RESEARCH ARTICLE

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Similar outcome of allogeneic stem cell transplantation after myeloablative and sequential conditioning regimen in patients with refractory or relapsed acute myeloid leukemia: A study from the Société Francophone de Greffe de Moelle et de Thérapie Cellulaire

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Abstract

Patients with acute myeloid leukemia (AML) in relapse or refractory to induction therapy have a dismal prognosis. Allogeneic hematopoietic stem cell transplantation is the only curative option. In these patients, we aimed to compare the results of a myeloablative transplant versus a sequential approach consisting in a cytoreductive chemotherapy followed by a reduced intensity conditioning regimen and prophylactic donor lymphocytes infusions. We retrospectively analyzed 99 patients aged 18-50 years, transplanted for a refractory (52%) or a relapsed AML not in remission (48%). Fifty-eight patients received a sequential approach and 41 patients a myeloablative conditioning regimen. Only 6 patients received prophylactic donor lymphocytes infusions. With a median follow-up of 48 months, 2-year overall survival was 39%, 95% confidence interval (CI) (24-53) in the myeloablative group versus 33%, 95% CI (21-45) in the sequential groups ($P = .39$), and 2-year cumulative incidence of relapse (CIR) was 57% versus 50% respectively ($P = .99$). Nonrelapse

mortality was not higher in the myeloablative group (17% versus 15%, $P = .44$). In multivariate analysis, overall survival, CIR and nonrelapse mortality remained similar between the two groups. However, in multivariate analysis, sequential conditioning led to fewer acute grade II-IV graft versus host disease (GVHD) (HR for sequential approach = 0.37; 95% CI: 0.21-0.65; $P < .001$) without a significant impact on chronic GVHD (all grades and extensive). In young patients with refractory or relapsed AML, myeloablative transplant and sequential approach offer similar outcomes except for a lower incidence of acute GvHD after a sequential transplant.

1 | INTRODUCTION

Refractory or relapsed acute myeloid leukemia (AML) is associated with a dismal prognosis. Approximately, one third of patients younger than 60 years with newly diagnosed AML fail to achieve complete remission (CR) after induction therapy.[1,2] For these resistant patients, chemotherapy alone does not offer any chance of cure.[3] Moreover, among complete responders to induction therapy, half of patients experiences relapse.[1,2] The probability to obtain a second remission with salvage chemotherapy is lower and patient prognosis is poor[2] with a 5-year overall survival (OS) rate close to 5% to 20%.[4–8]

To date, allogeneic hematopoietic stem cell transplantation (HSCT) is the only potentially curative approach in patients with primary induction failure (PIF) or patients who relapsed, and failed to achieve a second remission.[5–11] In this setting, conventional myeloablative conditioning (MAC) transplants are usually associated with nonrelapse mortality (NRM) greater than 40% in historical series.[12–17] More recently, promising results have been reported in patients with refractory or relapsed AML, using a sequential (SEQ) treatment approach. This strategy consists in an initial cytoreductive chemotherapy followed by a reduced-intensity conditioning regimen transplant (RIC) to limit toxicity. Prophylactic donor lymphocyte infusions (DLI) are systematically planned, in the absence of graft versus host disease (GVHD) to reinforce the graft versus leukemia (GVL) effect.[18] Sequential approaches are associated to 30%-45% long-term survival in advanced AML patients.[19–23] Conversely, major advances in the supportive care of HSCT, such as fungal and viral infections prevention and treatment, or HLA-typing in unrelated transplants, have reduced NRM after MAC transplants.[24–27] This evolution leads to reassess the role of MAC transplants in relapsed or refractory AML.

In the present study, we retrospectively analyzed data from the Société Francophone de Greffe de Moelle et de Thérapie Cellulaire (SFGM-TC) to compare the results of SEQ and MAC approaches in patients transplanted for relapsed or refractory AML.

2 | PATIENTS AND METHODS

2.1 | Study design

The inclusion criteria were (a) non-ex vivo T-cell depleted HSCT performed from January 2006 to December 2013 in SFGM-TC centers, (b) patients aged 18–50 years at transplant, (c) relapsed AML with active disease[28] or PIF, defined as (i) $\geq$5% bone marrow blasts, or (ii) persistence

of blood blasts at least 28 days after the first or second induction treatment, or lack of hematopoietic recovery at 50 days post induction without bone marrow blasts, (d) previous treatment with at least one cycle of induction chemotherapy course including anthracycline and cytarabine, (e) transplant from a HLA matched related or unrelated donor or a HLA 9/10 unrelated donor. Patients included in the prospective "SETRIC" study (NCT01188174) were excluded.

