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ABSTRACT

The prognosis of patients with acute myeloid leukemia in whom primary treatment fails remains very poor. In order to improve such patients' outcome, we conducted a phase 2, prospective, multicenter trial to test the feasibility of a new sequential regimen, combining a short course of intensive chemotherapy and a reduced intensity-conditioning regimen, before allogeneic stem-cell transplantation. Twenty-four patients (median age, 47 years) with acute myeloid leukemia in primary treatment failure were included. Cytogenetic risk was poor in 15 patients (62%) and intermediate in nine (38%). The sequential regimen consisted of clofarabine (30 mg/m^2/day) and cytosine arabinoside (1 g/m^2/day) for 5 days, followed, after a 3-day rest, by reduced-intensity conditioning and allogeneic stem-cell transplantation combining cyclophosphamide (60 mg/kg), intravenous busulfan (3.2 mg/kg/day) for 2 days and anti-thymocyte globulin (2.5 mg/kg/day) for 2 days. Patients in complete remission at day +120 received prophylactic donor lymphocyte infusion. Eighteen patients (75%) achieved complete remission. With a median follow-up of 24.6 months, the Kaplan-Meier estimate of overall survival was 54% (95% CI: 33-71) at 1 year and 38% (95% CI: 18-46) at 2 years. The Kaplan-Meier estimate of leukemia-free survival was 46% (95% CI: 26-64) at 1 year and 29% (95% CI: 13-48) at 2 years. The cumulative incidence of non-relapse mortality was 8% (95% CI: 1-24) at 1 year and 12% (95% CI: 3-19) at 2 years. Results from this phase 2 prospective multicenter trial endorsed the safety and efficacy of a clofarabine-based sequential reduced-toxicity conditioning regimen, which warrants further investigation. This study was registered at www.clinicaltrials.gov, identifier number: NCT01188174.

Introduction

The prognosis of patients with acute myeloid leukemia (AML) in primary treatment failure remains very poor. Primary treatment failure or primary refractory AML is defined by failure to achieve a complete hematologic remission (CR) after one or two courses of induction chemotherapy.[1,2] For these patients, the chance of achieving a CR with another standard or conventional treatment, including the use of intermediate- or high-dose cytosine arabinoside (Ara-C) alone or in combination with an

anthracycline, fludarabine or gemtuzumab-ozogamicin, is 10-20% at best. The overall survival rate at 1 year is less than 20%, with a median survival of less than 6 months.[3-5] In light of these poor results with conventional chemotherapy, allogeneic stem cell transplantation (SCT) represents a potential therapeutic tool for the treatment of patients with refractory AML.[6] However, standard myeloablative conditioning (MAC), combining cyclophosphamide and either total body irradiation or busulfan, is associated with a very high day +100 overall mortality rate, around 40%[7-10] in refractory AML patients, precluding its use. Reduced-intensity conditioning (RIC) regimens allowed a significant reduction of non-relapse mortality (NRM), expanding the transplant option to those patients ineligible for standard MAC allogeneic SCT. The RIC regimen aims to take advantage of the graft-*versus*-leukemia (GvL) effect mediated by the donor's immunocompetent cells, rather than the upfront cytoreductive effect of the conditioning chemotherapy.[11] However, in refractory AML, RIC may not control the disease sufficiently to allow a GvL effect to occur. In an attempt to overcome this problem, the so-called "sequential" transplant approach was developed, which combines a short, intensive course of salvage chemotherapy to decrease the leukemia cell burden with RIC. The results achieved with the sequential FLAMSA strategy (fludarabine, intermediate dose Ara-C, amsacrine followed by 4 Gy total body irradiation, cyclophosphamide and anti-thymocyte globulin) developed by the Munich group are among the most promising published so far.[12,13] However, the results are still unsatisfactory in terms of relapse incidence and long-term leukemia control, and the high incidence of severe toxicities, related to amsacrine (mainly cardiotoxicity) and total body irradiation hamper the overall safety and success of the procedure.

