All drugs were administered for five consecutive days. Only three patients received clofarabine in association with another chemotherapy. Nine patients received concomitantly intrathecal chemotherapy. The majority of the patients (27 out of 38) received only one course of clofarabine. Seven out of the 38 patients received 2 courses of clofarabine and four of them received 3 courses.

Response and toxicity criteria

Complete remission (CR) was defined as no detectable leukaemia cells on peripheral blood, M1 bone marrow (≤ 5% blasts) and recovery of peripheral counts (platelets ≥ 100 x 10^9/l and an absolute granulocyte count (ANC) ≥ 1.0 x 10^9/l). CR in the absence of total platelet recovery (CRp) was defined as patients who met all the criteria for a CR except for recovery of platelet counts (platelets < 100 x 10^9/l). Partial remission (PR) was defined as complete disappearance of circulating blasts and either a M2 bone marrow (> 5% and ≤ 25% blasts) and appearance of normal progenitor cells or a M1 bone marrow that did not qualify for CR or CRp. The overall remission rate (ORR) was defined as the number of patients who achieved CR or CRp divided by the number of treated patients. MRD was analyzed by real time quantitative-polymerase chain reaction (RQ-PCR) analysis of "leukaemia-specific" junctional regions of rearranged immunoglobulin (Ig) genes and T-cell receptor (TCR) genes, according to the ESG-MRD-ALL guidelines 2007 (Van der Velden *et al*, 2007). Adverse events (AEs) were evaluated using National Cancer Institute's Common Terminology Criteria for Adverse Events (NCI CTCAE v4.0).

Statistical analysis

This retrospective study involved 38 children treated with clofarabine outside clinical trials between January 1st, 2006 and January 1st, 2010 in 17 French centres. We reported quantitative variables as median and range, and qualitative variables as percentages. Comparison of response rates were based on the exact Fisher test. Predictive factors of remission were assessed through univariable logistic regression models, with reported odds ratio (OR) with ninety-five percent confidence intervals (95%CI).

Survival and response durations were estimated by the Kaplan-Meier method, where patients who did not experience the event of interest were censored at the date of last follow-up. Survival curves were compared using the log-rank test.

Finally, owing to these non-randomized available data, there was a need to correct for potential recruitment bias. Therefore, to get some comparison with historical controls, we performed a propensity score-matched analysis, using 330 controls with first relapse selected from the FRALLE93 trial data base. The idea was to model, for each patient alive after relapse, the probability of

receiving clofarabine, according to a set of baseline characteristics (namely, gender, baseline age, T or B ALL, WBC count, refractory relapse, and relapse-free interval) using logistic regression. It was then used to match 1:1 patients with similar propensity to receive clofarabine, based on nearest neighbour matching using callipers of width 0.2 as recommended. Survival benefit of clofarabine is then estimated based on the matched dataset.

All statistical analyses were performed on R software (http://www.R-project.org). All statistical tests were two-sided with type I error of 0.05.

Results

Patients and treatment

The patient characteristics at initial diagnosis are shown in Table 1. Median age at diagnosis was 4 years (range, 0-16), including three infants. There were 22 boys and 16 girls. The initial diagnosis was B lineage ALL for 33 patients, T cell ALL for 2 patients and a biclonal ALL for the remaining three. The median white blood cell count was 7.45 x 10^9/l (range, 0.9-675). Only 3 patients with B lineage ALL had initial high-risk cytogenetic features (translocation t(4; 11): one patient, hypodiploidy: two patients). Two patients had a central nervous system (CNS) involvement at diagnosis. The first line protocol was the EORTC 58951 (17 patients) or the FRALLE 2000 (17 patients). Three patients were included in the Interfant 2006 protocol. The last patient (biclonal T cell and myeloid leukaemia) was treated in the acute myeloid leukaemia protocol (ELAM02). All patients were in remission after the first course of chemotherapy.

Thirty-seven out of 38 patients were treated with clofarabine at bone marrow relapse of ALL (including 6 patients for a combined relapse). Thirty patients received clofarabine for a bone marrow relapse of ALL and 7 for a high MRD after relapse treatment. Only one patient did not relapse and received clofarabine for a high MRD in CR1 (Fig 1). If we consider the 30 patients treated for a relapse of ALL, nine patients (30%) received clofarabine as the first treatment for relapse: 5 for a first relapse,

3 for a second relapse and 1 for a third relapse[1]. Twenty-one patients (70%) had a refractory relapse with at least one previous treatment for the relapse: 14 patients received clofarabine for a refractory first relapse, 6 for a refractory second relapse and 1 for a refractory third relapse. For the remaining seven patients, clofarabine was given in condition of remission after a relapse but with a high MRD with the aim of decreasing tumour burden before transplantation.