2.2 | Treatment

MAC consisted of 12 Gy-fractionated total body irradiation (TBI) or 12.8 mg/kg intravenous (IV) busulfan, both associated with cyclophosphamide. Sequential conditioning regimen was administered as previously described,[19,20] including: FLAMSA (fludarabine (120 mg/m^2 total dose) plus cytarabine (8 g/m^2 total dose) plus amsacrine (400 mg/m^2 total dose)) or an association of clofarabine (150 mg/m^2 total dose) and cytarabine (5 g/m^2 total dose) followed by a RIC (4 Gy TBI, or 6.4 mg/kg IV busulfan plus cyclophosphamide 80–120 mg/kg and mostly with antithymocyte globulin (ATG) (Genzyme, Cambridge MA, USA) 5 mg/kg). In this retrospective study, the type of conditioning regimen depended on the physician choice. GVHD prophylaxis consisted of cyclosporine A with mycophenolate mofetil in the SEQ group and mostly with methotrexate in the MAC group. Prophylactic DLI were to be administered systematically in the SEQ group in patients without GVHD after cessation of immunosuppressive therapy. The study was accepted by the scientific council of the SFGM-TC. Patients and donors gave informed consent for data analyses, in accordance with the declaration of Helsinki.

2.3 | Outcomes

The neutrophil recovery was defined as three consecutive days with a neutrophil count $>0.5 \times 10^6$/L. Platelet recovery was defined as three consecutive days with a platelet count above 50×10^9/L at least 7 days after the last platelet transfusion. Acute GVHD (aGVHD) and chronic GVHD (cGVHD) were defined and staged as previously reported.[29–31]

2.4 | Statistics

Patient-, disease- and transplantation related characteristics were compared using the chi-square test for categorical variables, Kendall's tau for ordinal variables and the Student t test for continuous variables. The Kaplan-Meier method was used to calculate the probability of OS

(from the day of transplant to the day of death) and the probability of disease free survival (DFS) (relapse or death being considered as events). Cumulative incidence of relapse (CIR) was defined considering NRM as a competing event. Cumulative incidence of aGVHD and cGVHD were estimated considering both death and relapse as competing risks. Patients were censored at last follow-up. OS and DFS curves were compared using the log-rank test and CIR, NRM and cumulative incidence of GVHD using the Fine and Gray test. The univariate analysis had included the following variables: relapse risk according to the European LeukemiaNet (ELN) classification, number of pretransplant chemotherapy lines, age at transplant, time between diagnosis and day of transplant (shorter or longer than 6 months), status at transplant (refractory or relapse), percentage of blood blasts at transplant, percentage of bone marrow blasts at transplant, white blood cell count at transplant, conditioning regimen type, use of ATG in the conditioning regimen, hematopoietic stem cell source (bone marrow versus peripheral blood), donor type (matched vs mismatched), donor gender, cytomegalovirus (CMV) status and CD34+ cell dose. The number of pretransplant chemotherapy lines, the age at transplant, the percentage of blood blasts at transplant, the percentage of bone marrow blasts at transplant, the white blood cell count at transplant were analyzed as continuous variables. Cox regression models were used to perform the multivariate analyses for censored outcomes and Fine and Gay models for competing risks. P value $\leq .10$ in univariate analysis was the criterion for a covariate to be included in multivariate models, followed by backward selection; type of conditioning regimen was forced into the models. Type I error rate was fixed at 0.05. Statistical analyses were realized with STATA 12.0 (Stata Corp) and the 3.2.3 version of R (https://cran.r-project.org).

3 | RESULTS

3.1 | Patients characteristics

Ninety-nine patients were included, 41 in the MAC and 58 in the SEQ groups. At transplant, 51 patients were in PIF. Two of these patients lacked hematopoietic recovery at 50 days post induction and 4 received only one intensive chemotherapy course before the transplant, including one who also received additional hypomethylating agent therapy courses. Forty-eight patients were in relapse with active disease. Characteristics are summarized in Table 1. Median age at transplant was 40 in both groups. There was no difference between the 2 groups regarding the ELN classification, the number of lines of chemotherapy prior to HSCT and the status of disease at transplant. There was a trend for a longer time between diagnosis and day of transplant in the sequential approach group (7.5 months vs 5 months, $P = .055$). Peripheral, but not bone marrow blast percentages at transplant were higher in the SEQ group (7 vs 1% ($P = .01$)).