Strategies for improving the sequential approach are warranted. Such improvement could be made possible through avoidance of amsacrine and the use of clofarabine and intravenous (i.v.) busulfan in replacement of fludarabine and total body irradiation, respectively. Clofarabine is a next-generation purine analog with significant antileukemic activity, particulary in relapsed AML and acute lymphoblastic leukemia.[14-18] We found that a RIC regimen combining clofarabine, bulsulfan and anti-thymocyte globulin was safe, with a 1-year NRM rate of 6%, and allowed efficient disease control in AML patients in CR.[19] Thus, the use of clofarabine as an alternative to fludarabine in the setting of RIC allogeneic SCT appears to be very attractive. On the other hand, the antileukemic activity of bulsulfan is widely recognized, and the favorable safety profile associated with the use of i.v. bulsufan is well established.[20-22]

We, therefore, conducted a multicenter prospective phase 2 study aiming to assess the efficacy of a sequential regimen combining a short course of intensive chemotherapy based on clofarabine, Ara-C and RIC allogeneic SCT with cyclophosphamide, i.v. busulfan and anti-thymocyte globulin, followed by delayed prophylactic donor lymphocyte infusion (pDLI) in patients with AML in primary treatment failure.

Methods

Study design and inclusion criteria

This prospective multicentre phase 2 study included 24 patients diagnosed with AML in primary treatment failure who underwent allogeneic SCT between 2010 and 2013. The trial was approved by each participating center's institutional review board, the *Comité de Protection des Personnes de Tours* (CPP Ouest-1, ref. 2009-R29) and the national *Agence Française de Sécurité Sanitaire des Produits de Santé* (AFSSAPS). This study was registered with ClinicalTrials.gov, identifier number: NCT01188174. Informed consent from patients and donors was obtained before their inclusion in the study.

Patients aged between 18 and 55 years were eligible for the study if they had a confirmed diagnosis of AML in the status of primary induction failure, i.e. patients alive after induction treatment but who failed to fulfill the criteria for CR or CR with incomplete recovery.[1,2,23] This was defined by either: (i) the persistence of leukemic blasts in the peripheral blood; or by the persistence of ≥5% leukemic blasts in a representative bone marrow aspiration after a second course of induction chemotherapy; or (ii) persisting hypoplasia, defined by a hypocellular bone marrow and incomplete reconstitution of the cell counts in the peripheral blood, i.e. absolute neutrophil count $<0.5\times10^9$/L or platelet count $<50\times10^9$/L at day 100 after starting chemotherapy. Induction chemotherapy itself was administered according to the participating institutions' preference. Additional inclusion criteria were the availability of a matched sibling donor or unrelated stem-cell donor (10-HLA match or a single HLA antigen or allele mismatch).

Exclusion criteria were documented chloroma or leukemic infiltration of the central nervous system, M3 AML, Karnofsky performance score below 60%, serum creatinine above 1.0 mg/dL or creatinine clearance less than 60 mL/min, bilirubin above 1.5 mg/dL, aminotransferases or alkaline phosphatase above 2.5 times the upper normal limit, acute or chronic heart failure, and pregnancy.

Treatment

Patients included in the study proceeded directly to the sequential allogeneic SCT; however, additional cytoreductive chemotherapy, at the treating physician's discretion, was allowed to control leukemic proliferation while preparation for allogeneic SCT was ongoing. The preparative regimen consisted of 30 mg/m^2/day clofarabine and 1 g/m^2/day Ara-C for 5 consecutive days (days -13 to -9) and, after a 3-day rest, 60 mg/Kg cyclophosphamide for 1 day (day -5), 3.2 mg/Kg/day i.v. busulfan for 2 consecutive days (days -5 and -4), and 2.5 mg/Kg/day anti-thymocyte globulin for 2 consecutive days (days -3 and -2) (Figure 1). All patients received this treatment as in-patients in private rooms, and remained in hospital until hematopoietic recovery. Day 0 was designated as the day of graft infusion. Supportive care and antimicrobial prophylaxis were as reported previously.[24] Of note, supportive care and management of cytomegalovirus infection were homogeneous during the period of the study. Blood products were filtered, irradiated and screened for cytomegalovirus. In the first 100 days after their transplant, patients were assessed at least once a week for cytomegalovirus reactivation, in order to initiate pre-emptive ganciclovir therapy if necessary.