According to treatment received, nine patients were treated with clofarabine as a monotherapy, 2 for a high MRD and 7 for a relapse of disease. The 29 other patients received clofarabine in combination, 6 for a high MRD and 23 for a relapse (Fig 2).

In total, 8 patients received clofarabine for a high MRD (one in CR1, six in CR2 and one in CR3) and 30 patients received clofarabine for a relapse of ALL. The median interval between initial diagnosis and clofarabine administration was 1.8 year for the 30 patients treated for a relapse of ALL and 2.7 years for the 8 patients treated for a high MRD.

Finally, most of the patients were heavily pre-treated with a mean of 2.5 prior therapies (range, 1-4). Ten patients had received a prior HSCT (five in CR1 and five in CR2).

Response and outcome

To describe the response to treatment the population was separated in two groups: one group of 30 patients treated for ALL in relapse and a second group of 8 patients treated for a high MRD.

In the first group of 30 patients, eight achieved CR and 3 CRp, giving an overall remission rate (ORR) of 37% (Table 2). It is worth noting that these 30 patients were heavily pre-treated with a mean of 2.5 prior therapies. Eight patients achieved remission after the first cycle of clofarabine, two patients after two cycles and one after three cycles. The initial diagnosis was a B lineage ALL for 26 patients, a T cell ALL for 2 patients and a biclonal ALL for the last two ones. Ten out of 26 children (38%) with B ALL and one out of two children with T ALL have achieved CR or CRp. The two cases of biphenotypic leukaemia failed to respond. A remission was obtained for 8 out of 21 patients with refractory

[1] This patient received clofarabine for a third combined relapse (bone marrow and CNS) but in fact it was the eighth one (6 isolated CNS relapses previously).

relapse (7 CR and 1 CRp; ORR 38%). For the 9 patients who received clofarabine as a first treatment for a relapse, the ORR was 33% (1 CR and 2 CRp). Seven patients received clofarabine as a monotherapy and 23 clofarabine in combination. ORR was not different in combination regimens (35%) compared with single agent use (43%) (p=1.00) (Fig 2). In this first group of 30 patients, 3 had adverse cytogenetic abnormalities and failed to respond to clofarabine treatment. The probability to respond to treatment was not influenced by age at diagnosis (p=0.94) nor WBC count at diagnosis (p=0.63) but only by the time interval between the diagnosis and the first relapse (p=0.02) (Table 3). Two out of 8 patients (25%) who had previously received HSCT responded to clofarabine treatment as compared to nine (41%) out of 22 patients who had not been previously transplanted.

Among these 30 patients, 5 have received clofarabine as a first treatment for a first bone marrow relapse. These 5 patients presented an ORR of 40% (1CR and 1CRp). They had especially serious ALL (3 of them with a prior HSCT in CR1, early relapses for all the patients, and one patient with a severe hypodiploidy). However, one out of these five patients who was in a PR after clofarabine treatment has reached a CR using a conventional chemotherapy.

Ten out of 11 responding patients subsequently received HSCT. The median interval between clofarabine treatment and HSCT was 3.5 months (range, 3.1-4.3). Five out of ten transplanted patients are alive (range, 4.5-22 months).

For the 19 non-responding patients, 2 were alive at the end of the study (one was in PR after clofarabine and the second one was in palliative treatment).

In the second group of eight patients treated for a high MRD, was obtained, as expected, the best survival rate. But the MRD levels did not decrease significantly after clofarabine (Table 4). Only one patient really has his MRD improved by more than one log ($\geq 10^{-2}$ before clofarabine and $<10^{-3}$ after clofarabine). This patient was treated by clofarabine in first remission. Seven among these eight patients received HSCT and six of them are alive at the end of the study. Another patient is alive without receiving HSCT.

