Cukurova Medical Journal
ÇUKUROVA ÜNİVERSİTESİ TIP FAKÜLTESİ

Cukurova Med J 2023;48(2):535-540
DOI: 10.17826/cumj.1274325

RESEARCH

Clofarabine, cyclophosphamide, and etoposide (CLOVE) in adult patients with relapsed/refractory acute T-cell lymphoblastic leukemia: single-center experience

Relaps/refrakter T hücreli akut lenfoblastik lösemili erişkin hastalarda klofarabin, siklofosfamid, etoposid (CLOVE): tek merkez deneyimi

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Abstract

Purpose: Clofarabine is a second-generation purine analog that inhibits DNA synthesis. It is used as an effective new agent in relapsed refractory acute leukemia. We aimed to report our single center experience about CLOVE protocol as a clofarabine-based regimen in patients with relapsed or refractory T-cell acute lymphoblastic leukemia.

Materials and Methods: Thirteen patients with relapsed or resistant T-cell acute lymphoblastic leukemia were included in this study. Patients were administered clofarabine (40 mg/m^2/day), etoposide (100 mg/m^2/day), and cyclophosphamide (440 mg/m^2/day) (5 days chemotherapy). The patients' data were reviewed retrospectively, the demographic and clinical characteristics of patients were recorded.

Results: The patients' median age was 47, and 77% (n:10) of them were male. The median number of chemotherapy regimens they received before clofarabine was 3. Of the patients, 54% (n=7) presented with relapse, and 46% (n=6) with refractory disease. Hematological side effects were observed in all patients during treatment, and 4 (31%) patients were complicated by febrile neutropenia. Other side effects were hepatotoxicity 39%, skin reaction 2%, nausea 54%, and mucositis 31%. Three (23%) patients were unresponsive to treatment. A response was obtained in 10 (77%) patients. Allogeneic stem cell transplantation was performed in 4 of 5 patients with complete response. The median follow-up time after CLOVE was 2.3 (0.69-26.02) months. The median estimated survival time was 21.04 ± 3.88 (95%CI: 13.43 -28.64) months. Overall

Öz

Amaç: Klofarabin, DNA sentezini inhibe eden ikinci nesil bir pürin analoğudur. Nükseden, dirençli akut lösemide etkili yeni bir ajan olarak kullanılır. Nükseden veya dirençli T hücreli akut lenfoblastik lösemili hastalarda klofarabin bazlı bir rejim olarak CLOVE protokolü ile ilgili tek merkez deneyimimizi bildirmeyi amaçladık.

Gereç ve Yöntem: Nükseden veya dirençli T hücreli akut lenfoblastik lösemili 13 hasta bu çalışmaya dahil edildi. Hastalara klofarabin (40 mg/m2/gün), etoposid (100 mg/m2/gün) ve siklofosfamid (440 mg/m2/gün) (5 gün süre ile) uygulandı. Hastaların verileri retrospektif olarak incelendi, hastaların demografik ve klinik özellikleri kaydedildi.

Bulgular: Hastaların ortanca yaşı 47 olup, %77'si (n:10) erkekti. Klofarabinden önce aldıkları ortanca kemoterapi rejimi sayısı 3 idi. Hastaların %54'ü (n=7) nüks, %46'sı (n=6) dirençli hastalık ile başvurdu. Tedavi sırasında tüm hastalarda hematolojik yan etkiler gözlendi ve 4 (%31) hastada febril nötropeni gelişti. Diğer yan etkiler %39 hepatotoksisite, %2 cilt reaksiyonu, %54 bulantı ve %31 mukozit idi. Üç (%23) hasta tedaviye yanıtsızdı. On (%77) hastada tedaviye yanıt (tam yanıt ve kısmi yanıt) alındı. Tam yanıt alınan 5 hastanın 4'üne allojenik kök hücre nakli yapıldı. CLOVE sonrası ortanca takip süresi 2,3 (0,69-26,02) aydı. Ortanca tahmini hayatta kalma süresi 21,04 ± 3,88 (%95 GA: 13,43 -28,64) aydı. Genel sağkalım üç ayda %85.7 ve bir yılda %57.1 idi. Çalışmamızın sonunda üç hasta hayattaydı.

Sonuç: Klofarabin, etoposid ve siklofosfamid (CLOVE) kombinasyonu, tekrarlayan veya dirençli akut lösemide

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Received: 01.04.2023 Accepted: 25.05.2023

survival was 85.7% at three months and 57.1% at one year. Three patients were alive at the end of our study.
Conclusion: The combination of clofarabine, etoposide, and cyclophosphamide (CLOVE) appears to be successful in achieving a response in relapsing or resistant acute leukemia. However, more effective regimens are still needed.
Keywords: Clofarabine, relapsed/resistant leukemia, T-cell acute lymphoblastic leukemia

yanıt elde etmede başarılı görünmektedir. Bununla birlikte, daha etkili rejimlere hala ihtiyaç vardır.

