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ARTICLE

The safety and efficacy of clofarabine in combination with high-dose cytarabine and total body irradiation myeloablative conditioning and allogeneic stem cell transplantation in children, adolescents, and young adults (CAYA) with poor-risk acute leukemia

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Abstract

Acute leukemias in children with CR3, refractory relapse, or induction failure (IF) have a poor prognosis. Clofarabine has single agent activity in relapsed leukemia and synergy with cytarabine. We sought to determine the safety and overall survival in a Phase I/II trial of conditioning with clofarabine (doses 40 – 52 mg/m^2), cytarabine 1000 mg/m^2, and 1200 cGy TBI followed by alloSCT in children, adolescents, and young adults with poor-risk leukemia. Thirty-seven patients; Age 12 years (1–22 years); ALL/AML: 34:3 (18 IF, 10 CR3, 13 refractory relapse); 15 related, 22 unrelated donors. Probabilities of neutrophil, platelet engraftment, acute GvHD, and chronic GvHD were 94%, 84%, 49%, and 30%, respectively. Probability of day 100 TRM was 8.1%. 2-year EFS (event free survival) and OS (overall survival) were 38.6% (CI$_{95}$: 23–54%), and 41.3% (CI$_{95}$: 25–57%). Multivariate analysis demonstrated overt disease at time of transplant (relative risk (RR) 3.65, CI$_{95}$: 1.35–9.89, $P = 0.011$) and umbilical cord blood source (RR 2.17, CI$_{95}$: 1.33–4.15, $P = 0.019$) to be predictors of worse EFS/OS. This novel myeloablative conditioning regimen followed by alloSCT is safe and well tolerated in CAYA with very poor-risk ALL or AML. Further investigation in CAYA with better risk ALL and AML undergoing alloSCT is warranted.

Introduction

Despite excellent outcomes in pediatric ALL, 20–30% of patients relapse or become refractory to front-line therapies. Current re-induction regimens are associated with approximately a 40% CR, yet survival in third complete remission

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(CR3) remains <10%. Allogeneic stem cell transplantation is the treatment of choice for patients in second CR with early relapse and patients in CR3, once thought to be incurable, are now felt to have some promise for sustained remission [1].

In pediatric AML, only around 50% are cured with current regimens. Among those who are refractory or relapse, outcome is dismal. Reported 5-year disease-free survival for patients in second CR, first relapse, or with refractory disease is generally poor [2, 3].

Clofarabine is approved for treatment of refractory/relapsed pediatric acute leukemias. Early studies of clofarabine in combination with cytarabine in adult patients with relapsed/refractory acute leukemias reported an overall response rate of 38%, with 22% of patients achieving CR [4]. Clofarabine-based combination therapy has also demonstrated superiority to high dose cytarabine alone in younger adults with AML in first remission [5, 6] and reports of the combination of the two have been promising in children with acute leukemias [7].

Overall there is a lack of studies utilizing a leukemia-targeted conditioning approach prior to alloSCT in pediatric patients with poor-risk relapsed or refractory ALL/AML. Traditional regimens are broadly myeloablative without targeting the underlying malignancy. We decided to investigate an anti-leukemic conditioning regimen prior to alloSCT in poor risk pediatric, adolescent, and young adult patients with acute leukemia. We hypothesized that conditioning with clofarabine, cytarabine, and TBI followed by alloSCT would be safe and well-tolerated.

Materials and methods

This research protocol was conducted in compliance with the Declaration of Helsinki. The protocol was approved and facilitated by the Pediatric Blood and Marrow Transplant Consortium and opened at 10 centers across the United States over an 8 year period. The protocol and informed consent documents were approved by the respective Cancer Centers (if applicable) and Institutional Review Boards. Informed consent and assent (when applicable) were obtained prior to enrollment. The protocol was registered with clinicaltrials.gov (NCT00529360).

Patient eligibility criteria

Patients between 1–30 years-old with ALL or AML in refractory relapse, induction failure (IF), or CR3 were eligible. Refractory relapse was defined as those patients unable to achieve subsequent remission (<5% bone marrow (BM) blasts) following retrieval therapy after a documented relapse. IF was defined as patients with at least >5% BM blasts at the end of primary induction or persistent minimal residual disease (>0.01%) following consolidation. Patients in relapse or IF must have demonstrated <25% BM blasts within 14 days of initiation of conditioning. Adequate organ function and performance score ≥ 60 were required. Exclusion criteria included: patients with a prior alloSCT; active central nervous system leukemia; uncontrolled infection; and Down syndrome.

Allogeneic donor eligibility criteria

Sibling and unrelated cord blood donors were 5-6/6 HLA matched (intermediate resolution HLA-A and -B, high resolution HLA-DRB1). Unrelated donors were 9-10/10 HLA matched (high resolution HLA-A, -B, -C, -DRB1, and -DQB1). Pre-cryopreserved total nucleated cell count for cord blood units was $\geq 3 \times 10^7$/kg for single units and $\geq 5 \times 10^7$/kg for two combined units.