The Kaplan-Meier curves for overall survival (OS) are shown in Figure 3. The probability of survival one year from the beginning of clofarabine treatment for the entire cohort of patients was 38.1% (95%CI: 13.7%-100%). Patients treated for a high MRD had a significantly increased probability of survival in comparison to those treated for a relapsed ALL (with 1 year OS estimated at 87.5%, 95%CI: 67.3%-100% versus 29.1%, 95%CI: 16.5%-51.4%) (p=0.003). Among the 30 patients treated for a

relapse, the 1 year survival rate for patients who have or have not achieved a CR or CRp was respectively of 63.6% (95%CI: 40.7%-99.5%) and 6.6% (95%CI: 1%-42.1%) (p=0.0015).

Finally, the survival of the entire cohort was compared to that of a matched cohort of patients with first relapse after inclusion in the FRALLE93 trial. Survival after relapse appeared non significantly reduced in the clofarabine group as compared to the control group (estimated hazard ratio= 0.70, 95%CI: 0.18-2.76, p= 0.18). Numbers were too small to compare only patients receiving clofarabine in first relapse.

Toxicity

Treatment related toxicities are presented in Table 5. Only side effects grade ≥ 3 are reported. Febrile neutropenia grade ≥ 3 was reported in 79% of patients. Documented infections grade ≥ 3 occurred in 9 patients (24%). Among these 9 patients, six patients developed septicaemia (16%) and 3 had pneumonia (8%). Infectious complications were more frequent in patients treated with clofarabine in combination than alone (27.5% versus 11%). The median time to neutrophil recovery (defined as an absolute granulocyte count > 0.5 x 10^9/l) was only known for 10 out of 11 patients who achieved CR or CRp and was 22.5 days (range, 15.75-31). Ten patients (26%) had hepatotoxicity grade 3 (nine elevated transaminases, and one hyperbilirubinemia) but no case of VOD was described. The frequency of hepatic complications was more important for patients who have received an intrathecal chemotherapy concomitantly with clofarabine (Table 6). Five cases (56%) of hepatotoxicity grade 3 (elevation of transaminases) have been identified in this sub group of 9 patients, as compared to 5 out of 29 patients (17%) without intrathecal therapy (p=0.02). Hepatotoxicity was not statistically different for patients treated with clofarabine alone or in combination. Acute renal failure was diagnosed in four patients, one patient with grade 3 and three patients with grade four (2 of them had a concomitant multi-organ failure). Four patients (10.5%) died due to potential treatment related complications. All four patients developed a fatal multi-organ failure. One patient presented a refractory first relapse despite two treatments and was in a bad general status before starting clofarabine treatment. Another patient received clofarabine for a first refractory relapse. He had a severe hypodiploidy with 25 chromosomes and had previously presented a serious toxicity during first line therapy after methotrexate treatment (MTHFR mutation). After the start of the clofarabine treatment, he presented a multi-organ failure and died.

The third patient had received clofarabine in first line treatment for a second bone marrow relapse and had undergone previous HSCT in CR2. The last patient presented with a second combinated relapse (medullary and CNS) with paraplegia and blastic meningitis before the beginning of clofarabine treatment. He died in a context of multi-organ failure and status epilepticus. All these patients had received clofarabine in combination and 2 out of them had received concomitantly intrathecal chemotherapy.

Only two patients have needed a premature discontinuation of treatment with four days of clofarabine instead of five days planned (one patient for a multi-organ failure and the second one for uncontrolled fever, headache and abdominal pain). Finally, 95% of patients received a complete first course treatment.

Discussion

This multicenter French retrospective study of 38 patients treated by clofarabine for ALL shows worse results than expected. In the group of 30 patients who received clofarabine for an ALL in relapse, the ORR obtained is only of 37%. This result is lower than the ones reported by Hijiya *et al* (2009) and Locatelli *et al* (2009) for patients treated with the combination of clofarabine, cyclophosphamide and etoposide. These authors respectively report ORR of 56% and 55%. Nevertheless, these phases I and/or II studies have been conducted with selected patients. On the other hand, we have obtained better results than in studies with clofarabine used alone where remission rates are about 20% (Kantarjian *et al*, 2003a; Jeha *et al*, 2004; Jeha *et al*, 2006; Kearns *et al*, 2006). A similar study, which presents the UK experience of clofarabine in the treatment of relapsed and refractory paediatric ALL, has been recently published by O'Connor *et al* (2011). Their results are better than ours with an ORR of 52%. However, in our study, most of patients had an advanced disease when clofarabine was administered. They were heavily pre-treated with an average of 2.5 treatments before clofarabine, and 70% of them were refractory to their most recent prior treatment. All these factors may have contributed to the low ORR obtained in our study. In O'Connor study, patients have received a mean of only 2 prior treatments and no information was provided about refractory patients (O'Connor *et al*, 2011).

