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Original Article

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Clofarabine in Pediatric Acute Relapsed or Refractory Leukemia: Where Do We Stand on the Bridge to Hematopoietic Stem Cell Transplantation?

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Abstract

Background: Despite pronounced improvement in overall survival (OS) in pediatric leukemia, a proportion of patients continue to suffer from lack of response or relapse, and the management of such patients is exceedingly difficult. Immunotherapy and engineered chimeric antigen receptor (CAR) T-cell therapy have shown promising results in the course of relapsed or refractory acute lymphoblastic leukemia (ALL). However, conventional chemotherapy continues to be utilized for re-induction purposes whether independently or in combination with immunotherapy.

Methods: Forty-three pediatric leukemia patients (age < 14 years at diagnosis) consecutively diagnosed at our institution and got treated with clofarabine based regimen at a single tertiary care hospital between January 2005 and December 2019 were enrolled in this study. ALL comprised of 30 (69.8%) patients of the cohort while the remaining 13 (30.2%) were with acute myeloid leukemia (AML).

Results: Post-clofarabine bone marrow (BM) was negative in 18 (45.0%) cases. Overall clofarabine failure rate was 58.1% (n = 25) with 60.0% (n = 18) in ALL and 53.8% (n = 7) in AML (P = 0.747). Eighteen (41.9%) patients eventually underwent hematopoietic stem cell transplantation (HSCT); 11 (61.1%) were from ALL group and remaining seven (38.9%) were AML (P = 0.332). Three- and 5-year OS of our patients was 37.7±7.6% and 32.7±7.3%. There was a trend of better OS for ALL patients compared to AML (40.9±9.3% vs. 15.4±10.0%, P = 0.492). Cumulative probability of 5-year OS was significantly better in transplanted patients (48.1±12.1% vs. 21.4±8.4%, P = 0.024).

Conclusions: Though almost 90% of our patients proceeded to HSCT with complete response post-clofarabine treatment, yet clofarabine-based regimens are associated with the significant burden of infectious complications and sepsis-related deaths.

Keywords: Clofarabine; Stem cell transplantation; Bridging chemotherapy

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Introduction

The collaborative research efforts over the past 60 years have resulted in dramatic improvement in outcomes for children with acute leukemia, particularly for B lymphoblastic leukemia where the overall survival (OS) is approaching 90% utilizing state of the art protocols [1]. Meanwhile, the OS for myelocytic and T lymphoblastic leukemia has also improved and is currently reported at around 70-80% respectively [2, 3]. Despite this pronounced improvement, a proportion of patients continue to suffer from lack of response or relapse, and the management of such patients is exceedingly difficult. There have been many promising advances with the incorporation of immunotherapy and engineered chimeric antigen receptor (CAR) T-cell therapy in the course of relapsed or refractory acute lymphoblastic leukemia (ALL). However, conventional chemotherapy continues to be utilized for re-induction purposes whether independently or in combination with immunotherapy [4].

Different chemotherapy protocols are used for re-induction with variable efficacy reported. One of the agents frequently utilized in salvage regimes is clofarabine (CLOF). CLOF is a second-generation purine nucleoside analogue, which was designed to overcome the limitations of its counterpart; fludarabine and cladribine with enhanced efficacy and decreased toxicity [5]. Initially, it showed promising results in inducing remission in refractory/relapsed leukemia as a single agent, prompting accelerated Food and Drug Administration (FDA) approval in the early 2000s for the treatment of refractory and relapsed leukemia in children ages 1 - 21 years [5-7]. Subsequent trials incorporated CLOF in multi-agent chemotherapy backbone with improved efficacy reported. Hijiya et al investigated the combination of CLOF, cyclophosphamide (CPM) and etoposide (ETOP) in the CLO218 phase I and II trials, which enrolled

both relapsed ALL and acute myeloid leukemia (AML) [8]. The dose escalation part of the phase I trial recommended a phase II evaluation of CLOF 40 mg/m^2/day, CPM 440 mg/m^2/day and ETOP 100 mg/m^2/day for 5 consecutive days. Subsequently, the phase II trial reported an overall response rate of 64%; noted in 20 ALL and five AML patients. The overall response rate for ALL patients was 55%. A very similar dose combination of CLOF 40 mg/m^2/day, CPM 400 mg/m^2/day and ETOP 150 mg/m^2/day for 5 days was also studied in refractory and relapsed T-cell ALL and B-cell ALL in Italy, with a reported overall response rate of 56%. The response was found to be strikingly higher in the B-cell ALL group, where 13 out of 17 patients responded to treatment [9]. Among reported toxicities, the most significant adverse events are hepatic toxicity, risk of sinusoidal obstruction syndrome and capillary leak syndrome. Severe hepatic toxicity was more evident in patients post-hematopoietic stem cell transplantation (HSCT). Another CLOF-based regimen utilized in acute leukemia includes CLOF and cytarabine, which has been investigated in preclinical and clinical models. CLOF is a potent inhibitor of ribonucleotide reductase, which can increase the accumulation of the cytotoxic triphosphate form of cytarabine (triphosphate cytarabine (Ara-CTP)) in leukemia cells [10, 11]. The Children's Oncology Group evaluated this regimen in their AAML0532 study including both dosing and efficacy phases. The study concluded with a recommended dosing regimen of CLOF 52 mg/m^2 and cytarabine 1 g/m^2 for 5 days with demonstrated acceptable toxicity profile. This combination was shown to be more efficacious in the AML group with an overall response of 48% compared to a significantly inferior response of 14% in the ALL group [12, 13]. As such, we report our experience with utilizing aforementioned CLOF-based regimens in 30 patients with relapse/refractory ALL and 13 patients of relapse/refractory AML at our institution.