Conditioning regimen

All patients received a myeloablative conditioning regimen beginning on day -10 consisting of clofarabine, cytarabine, and TBI, followed by alloSCT on day 0. Cytarabine 1000 mg/m^2 was given as a 3h IV infusion on days –10 to –5. Clofarabine was given as a 2h IV infusion on days –9 to –5. The starting dose of clofarabine was 40 mg/m^2 at dose level 1 escalating to 46 mg/m^2 and 52 mg/m^2. Hydrocortisone IV was given immediately prior to each dose of clofarabine. On the days when both cytarabine and clofarabine were administered, clofarabine was given as a 2h IV infusion followed by a 4h rest. The TBI treatment dose was 1200 cGy given twice daily on days –4 to –2. Rabbit ATG was administered to patients with an unrelated donor at a dose of 2.5 mg/kg/dose on days –5 to –2.

Clofarabine administration

Part A dose escalation of clofarabine utilized a standard 3 + 3 design. Dose level 1 of clofarabine was 40 mg/m^2. Dose level 2 was 46 mg/m^2, and dose level 3 was 52 mg/m^2. Toxicity was scored according to NCI/CTC version 4 (http://ctep.cancer.gov/reporting/ctc.html). Patients were monitored for 100 days following alloSCT for non-hematologic toxicity related to clofarabine. The maximum tolerated dose (MTD) was defined as the highest dose level for which the incidence of dose limiting toxicity was less than 33%. Any grade V toxicity was considered a DLT. Grade III-IV hematologic toxicities were not considered DLT. Grade III or IV non-hematologic toxicities persisting for more than 24 h were considered DLT with the exception of the following: fever or infection, toxicities attributable to infection, alopecia, anorexia, elevations in hepatic

transaminases or alkaline phosphatase that returned to ≤grade II elevations within 14 days, nausea, vomiting, diarrhea, or mucositis, elevations in amylase, lipase, or total bilirubin that are asymptomatic and that return to ≤ grade II elevations within 7 days. The Data Safety Monitoring Board reviewed data quarterly. The study was designed to not exceed a 20% transplant-related mortality rate in the first 100 days after alloSCT. Once the MTD and/or safe, tolerated dose of clofarabine had been established, additional patients were enrolled on Part B to further define the event free, disease free, and overall survival at the MTD or safe/tolerable dose of clofarabine in combination with ARA-C and TBI followed by SCT.

Infection prophylaxis and supportive care

Hematopoietic growth factor support, blood product support, isolation, and infection prophylaxis was administered as we have previously described [8].

Graft-versus-host disease prophylaxis and grading

GvHD prophylaxis consisted of tacrolimus and mycophenolate mofetil as we have previously described [9, 10]. Tacrolimus was given starting on day -1 with dosage adjustments to maintain blood levels between 10 and 20 ng/ml. Tacrolimus was tapered as clinically indicated after day +60. Mycophenolate mofetil was administered beginning on day +1. For patients with a matched sibling donor and ≤grade I aGvHD, mycophenolate mofetil was stopped at day +30. Patients receiving unrelated or umbilical cord blood donor transplants with ≤grade I aGvHD had mycophenolate mofetil tapered as clinically indicated after day +60. aGvHD was graded per the modified Glucksberg criteria [11].

Statistics

Cumulative incidence of neutrophil and platelet engraftment, aGvHD and chronic GvHD, and relapse were estimated by Kaplan–Meier method. Event-free survival and OS were similarly estimated by Kaplan–Meier method. Whole blood donor chimerism levels were summarized as mean ± standard deviation. Differences in time-dependent outcomes (neutrophil and platelet engraftment, aGvHD and cGvHD, relapse, EFS, OS) between groups were assessed using log rank tests as part of an exploratory analysis. A p value of <0.05 was considered statistically significant. Both univariate and multivariable regression analysis was performed looking at stem cell source and disease status prior to alloSCT as variables.

The primary endpoints of the study were determination of the MTD of clofarabine in combination with ARA-C and TBI followed by alloSCT (Part A), and assessment of the safety and toxicity of the regimen in the study population. Under the standard 3 + 3 design used in part A of the study, the probability of escalation to the next dose level is probable if the risk of DLT is low and likelihood of escalation decreases as likelihood of DLT increases. A treatment-related mortality rate of 20% was considered acceptable. A one-sided 95% lower confidence bound for treatment-related mortality was calculated after enrollment of 6 patients and after enrollment of every 3 patients thereafter, with consideration of premature study termination if this lower bound exceeded 20%. Target enrollment for Parts A and B was 12 and 18 patients, respectively, and was exceeded. At this enrollment target ($n = 30$), which was exceeded, we had >80% power to exclude a treatment-related mortality rate of ≥40% with 95% confidence, presuming treatment-related mortality is independent of clofarabine dose.