For graft-versus-host disease (GvHD) prophylaxis, patients were given either cyclosporine A alone, in the case of an HLA-matched sibling donor, or cyclosporine A and mycophenolate mofetil, in the case of an HLA-matched unrelated donor.[25,26] Per protocol, the dose of mycophenolate mofetil was decreased progressively over 4 weeks, starting from day 35, and that of cyclosporine A from day 62, to be stopped at day 90 if no GvHD appeared. Patients received pDLI if they were in CR in the absence of a history of grade II-IV GvHD after >30 days without immunosuppressive therapy, were free of active infections, and had a documented donor cell chimerism of >80% in peripheral blood T

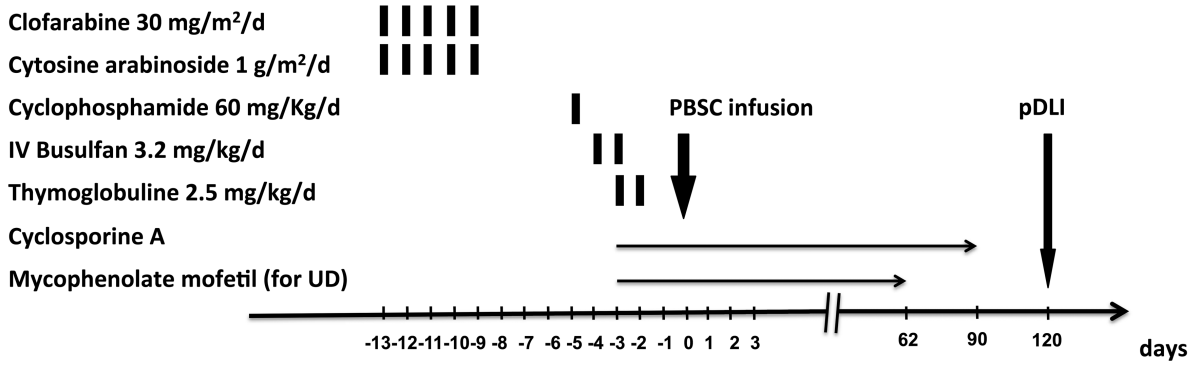

Figure 1. Sequential conditioning regimen schema. PBSC: peripheral blood stem cells; pDLI: prophylactic donor lymphocyte infusion; IV: intravenous; UD: unrelated donor.

cells. The first pDLI was given at day +120 or 30 days after discontinuation of immunosuppression. The initial dose was 1×10^7 $CD3^+$ cells/kg for patients with a matched sibling donor, and 1×10^6 $CD3^+$ cells/kg for those with an unrelated donor. In the absence of GvHD, the pDLI dose was escalated (5-fold increase/transfusion), up to three times, every 4 to 6 weeks.

All donor/recipient pairs were typed at the allelic level. A single HLA mismatch out of ten (HLA-A, HLA-B, HLA-Cw, HLA-DRB1 and HLA-DQB1) was allowed at the antigen or allele level. For graft source, granulocyte - colony-stimulating factor-mobilized peripheral blood stem cells were the graft source recommended per protocol, but unmanipulated bone marrow was accepted when peripheral blood stem cells were not available.

Clinical outcomes and graft-*versus*-host disease assessment

The overall survival rate at 2 years after transplantation was the primary endpoint. Secondary endpoints included engraftment, leukemia-free survival, leukemia response rate (remission status), relapse rate, treatment-related toxicity, NRM, discontinuation of immunossupressive therapy, pDLI, acute GvHD and chronic GvHD. Time to neutrophil recovery was defined as the first of 3 consecutive days in which the absolute neutrophil count exceeded 0.5×10^9/L, and engraftment failure as an absolute neutrophil count above 0.5×10^9/L at day +42 after allogeneic SCT. Leukemia response rate was evaluated at days +30 and +60, and 6, 12 and 24 months after the transplant. GvHD was evaluated according to the Seattle standard criteria.[27]

Statistical methods

The hypothesis for the primary endpoint was an improvement in overall survival at 2 years from 15% to 40%. Using a one step A'Hern procedure, 21 patients would be needed for the statistical analysis to be meaningful. In all, inclusion of 24 patients was planned in order to take account of possible drop outs after inclusion, i.e. patients who would not receive a transplant due to infection or other events occurring after identification of the donor etc., but before the start of conditioning. If the number of surviving patients is seven or more, the hypothesis that the 2-year overall survival rate is less than 15% is rejected with a target error rate of 0.05. If the number of surviving patients at 2 years is six or less, the hypothesis that 2-year overall survival is >40% is rejected with a target error rate of 0.20. Overall and leukemia-free survival rates were calculated using the Kaplan-Meier method. Overall survival was defined as the time from allogeneic SCT to death, regardless of the cause. Leukemia-free survival was defined as survival with no evidence of leukemia after achieving complete remission. The probability of NRM was calculated using the cumulative incidence procedure. We defined NRM as death with no evidence of leukemia relapse or progression. Data were computed using SPSS and EZR version 1.27 (Saitama Medical Center, Jichi Medical University, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, version 3.1.1).[28]