Materials and Methods

Patients

Forty-three pediatric leukemia patients (age < 14 years at diagnosis) consecutively diagnosed at our institution and got CLOF during the course of their treatment at a single tertiary care hospital between January 2005 and December 2019 were enrolled in this study. Our first patient received the very first cycle of CLOF in February 2011. ALL comprised of 30 (69.8%) patients of the cohort while the remaining 13 (30.2%) were with AML. Clinical data including demographic characteristics, treatment and transplant outcomes were obtained through the review of paper and electronic medical records of the patients as well as downloaded from the prospectively maintained databases at our institution.

Definitions

Disease-related characteristics including classification of cytogenetics and risk group stratification were extrapolated from the Children's Oncology Group criteria. Negative bone marrow (BM) result was defined as per institutional parameters for negative minimal residual disease (MRD) below 0.1-0.01% for lymphoblastic leukemia and M1 marrow for myelocytic leukemia. Complete response (CR) was defined as the attainment of negative BM after treatment with complete or partial hematological recovery. Complete hematological recovery was defined as attainment of absolute neutrophil count $\geq 0.75 \times 10^9$ and platelet count $\geq 75 \times 10^9$. Partial hematological recovery was defined as not meeting absolute neutrophil count and/or platelet count recovery threshold. Adverse events were graded as per Common Terminology Criteria for Adverse Events (CTCAE) version 5.0.

OS was defined as survival time in months from the date of initial diagnosis to last follow-up or death. Death from any cause was considered as an event. The last follow-up on the patients was carried out in February 2022.

Treatment and transplant details

As per institutional guidelines, patients with relapsed ALL received CLOF 40 mg/m^2/day, CPM 440 mg/m^2/day and ETOP 100 mg/m^2/day for 5 consecutive days and patients with relapsed AML received CLOF 52 mg/m^2 and cytarabine 1 g/m^2 for 5 days for one to two cycles.

Data management and statistical analyses

IBM-SPSS version 20.0 (IBM, Armonk, USA) was used to collect, manage and statistical analysis of the data.

Institutional Review Board approval

This clinical research study was approved by the Institutional Review Board (IRB) of the hospitals via approval number 2201279, which was to be conducted under the international guidelines for the enrollment of human subjects. The data from patients' medical records were collected and maintained in accordance with institutional policy on data confidentiality, security, and safety. As the study was designed as a retrospective review, no consent/assent was taken from patients/parents. A waiver of informed consent/assent was sought from the IRB and was duly granted.

Ethical compliance with human/animal study

This study was conducted in compliance with the ethical standards of the institution on human subjects as well as with the Helsinki Declaration.

Results

Clinical presentation

Clinical characteristics of patients enrolled in this study are

provided in Table 1 and Supplementary Material 1 (www.thejh.org). Median age at diagnosis was 5.4 years whereas majority of the patients were male (n = 31, 72.1%). Median time in months to receive the first cycle of CLOF was lower in AML as compared to ALL patients; however, it did not reach statistical significance (11.2 vs. 15.8, P = 0.428). Majority of our ALL patients were B cell (n = 25, 83.3%) and AML morphology was unknown for 76.9% (n = 10). In terms of cytogenetics, favorable cytogenetics were almost similar to unfavorable in ALL cases. However, our AML subgroup comprised of 76.9% of high-risk cytogenetics (10 out of 13). Majority of the cases were central nervous system (CNS)-1 for both the subgroups. Relapse of primary disease was the significant indication for CLOF, whereas 17 (68.0%) of these 25 relapsed patients had experienced second relapse before getting treated with CLOF. One of our patients had already been a failure of HSCT before receiving CLOF and then undergoing CAR T-cell therapy. This patient is alive and doing well at 51.3 months of follow-up. BM remained the most common site of relapse while median time to first cycle of CLOF was 13.4 months (1.1 - 77.8) from diagnosis. Around 60% of the cases in both the subgroups received only one cycle of CLOF (Table 1).

CLOF bridging to HCT

Post-CLOF BM was negative in 18 (45.0%) cases. Overall CLOF failure rate was 58.1% (n = 25) with 60.0% (n = 18) in ALL and 53.8% (n = 7) in AML (P = 0.747) (Table 2). Number of CLOF cycles (one vs. two), pre-treatment disease status (relapse vs. refractory disease), initial risk assignment, cytology (favorable vs. unfavorable), CNS disease (CNS-1 vs. CNS-2 vs. CNS-3), site and number of pre-CLOF relapse of primary disease were not found to be significantly associated with CLOF treatment failure. Eighteen (41.9%) patients eventually underwent HSCT; 11 (61.1%) were from ALL group and remaining seven (38.9%) were AML (P = 0.332). Median time to HSCT from first cycle of CLOF was 2.7 months (range: 1.2 - 6.4) (Table 2).