To get some insight in the potential survival benefit of clofarabine, we used a propensity score matching procedure that allows controlling for potential source of selection bias of the cohort. This

allowed to suggest that patients treated with clofarabine had a non significantly reduced survival as compared to first relapsing patients who were administered other chemotherapies. Obviously, this analysis should be interpreted cautiously given notably that only first relapses were considered in the controls.

Surprisingly, the ORR was similar for refractory patients (ORR 38%) to the one for patients treated with clofarabine in first line for a relapse (33%). Also, the ORR was not better for the 5 patients treated by clofarabine in first line for a first relapse (ORR 40%). The results obtained for these 5 patients are in contradiction with the ones of O'Connor (O'Connor *et al*, 2011). Effectively, in the same conditions, O'Connor presented an ORR of 86% (7 patients, 6 RC). Obviously, our results must be interpreted with caution due to the small number of patients and the severity of disease they presented with.

If we consider the 11 patients in remission after clofarabine among the 30 patients studied, 10 of them have received an HSCT (33%) and 5 were alive (17%) at the end of our study with a median follow-up post HSCT of 13 months (range; 4.5-22). These data are similar to the ones presented in other studies with transplantation rates after clofarabine of 28% for Locatelli *et al* (2009), 30% for Hijiya *et al* (2009) and 43% for O'Connor *et al* (2011). Regarding the survival rate after clofarabine treatment, Locatelli has obtained a survival rate of 16% with a median follow-up of 8 months after HSCT (Locatelli *et al*, 2009). In his study, O'Connor presented a survival rate of 26% with a median follow-up of 13 months post HSCT (O'Connor *et al*, 2011). Finally, fewer patients in our cohort were in remission after clofarabine in comparison with the other studies, but the post clofarabine transplantation rate and survival rate were similar to the ones of other published studies.

Finally, 8 patients were treated in complete remission but with a high MRD level. The use of clofarabine for such an indication has not yet been described in the literature. Unexpectedly, clofarabine treatment has only moderately improved (one log or less) the MRD levels in 4 out of 8 patients. Nevertheless, the majority of these patients were transplanted after clofarabine treatment and the survival rate in this sub-group is high (87.5% one year after clofarabine). However, the limited number of patients studied in this subset analysis limits our possible conclusions.

Adverse events with clofarabine treatment are frequent. In our study clofarabine was generally well tolerated, even if we report 4 deaths (10.5%) potentially attributed to the treatment. These 4 patients were all in severe condition and developed a multi-organ failure after clofarabine treatment. In the literature, Hijiya *et al* (2009) report an 8% death rate (2 patients) after clofarabine treatment,

but no death was reported by Locatelli *et al* (2009) and O'Connor *et al* (2011). Several cases such as systemic inflammatory response syndrome (SIRS) or capillary leak syndrome which both could evolve in a multi-organ failure, have already been reported with clofarabine treatment (Jeha *et al*, 2006).

For others patients, we report similar adverse events to published data. The incidence of febrile neutropenia in our study (79%) is comparable with previous reported rates of 64% and 65% (Hijiya *et al*, 2009; O'Connor *et al*, 2011). However, we report less documented infections (grade ≥ 3) than in the literature, with only 9 cases (24%) including 6 sepsis. As comparison, infections (grade ≥ 3) are described in the literature with higher rates: 72%, 32% and 30% (respectively Hijiya *et al*, 2009; Locatelli *et al,* 2009; O'Connor *et al,* 2011). Infectious complications are frequent with clofarabine treatment. In order to decrease their incidence, prophylactic treatments against bacterial and fungal infections with an active monitoring of patients are essential.

Literature reports frequent cases of hepatic toxicity when clofarabine is used as single agent or in combination (Kantarjian *et al*, 2003a; Jeha *et al*, 2004; Karp *et al*, 2007). Some severe and life-threatening cases of hepatic toxicity have also been described by Hijiya *et al* (2009) (3 veno-occlusive disease, 1 hyperbilirubinemia). In our study, hepatotoxicity grade 3 occurred in 10 patients (26%; 1 hyperbilirubinemia and 9 elevated transaminases), but no case of VOD have been recorded. Nevertheless, we found more frequent hepatic complications in a sub-group of the cohort made of 9 patients who received intrathecal chemotherapy concomitantly with clofarabine. Five of them (56%) developed hepatotoxicity grade 3 with elevated transaminases. Moreover, in Hijiya study where severe cases of hepatotoxicity with VOD have been described all the patients had received intrathecal prophylaxis (Hijiya *et al*, 2009). However, in our patients we only found transaminases elevation. Therefore, we cannot conclude on a possible link between clofarabine associated to intrathecal chemotherapy and increased risk of VOD.