Anahtar kelimeler: Klofarabin, tekrarlayan/dirençli lösemi, T-hücreli akut lenfoblastik lösemi

INTRODUCTION

T-cell acute lymphoblastic leukemia (T-ALL) is a severe hematologic cancer marked by abnormal thymocyte proliferation. T-ALL is responsible with 10% to 15% of pediatric ALL cases and 25% of adult ALL cases. Because of the severe side effects of treatment, adult T-ALL patients' overall survival (OS) rates are fewer than 50%[1]. The prognosis is dismal for patients unresponsive to first-line therapy or who relapse after intensive second-line therapies. Just 10% of adult patients with the recurrent disease survive over the long term, and their results are poor[2]. For patients with refractory or relapse acute leukemia, the only curative treatment option is allogeneic stem cell transplantation (ASCT). Bone marrow transplantation can lead in a cure; however, the 4-year survival rate is just 25–30%[3]. The best results with allogeneic bone marrow transplantation have been obtained in patients with a complete response to salvage therapy. Salvage chemotherapy regimens are high-dose treatments that are challenging to administer and difficult for patients to tolerate. There are no comparative studies with each other, and a regimen with proven superiority could not be demonstrated[4].

Clofarabine, a new-generation nucleoside analog, is a component of salvage regimens for relapsed/refractory ALL, especially in pediatric patients. There are studies in which clofarabine was used as a single agent in patients with relapsing/refractory leukemia[5,6]. These studies demonstrated the safety and efficacy of clofarabine in the pediatric patients[6,7]. Due to the synergistic effect, combination treatments with cyclophosphamide and cytarabine have been tried frequently[8-10].

There are a limited number of studies in the literature conducted in the adult patient group. In this study, we sought to assess the efficacy and side effects of the CLOVE protocol, a clofarabine-based treatment regimen, in patients with relapsed or refractory T-cell acute lymphoblastic leukemia. We think that our experience with these cases, whose management is quite challenging, will contribute to the literature.

MATERIALS AND METHODS

Study group

The data of patients with relapsed or refractory T-ALL between September 2019 and September 2022, followed in the Erciyes University Bone Marrow Transplantation and Stem Cell Center, were retrospectively analyzed. The target population of the study consisted of adults with r/r T-ALL aged >18 years at the time of enrollment. None of the patients had previously received a chemotherapy protocol containing clofarabine. Patients with pre-treatment serum creatinine elevation (>2X ULN), total bilirubin elevation (>1.5X ULN), severe infection findings, and central nervous system involvement were not included in the study. Patients who had undergone ASCT within three months and were diagnosed with active graft-versus-host disease were also ineligible.

Written and signed consents were obtained from the patients included in the study, which was approved by the Ethics Committee of Erciyes University (2022/42-05.01.2022). For concerns of patient privacy and security, none of the data gathered during our study has been disclosed. The Declaration of Helsinki was followed in the conduct of this investigation.

Treatment plan

We treated our patients with the CLOVE protocol (clofarabine (40 mg/m2), cyclophosphamide (440 mg/m2), and etoposide (100 mg/m2) for five consecutive days)[11]. All patients received antibiotics and antifungal prophylaxis. A colony-stimulating factor (G-CSF) was not routinely used during follow-up. However, when neutropenia (neutrophil

<1.000/mm^3) developed, G-CSF treatment was started. According to institutional policies, infection prophylaxis was advised.

Response and toxicity criteria

A hematopathologist evaluated the treatment responses of the patients from the bone marrow sample. Patients underwent bone marrow aspiration to determine treatment response around day 21 of the first induction cycle. Complete response was defined as no circulating blasts, no extramedullary disease, and <5% blasts in the bone marrow. The absence of circulating blasts and the presence of >5% and <25% blasts in the bone marrow were considered to be signs of a partial response.

The Common Terminology Criteria of the National Cancer Institute were used to define treatment toxicity (NCI CTCAE v3.0).

Statistical analysis

Continuous data with a normal distribution were expressed as the mean standard deviation, whereas continuous data with a non-normal distribution were expressed as the median and min-max, and categorical data were expressed as percentages (%). The Chi-square test was used to compare categorical data. OS was measured as the time from the onset of clofarabine to death, regardless of any cause. Using the Kaplan-Meier method, survival curves were produced. Power=80%, confidence interval=95%, and the minimum number to be reached in the sample size analysis was found to be 12. The SPSS for Windows set of software was used to examine the data (v. 22.0, SPSS Inc., Chicago, IL, USA). A p-value < 0.05 was considered significant.