Pre- and post-CLOF hematological parameters are provided in Table 1. Post-treatment white blood cell counts (WBC) and platelets were significantly lower in ALL subgroup (P < 0.001) compared to pre-treatment values as well as in AML cases (P = 0.001 and P = 0.001, respectively).

CLOF regimen-related toxicity

Toxicity profile of CLOF is provided in Table 3; febrile neutropenia followed by bacterial infections were the most common treatment-related toxicity. Intensive care unit (ICU) admissions were recorded for 19 (44.2%) patients due to septic shock (n = 13).

Survival

With a median follow-up time of 26 months (range, 4.7 - 98.6) and an overall mortality rate of 81.4% (n = 35), 3- and 5-year OS of our patients was 37.7±7.6% and 32.7±7.3% for the whole cohort. There was a trend of better OS at 5-year for ALL patients compared to AML (40.9±9.3% vs. 15.4±10.0%, P = 0.492) (Table 2, Fig. 1). Cumulative probability of 5-year OS was significantly better in transplanted patients (48.1±2.1% vs. 21.4±8.4%, P = 0.024) (Fig. 2). Similarly, survival benefits were observed within ALL and AML subgroups in transplanted vs. non-transplanted patients (Tables 2, 4, Figs. 3, 4).

Disease progression was the primary cause of death in majority of the patients (n = 26, 74.3%), followed by transplant related (n = 6, 17.1%) and CLOF-associated toxicity in three (8.6%) patients. Twelve out of 18 patients who underwent HSCT died, making 34.3% of the overall mortality (n = 35) of our cohort.

Discussion

Our results are concordant with prior published retrospective and prospective reports, demonstrating 40% CR in the ALL group receiving CLOF/CYTOX/ETOP and 46% CR in the AML group receiving CLOF/cytosine arabinoside (Ara-C). Additionally, CLOF-based regimens demonstrated noteworthy response in our cohort of heavily treated patients in second relapse, where CR was achieved in eight out of 17 patients. CR was also achieved in two out of four patients with T-cell ALL, who were able to proceed to HSCT after but eventually, succumbed to disease progression. On the other hand, primary refractory AML had strikingly poor response, where none of the four patients achieved response. Durable remissions permitted all patients to proceed to definitive cellular therapy, where 90% of patients proceeded with HSCT and two patients who received CLOF post-HSCT proceeded to CAR T-cell therapy. In the HSCT group, 5-year OS was found to be 48.1%, with proportionally higher 5-year OS in the ALL group at 62% compared to 28% in the AML group. The rate of HSCT post-CLOF in our cohort is amongst the highest reported in the literature.

In our experience toxicity was common and significant (Table 3). Bacterial infections and ICU admissions were encountered at 62.8% and 44.2% consecutively. In contrast to reports of high-grade hepatotoxicity with CLOF regimens, hepatic failure in the context of multi-organ failure was encountered in three patients and grade II sinusoidal obstruction syndrome was encountered in one patient. Otherwise, transient transaminitis and hyperbilirubinemia were observed as detailed in Table 3. Additionally, CLOF was tolerated well in post-HSCT cases where only one recipient reported sinusoidal obstruction syndrome while two patients developed fatal acute respiratory distress syndrome. There were three deaths that were attributed to treatment with CLOF, which is comparable to reported treatment-related deaths by Inaba et al and Hijiya et al [14, 15].

Our report is limited by the small cohort which is often the case for refractory/relapsed leukemia studies. However, to our knowledge it is the first report from the region demonstrating comparable efficacy of CLOF-based regimens with

Table 1. Patient and Underlying Disease Characteristics (N = 43)

	ALL (30, 69.8%)	AML (13, 30.2%)	Total	P value
Median age at diagnosis, years, (range)	5.1 (0.4 - 13.0)	6.0 (1.1 - 11.7)	5.4 (0.4 - 13.0)	0.979
Gender n (%)				0.460
Female	7 (23.3%)	5 (38.5%)	12 (27.9%)	
Male	23 (76.7%)	8 (61.5%)	31 (72.1%)	
Leukemia subtypes, n (%)				
B-cell	25 (83.3%)	-		
T-cell	4 (13.3%)	-		
Biphenotypic	1 (3.3%)	-		
M0	-	None		
M1	-	None		
M2	-	None		
M3	-	None		
M4	-	None		
M5	-	1 (7.7%)		
M6	-	None		
M7	-	2 (15.4%)		
Unknown	-	10 (76.9%)		
Cytogenetics				
Favorable	14 (46.7%)	-		
Unfavorable	13 (43.3%)	-		
Unknown	3 (10.0%)	-		
Low risk	-	1 (7.7%)		
Intermediate risk	-	2 (15.4%)		
High risk	-	10 (76.9%)		
Risk group				< 0.001
Low	None	1 (7.7%)	1 (2.3%)	
Intermediate	None	2 (15.4%)	2 (4.7%)	
Standard	15 (50.0%)	None	15 (34.9%)	
High	15 (50.0%)	9 (69.2%)	24 (55.8%)	
Very high	None	1 (7.7%)	1 (2.3%)	
CNS disease				0.841
CNS-1	22 (73.3%)	11 (84.6%)	33 (76.7%)	
CNS-2	6 (20.0%)	1 (7.7%)	7 (16.3%)	
CNS-3	2 (6.7%)	1 (7.7%)	3 (7.0%)	
Hematological profile, median (range)				
Pre-treatment WBC	32.6 (1.5 - 440.0)	11.6 (0.9 - 226.0)	-	
Post-treatment WBC	2.6 (0.1 - 20.8)	4.9 (0.1 - 6.6)	-	
Pre-treatment platelets	44.0 (0.6 - 279.0)	66.0 (0.7 - 252.0)	-	
Post-treatment platelets	38.5 (6.0 - 482.0)	27.0 (6.0 - 220.0)	-	
Pre-treatment Hb	86.5 (49.0 - 123.0)	93.0 (70.0 - 120.0)	-	
Post-treatment Hb	87.0 (69.0 - 124.0)	85.0 (74.0 - 109.0)	-	
Clofarabine indications				0.503
Refractory disease	14 (46.7%)	4 (30.8%)	18 (41.9%)	