Results

Patient and donor characteristics

The characteristics of the patients and the donors are summarized in Tables 1 and 2. The median age of recipients was 47 years (range, 20-57). One patient aged >55 years was included in the trial after approval of the protocol steering committee. AML cytogenetic status, classified according to the European Leukemia Net,[23] was intermediate in 9 patients (38%) and adverse in 15 (62%); no patients had AML with favorable cytogenetics. Seven patients (29%) had a secondary AML (5 secondary to myelodysplastic syndrome and 2 to others malignancies treated by chemotherapy or radiotherapy). The Duval score[7] was 0 in six patients (25%), 1 in five patients (21%), 2 in six patients (25%) and 3 in seven patients (29%). The diagnosis of AML in primary induction failure was confirmed by the persistence after a second course of induction chemotherapy of leukemic blasts in the peripheral blood in two patients (81% and 89.5%) or of ≥5% leukemic blasts in the bone marrow in 21 patients, while only one patient had persistent hypoplasia. Beside the patient with persistent hypoplasia, the median marrow blast percentage at transplantation was 20% (range, 6-82%). Fifteen donors (62%) were HLA-identical siblings and nine (38%) were unrelated donors. The stem cell source was bone marrow in one case (4%) and peripheral blood stem cells in the remaining 23 (96%). In 11 patients (46%) the Karnofsky score was <90%, whereas in 13 (54%) it was ≥90%.

Engraftment and chimerism

All patients developed pancytopenia after the sequential conditioning regimen. Twenty-two engrafted (92%) and

two died in aplasia. The median time to neutrophil recovery was 15 days (range, 13-56), while the median time to platelet recovery (>50×10^9/L) was 13 days (range, 7-42). Chimerism was evaluated on CD3$^+$ T cells on day +28. Full donor chimerism (donor CD3$^+$ T cells ≥95%) was found in 14 patients, while eight patients had mixed chimerism.

Acute and chronic graft-*versus*-host-disease

Grade II-IV acute GvHD developed in four patients of whom three had grade II disease at days 9, 12, and 83, and one had grade III GvHD at day 62 after transplantation: no patient developed grade IV acute GvHD. Skin, gut and liver were affected in three, three and one patient, respectively. Acute GvHD resolved in all patients upon corticosteroid treatment. Chronic GvHD developed in nine patients, of whom five had limited disease and four had extensive chronic GvHD.

Infection and toxicity

Septicemia was encountered in nine patients, caused by bacteria in seven and *Candida albicans* in two. Septicemia led to sepsis or septic shock in three patients. Eight patients developed pneumonia, caused by *Aspergillus* spp. in three patients, bacteria in two, viruses in two, and was of unknown etiology in one patient. Besides viral pneumonia, two patients had cytomegalovirus reactivation, two had Epstein-Barr virus reactivation, one had coronavirus infection and one patient had BK virus-associated hemorrhagic cystitis.

Non-hematologic side effects not related to GvHD or infections were classified according to World Health Organization criteria. Fifteen grade III adverse events were reported in 12 patients; no grade IV adverse event occurred in this study (Table 3). Protocol-related grade III-IV hematologic adverse events were reported in five patients including neutropenia (grade III, n=3; grade IV, n=1), lymphopenia (grade III, n=1; grade IV, n=1), anemia (grade III, n=1), bone marrow failure (grade III, n=1) and pancytopenia (grade III, n=1), with some patients having more than one severe hematologic adverse event.

