



Full Length Article

Allogeneic – Adult

Late relapse after hematopoietic stem cell transplantation for acute leukemia: a retrospective study by SFGM-TC



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Late relapse (LR) after allogeneic hematopoietic stem cell transplantation (AHST) for acute leukemia is a rare event (nearly 4.5%) and raises the questions of prognosis and outcome after salvage therapy. We performed a retrospective multicentric study between January 1, 2010, and December 31, 2016, using data from the French national retrospective register ProMiSe provided by the SFGM-TC (French Society for Bone Marrow

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Transplantation and Cellular Therapy). We included patients presenting with LR, defined as a relapse occurring at least 2 years after AHSCT. We used the Cox model to identify prognosis factors associated with LR. During the study period, a total of 7582 AHSCTs were performed in 29 centers, and 33.8% of patients relapsed. Among them, 319 (12.4%) were considered to have LR, representing an incidence of 4.2% for the entire cohort. The full dataset was available for 290 patients, including 250 (86.2%) with acute myeloid leukemia and 40 (13.8%) with acute lymphoid leukemia. The median interval from AHSCT to LR was 38.2 months (interquartile range [IQR], 29.2 to 49.7 months), and 27.2% of the patients had extramedullary involvement at LR (17.2% exclusively and 10% associated with medullary involvement). One-third of the patients had persistent full donor chimerism at LR. Median overall survival (OS) after LR was 19.9 months (IQR, 5.6 to 46.4 months). The most common salvage therapy was induction regimen (55.5%), with complete remission (CR) obtained in 50.7% of cases. Ninety-four patients (38.5%) underwent a second AHSCT, with a median OS of 20.4 months (IQR, 7.1 to 49.1 months). Nonrelapse mortality after second AHSCT was 18.2%. The Cox model identified the following factors as associated with delay of LR: disease status not in first CR at first HSCT (odds ratio [OR], 1.31; 95% confidence interval [CI], 1.04 to 1.64; $P = .02$) and the use of post-transplantation cyclophosphamide (OR, 2.23; 95% CI, 1.21 to 4.14; $P = .01$). Chronic GVHD appeared to be a protective factor (OR, .64; 95% CI, .42 to .96; $P = .04$). The prognosis of LR is better than in early relapse, with a median OS after LR of 19.9 months. Salvage therapy associated with a second AHSCT improves outcome and is feasible, without creating excess toxicity.

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INTRODUCTION

Relapse of the original disease after allogeneic hematopoietic stem cell transplantation (AHSCT) remains the main cause of graft failure. It is the leading cause of death after AHSCT, with little improvement in recent decades. Post-AHSCT acute leukemia relapse occurs in 20% to 50% of cases in the first 2 years. These post-transplantation relapses occur early, at a median of 6 months post-AHSCT. The prognosis is poor, with a 20% survival rate at 2 years [1,2].

Reported risk factors for post-transplantation acute leukemia relapse include poor cytogenetics (complex or monosomal karyotype), molecular features (TP53 status), and nonachievement of first complete remission (CR) or detectable minimal residual disease (MRD) at AHSCT. Factors associated with a better prognosis after a post-AHSCT relapse of acute myeloid leukemia (AML) included low proliferative features, cytogenetics and molecular features, and donor availability for donor lymphocyte infusion (DLI) or subsequent second AHSCT [3–5]. The absence of acute graft-versus-host disease (aGVHD) is associated with a better outcome [3]. Regarding acute lymphoblastic leukemia (ALL), factors associated with longer survival after relapse include undergoing AHSCT while in first CR and lower bone marrow and blood blast content (<60% and <10%, respectively) [6]. However, the main prognostic factor identified in previous studies is delayed relapse, with improved outcomes associated with relapse occurring more than 2 years after AHSCT. In a large observational study, the cumulative incidence of relapse after 2 years was 10% for patients with AML and 9% for those with ALL [7]. This study reports that late relapse (LR) remains the main cause of death after 2 years post-AHSCT, accounting for 47% of deaths from AML and 55% of deaths from ALL. Risk factors for LR are later stage of disease before transplantation, absence of aGVHD, lower performance score at the time of transplantation (Hematopoietic Cell Transplantation Comorbidity Index), and T cell depletion (in vivo or ex vivo) for AML.