Table 1. Patient and Underlying Disease Characteristics (N = 43) - *(continued)*

	ALL (30, 69.8%)	AML (13, 30.2%)	Total	P value
Relapse	16 (53.3%)	9 (69.2%)	25 (58.1%)	
BM	8 (50.0%)	8 (88.9%)		
CNS	1 (6.2%)	1 (11.1%)		
Extramedullary	1 (6.2%)	None		
BM + CNS	5 (31.2%)	None		
BM + testicular	1 (6.2%)	None		
Relapse I	5 (31.2%)	3 (33.3%)	8 (32.0%)	
Relapse II	11 (68.8%)	6 (66.7%)	17 (68.0%)	
Median time to cycle 1 of clofarabine months, (range)	15.8 (1.1 - 77.8)	11.2 (1.1 - 36.0)	13.4 (1.1 - 77.8)	0.428
Minimum residual disease (+)				
Day 14	10 of 25 (40.0%)	-		
Day 28	15 of 30 (50.0%)	-		
Post-consolidation	5 of 11 (45.5%)	-		
Post-induction I	-	11 of 12 (91.7%)		
Post-induction II	-	5 of 10 (50.0%)		
Post-intensification I	-	3 of 7 (42.9%)		
Number of clofarabine cycles (per case)				1.000
One cycle	19 (63.3%)	8 (61.5%)	27 (62.8%)	
Two cycles	11 (36.7%)	5 (38.5%)	16 (37.2%)	

Values are presented as median (range) for continuous and numbers (percentage) for discrete data. ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; BM: bone marrow; WBC: white blood cell; Hb: hemoglobin.

Table 2. Treatment Response, Outcome and Clofarabine Regimen Related Toxicity

	ALL (30, 69.8%)	AML (13, 30.2%)	Total	P value
Post-clofarabine bone marrow response[a]				0.498
Positive	17 (58.6%)	5 (45.5%)	22 (55.0%)	
Negative	12 (41.4%)	6 (54.5%)		
After cycle 1 (negative BM)	13 of 29 (44.8%)	6 of 11 (54.5%)	19 of 40 (47.5%)	
After cycle 2 (negative BM)	5 of 11 (45.4%)	4 of 5 (80.0%)	9 of 16 (56.3%)	
Overall response to clofarabine treatment				0.747
Failure	18 (60.0%)	7 (53.8%)	25 (58.1%)	
CR	12 (40.0%)	6 (46.2%)	18 (41.9%)	
Hematopoietic recovery	13 (68.4%)	6 (31.6%)	19 (44.2%)	1.000
Incomplete	7 (53.8%)	3 (50.0%)	10 (52.6%)	
Complete	6 (46.2%)	3 (50.0%)	9 (47.4%)	
Treatment (clofarabine) failure by				
Treatment cycles				0.262[b]
One cycle only	12 (63.2%)	6 (75.0%)	18 (66.7%)	
Two cycles	6 (54.5%)	1 (20.0%)	7 (43.8%)	
Pre-treatment disease status				0.557[b]
Relapse	10 (62.5%)	3 (33.3%)	13 (52.0%)	
Refractory disease	8 (57.1%)	4 (100.0%)	12 (66.7%)	
Initial risk assignment				0.060/1.000
Low	-	1 (100.0%)	1 (100.0%)	
Intermediate	-	1 (50.0%)	1 (50.0%)	
Standard	6 (40.0%)	-	6 (40.0%)	

Table 2. Treatment Response, Outcome and Clofarabine Regimen Related Toxicity - *(continued)*