3.2 | Transplant modalities

MAC regimen consisted of busulfan plus cyclophosphamide in 59% of patients and in TBI plus cyclophosphamide in 41% of patients.

Cytoreductive chemotherapy of the sequential approach was FLAMSA in 67% of patients (RIC conditioning regimen comprising TBI in 18 patients and busulfan in 21 patients) and the association of clofarabine and cytarabine in the remaining 33% (RIC conditioning regimen comprising TBI in 1 patient and busulfan in 18 patients). Sequential approaches included ATG in 84% of patients versus only 10% in the MAC transplants ($P < .001$).

Both groups appear to be similar with regards to the stem cell source, which was mostly peripheral stem cells (71% in the MAC group and 85% in the SEQ group, $P = .10$). Donor type was also distributed similarly with a predominance of unrelated transplant (59% in the MAC group and 60% in the SEQ group, $P = .79$). HLA mismatched unrelated transplants (9/10) represented 12% of MAC transplant and 17% of sequential approaches. Numbers of CD34 transplanted cells were close in both groups: median 5.86×10^6/kg (range 1.7–21.19) in the MAC group and 6.35×10^6/kg (range 0.85-10.59) in the SEQ group. Only 5 (8.6%) patients of the SEQ group received prophylactic DLI. Reasons of nonadministration were early deaths in 16 patients, early relapse in 8 patients, occurrence of GVHD in 11 patients and unknown in 18 patients. Prophylactic DLI were administered in one patient of the MAC group. Eight (13.7%) patients from the SEQ and 5 (12.1%) from the MAC groups received DLI after AML relapse.

3.3 | Impact of conditioning regimen on outcomes

Seven patients died before Day +30 from infectious complications without neutrophil and platelet recovery. The remaining 92 patients reached a neutrophil count over 0.5×10^6/L with a median time of 17 days (range, 6–33) in the MAC group and 15 days (range, 9–41) in the SEQ group ($P = .78$). Ninety two percent of patients reached a platelet count above 50×10^6/L in the MAC group with a median of 20 days (range, 7–379) and 98% in the SEQ group with a median of 16 days (range, 0–60) ($P = .22$).

Cumulative incidence of acute and chronic GVHD were significantly higher in the MAC group: aGVHD grade II-IV and III-IV occurred in 68% of the MAC group vs 36% in the SEQ group ($P = .002$) and in 29% of the MAC group vs 14% in the SEQ group respectively ($P = .056$). Cumulative incidences of all grade cGVHD and extensive cGVHD at 2 years were 44% 95%CI (28–60) in the MAC group vs 24% 95%CI (13.0–35.0) in the SEQ group ($P = .048$) and 22% 95%CI (9.0–36.0) in the MAC group vs and 7% 95%CI (1.0–14.0) in the SEQ group ($P = .048$) respectively. In univariate analysis, use of ATG was strongly associated with the incidence of GVHD: HR: 0.35, 95%CI (0.22–0.48); $P = .017$ for grade II-IV aGVHD, and HR: 0.23, 95%CI (0.11–0.35); $P = .043$ for cGVHD (Table 2).

There were no differences between the two approaches in terms of OS, DFS, CIR and NRM (Figure 1). With a median follow-up of 4 years, two year-OS was 39%, 95%CI (24–53) in the MAC group and 33%, 95%CI (21.0–45.0) in the SEQ group ($P = .39$). Estimated 2-year DFS was 29%, 95%CI (16.0–43.0) in the MAC group and 33%, 95%CI (21.0–45.0) in the SEQ group ($P = .48$). The 2-year CIR was 57%, 95%CI (41–72) in the MAC group and 50%, 95%CI (37–63) in the SEQ group ($P = .99$). The 2-year NRM was 15% (95%CI (4.0–26.0)) in the