Results

Patient demographics and disease status

Part A

A total of 12 patients (10 ALL, 2 AML) were enrolled. Median age of 9 years (range: 5–14. Median follow-up for surviving patients is 2014 days (range 1479–2058). Two of 12 patients (17%) had IF, 3 of 12 (25%) were transplanted in relapse, and 7 of 12 (58%) were transplanted in CR3. We utilized 6 related donors (50%) and 6 unrelated donors (50%); of the sources, 8 were from marrow (67%), 2 were from UCB (17%), and 2 were from peripheral blood stem cells (PBSC) (16%). Seven were complete HLA matches (58%), 5 were mismatched at one locus (42%).

Part B

In Part B, a total of 25 patients (24 ALL, 1 AML) were enrolled. Median age of 12 years (range: 1–22 years). Median follow-up for surviving patients is 1238 days (range: 193–1718 days). Sixteen of 25 patients (64%) had IF, 1 of 25 (4%) were transplanted due to refractory relapse, and 3 of 25 (12%) were transplanted due to CR3. Of all patients with IF, 27% achieved CR prior to alloSCT; the remainder underwent alloSCT with refractory disease. We utilized 9 related donors (36%) and 16 unrelated donors (64%); of the sources, 13 were from marrow (52%), 11 were from UCB (44%), 1 was from PBSC (4%). Twelve were complete HLA matches (48%), 10 were mismatched at one locus (40%), and 3 were mismatched at two (12%). The complete demographics and key outcome variables for Part A and B patients are depicted in Table 1.

Table 1 Patient demographics donor source and outcomes

ID#	CLO dose mg/m^2	Age (years) / sex	Disease	Cytogenetic abnormalities	Indication for alloSCT	HLA match	Donor/source	Outcome
1	40	13.6 / F	AML	None	IF	9/10	Unrelated/BM	NED day + 2058
2	40	6.8 / M	Pre-B ALL	MLL rearrangement	Relapse 1	6/6	Sibling/BM	NED day + 2014
3	40	10.4 / M	T-cell ALL	None	CR3	5/6	Sibling/BM	NED day + 2021
4	46	18.5 / M	Pre-B ALL	None	CR3	6/6	Sibling/BM	Dead day + 182, candidal meningoencephalitis
5	46	13.1 / M	T-cell ALL	None	Relapse 3	6/6	Sibling/BM	Dead day + 150, progressive disease
6	46	6.4 / M	ALL	None	CR3	6/6	Father/BM	NED day + 1713
7	52	10.8 / F	AML	MLL rearrangement	CR3	10/10	Unrelated/BM	Dead day + 137, klebsiella sepsis
8	52	5.6 / M	Pre-B ALL	None	CR3	10/10	Unrelated/BM	Dead day + 216, progressive disease
9	52	11.9 / M	Pre-B ALL	Monosomy 5 & 7	CR3	5/6	Unrelated/UCB	Dead day + 405, multi-organ failure
10	52	7.2 / M	T-cell ALL	14q11 rearrangement	Relapse 1	5/6	Father/PBSC	Dead day + 333, progressive disease
11	52	14.4 / M	AML	Trisomy 4 and 17q22 deletion	IF	5/6	Unrelated/UCB	Dead day + 544, cGvHD liver
12	52	12.5 / M	Pre-B ALL	MLL rearrangement	CR3	10/10	Unrelated/PBSC	NED day + 1479
13	52	5.2 / F	Pre-B ALL	TEL/AML	CR3	9/10	Unrelated/PBSC	Dead day + 241, progressive disease
14	52	8.4 / F	T-cell ALL	None	CR3	5/6	Unrelated/UCB	Dead day + 129, progressive disease
15	52	16.9 / M	Pre-B ALL	None	Refractory disease	4/6	Unrelated/UCB	Dead day + 173, multi-organ failure
16	52	9.6 / M	Pre-B ALL	None	IF	9/10	Unrelated/BM	NED day + 1238
17	52	14.4 / M	T-cell ALL	None	Refractory disease	10/10	Unrelated/BM	Dead day + 150, resp failure and CMV
18	52	20.7 / F	Pre-B ALL	9p16 deletion	IF	4/6	Unrelated/UCB	Dead day + 68, multi-organ failure
19	52	16.0 / M	Pre-B ALL	None	Refractory disease	10/10	Unrelated/BM	NED day + 1718
20	52	9.3 / M	T-cell ALL	None	Refractory disease	6/6	Father/BM	Dead day + 196, progressive disease
21	52	1.5 / M	Pre-B ALL	None	Refractory disease	6/6	Sister/UCB	NED day + 1657
22	52	19.9 / M	T-cell ALL	None	IF	6/6	Sister/BM	NED day + 1586
23	52	7.0 / F	Pre-B ALL	None	Refractory disease	5/6	Unrelated/UCB	Dead day + 110, adenovirus pneumonia and multi-organ failure
24	52	11.8 / M	ALL	TEL/AML	CR3	9/10	Unrelated/BM	AWD day + 1399
25	52	17.8 / M	ALL	9p deletion, 11q deletion	IF	6/6	Sibling/BM	Dead day + 29, sepsis
26	52	21.8 / M	T-cell ALL	None	Refractory disease	6/6	Sister/BM	Dead day + 381, cGvHD
27	52	11.3 / M	Pre-B ALL	None	Refractory disease	5/6	Unrelated/UCB	Dead day + 198, late-stage aGvHD
28	52	4.6 / F	AML	gain of AML1 gene locus	Refractory disease	5/6	Unrelated/UCB	Dead day + 14, sepsis
29	52	12.5 / M	Pre-B ALL	None	Refractory disease	9/10	Unrelated/BM	NED day + 1424
30	52	14.1 / M	ALL	None	Refractory disease	6/6	Sibling/BM	Dead day + 297, thrombotic angiopathy and cGvHD
31	52	22 / F	Pre-B ALL	None	IF	5/6	Unrelated/UCB	Dead day + 129, ARDS due to fungal infection
32	52	17 / F	Pre-B ALL		IF	4/6	Unrelated/UCB	Dead day + 109, DAH & disseminated aspergillosis