Regarding ALL, later stage of disease before transplantation (not in first CR), older age, absence of aGVHD, and the use of busulfan plus cyclophosphamide as a conditioning regimen are associated with an increased risk of LR. Conditioning without irradiation in ALL is another risk factor. A retrospective study by the Center for International Blood and Marrow Transplant Research reported a cumulative risk of relapse at 5 years post-AHSCT of 11% among patients in CR after 2 years. The disease status of AML and

ALL at AHSCT also has been reported as a risk factor for LR. Particularly in ALL, age >40 years and conditioning without irradiation are associated with an increased rate of LR [8].

Little is known about the features and outcomes of LR occurring >2 years post-AHSCT. The present study, conducted with the French Society for Bone Marrow Transplantation and Cellular Therapy (SFGM-TC), aimed to describe the survival of patients after acute leukemia LR post-AHSCT, their clinical and biological features, and their outcomes after salvage therapy and to identify some of the risk factors for LR.

METHODS

Patients and Data Collection

Study design

The study was conducted between January 2010 and December 2016 in 29 centers belonging to the SFGM-TC. Data were extracted from the French national retrospective register, ProMISe. All patients who underwent AHSCT provided signed informed consent authorizing the collection and use for research purposes of their laboratory and clinical data regarding AHSCT. The French National Ethics Board from the SFGM-TC approved this study.

We retrospectively included adult patients in each center with post-AHSCT acute leukemia LR, which was defined as cytologic relapse occurring >2 years after AHSCT. Very late relapse (VLR) was defined as relapse occurring >5 years after AHSCT. The diagnosis of hematologic malignancy was determined according to the 2016 World Health Organization classification.

Data collection

Data on 319 patients from 29 SFGM-TC transplant centers included details of patients and disease characteristics at diagnosis and LR. Disease status assessment was performed by bone marrow aspiration. Cytologic relapse was defined as $\geq 5\%$ blasts in bone marrow or granulocytic sarcoma. Cytology of relapse and cytogenetics were compared with the initial diagnosis to identify donor-derived leukemia. High-risk cytogenetics was defined according to the European LeukemiaNet 2017 risk stratification [9].

Molecular MRD assessment was done by real-time quantitative polymerase chain reaction (qPCR) when the target was available, in bone marrow for core binding factor AML (RUNX1-RUNX1T1 or CBFB-MYH11), acute promyelocytic

leukemia (PML-RARA), NPM1 mutants, and BCR-ABL and in peripheral blood for WT1 [10]. Regarding ALL, MRD was assessed by detection of Ig/T cell receptor rearrangements and fusion transcripts (BCR-ABL) by qPCR [11].

A CR to salvage therapy was defined as the presence of <5% blasts in the bone marrow with no extramedullary disease, associated with peripheral hematologic recovery (defined as a platelet count $>100 \times 10^9/L$ and/or an absolute neutrophil count $>1 \times 10^9/L$). CRi was established as CR with incomplete hematologic recovery. MRD response was defined as CR with undetectable disease, irrespective of the surrogate marker [9].

Regarding AHSCT, donor stem cell sources included peripheral blood, bone marrow, and umbilical cord blood. Conditioning was classified conventionally as either myeloablative (MAC) or non-myeloablative (NMA). MAC included total body irradiation (TBI) at 8 Gy or a total busulfan dose of >8 mg/kg orally or >6.4 mg/kg i.v. All other regimens were considered NMA.

Chimerism was performed by qPCR, based on single nucleotide polymorphisms. Full donor chimerism was defined as $>95\%$ donor-derived cells in all lineages, and mixed chimerism was defined as $>5\%$ but $<95\%$ donor-derived cells. GVHD was assessed using the modified Glucksberg criteria for aGVHD [12] and the 2014 revised National Institutes of Health Consensus Conference criteria for chronic GVHD (cGVHD) [13].

Therapeutic strategies included palliative treatment (low-dose cytarabine, hypomethylating agents, supportive care) and salvage therapy (intensive chemotherapy, DLI combined with intensive chemotherapy or hypomethylating agents, monoclonal antibodies associated with tyrosine kinase inhibitors [TKIs]) with or without subsequent second AHSCT.

Statistical Analysis

The study's primary endpoint was overall survival (OS), defined as the time from the onset of LR to death or last follow-up. Patients alive at last follow-up were censored. Cumulative incidence was used to estimate the endpoints of relapse/progression, with death as the competing event. Relapse-free survival (RFS) was defined as survival after LR with no evidence of cytologic relapse or progression. Nonrelapse mortality (NRM), based on competing events, referred to death from any cause without previous leukemia relapse/progression.