RESULTS

Thirteen patients were included in the study. Their demographic and clinical characteristics are listed in Table 1. The median age was 47 (22 to 61 years), and 77% were male. Seven patients had relapsed leukemia, and six were refractory to the previous treatment. The median number of prior induction treatments was three, and only three patients had previously undergone ASCT.

There was a 77% response rate after CLOVE. Five of the patients had complete response (CR), and five achieved partial response (PR). The remaining three patients (23%) were resistant to treatment. CR or PR was achieved in three patients who had previously undergone ASCT. ASCT was performed in four (31%) patients with complete response after CLOVE from a matched sibling donor. The median follow-up time after CLOVE was 2.3 (0.69-26.02) months. Median estimated survival time 21.04 ± 3.88 (95%CI: 13.43- 28.64) months. Survival was 85.7% at three months and 57.1% at one year. Patients who underwent ASCT after CLOVE had better survival outcomes (Figure 1). The prognosis of CLOVE refractory patients was poor (Figure 2). Three patients were alive at the end of our study.

Table-1. Baseline characteristics

Variables	**All population n=13**
Age, years	47 (22-61)
Gender, n (%)	
Female	3 (23)
Male	10 (77)
Pre-CLOVE regimen disease status, n (%)	
Relapsed	7 (54)
Refractory	6 (46)
ECOG, n (%)	
0	3 (23)
1	5 (38.5)
2	5 (38.5)
Number of cures pre-CLOVE regimen, n (%)	
2	3 (23)
3	7 (54)
4	3 (23)
ASCT before CLOVE regimen, n (%)	
No	10 (77)
Yes	3 (23)
ASCT after CLOVE regimen, n (%)	
No	9 (69)
Yes	4 (31)
Post-CLOVE regimen disease status, n (%)	
Refractory	3 (23)
Partial response	5 (38.5)
Complete response	5 (38.5)

ASCT: Allogeneic stem cell transplantation

Factors influencing the response status after CLOVE are shown in Table 2. Response rates were better in the group with ECOG performance of 0 and in patients who underwent ASCT before CLOVE. But it was not statistically significant.

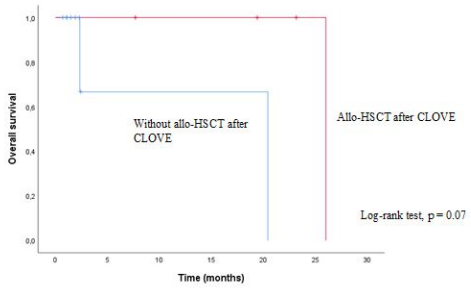

Figure-1. Overall survival in patients with and without allogeneic hematopoietic stem cell transplantation transplantation after Clove

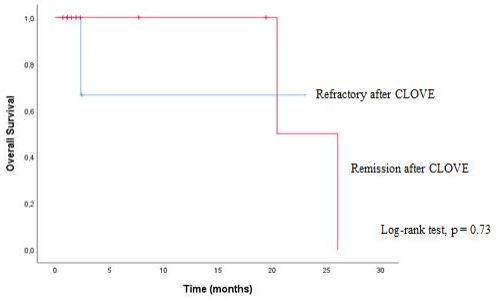

Figure-2. Overall survival in refractory and remission patients after CLOVE

Table-2. Factors influencing the response status after CLOVE.

	Refractory (n=3)	Partial response (n =5)	Complete response (n =5)	p
Gender, n (%) Male	3 (100)	1 (20)	2 (40)	0.42
Pre-CLOVE regimen disease status, n (%) Relapsed	2 (66.7)	2 (40)	3 (60)	0.72
ECOG, n (%)				
0 1 2	- 1 (33.3) 2 (66.7)	- 2 (40) 3 (60)	3 (60) 2 (40) -	0.09
Number of cures pre-CLOVE regimen, n (%) 2 3 4		 1 (20) 2 (40) 3 (60)	 1 (20) 3 (60) 1 (20)	 0.77
ASCT before CLOVE regimen, n (%)	-	1 (20)	2 (40)	0.42
Death, n (%)	2 (66.7)	5 (100)	3 (60)	0.29

ECOG: Eastern Cooperative Oncology Group -Performance Status
ASCT: Allogeneic stem cell transplantation

Side effects and infections after CLOVE regimen are listed in Table 3. Hematological side effects of any degree were observed in all patients. Severe (grade 3-4) neutropenia developed in all patients. However, febrile neutropenia was observed in 4 (31%) patients. The mean duration of neutropenia was 21 days. Apart from this, hepatotoxicity, nausea, and mucositis were observed. Skin reactions were grade 1-2 in 23% of the patients; It was observed as peeling of the skin and redness on the hands and feet. No severe skin reaction was observed. Two (15%) patients were infected with COVID-19.