Table 1 (continued)

ID#	CLO dose mg/m^2	Age (years) / sex	Disease	Cytogenetic abnormalities	Indication for alloSCT	HLA match	Donor/source	Outcome
33	52	3 / M	T-cell ALL		IF	6/6	Unrelated/UCB	NED day + 731
34	52	19 / M	Pre-B ALL		IF (MRD)	10/10	Sibling/BM	NED day + 622
35	52	11 / M	Pre-B ALL		Relapse 1	10/10	Sibling/BM	NED day + 531
36	52	1 / M	Pre-B ALL	MLL rearrangement	IF	10/10	Sibling/BM	NED day + 293
37	52	19 / F	Pre-B ALL		Relapse 1	5/6	Unrelated/UCB	NED day + 193

AML acute myelogenous leukemia, *ALL* acute lymphocytic leukemia, *IF* induction failure, *CR* complete response, *BM* bone marrow, *PBSC* peripheral blood stem cells, *UCB* umbilical cord blood, *NED* no evidence of disease, *AWD* alive with disease.

Part A clofarabine dose escalation and associated toxicities

In Part A of this study, three patients each at clofarabine doses of 40 mg/m^2 and 46 mg/m^2, and six patients at clofarabine dose 52 mg/m^2 were treated. Patients were monitored for 100 days following alloSCT for non-hematologic toxicity related to clofarabine. At dose level 40 mg/m^2, one patient developed grade II seizures unrelated to clofarabine and two patients developed infections which resolved with treatment. At dose level 46 mg/m^2, one patient died of fungal encephalitis on day +182 and two patients developed infections which resolved with treatment. There were no DLTs possibly, probably or directly related to clofarabine and the tolerated dose was determined to be 52 mg/m^2 daily x 5 days.

Part B associated toxicities

All 25 patients in Part B were treated at the MTD dose of clofarabine, 52 mg/m^2. One patient had grade IV hypotension and was unable to receive doses 3–5 of clofarabine; symptoms resolved with treatment. One patient died of bacterial sepsis on day + 137, one patient died of liver cGvHD on day +544, and three patients developed infections which resolved with treatment. During the first 100 days following this conditioning regimen, no patients developed grade III–IV renal or liver toxicity related to clofarabine. Two patients developed grade III hearing loss likely related to tacrolimus and prior nelarabine and 1 patient developed grade III demyelinating sensorimotor polyneuropathy likely related to prior chemotherapy. No DLTs secondary to clofarabine were observed. The day +100 treatment-related mortality was 8.1%.

Hematologic reconstitution and GvHD

Neutrophil engraftment was observed in 35 of 37 patients, at a median of 15 days (range: 11–51 days) among those who engrafted (Fig. 1a). A significant delay in neutrophil engraftment was observed among recipients of UCB vs. recipients of BM or PBSCs (hazard ratio [HR] 1.86, CI_{95} 1.10–4.14, $P = 0.0462$). Thirty-one of 37 patients engrafted platelets, at a median of 35 days (range: 15 – 236 days) (Fig. 1b). Similarly, a non-significant trend toward delayed platelet engraftment was observed among recipients of UCB vs. recipients of BM or PBSCs (HR 1.89, CI_{95} 0.94–3.87, $P = 0.0880$). The cumulative incidence of developing grade II–IV aGvHD or cGvHD was 48.9% (CI_{95} 31.1–64.5%) and 29.7% (CI_{95} 6.5–58.3%), respectively (Fig. 2a–c). The cumulative incidence of grade III–IV aGvHD was 16.8% (CI_{95} 1.89–45.03%) (Fig. 2b). The incidence of grade II–IV aGvHD and of cGvHD did not differ significantly between