TABLE 1 Patient characteristics

	Total	MAC	SEQ	P value
Number of patients, n (%)	**99**	**41 (41%)**	**58 (59%)**	
Age in years, median (range)	40 (18–50)	40 (19–50)	39 (18–49)	P = .97
Male gender, n (%)	48 (48%)	20 (49%)	28 (48%)	P = .96
ELN classification at diagnosis, n (%)				
Favorable	4 (4%)	2 (5%)	2 (4%)	P = .62
Intermediate-1	30 (34%)	14 (36%)	16 (32%)	
Intermediate-2	21 (24%)	9 (23%)	12 (24%)	
Adverse	34 (38%)	14 (36%)	20 (40%)	
Missing (n)	10	2	8	
Pretransplant chemotherapy lines, median (range)	2 (1–3)	2 (1–3)	2 (1–3)	P = .49
Time from diagnosis to day of transplant (months), median (range)	6.5 (2–121)	5 (2–18.5)	7.5 (2–121)	P = .055
Disease status at transplant, n (%)				
Refractory	51 (51%)	21 (51%)	30 (52%)	P = .96
Relapsed	48 (49%)	20 (49%)	28 (48%)	
First relapse	40 (83%)	16 (80%)	24 (86%)	
Second relapse	8 (17%)	4 (20%)	4 (14%)	
Biologic parameters at transplant				
Blood blasts (%), median (range)	2 (0–93)	1 (0–85)	7 (0–93)	P = .02
Missing (n)	3	0	3	
Bone Marrow blasts (%), median (range)	20 (0–96)	13 (0–86)	25 (0–96)	P = .34
Missing (n)	19	5	14	
White blood cells (G/L), median (range)	2.4 (0–88)	2.7 (0.1–84)	2.2 (0–88)	P = .96
Missing (n)	1	1	0	

ELN, European LeukemiaNet; HSCT, hematopoietic stem cell transplantation; MAC, myeloablative conditioning regimen transplant; SEQ, sequential conditioning regimen transplant.

MAC group and 17% 95%CI (7.0–27.0) in the SEQ group (P = .44). Variables associated with post-transplant outcomes in univariate analysis are provided in Table 2.

The patients who received a FLAMSA regimen and those who received the clofarabine/cytarabine regimen had similar OS (HR for FLAMSA: 1, 95%CI (0.53–1.88); P = 1.0), CIR (HR for FLAMSA: 1.01, 95%CI (0.51–2.0); P = .98) and NRM (HR for FLAMSA: 0.99, 95%CI (0.31–3.17); P = .98). There was also no difference in the MAC group between TBI or busulfan based regimens with respect to OS (HR for TBI: 0.85, 95%CI (0.40–1.8); P = .67), CIR (HR for TBI: 1.1, 95%CI (0.51–2.37); P = .81), NRM (HR for TBI: 0.27, 95%CI (0.03–2.32); P = .23) and cGVHD (HR for TBI: 1.89, 95%CI (0.76–4.75); P = .17).

3.4 | Risk factors for post-transplant outcomes in multivariate analysis

In multivariate analysis, sequential regimen remained associated with a lower incidence of grade II-IV aGVHD (HR: 0.37, 95%CI (0.21–0.65); P < .001) but not with cGVHD (all grades and extensive). Variable associated with all grades cGVHD in multivariate analysis were CMV donor/recipient status (HR: 0.46, 95%CI (0.43–0.49); P = .03) and ATG use in the conditioning regimen (HR: 0.41, 95%CI (0.20–0.87) P = .019). ATG use was the only variable associated with extensive cGVHD (HR: 0.15, 95%CI (0.03–0.66); P = .012) (Table 2).

In multivariate analysis including the conditioning regimen type, two variables were significantly associated with OS: relapse status (HR:

1.66, 95%CI (1.03–2.66); P = .036) and a higher blood blast percentage at HSCT analyzed as a continuous variable, (HR: 1.01, 95%CI (1.01–1.02); P < .001).

A worse DFS was observed in patients with (a) more than 6 months elapsing between diagnosis and treatment (HR: 1.65, 95%CI (1.04–2.61); P = .035), (b) a higher blood blast percentage (HR: 1.01, 95%CI (1.01–1.02); P = .001) and c) a lower CD34 infused cell dose analyzed as a continuous variable (HR: 0.93, 95%CI (0.86–1.0); P = .049).