Disease response and outcome

At day +30, 23 patients were evaluable for response and one patient had died. Eighteen patients were in CR (75%) and five had persistent leukemia (Tables 2 and 4). One patient with persistent disease at day +30 achieved CR at day +60, without any further treatment beside reduction of immunosuppressive therapy, and maintained it until the last follow-up. Disease relapse after achieving CR occurred in ten patients (42%) at a median of 115 days (range, 49 to 624) after allogeneic SCT. The overall median follow-up after transplant was 24.6 months (range, 23.7-28.6) among surviving patients. Of the 24 patients included in this study, 17 died and seven are still alive at last follow-up. The Kaplan-Meier estimates of overall survival and leukemia-free survival at 2 years were 38% (95% CI: 19-56%) and 29% (95% CI: 13-48%) (Figure 1A,B). Thirteen deaths were directly attributed to disease progression or relapse, whereas four cases were related to the transplant, of which two were related to infection and two to chronic GvHD. At 2 years, the cumulative incidences of relapse/progression and NRM were 54.2% (95% CI: 31.8-72.0) and 12% (95% CI: 3-29%), respectively (Figure 2C,D).

Table 1. Patient and donor characteristics.

Characteristic (%)	Study population (n=24)
Patient median age, years (range)*	47 (20-57)
Patient gender (female/male) (%)	9/15 (38%/62%)
Donor gender (female/male) (%)	10/14 (42%/58%)
Sex mismatch (female to male) (%)	8 (33%)
Median time from diagnosis to transplantation, days (range)	122 (73-312)
Median marrow blasts at transplantation, % (range)**	20% (6-82)
Diagnosis	
De novo AML	17 (71%)
AML secondary to MDS	5 (21%)
AML secondary to other malignancies	2 (8%)
Cytogenetics	
Intermediate	9 (38%)
Unfavorable	15 (62%)
Karnofsky score	
≥ 90%	13 (54%)
< 90%	11 (46%)
CMV serologic seronegative donor-recipient pairs	
Stem cell source	
Bone marrow	1 (4%)
PBSC	23 (96%)
Donor type	
Matched sibling donor	15 (63%)
Unrelated donor	9 (37%)***
Cell dose, median (range)****	
TNC 10^8/Kg	9.0 (3.9-27.5)
CD34$^+$ cells 10^6/Kg	7.1 (3.6-8.6)

AML: acute myeloid leukemia; MDS: myelodysplastic syndrome; CMV: cytomegalovirus; PBSC: peripheral blood stem cells; TNC: total nucleated cells. *One patient aged >55 years was included in the trial after approval of the protocol steering committee.- ** One patient had persistent BM hypoplasia and marrow blasts were not evaluable; in two patients BM blasts were not evaluated given the high number of circulating blasts (81% and 89.5%). ***Four patients had a donor with a single antigenic or allelic mismatch. ****Cell doses are indicated for the 23 patients who received PBSC, the patient who received BM, received 1.79×10^8/Kg TNC and 1.86×10^6/Kg CD34$^+$ cells.

Donor lymphocyte infusion

Six of 24 patients (25%) received DLI in our study, including three patients who fulfilled the criteria for pDLI, and three additional patients who received DLI before 30 days without immunosuppressive therapy because of mixed chimerism. Although pDLI could be given from day +120, patients received the first dose on days 132, 173 and 173, due to delayed cyclosporine withdrawal. Patients with mixed chimerism received the first DLI dose earlier, on days 93, 92 and 98. Overall, three patients received one transfusion, two received two transfusions and one received three transfusions in escalating doses. In the DLI group, patients received fewer than three transfusions because of relapse or development of GvHD.

Leukemic relapse occurred in one patient after pDLI and in two after DLI for mixed chimerism, and was the cause of death in all three. GvHD was the main complication after DLI: grade II acute GVHD developed in one patient after DLI for mixed chimerism, and chronic GvHD in all three patients who received pDLI and in one after DLI for mixed chimerism. One patient died from extensive chronic GvHD after pDLI. At the last follow-up, two patients

Table 2. Characteristics of the AML and patients' outcome.