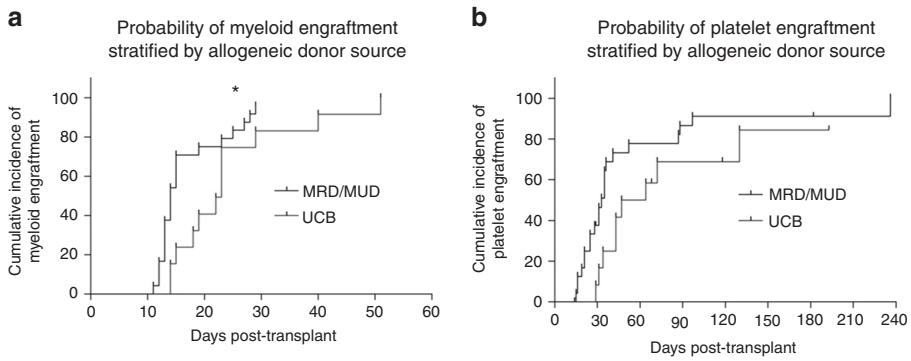

Fig. 1 Cumulative probability of **a** myeloid engraftment and **b** platelet engraftment by Kaplan–Meier method in patients receiving a myeloablative conditioning regimen of clofarabine in combination with cytarabine and TBI and alloSCT in CAYA with poor-risk acute leukemia (*$P <$ 0.05)

recipients of HLA-identical sibling-donor BM vs UCB vs. those transplanted from other donor sources. The median percent donor chimerism of whole blood at one year was 100%.

Relapse and survival

Two-year OS and EFS were 41.3% (CI_{95}: 25–56.8%) and 38.6% (CI_{95}: 22.8–54.2%), respectively (Fig. 3a, b). In total, 21 patients died on-study (range: day +14 to day +544) out of which six died of progressive disease (range: day +129 to day +333), eight from infection (three bacterial, two viral, and three fungal), three from multi-organ failure, and four from GvHD. Cumulative incidence of relapse for all patients following this conditioning regimen and alloSCT was 25.2% (CI_{95} 5.2–52.6%), and all relapses occurred prior to one year post-alloSCT (Fig. 4). A total of 18 of 28 (64%) patients who were considered IFs or CR3 were able to attain minimal residual disease (MRD) negative status prior to transplant. The cumulative incidence of relapse for patients who were MRD-negative, measured by flow cytometry with level of detection <0.01%, prior to alloSCT was 14.1% (CI_{95} 0.2–53.5%), and the incidence of relapse in MRD-positive patients prior to alloSCT was 45.3% (CI_{95} 7.4–78.6%). Thus, patients who were MRD negative prior to alloSCT were observed to have an overall decreased incidence of relapse using the log rank test (HR 0.29, CI_{95} 0.029 – 1.38), although this trend did not reach significance ($P = 0.1034$) (Supplementary Figure 1). The 1-year and 2-year incidence of non-relapse mortality, with relapse considered a competing risk were 36% (CI_{95} 16.14–56.5%) and 48.3% (CI_{95} 28.8–65.4%), respectively (Fig. 5).

Further analysis of predictors of EFS and OS yielded the following results: EFS for patients who were MRD-negative was 48.0%, while EFS for patients who were either MRD-positive or had overt leukemia prior to alloSCT was 22.2% (HR 2.01, CI_{95} 0.79–6.79) and was statistically significant on multivariable analysis (relative risk 3.65, $P = 0.011$, CI_{95}: 1.35–9.89; [Table 2]). OS for patients who were MRD-negative was 47.6%, while OS for patients who were either

MRD-positive or had overt leukemia prior to alloSCT was 22.2% (HR 1.85, CI_{95}: 0.72–5.89) and was significant on multivariable analysis (RR 2.95, $P = 0.032$, CI_{95}: 1.10–7·91; [Table 2]). The EFS and OS in patients who were in CR prior to alloSCT were significant versus those who had overt leukemia prior to alloSCT ($P = 0.0076$ and 0.0394, respectively) (Fig. 6a, b). Similarly, analysis of EFS and OS in patients who were MRD positive, MRD negative, or had overt leukemia prior to alloSCT showed significance or a trend toward significance ($P = 0.0247$ and 0.0968, respectively (Fig. 6c, d). Finally, comparison of patients whose donor source was UCB compared to all other donor sources had significantly worse EFS and OS (HR 2.2, $P =$ 0.0576 and HR 2.7, 0.0188, respectively) (Fig. 6e, f); multivariable analysis confirmed these results (RR 2.17, $P = 0.019$, $CI_{95:}$ 1.33–4.15 and RR 2.46, $P = 0.007$, $CI_{95:}$ 1.28–4.75, respectively; [Table 2]). Multivariable analysis did not demonstrate significant impact on EFS or OS in IF, first vs multiple relapse, or good vs poor risk cytogenetics.

Discussion

In this study, we demonstrate that clofarabine in combination with a high-dose cytarabine and TBI-based myeloablative conditioning regimen, followed by alloSCT is safe and feasible in a very poor risk group of patients, with a significant subset of patients (≥40%) achieving long-term EFS. Historically, this group has had only a 10–20% long-term survival rate. In our multivariate analysis, the following variables were demonstrated to be significant for worse EFS: overt disease at time of transplant and UCB stem cell source.