	ALL (30, 69.8%)	AML (13, 30.2%)	Total	P value
High	12 (80.0%)	4 (44.4%)	16 (66.7%)	
Very high	-	1 (100.0%)	1 (100.0%)	
Cytology (n = 23)				0.440/1.000
Favorable	7 (50.0%)		7 (50.0%)	
Unfavorable	9 (69.2%)		9 (69.2%)	
Low risk	-	1 (100.0%)	1 (100.0%)	
Intermediate risk	-	1 (50.0%)	1 (50.0%)	
High risk	-	5 (50.0%)	5 (50.0%)	
CNS disease				0.565/0.192
CNS-1	12 (54.5%)	7 (63.6%)	19 (57.6%)	
CNS-2	4 (66.7%)	None	4 (57.1%)	
CNS-3	2 (100.0%)	None	2 (66.7%)	
Site of relapse prior to clofarabine				0.502/0.333
BM	6 (75.0%)	2 (25.0%)	8 (50.0%)	
CNS	None	1 (100.0%)	1 (50.0%)	
Extramedullary	1 (100.0%)	None	1 (100.0%)	
BM + CNS	2 (40.0%)	None	2 (40.0%)	
BM + testicular	1 (100.0%)	None	1 (100.0%)	
Risk assignment before relapse				0.500/NA
High	8 (57.1%)	3 (33.3%)	11 (47.8%)	
Very high	2 (100.0%)	None	2 (100.0%)	
Number of relapses				1.000/1.000
Relapse I	3 (60.0%)	1 (33.3%)	4 (50.0%)	
Relapse II	7 (63.6%)	2 (33.3%)	9 (52.9%)	
Stem cell transplantation				0.332
Negative	19 (63.3%)	6 (46.2%)	25 (58.1%)	
Positive	11 (36.7%)	7 (53.8%)	18 (41.9%)	
Post-clofarabine CR				1.000
HSCT (-)	2 (16.7%)	None	2 (11.1%)	
HSCT (+)	10 (83.3%)	6 (100.0%)	16 (88.9%)	
Post-clofarabine treatment failure				0.529
HSCT (-)	17 (73.9%)	6 (85.7%)	23 (92.0%)	
HSCT (+)	1 (5.6%)	1 (14.3%)	2 (8.0%)	
Median time to HCT from cycle 1, months, (range)	2.7 (1.2 - 6.4)	2.6 (1.4 - 5.3)	2.7 (1.2 - 6.4)	0.930
Survival status				1.000
Alive	6 (20.0%)	2 (15.4%)	8 (18.6%)	
Expired	24 (80.0%)	11 (84.6%)	35 (81.4%)	
Post-clofarabine CR				0.363
Alive	5 (41.7%)	1 (16.7%)	6 (33.3%)	
Expired	7 (58.3%)	5 (83.3%)	12 (66.7%)	
Post-clofarabine treatment failure				0.490
Alive	1 (5.6%)	1 (14.3%)	2 (8.0%)	
Expired	17 (94.4%)	6 (85.7%)	23 (92.0%)	
Overall survival (5 year)	40.9±9.3%	15.4±10.0%	32.7±7.3%	0.492
HSCT (-), n = 25, events = 23	28.7±10.7%	0.0±0.0%	21.4±8.4%	
HSCT (+), n = 18, events = 12	62.3±15.0%	28.6±17.1%	48.1±12.1%	
	(P = 0.140)	(P = 0.009)	(P = 0.024)	

Values are presented as median (range) for continuous and numbers (percentage) for discrete data. [a]Bone marrow was not done for three cases post cycle 1. [b]Mantel-Haenszel. ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; HSCT: hematopoietic stem cell transplantation; BM: bone marrow; WBC: white blood cell; CR: complete response; HCT: hematopoietic cell transplantation.

Table 3. Clofarabine Regimen-Related Toxicity

	ALL (30, 69.8%)	AML (13, 30.2%)	Total (43, 100%)
Observed toxicity	26 (86.7%)	13 (100.0%)	39 (90.7%)
Mucosal infection	7 (23.3%)	5 (38.5%)	12 (27.9%)
Grade 1	-	-	-
Grade 2	-	1 (7.7%)	1 (2.3%)
Grade 3	5 (16.7%)	-	5 (11.6%)
Grade 4	2 (6.7%)	4 (30.8%)	6 (14.0%)
Hepatobiliary disorders	16 (53.3%)	7 (53.8%)	23 (53.5%)
Hepatic failure	3 (10.0%)	-	3 (7.0%)
Sinusoidal obstruction syndrome (grade 2)	1 (3.3%)	-	1 (2.3%)
Hyperbilirubinemia	5 (16.7%)	2 (15.4%)	7 (16.3%)
Grade 1	1 (3.3%)	1 (7.7%)	2 (4.7%)
Grade 2	-	1 (7.7%)	1 (2.3%)
Grade 3	4 (13.3%)	-	4 (9.3%)
Grade 4	-	-	-
Transaminitis	16 (53.3%)	7 (53.8%)	23 (53.5%)
Grade 1	2 (6.7%)	1 (7.7%)	3 (7.0%)
Grade 2	4 (13.3%)	-	4 (9.3%)
Grade 3	6 (20.0%)	6 (46.2%)	12 (27.9%)
Grade 4	4 (13.3%)	-	4 (9.3%)
Febrile neutropenia	24 (80.0%)	12 (92.3%)	36 (83.7%)
Encephalopathy (grade 2)	-	1 (100%)	1 (2.3%)
Renal and urinary disorders			
Acute kidney injury (grade 2)	-	2 (15.4%)	2 (4.7%)
Infections and infestations			
Bacterial infections	17 (56.7%)	8 (61.5%)	25 (58.1%)
Viral infections	5 (16.7%)	1 (7.7%)	6 (14.0%)
Fungal infections	8 (26.7%)	4 (30.8%)	12 (27.9%)
Sepsis (NOS)	9 (30.0%)	4 (30.8%)	13 (30.2%)
ICU admissions	13 (43.3%)	6 (46.2%)	19 (44.2%)