This French "real life" experience of clofarabine treatment for refractory or relapse ALL has shown lower response rates than expected. Our remission rates remain lower than the ones presented in similar studies. Nevertheless, in view of the limited number of patients and the severity of their diseases, additional studies are required. It could be interesting to use clofarabine at an earlier stage of the disease, in order to assess if clofarabine offers a better efficiency in such a context. In that line, the next International Study on Relapsed ALL will randomize the use of clofarabine in combination in patients at high risk.

For patients with high MRD levels, only a moderate gain was shown. Again, further prospective investigations are necessary.

Clofarabine is associated with a high risk of infection and hepatotoxicity. Deaths related to treatment have been observed. These risks must be taken into consideration before any prescription of clofarabine treatment.

Finally, the cost of this new drug renders comparative and prospective evaluation of its risk-benefit ratio mandatory.

Annex

Table 1. Patient characteristics (N=38)

Patient characteristics	**Number (%)**
Gender: male/female	22/16 (58%/42%)
Median age at diagnosis, years (range)	4 (0-16)
Median age at clofarabine treatment, years (range)	7 (0-18)
WBC at diagnosis $x10^9$/l (range)	7.45 (0.9-675)
Immunophenotype	
B lineage	33 (87%)
T lineage	2 (5%)
Biphenotypic	3 (8%)
CNS involvement at diagnosis	2
Adverse cytogenetics	
t(4;11)	1
Hypodiploid karyotype	2
First line protocol	
FRALLE 2000	17
EORTC 58951	17
ELAM 02	1
Interfant 2006	3
Refractory to first line therapy	0
Number of earlier therapies, mean (range)	2.5 (1-4)
Previous hematopoietic stem-cell transplantation	10

WBC: white blood cell count

CNS: central nervous system

Table 2. Response to treatment for the 30 patients treated with clofarabine for a relapse or refractory ALL

N=30 patients	Clofarabine alone	Clofarabine in combination	Total	ORR
Clofarabine for refractory relapse	Patients = 4 CR = 2 CRp = 0 Alive = 0	Patients = 17 CR = 5 CRp = 1 Alive = 2	Patients = 21 CR = 7 (33%) CRp = 1 (5%) Alive = 2	38%
Clofarabine as first treatment for relapse	Patients = 3 CR = 0 CRp = 1 Alive = 2	Patients = 6 CR = 1 CRp = 1 Alive = 3	Patients = 9 CR = 1 (11%) CRp = 2 (22%) Alive = 5	33%
Total	Patients = 7 CR = 2 CRp = 1 Alive = 2	Patients = 23 CR = 6 CRp = 2 Alive = 5	Patients = 30 CR = 8 CRp = 3 Alive = 7	37%

ORR: overall remission rate

CR: complete remission

CRp: complete remission in the absence of total platelet recovery

Table 3. Predictive factors of remission for 30 patients treated with clofarabine for a relapse or refractory ALL

Univariable models	**OR (95%CI)**	**P-value**
Age at diagnosis	0.99 (0.85-1.17)	0.94
WBC at diagnosis	0.87 (0.49-1.53)	0.63
Delay (years) between diagnosis and relapse	4.07 (1.25-13.3)	0.020

OR (95%CI): odds ratio (95% confidence interval)

WBC: white blood cell count

Table 4. Impact of clofarabine on patients with a high MRD (N=8)

MRD evolution	**Number (%)**
Improvement of one logarithm	1 (12.5%)
Improvement of less than one logarithm	3 (37.5%)
Stable	3 (37.5%)
Non evaluable	1 (12.5%)

MRD: minimal residual disease

Table 5. Treatment-related toxicity (N=38)

Adverse events (grade >=3)	**Number (%)**
Febrile neutropenia	30 (79%)
Hepatic dysfunction	10 (26%)
Diarrhea	8 (21%)
Vomiting	7 (18%)
Hypokalemia	7 (18%)
Bacterial sepsis	6 (16%)
Mucositis	4 (10.5%)
Headache	4 (10.5%)
Multi-organ failure	4 (10.5%)
Lung infection	3 (8%)