Prior Phase I trials of clofarabine as a single agent have demonstrated the MTD to be 52 mg/m^2/day [12]. In Part A of this study, we also found MTD of clofarabine to be 52 mg/m^2/day x 5 days in combination with high-dose cytarabine and TBI (1200 cGy). This study is most

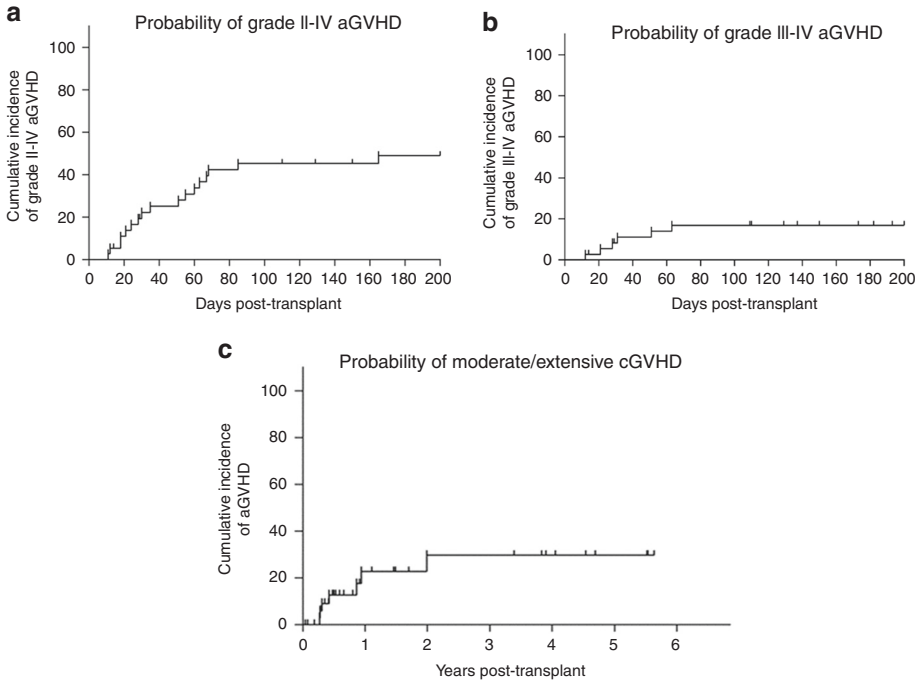

Fig. 2 Cumulative probability of **a** Grade II–IV aGvHD, **b** Grade III–IV aGvHD, and **c** cGvHD by Kaplan–Meier method in patients receiving a myeloablative conditioning regimen of clofarabine in combination with cytarabine and TBI and alloSCT in CAYA with poor-risk acute leukemia

remarkable in that we were able to add TBI and high dose cytarabine to the established single agent clofarabine MTD. We chose not to pursue a true MTD escalation above 52 mg/m^2/day due to the presumed risks of morbidity and mortality with a higher dose of clofarabine in this poor risk group of alloSCT recipients.

TBI-based alloSCT remains the standard of care for children with twice-relapsed ALL who achieve a third remission [13]. A recent CIBMTR retrospective study of 155 pediatric recipients of unrelated donor alloSCT for management of ALL in CR3 noted 5-year leukemia-free survival of 30%, with a 25% incidence of relapse and 45% non-relapse mortality [14, 15]. Myeloablative conditioning and alloSCT has also previously been the standard of care for patients with ALL achieving CR1 after initial IF [12]. In an open-label study, pediatric patients with refractory or relapsed AML received clofarabine with an overall complete response rate of 26% [16]. However, there were too few patients with high-risk AML in this study to make any definite conclusions regarding the efficacy of this approach.

Despite significant advances in supportive care, myeloablative conditioning followed by alloSCT in heavily pretreated patient subgroups carries significant risk of non-relapse mortality. In the aforementioned retrospective report from Nemecek et al. reporting on children and adolescents with ALL in CR3 undergoing unrelated donor alloSCT, NRM was estimated to be 19% at 100 days, 41% at 1 year, and 45% at 5 years post-transplant [17–19]. In our study, day 100 NRM was encouragingly only 8.1%, though significant NRM was observed following this time point as well, most frequently due to complications related to infection and/or GvHD. The transplant related mortality is consistent with the degree of HLA disparity in donors as well as the high-risk refractory leukemia in the patients in this study.