Patient	AML	Karyotype	Bone marrow blasts at transplant	Peripheral blood blasts at transplant	WBC at transplant × 10^9/L	Duval score	CR at days 30	Relapse after CR	Death (yes/no)	Cause of death
01001	*De novo*	Complex	51	2	6.9	3	Yes	Yes	Yes	AML
01002	*De novo*	Normal	40.5	0	0.8	1	Yes	Yes	Yes	AML
01003	*De novo*	-7; t(3;3)	-	89.5	6.6	3	No	-	Yes	AML
01004	Secondary to MDS	Del(2q)	7.5	0	0.7	0	Yes	Yes	Yes	AML
01005	*De novo*	Complex	39	0	1.3	1	Yes	Yes	Yes	AML
01006	*De novo*	Complex	-	81	43.7	3	No	-	Yes	AML
02001	Secondary to MDS	abn 7	14	0	4.6	0	-	-	Yes	Infection
02002	Secondary to solid tumor	Complex	52	54	1.6	3	No	-	Yes	AML
02003	*De novo*	Normal	7	0	1.1	0	Yes	No	Yes	Infection
02004	*De novo*	Complex	24	0	.7	2	Yes	Yes	Yes	AML
06001	*De novo*	Normal	8	0	4.2	0	No*	No	No	-
07001	Secondary to lymphoma	+4, t(7;14)	51	0	1.3	1	Yes	No	No	-
08001	*De novo*	Complex	20	5	1.6	2	Yes	Yes	Yes	AML
08002	*De novo*	Complex	39	55	3.3	2	Yes	No	No	-
09001	Secondary to MDS	-7	6	10	1.2	3	Yes	Yes	Yes	AML
09002	*De novo*	Complex	72	65	6.6	3	Yes	Yes	Yes	AML
09003	*De novo*	Complex	1	0	1.0	1	Yes	No	No	-
09004	Secondary to MDS	Complex	12	0	0.7	3	Yes	No	Yes	cGvHD
09005	*De novo*	t(6;9)(p23;q34)	11	0	5.6	1	Yes	Yes	Yes	AML
09006	*De novo*	-7	82	0	0.3	2	Yes	No	Yes	cGvHD
09007	*De novo*	Normal	8	0	1.7	0	Yes	No	No	-
10001	*De novo*	Complex	6	0	1.7	2	Yes	No	No	-
10002	*De novo*	+8	60	2	1.3	2	Yes	No	No	-
10004	Secondary to MDS	-7q	12	0		0	No	Yes	Yes	AML

AML: acute myeloid leukemia; WBC: white blood count; CR: complete remission; MDS: myelodysplastic syndrome; abn: abnormality; cGvHD: chronic graft-*versus*-host disease. *Patient 06001 achieved complete remission at day +60.

were alive in CR. After DLI for mixed chimerism, all three patients successfully converted to full donor chimerism. Among 13 patients alive at day +120, the reasons for not giving pDLI were disease progression or relapse (n=4), a history of grade II-IV acute GvHD (n=4), current chronic GvHD (n=2), infections (n=1), refusal by donor or patient (n=1) or the decision of the physician in charge of the patient (history of grade I acute GvHD; n=1).

Discussion

The sequential transplant approach combining a short course of intensive chemotherapy based on clofarabine and Ara-C and a RIC allogeneic SCT, evaluated in the current phase 2 prospective study, has successfully fulfilled its objective to achieve limited toxicity while retaining substantial antileukemia activity. In a heavily pretreated population, having received two induction chemotherapy courses, with a median age of 47 years (range, 20-57) and a Karnofsky score <90% in 46% of patients, we found a 2-year NRM rate of 12% and no grade IV non-hematologic adverse events. This favorably compared with standard MAC regimens,[9,10,29] but also with the sequential FLAMSA strategy. In the long-term update of the FLAMSA study, Schmidt *et al.* reported a 2-year NRM incidence of 22.2%,[13] consistent with further reports evaluating the FLAMSA strategy.[30,31] A retrospective study recently reported a 1-year NRM incidence of 24% after allogeneic SCT for AML in primary induction failure, with no significant difference, according to multivariate analysis, in NRM between patients treated with MAC, RIC or FLAMSA regimens.[32] Thus, our approach avoiding amsacrine and replacing fludarabine and total body irradiation by clofarabine and i.v. bulsufan, respectively, fulfilled its objective to decrease toxicity with a very low incidence of NRM, comparable to that observed after the reduced toxicity regimen based on fludarabine and i.v. busulfan.[33,34]

Decreased toxicity was not achieved at the expense of less antitumor efficacy. Our sequential conditioning regimen approach still exerts an antileukemic effect in patients with AML in primary treatment failure, 75% of whom achieved CR by day 30 after their transplant. This may appear lower than the 88% CR rate achieved by day 30 using the FLAMSA approach;[12] however, the latter study included both patients with refractory and relapsed AML and 21% (16/75) of patients were already in CR before allogeneic SCT in that study. In contrast, in our trial, all patients had primary refractory AML and, therefore, active disease at the time of transplantation. Furthermore, 62% of our patients had unfavorable cytogenetics and 29% had secondary AML. Thus, our sequential approach