Table-3. Side effects and infections after the CLOVE regimen

Variables	All populationn=13
Hematological side effects n (%)	13 (100)
Anemia	
Grade 1-2	6 (46)
Grade 3-4	7 (54)
Neutropenia	
Grade 3-4	13 (100)
Thrombocytopenia	
Grade 1-2	3 (23)
Grade 3-4	10 (77)
Hepatotoxicity, n (%)	
Grade 1-2	2 (15.4)
Grade 3-4	3 (23)
Skin reaction, n (%)	
Grade 1-2	3 (23)
Nausea, n (%)	
Grade 1-2	3 (23)
Grade 3-4	4 (31)
Oral mucositis, n (%)	
Grade 1-2	3 (23)
Grade 3-4	1 (7.7)
Febrile neutropenia, n (%)	4 (31)
Mean duration of neutropenia, days	21 (12-35)
Septic shock, n (%)	1 (7.7)
Neutropenic colitis, n (%)	1 (7.7)
COVID-19 infection, n (%)	2 (15.4)

DISCUSSION

It is known that the prognosis of patients affected by relapsed/refractory acute leukemia is very poor. The agents to be tried are limited in these patients, who usually receive several lines of treatment. Concomitant use of clofarabine, etoposide, and cyclophosphamide is particularly promising in pediatric patients. However, the number of studies on the adult population is small. Studies usually consist of people that include both acute myeloblastic leukemia (AML) and acute lymphoblastic leukemia (ALL) patients[11-13].

In a study by Miano et al., 24 pediatric patients diagnosed with relapsed, and refractory ALL were treated with CLOVE, and a response of 42% was obtained. The same study's 24-month overall survival was 25%[12]. In another study conducted with pediatric patients, a response of 66% was found in 9 patients with ALL[13]. In our study, the response rate after CLOVE was 77%. Since there are limited studies in adult patients, a comparison was made with pediatric studies, and similar results were found. Patients who achieved remission after CLOVE and underwent ASCT had better outcomes, as expected. At the conclusion of this study, only three patients were still alive. Relapse of the disease was the main reason for death. Although clofarabine successfully induces remission, we think it should be supported with ASCT if possible since patients relapsed in the long term.

The combination of clofarabine, etoposide, and cyclophosphamide was investigated in a phase 1/2 dose escalation study in adult patients by the Japan Adult Leukemia Study Group[14]. In this study, it was aimed to determine the maximum tolerated dose and efficacy of clofarabine, etoposide and cyclophosphamide combination in adult patients with relapsing/refractory acute lymphoblastic leukemia (ALL). The overall response rate was 44%.

The most common side effect observed in the studies was hematological toxicity[15,12,13]. Similarly, hematological side effects were observed in all our patients. All patients had neutropenia, but only 4 (31%) were complicated by febrile neutropenia. Hepatotoxicity was seen as an important and challenging side effect in some studies. Our study observed grade 3-4 hepatotoxicity in 3 patients. The veno-occlusive disease was not observed in any patient, and treatment was not required to be discontinued. The side effects observed in our study were like those in the literature. Several nucleoside analogs have been found to cause significant neurotoxicity, but clofarabine has not been associated with any neurotoxicity[16]. No drug-related neurotoxicity was observed in our patients either.

Our study has some limitations as it included a limited number of patients. It also reflects the experience of a single center. To obtain more accurate results, studies with larger and multicenter patient groups are needed.

Response rates are still low with current treatments in patients with relapsed or refractory acute leukemia. There is a need to develop new treatment agents that will provide a survival advantage. Our study has encouraging results for adult patients with relapsed/refractory T-ALL. CLOVE is an effective and safe combination that can be used as a bridge therapy to transplantation in patients with complete responses after induction.

Author Contributions: Concept/Design : ZTG,MK; Data acquisition: ZTG, ŞET; Data analysis and interpretation: ZTG, SÇ; Drafting manuscript: ZTG; Critical revision of manuscript: MK, AÜ; Final approval and accountability: ZTG, ŞET, SC, MK, AÜ; Technical or material support: ŞET, ZTG; Supervision: ZTG, SÇ, AÜ; Securing funding (if available): n/a.
Ethical Approval: The approval of the ethics committee was obtained from the Erciyes University Clinical Research Ethics Committee with the date of 05.01.2022 and the number of 2022/42.
Peer-review: Externally peer-reviewed.
Conflict of Interest: Authors declared no conflict of interest.
Financial Disclosure: Authors declared no financial support

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