Results of the BRIDGE trial using clofarabine salvage therapy as a bridge to HSCT in refractory AML patients demonstrated disease-free survival at 1 year of 51% (CI_{95} 39–63%), dropping to around 40% at the 2 year mark, similar to our results [20–22]. Other studies in relapsed/refractory leukemia and myelodysplastic syndrome patients have also demonstrated tolerability and low transplant-related mortality rates with 2 year OS of 31% (CI_{95} 14–48) utilizing single agent clofarabine with HSCT or clofarabine in combination [23]. The recent CLORIC as well as other trials investigated reduced toxicity conditioning regimen with clofarabine, busulfan, and ATG in high risk leukemia and myelodysplastic syndrome patients [24]. The CLORIC study reported a 1-year OS of 63 ± 9%, with a relapse incidence of 40 ± 9% and low regimen-related toxicity of 3.3 ± 3% [25]. Overall, these adult trials have demonstrated similar tolerability, short- and long-term outcomes utilizing clofarabine directed therapy prior to and during HSCT conditioning as our results demonstrate here. The recently published TACL study also showed similarly promising results utilizing clofarabine and TBI in a pediatric cohort [6, 26].

The results of this study should be interpreted with some caution. While all patients enrolled had poor-risk disease, there remains heterogeneity with respect to underlying disease (AML vs. ALL) and disease status at time of enrollment (CR3 vs. refractory relapse vs. IF). Our multivariate analysis confirms established reports

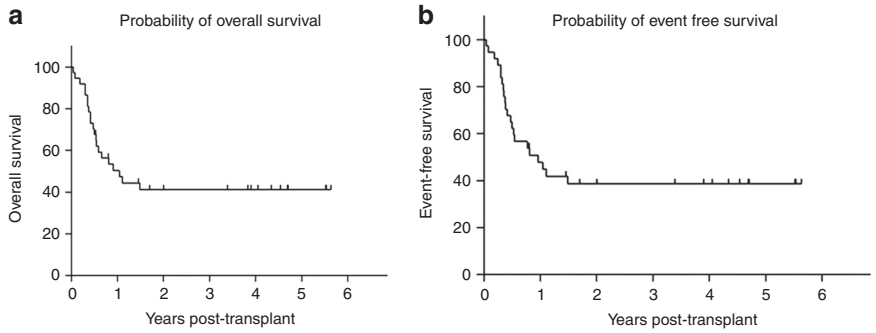

Fig. 3 Cumulative probability of **a** OS and **b** event-free survival by Kaplan–Meier method, in patients receiving a myeloablative conditioning regimen of clofarabine in combination with cytarabine and TBI and alloSCT in CAYA with poor-risk acute leukemia

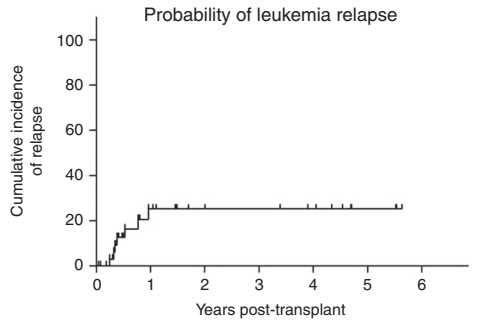

Fig. 4 Cumulative incidence of relapse by Kaplan–Meier method in patients receiving a myeloablative conditioning regimen of clofarabine in combination with cytarabine and TBI and alloSCT in CAYA with poor-risk acute leukemia

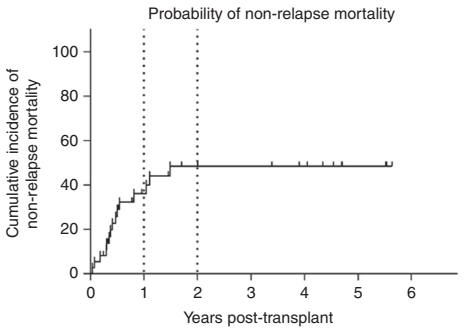

Fig. 5 Cumulative incidence of non-relapse mortality, with relapse considered a competing risk by Kaplan–Meier method in patients receiving a myeloablative conditioning regimen of clofarabine in combination with cytarabine and TBI and alloSCT in CAYA with poor-risk acute leukemia

Table 2 Multivariable Cox Regression analysis of predictors of EFS and OS in patients receiving a myeloablative conditioning regimen of clofarabine in combination with cytarabine and TBI and alloSCT in CAYA with poor-risk acute leukemia

Variable	RR	95% CI		*p* value
		Upper	Lower	
Risk for relapse or death				
Matched related donor	Ref	–	–	–
Matched unrelated donor	0.734	0.313	1.720	0.476
Umbilical cord blood	2.170	1.133	4.154	0.019
MRD negative	Ref	–	–	–
MRD positive	0.671	0.297	1.517	0.338
Overt disease	3.653	1.349	9.890	0.011
Risk for death				
Matched related donor	Ref	–	–	–
Matched unrelated donor	0.618	0.263	1.451	0.269
Umbilical cord blood	2.46	1.275	4.745	0.007

demonstrating BM transplantation with overt disease or MRD positivity to have inferior outcomes overall when compared to those that are MRD negative at time of transplant. In addition, the decrease in survival with UCB is consistent with what we and others have published showing increased infectious complications due to delayed immune reconstitution with UCB transplantation and a 30% risk of transplant-related mortality by day 100. In particular, for this high risk subset of patients, the current literature on non-transplant mortality with umbilical cord blood transplant is consistent with what we have found and we do not feel that our findings reflect regimen related toxicity. In addition, in this study we saw an increase in GvHD among patients having received UCB which further complicated the toxicity profile with UCB transplantation. The design of the Part B portion of the study was single-arm without an associated control group employing a more standard myeloablative conditioning regimen. Despite these limitations, the study was able to meet its primary objectives of defining a tolerable dose of clofarabine in combination with high-dose cytarabine and TBI (1200 cGy) myeloablative conditioning regimen, as well as establishing the safety profile of this regimen and early data suggesting efficacy in a high-risk group of children with poor risk acute leukemia.