Table 6. Hepatotoxicity for patients treated concomitantly with clofarabine and intrathecal chemotherapy

Criterion	Non intrathecal	Intrathecal
Number of patients	29	9
Hyperbilirubinemia, grade 3	1 (3.5%)	0 (0%)
Transaminases elevation, grade 3	4 (13.8%)	5 (55.6%)
Either one toxicity	5 (17.3%)	5 (55.6%)

Fig 1. Study flowchart

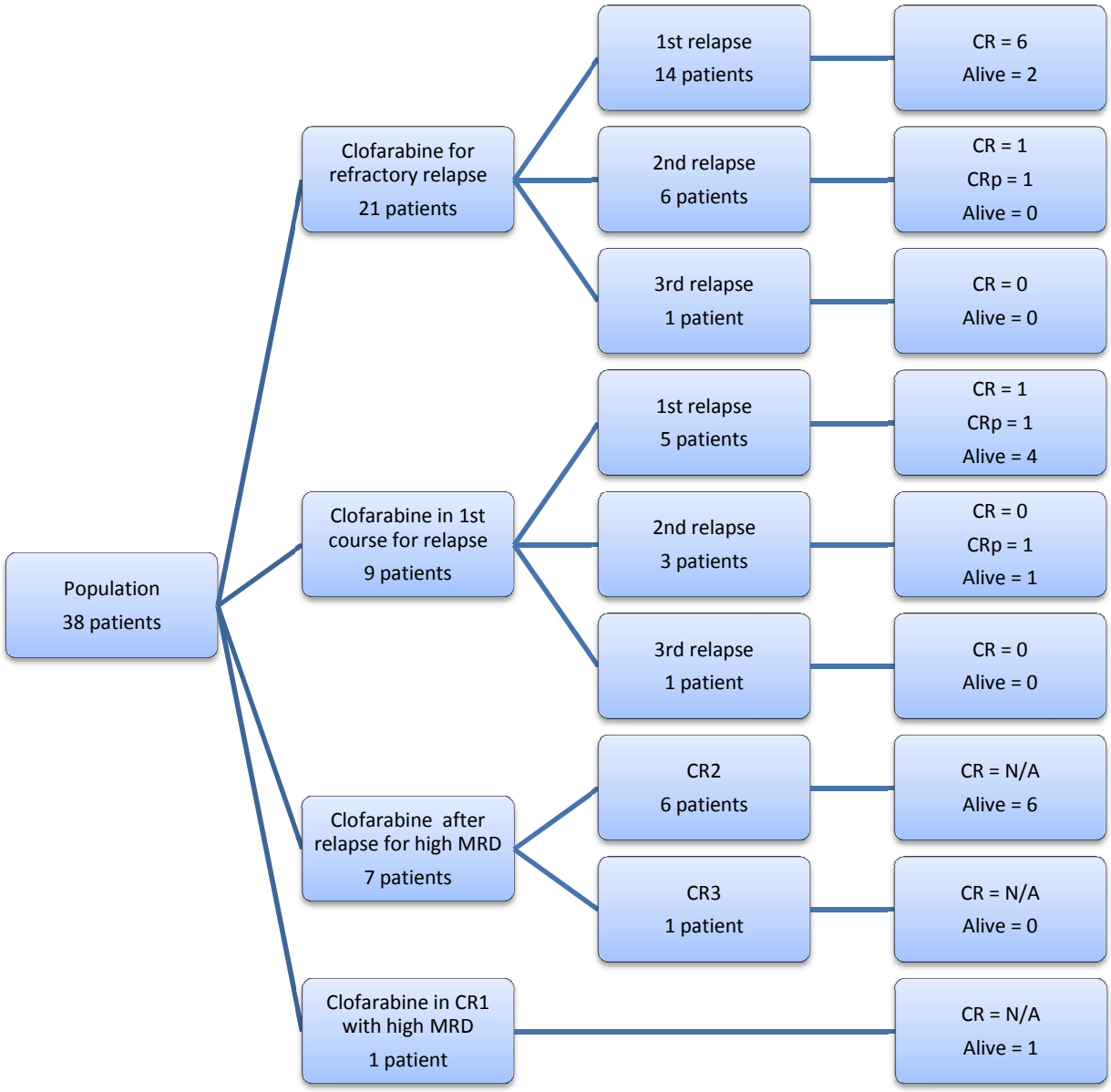

Fig 2. Treatment flowchart

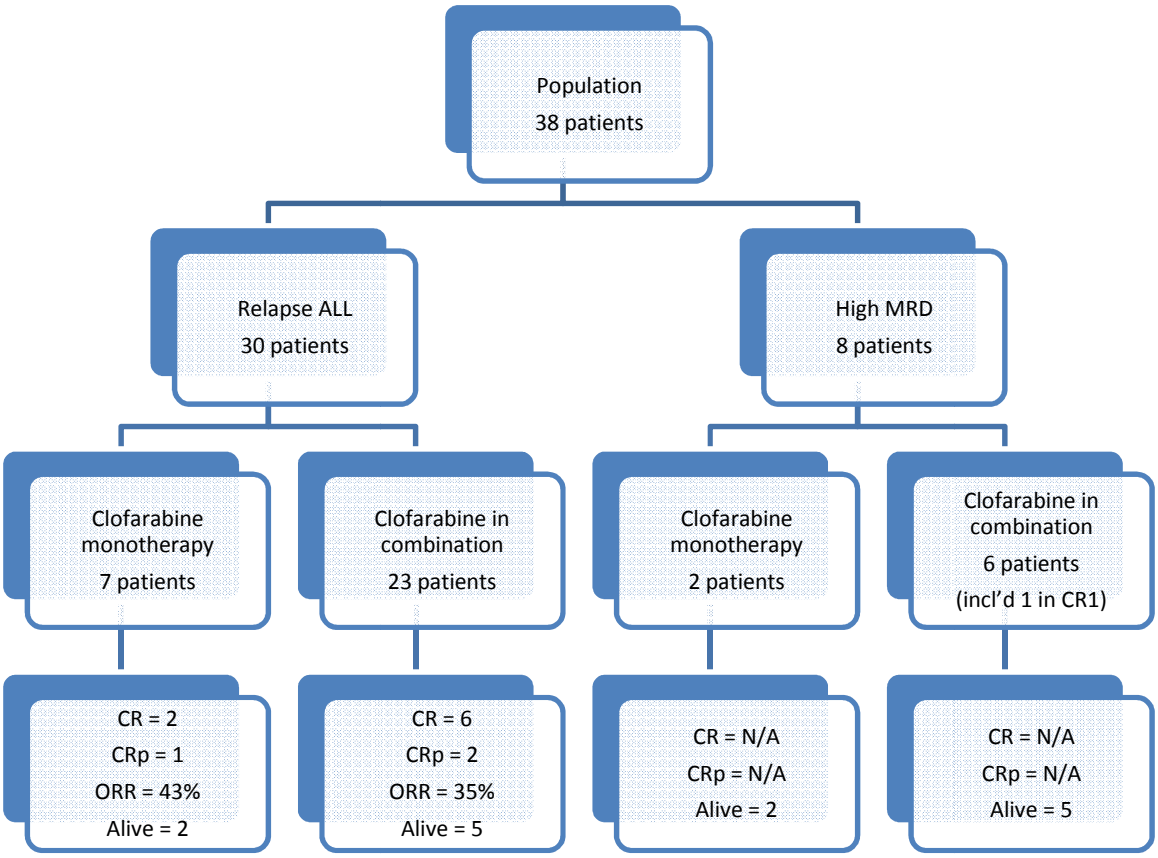

Fig 3. Overall survival (Kaplan-Meier)