Relapse status at transplant (HR: 2,03, 95%CI (1.2–3.46); P = .009), a higher number of pretransplant chemotherapy lines (HR: 1.56, 95%CI (1.08–2.23); P = .017), and a higher blood blast percentage at transplant (HR: 1.01, 95%CI (1.01–1.02); P = .004) were independently associated with higher post–transplant CIR, while there was no significant prognostic factor for NRM in our cohort.

After excluding from the analyses the four patients, who had received only one induction therapy before transplant and the two patients lacking a hematopoietic recovery 50 days after induction therapy, the conditioning regimen remained not statistically associated with OS, DFS, NRM, CIR, cGVHD but is still significantly associated with aGVHD (Supporting Information Table S1).

3.5 | Subgroup analysis

To identify a subgroup of patients who may benefit from the SEQ or the MAC approaches, we analyzed the impact of the conditioning regimen according to the presence of blood blasts at transplant and to the

TABLE 2 Risk factors for GVHD, OS, DFS, CIR

	Univariate analysis[a]			Multivariate analysis		
	HR	95% CI	P value	HR	95% CI	P value
aGVHD (grade II–IV)						
Conditioning type: Sequential	0.37	0.21–0.65	<.001	0.37	0.21–0.65	<.001
Female donor to male recipient	1.63	0.94–2.82	.081			
Use of ATG	0.40	0.23–0.71	.0017			
cGVHD (all grades)						
Conditioning type: Sequential	0.50	0.25–0.99	.049			
Use of ATG	0.48	0.24–0.98	.043	0.41	0.20–0.87	.019
CD34+ cell dose[§]	1.06	0.99–1.13	.086			
All CMV status vs CMV recipient -/donor -	0.46	0.23–0.92	.029	0.46	0.43–0.49	.029
cGVHD (extensive)						
Conditioning type: Sequential	0.30	0.09–0.96	.043			
Female donor to male recipient	2.70	0.89–8.06	.08			
Use of ATG	0.15	0.03–0.66	.012	0.15	0.03–0.66	.012
CD34+ cell dose[§]	1.09	0.99–1.20	.096			
OS						
Time diagnosis/transplant > 6 months	1.64	1.03–2.61	.037			
Status at transplant: Relapse	1.8	1.13–2.9	.013	1.66	1.03–2.66	.036
Blood blasts at transplant[§]	1.01	1.01–1.02	<.001	1.01	1.01–1.02	<.001
Bone marrow blasts at transplant[§]	1.01	1.01–1.02	.047			
White blood cells count at transplant[§]	1.16	0.97–1.39	.09			
CD34+ cell dose[§]	0.91	0.85–0.99	.02			
DFS						
Time diagnosis/transplant > 6 months	1.51	0.96–2.38	.076	1.65	1.04–2.61	.035
Status at transplant: Relapse	1.78	1.13–2.81	.013			
Blood blasts at transplant[§]	1.01	1.01–1.02	<.001	1.01	1.01–1.02	.001
White blood cells count at transplant[§]	1.03	1.01–1.04	.003			
CD34+ cell dose[§]	0.92	0.85–0.99	.026	0.93	0.86–1	.049
CIR						
Number of pre transplant chemotherapy lines[§]	1.64	1.16–2.32	.005	1.56	1.08–2.23	.017
Status at transplant: Relapse	1.89	1.13–3.17	.016	2.03	1.2–3.46	.009
Blood blasts at transplant[§]	1.01	1.01–1.02	.005	1.01	1.01–1.02	.004
Bone marrow blasts at transplant[§]	1.01	1.01–1.02	.01			

aGVHD, acute graft versus host disease; cGVHD, chronic graft versus host disease; CMV, cytomegalovirus; CI, confidence interval; CIR, cumulative incidence of relapse; DFS, disease free survival; HR, hazard ratio; OS, overall survival.

[a]The univariate analysis had included the following variables: ELN classification, number of pretransplant chemotherapy lines §, age at transplant §, time between diagnosis and transplant (shorter or longer than 6 months), status at transplant (refractory or relapse), percentage of blood blasts at transplant §, percentage of bone marrow blasts at transplant §, white blood cell count at transplant §, conditioning regimen type at transplant, hematopoietic stem cell source, donor type, donor gender, ATG, CMV status and CD34+ cell dose §.