In summary, a myeloablative conditioning regimen consisting of clofarabine in combination with high-dose cytarabine and TBI prior to alloSCT appears to be a safe and effective strategy in CAYA with poor-risk relapsed and refractory acute leukemias. Further comparisons with this conditioning regimen versus other myeloablative

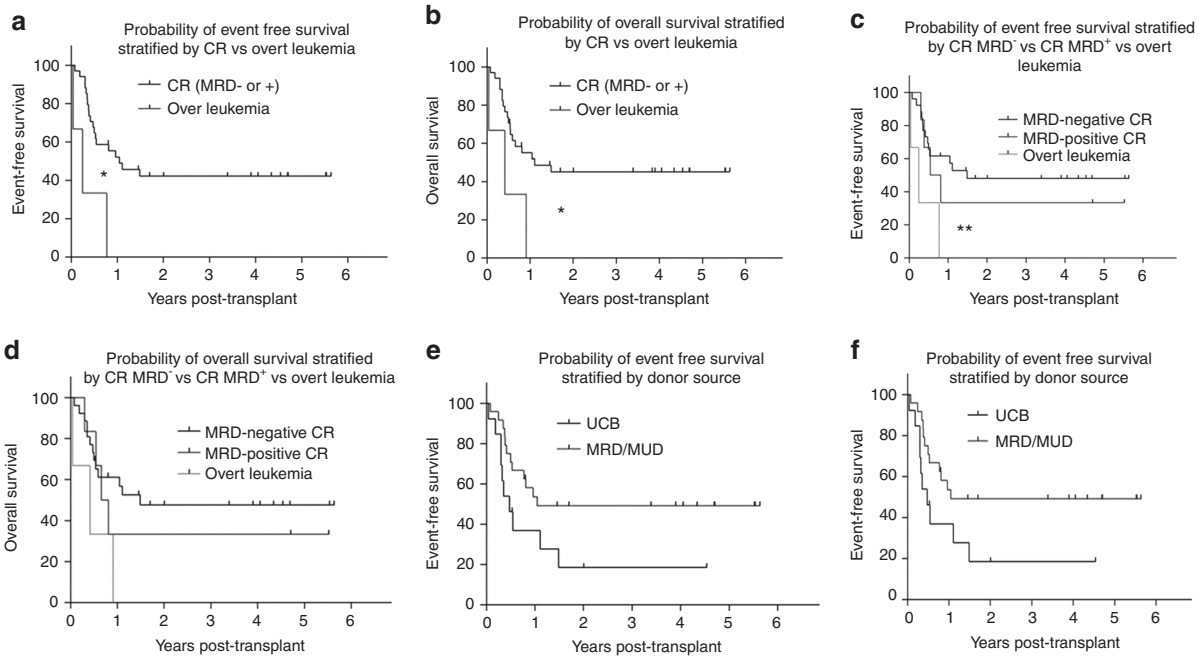

Fig. 6 Probability of **a**–**e** EFS and **b**–**f** OS by Kaplan–Meier method in patients who were in CR (MRD + or -) vs patients who had overt leukemia at time of transplant (**a**, **b**) ($*P < 0.05$), who were MRD negative, MRD-positive or had overt leukemia (**c**, **d**) ($**P < 0.05$) at time of transplant, or who received umbilical cord blood (UCB) donors vs others at transplant (**e**, **f**) ($***P < 0.05$) in patients receiving a myeloablative conditioning regimen of clofarabine in combination with cytarabine and TBI and alloSCT in CAYA with poor-risk acute leukemia ($*P < 0.05$)

conditioning regimens will be needed to assess efficacy of this approach in this population. Future studies will additionally be required to determine whether the anti-leukemic activity of clofarabine, in combination with other agents, might obviate the need for TBI in subsets of pediatric acute leukemia patients. Furthermore, our results support the potential use of a clofarabine-containing myeloablative conditioning regimen prior to alloSCT in lower-risk groups of pediatric patients with acute leukemias.

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Compliance with ethical standards

Conflict of interest M.B.G. reports grants and non-financial support from Doris Duke Charitable Foundation, during the conduct of the study; and grants from Lymphoma Research Foundation, grants from NIH/National Center for Advancing Translational Sciences, outside the submitted work. The remaining authors declare that they have no conflict of interest.

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