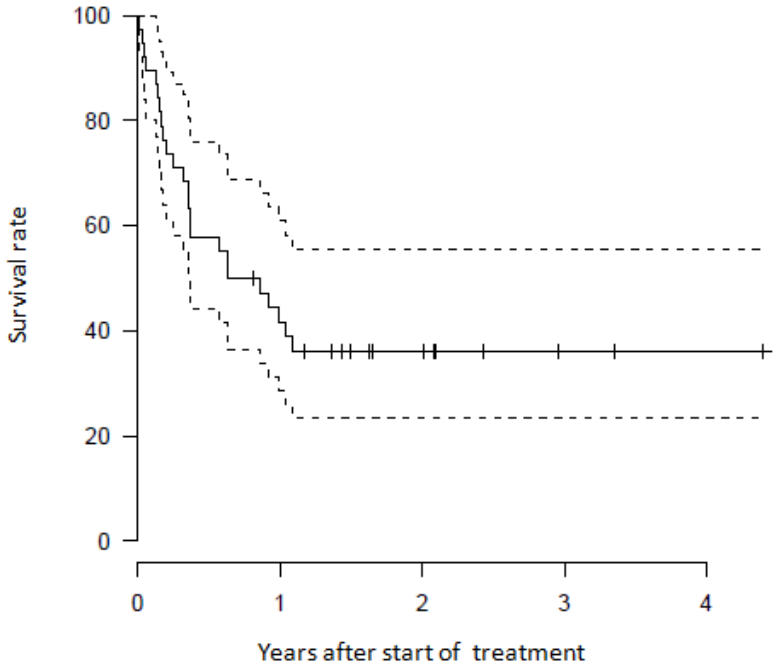

Fig 3a. Overall survival for the 38 patients from the beginning of clofarabine treatment

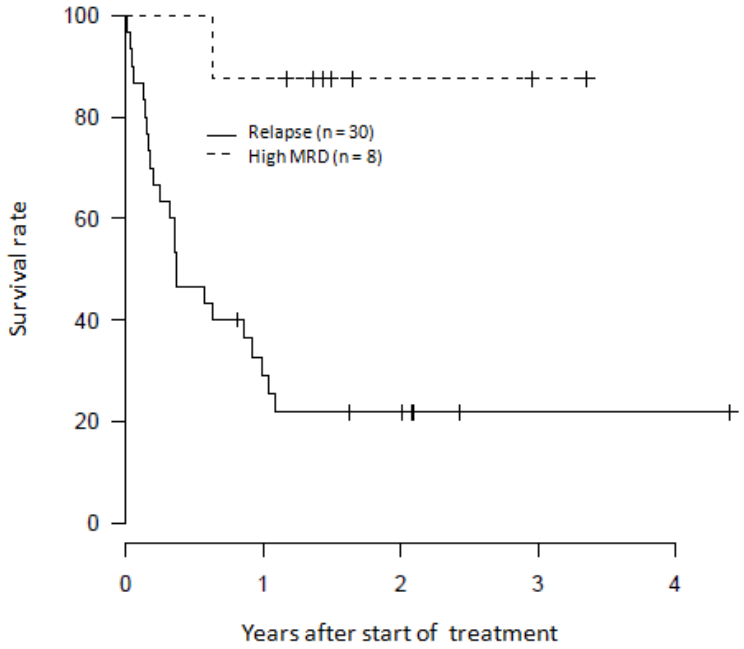

Fig 3b. Overall survival for the 30 patients with relapse of ALL versus the 8 patients treated for a high MRD

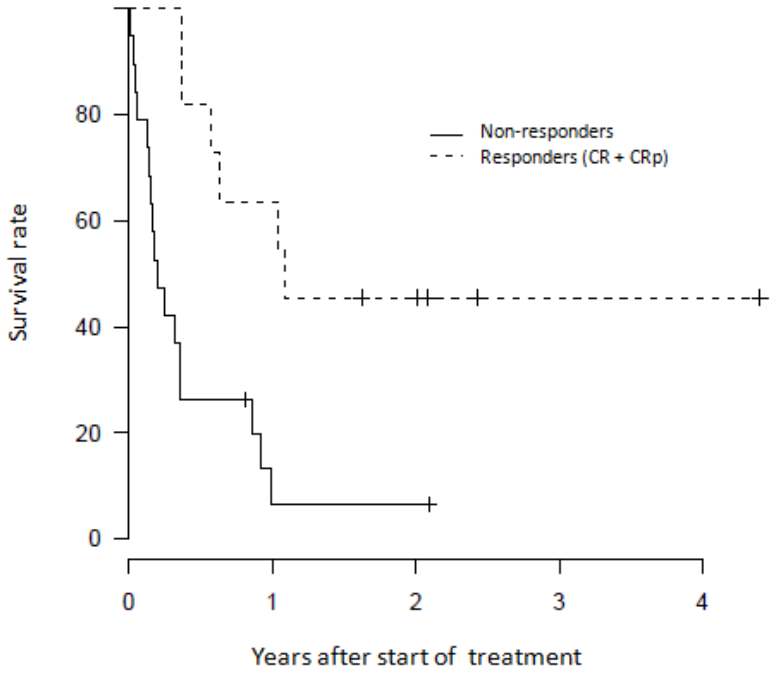

Fig 3c. Overall survival for the 30 patients with relapse of ALL from start of treatment by response to treatment

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