Values are presented as numbers (percentage). Grading is as per CTCAE 5.0. Resolution of hepatic toxicity was seen in 20 cases while three patients succumbed to their disease. ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; CTCAE: Common Terminology Criteria for Adverse Events; ICU: intensive care unit; NOS: not otherwise specified.

international reports. Even more remarkable in our experience is the successful HSCT in 90% of patients with CR. While efficacious, CLOF-based regimens are associated with the significant burden of infectious complications and sepsis-related deaths. Thus, rigorous protocols of antimicrobial prophylaxis (antibiotic, antifungal), surveillance for organisms, support with granulocyte colony-stimulating factor (G-CSF), and aggressive management of infections or febrile episodes are warranted. CLOF continues to be investigated in salvage regimens in refractory leukemias (NCT04002115, NCT03136146, NCT02441803), however its role in the evolving immunotherapy era is yet to be determined. It remains to be considered a powerful agent for refractory disease, particularly when immunotherapy is not readily available.

We continue to hope for further advances in the outcome for this fragile group of patients, and the facilitation of novel agents with enhanced benefit and reduced toxicities.

Supplementary Material

Suppl 1. Cytogenetics and molecular expressions of the cohort.

Acknowledgments

None to declare.

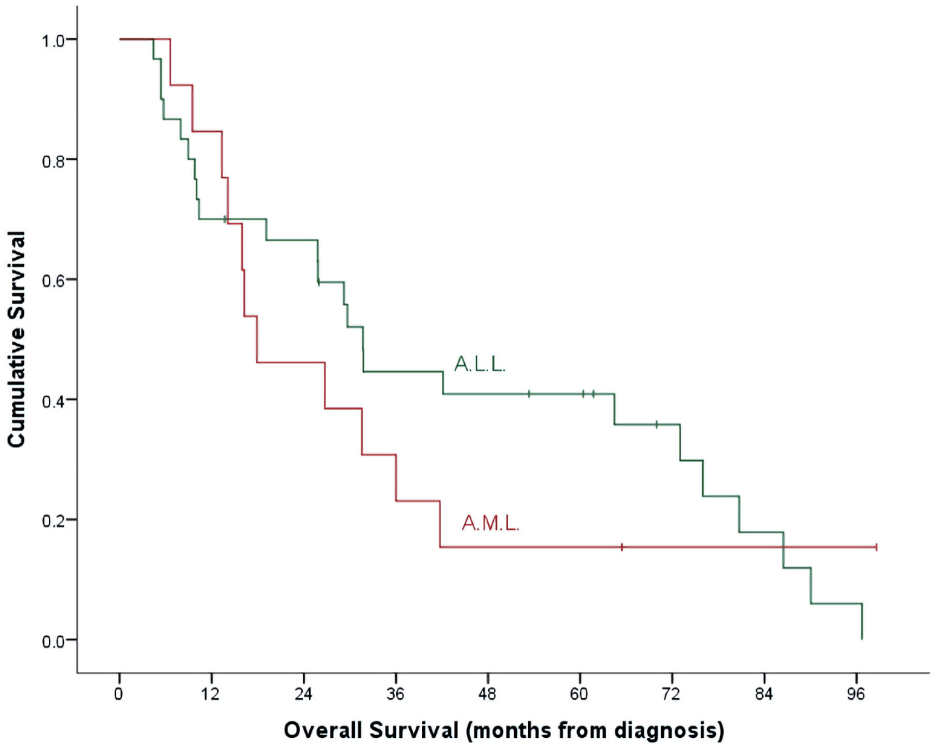

Figure 1. OS by leukemia subtypes. ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; OS: overall survival.