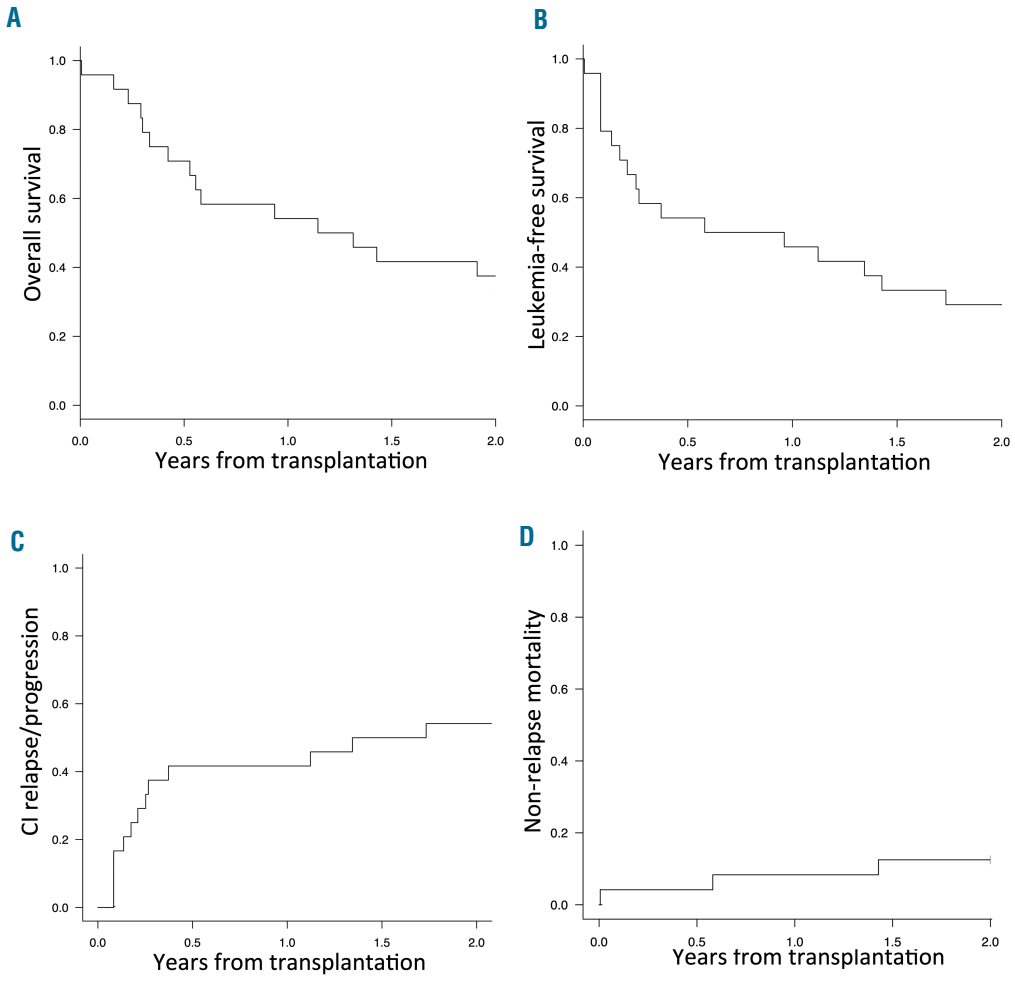

Figure 2. Outcome after allogeneic SCT. (A) Two-year overall survival; (B) 2-year leukemia-free survival; (C) cumulative incidence of relapse/progression; and (D) cumulative incidence of non-relapse mortality.

significantly enhanced the antileukemic effect compared to RIC allogeneic SCT, after which only 45% of patients with active AML achieved CR.[35]

The 2-year overall survival rate was 38%, similar to the 42% reported by Schmid *et al.* using the FLAMSA sequential regimen,[12] and superior to the 20-30% reported after standard MAC allogeneic SCT.[8,10,29] Similarly, Middeke *et al.* reported a 2-year overall survival rate of 43% in relapsed/refractory AML after clofarabine salvage therapy and allogeneic SCT in patients achieving a response.[16] Despite these encouraging results, relapse remains the principal complication, leading to a 2-year leukemia-free survival rate of 29%. We acknowledge that survival curves do not reach a plateau and relapses may continue to occur. However, persistent CR after 2 years in these very high-risk patients is an important achievement since retreatment is possible after late relapse. Of note, a longer interval from transplantation to relapse is associated with an improved survival.[36,37]