[§]Variables analyzed as continuous variables.

status at transplant. There was no difference between the two conditioning regimen neither for patients transplanted in absence of blood blasts at transplant (HR for the SEQ group: 0.95, 95%IC (0.40–2.24); P = .91), nor for patients transplanted with ≥1% of blood blasts (HR for the SEQ group: 1.42, 95%IC (0.80–2.55); P = .22). Likewise, there was no advantage of one or the other conditioning regimen for refractory patients (HR for the SEQ group: 1.34, 95%CI (0.66–2.72); P = .41) or for relapsed patients (HR for the SEQ group: 1.11, 95%CI (0.59–2.1); P = .74).

4 | DISCUSSION

As promising results have been reported with sequential transplants in refractory or relapsed AML, this approach is usually preferred to MAC

transplants traditionally associated with high NRM in this population. However, the optimal choice of conditioning regimen is still an open question. The improvement of supportive care in HSCT over the last decades, translating into reduced NRM in MAC transplants, leads to reassess the place of MAC versus sequential approach in refractory or relapsing AML patients. The main objective of this study was to compare the outcome of relapsed/refractory AML patients transplanted after a conventional MAC regimen or a sequential approach. As MAC transplant is not usually proposed to patients over 50 years, we have limited the inclusions to younger patients who were transplanted since 2006, which corresponds to the onset of the sequential transplant approach in France. To our knowledge, this is the first study designed to compare these two approaches of transplant. In a recent report evaluating long-term outcomes of patients transplanted for refractory AML, no difference in survival was observed after a FLAMSA-RIC, and a MAC regimen.[32]
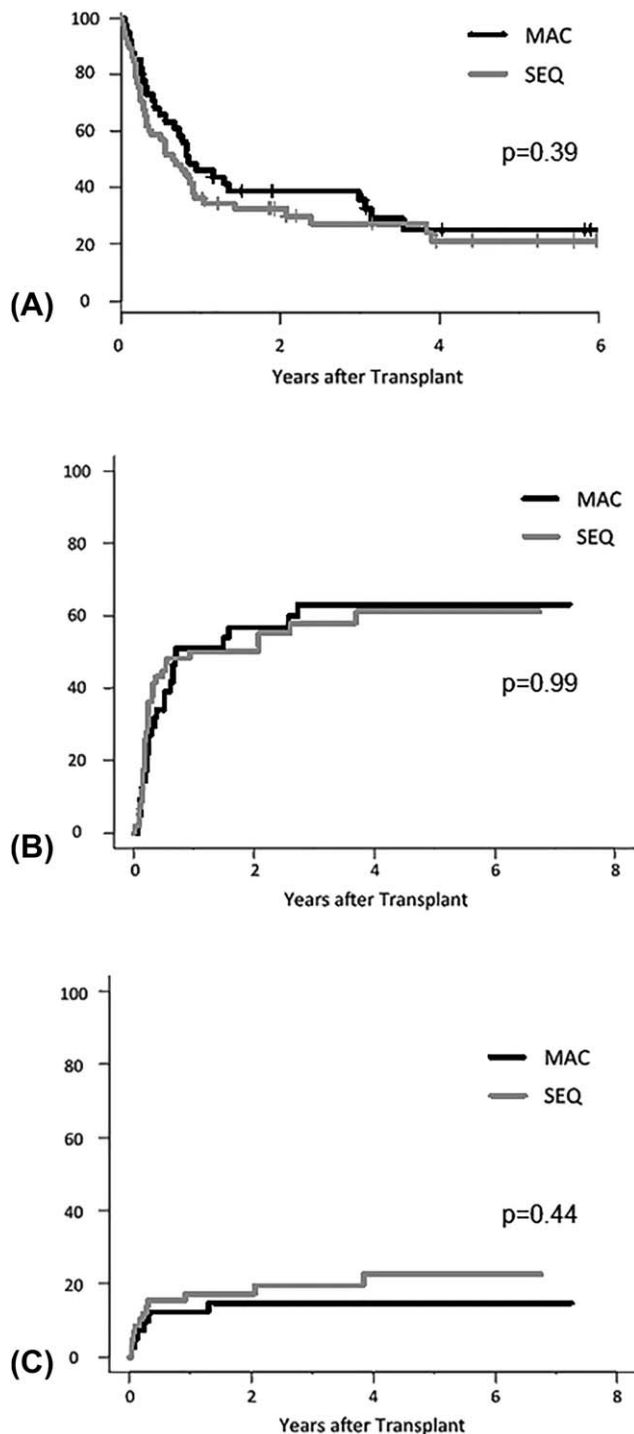
FIGURE 1 Post transplant outcomes according to the treatment group Black curve: myeloablative transplant, gray curve: sequential approach. (A) Overall Survival (B) Cumulative incidence of relapse (C) Non relapse mortality