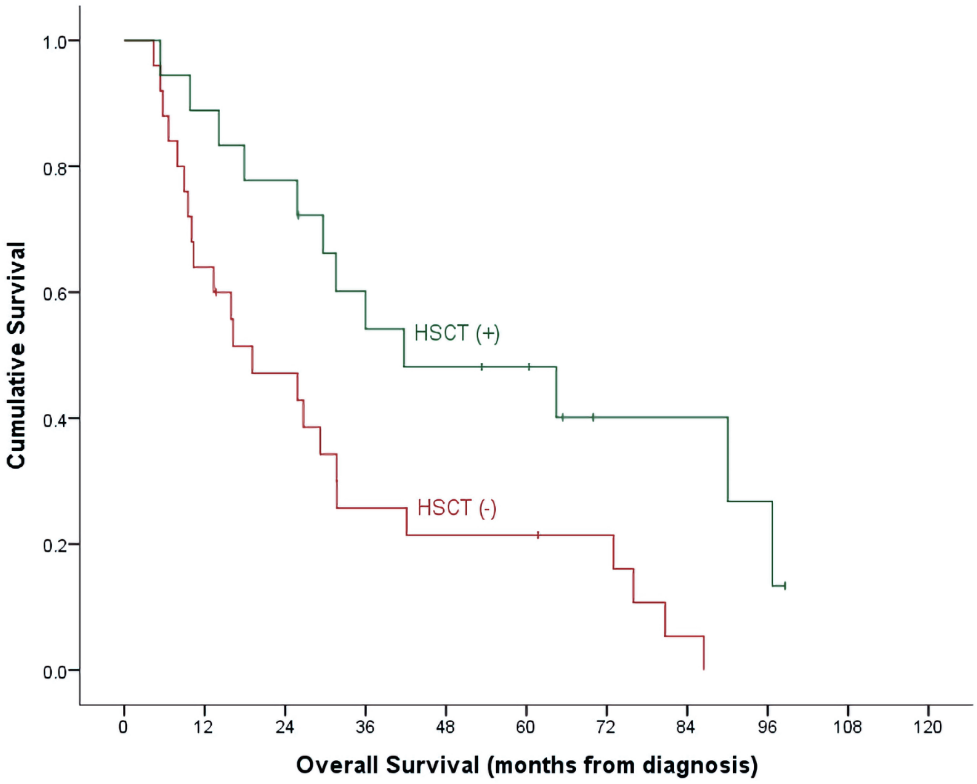

Figure 2. OS by HSCT and non-HSCT groups. OS: overall survival; HSCT: hematopoietic stem cell transplantation.

Table 4. Hematopoietic Stem Cell Transplant-Related Parameters and Outcome

	ALL (30, 69.8%)	AML (13, 30.2%)	Total (43,100%)
Transplanted	11 (36.7%)	7 (53.8%)	18 (41.9%)
Time to transplant from clofarabine cycle 1	2.7 (1.2 - 6.4)	2.6 (1.4 - 5.3)	2.7 (1.2 - 6.4)
Donor type			
Matched related	9 (81.9%)	6 (85.7%)	15 (83.3%)
Haploidentical	2 (18.2%)	1 (14.3%)	3 (16.7%)
GVHD prophylaxis			
CSA, MTX ± ATG	7 (63.6%)	5 (71.4%)	12
CSA, MMF ± Cy	2 (18.2%)	1 (14.3%)	3
MMF, Cy	1 (9.1%)	1 (14.3%)	2
MTX	1 (9.1%)	-	1
Conditioning regimen			
Bu, Cy	-	2 (28.6%)	2
Bu, Cy, VP-16	1 (9.1%)	-	1
Cy, ATG, TBI	1 (9.1%)	-	None
Cy, TBI	6 (54.5%)	3 (42.9%)	9
Flu, Cy, TBI	1 (9.1%)	-	1
Flu, TBI	1 (9.1%)	1 (14.3%)	2
Thiotepa, Flu, TBI	1 (9.1%)	1 (14.3%)	2
Time to ANC engraftment, days, median (range)	16 (13 - 38), n = 11	19 (13 - 26), n = 5	17 (13 - 38), n = 16
Time to ANC engraftment, days, median (range)	23 (14 - 96), n = 9	22 (20 - 26), n = 5	22.5 (14 - 96), n = 14
Acute GVHD (+)	3 of 11 (27.3%)	2 of 7 (28.6%)	5 of 18 (27.8%)
Skin	3	1	4
Liver	1	-	1
Gut	1	1	2
Transplant related toxicity (within day + 100)			
Mucositis	5 (45.5%)	1 (14.3%)	6 (33.3%)
Interstitial pneumonia	1 (9.1%)	1 (14.3%)	2 (11.1%)
Infections			
Bacterial	3 (27.3%)	3 (42.9%)	6 (33.3%)
Viral	7 (63.6%)	1 (14.3%)	8 (44.4%)
Fungal	-	-	None
Seizures	-	-	None
Veno-occlusive disease	1 (9.1%)	-	1 (5.6%)
Hemorrhagic cystitis	3 (27.3%)	-	3 (16.7%)

Values are presented as numbers (percentage). TBI: total body irradiation; Flu: fludarabine; Bu: Busulfan; Cy: cyclophosphamide; MTX: methotrexate; ATG: antithymocyte globulin; CSA: cyclosporine; MMF: mycophenolate mofetil; ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; GVHD: graft-versus-host disease; ANC: absolute neutrophil count.

Financial Disclosure

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Conflict of Interest

The authors declare no conflict of interest.

Informed Consent

As the study was designed as a retrospective review, no consent/assent was taken from patients/parents. A waiver of informed consent/assent was sought from the IRB and was duly granted.