Our study was designed to decrease relapse risk in patients achieving CR, with a planned pDLI infusion. However, among 19 patients alive at day +120, only a minority - six patients - received DLI in our study because of early disease relapse/progression or a history of GvHD. Furthermore, among patients treated with DLI, half died from relapse. The use of DLI to enhance the antileukemic effect and decrease relapse in our study was, therefore, a disappointing strategy. In addition, as previously described,[38] we observed a relatively high incidence of GvHD after pDLI. It does, therefore, seem indispensable to develop new strategies to prevent relapse after allogeneic SCT. One way to overcome these difficulties would be to perform earlier pDLI while patients are still under immunosuppressive therapy. Furthermore, Goodyear *et al.* reported that early administration of the hypomethylating agent azacytidine in AML patients was

Table 3. Non-hematologic organ toxicity according to WHO criteria.

	Grade III	Grade IV
Esophagitis	2	0
Asthenia	2	0
Liver	5	0
Hypokalemia	1	0
Renal	1	0
Central nervous system	1	0
Lung	1	0
Skin	2	0

well tolerated, and was associated with a low incidence of GvHD.[39] This strategy appears promising and a prospective trial evaluating preemptive azacytidine and DLI in high-risk AML patients is ongoing (NCT01541280). Targeted therapy with *FLT3*-specific tyrosine kinase inhibitors may also be relevant in specific cases. Preliminary data and case reports suggest that *FLT3* tyrosine kinase inhibitors can be effective in the post-transplant setting, particularly for patients who have *FLT3* internal tandem duplication.[40] We may consider early use of these new therapeutic strategies after allogeneic SCT, alone or in combination with pDLI, in order to enhance the GvL effect in patients with AML in primary induction failure. Our clofarabine-based, sequential reduced-toxicity conditioning approach seems to be the best setting in which to evaluate such therapeutic strategies, in order to minimize the risk of cumulative toxicity.

Since this was a phase 2 study, only a limited number of patients were enrolled, precluding the realization of subgroup analyses to evaluate the impact of disease characteristics (cytogenetic, secondary AML…) on patients' outcome. Of note, despite the inclusion and exclusion criteria, we believe that our population is an unselected, representative population of patients with refractory AML as shown by the inclusion of a majority of patients with unfavorable cytogenetics and of patients with proliferative, refractory AML. However, we acknowledge that identifying a suitable donor in a timely manner may be difficult in these patients with very aggressive disease, limiting access to transplantation. Use of haploidentical donors, readily available for nearly all patients,[41] may be promising in this setting.

Overall, in view of the results of the current prospective multicenter study, this sequential conditioning regimen combining clofarabine and Ara-C-based chemotherapy followed by a cyclophosphamide, i.v. bulsufan and anti-thymocyte globulin-based RIC allogeneic SCT appears to be a valid approach/platform in patients with AML with primary treatment failure. This regimen represents a significant improvement in terms of toxicity, with a low NRM. However, relapse remains the principal complication in these patients. Collectively, these results provide a framework for further refinement of the sequential approach designed to improve disease control without increasing toxicity. Although this approach should, ideally, be validated in a phase 3 randomized trial, the choice of the control arm – MAC, FLAMSA or other reduced toxicity regimen – may be tricky. Finally, any such approaches should include enhanced strategies to prevent relapse after allogeneic SCT.

Table 4. Transplant-related events.

Characteristic	Study population (n=24)
Median follow-up, months (range)	25 (24-29)
Overall survival	
At 1 year, % (95% CI)	54% (33-71)
At 2 years, % (95% CI)	38% (18-56)
Leukemia-free survival	
At 1 year, % (95% CI)	46% (26-64)
At 2 years, % (95% CI)	29% (13-48)
Non-relapse mortality	
At 1 year, (cumulative incidence, 95% CI)	8% (1-24)
At 2 years, (cumulative incidence, 95% CI)	12% (3-19)
Relapse/progression	
At 1 year, (cumulative incidence, 95% CI)	41.7% (21.7-60.6)
At 2 years, (cumulative incidence, 95% CI)	54.2% (31.8-72.0)
Response to treatment at day +30	
No response	5
Complete remission	18
Not evaluable	1

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