Our results showing a 2-year OS of about 40% compared favorably with previous series of MAC and sequential HSCT in relapsing or refractory AML.[9,10,12,15,19–21,23,32–35] Patients from two groups did not differ in regards to their major characteristics, especially the distribution between refractory and relapsed leukemia. Types of donor and stem cell sources were not significantly different between the 2 groups, but most patients in the SEQ group received ATG in their conditioning regimen, unlike patients of the MAC group (84% versus 10%, $P < .001$). We observed very close OS, DFS, relapse and NRM after a SEQ or a MAC transplant, and we failed to identify a subset of patients benefiting from one of these approaches rather than the other. The 2-year NRM of 17% we observed in the MAC group, was lower than reported in the historical series,[12,13,17] reflecting the progress in supportive care during the last decades.[25,27] We confirm here that refractory status is associated with a better OS than relapse status, as previously reported in the setting of sequential transplant.[19] Furthermore, Holtick et al recently showed that PIF and high-risk AML transplanted in first CR display identical OS and DFS after FLAMSA regimen, suggesting that patients in PIF seem to really benefit from early stem cell transplantation.[22] The main difference between the 2 approaches was a lower incidence of grade II-IV aGVHD observed in the SEQ group. This difference remained significant in multivariate analysis including ATG, but did not translate into lower cGVHD. Interestingly, RIC regimen seems to be less toxic to gonads. In our experience, two spontaneous pregnancies were observed a few years after SEQ transplant in two young females (N. Dhedin et al., unpublished data).

One limit of this retrospective study is to ignore the reason why physicians choose a SEQ approach or a MAC conditioning regimen. Moreover, each group of treatment featured different types of conditioning regimen: TBI or busulfan based regimen in the MAC group and clofarabine plus aracytine versus FLAMSA regimens in the SEQ group. Finally, the main difference was a more frequent use of ATG in the SEQ group, which had a major impact on chronic GVHD occurrence, as previously reported.[36–38]

In high-risk AML, relapse after transplant remains the major cause of death, leading to the recommendation of prophylactic DLI in the sequential approach. In several series of SEQ transplants, the addition of prophylactic DLI was associated with prolonged OS achieving 67% at 7 years in a large report from the German group.[19,39–41] In our retrospective study, only 8.6% of patients transplanted after a SEQ approach actually received prophylactic DLI. That is lower than previously reported in the experience of the German group.[18–20] However, in most studies of SEQ transplant, prophylactic DLI did not exceed 25%,[18–20,23,34] due to early transplant mortality, early relapse or GVHD occurrence, actually showing the poor feasibility of this strategy.

Another way of reducing post-transplant relapse in SEQ approach could be to reinforce the pretransplant cytoreduction by using news drugs. A second generation of purine analog, clofarabine, which also shown to be relevant in first line or in relapsed of AML,[42–44] was thus evaluated in the setting of sequential transplant.[21,35] In our series there is no superiority of a clofarabine/cytarabine-RIC compared with a more classic FLAMSA-RIC regimen. These data are consistent with the results of the prospective SETRIC trial reporting a 2-year OS of 38% after a clofarabine/cytarabine-RIC in refractory AML patients.[21] Finally, new strategies of maintenance therapies are being developed, alone or in combination with prophylactic DLI to avoid post-transplant relapse. The hypomethylating agent azacitidine or the tyrosine kinase inhibitor sorafenib for FLT3-ITD patients have shown tolerability and feasibility as maintenance therapies after transplant and seem to improve OS and

DFS rates.[45,46] Such therapies, including also new tyrosine kinase inhibitors that can target a broader spectrum of patients, have to be evaluated in these patients, especially in those at a very high risk of relapse, such as patients with persisting blood blasts.

In conclusion, our study shows that, for young patients with refractory or relapsed AML, OS, DFS, NRM and CIR appear to be similar after sequential and myeloablative transplants. Lower acute GVHD incidence in the sequential group did not lead to lower cGVHD. Post-transplant relapse remains a major issue and maintenance therapy after transplant is a promising possibility, which has to be prospectively evaluated.

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