Author Contributions

IG, OE and KS has designed and performed the study. SR, OE,

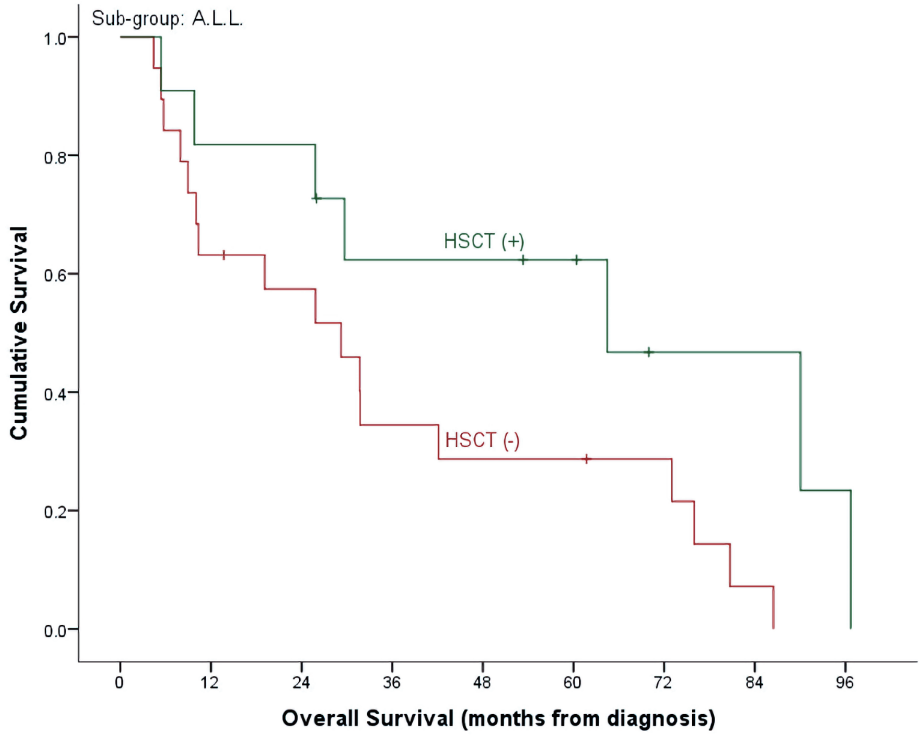

Figure 3. OS by HSCT and non-HSCT groups in ALL patients. OS: overall survival; HSCT: hematopoietic stem cell transplantation; ALL: acute lymphoblastic leukemia.

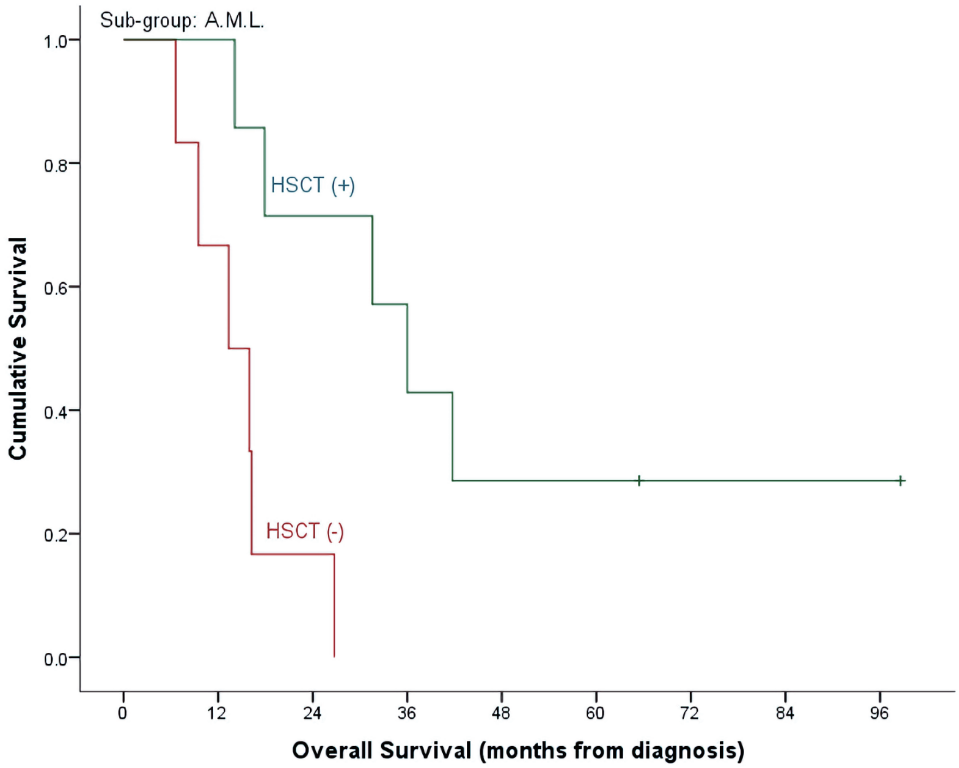

Figure 4. OS by HSCT and non-HSCT groups in AML patients. OS: overall survival; HSCT: hematopoietic stem cell transplantation; AML: acute myeloid leukemia.

IG and KS have drafted the manuscript and did critical editing. OE and OS have assisted and supported in sample collection while subsequent analysis with statistics was done by KS. SK, HA, AA, AAA, AAJ and MA have carefully supervised this manuscript preparation and writing.

Data Availability

The data used in this work is available from the corresponding author upon reasonable request. However, some restrictions may apply.

Abbreviations

ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; CAR: chimeric antigen receptor; HSCT: hematopoietic stem cell transplantation; BM: bone marrow; CLOF: clofarabine; WBC: white blood cell; Ara-CTP: triphosphate cytarabine; MRD: minimum residual disease; CR: complete response; G-CSF: granulocyte colony-stimulating factor; CTCAE: Common Terminology Criteria for Adverse Events; OS: overall survival; IRB: Institutional Review Board; ICU: intensive care unit

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