Qualitative variables are presented as number and percentage, and quantitative variables as median and interquartile range (IQR). A P value $<.05$ was considered to indicate significance. All analyses were performed using R version 4.1.2. A Cox proportional hazards model was used for multivariate regression. Results are expressed as odds ratio (OR) with 95% confidence interval (CI). All tests were 2-sided. The type 1 error rate was fixed at .05 for the determination of factors associated with time-to-event outcomes.

For our multivariate analysis model, we incorporated factors associated with LR RFS. We included variables known to be associated with post-AHSCT relapse: high-risk cytogenetic or molecular categories, disease status at first HSCT, NMA, and prior cGVHD [14,15]. We aimed to analyze the impacts of HLA matching, use of post-transplantation cyclophosphamide, and cytomegalovirus (CMV) disease on LR [16,17].

RESULTS

Patient Characteristics at First AHSCT

During the study period, 7,582 AHSCTs were performed, and 2565 relapses were reported (33.8%). Among them, 319 (12.4%) were considered LR (Figure 1). The incidence of LR was 4.2% in the entire cohort. Full data analysis was available for

290 patients (Figure 1), including 250 (86.2%) with AML and 40 (13.8%) with ALL. Data on characteristics of acute leukemia at diagnosis and AHSCT are provided in Tables 1 and 2. The median age at diagnosis was 49 years (IQR, 37.1 to 59.7 years). Cytogenetics was normal for 41.9% of patients and adverse (complex or monosomal) for 23.5%.

At diagnosis, extramedullary disease and central nervous system involvement were present in 12.2% and 4.7% of patients, respectively. At AHSCT, most patients (60.9%) were in first CR, 12.1% had refractory/evolutionary disease, and 52.6% were MRD-positive. Peripheral blood was the most common source of hematopoietic stem cells (75.8%), and an HLA-matched sibling donor was chosen in 47.7% of cases. An MAC regimen was provided in 42.6%.

Data on post-AHSCT follow-up and complications are presented in Supplementary Table S1. Most patients had complete donor chimerism at day 30 and day 100 post-AHSCT (79.5% and 77.3%, respectively). aGVHD occurred in 122 patients (42.1%), including 13 (10.9%) with grade III-IV, and 126 patients (43.4%) experienced cGVHD.

Characteristics of LR in Acute Leukemia

The incidence of LR in the whole cohort was 4.2%, with a median delay from AHSCT of 38.2 months (IQR, 29.2 to 49.7 months). The maximum delay of relapse was 113.8 months. Clinical and biological details of LR are presented in Table 3. Clinically, 17.2% of patients experienced exclusively extramedullary symptoms, and 10% had both medullary and extramedullary relapses.

Most patients lost complete chimerism at LR, with mixed chimerism in 37.4% and recipient chimerism in 25.2%. Persistence of full donor chimerism in the whole blood was observed in 37.4% of LRs and in 60% of patients with exclusively extramedullary LR. For these patients, morphologic and cytogenetic features were comparable to the primary diagnosis of the pretransplantation leukemia, refuting the hypothesis of donor cell leukemia. A comparison of cytogenetics between initial diagnosis and at LR was available for 61 patients, the majority of whom (72.6%) had clonal evolution (Figure 2).

Regarding data on molecular evolution, available for 132 patients, 66% lost an *FLT3* internal tandem duplication (ITD) at LR (31/47). Four patients acquired a *TP53* mutation, and 1 patient lost a *TP53* mutation found in the initial leukemic clone. Of the 16 patients with an *NPM1* mutation, 14 (87.5%) kept the same *NPM1* mutation at LR as at diagnosis.

Outcome of Patients after LR

The Mean OS after LR was 19.9 months (IQR, 5.6 to 46.4 months), with a 2-year survival after LR of 44% (Figure 3). Relapse was the main cause of death (84.5%), with deaths related to therapy representing 10.4%. Nine patients died from aGVHD (3 after salvage therapy by DLI and 6 after a second AHSCT), and 4 (4.3%) died from a post-transplantation lymphoproliferative disorder. We did not observe any differences in survival between LR and VLR ($n = 33$), with OS at 2 years of 42.1% and 58.2%, respectively ($P = .077$) (Supplementary Figure S1).

Comparison of Underlying Disease: AML versus ALL

At baseline, ALL patients were younger than AML patients (median, 36.9 years [IQR, 23.6 to 46.9 years] versus 51.6 years [IQR, 39.8 to 60.8 years]; $P < .001$). At diagnosis, ALL patients presented more frequently with central nervous system involvement (12.5% versus 2.9%; $P = .03$) and tended to have more extramedullary disease (21.4% versus 10.2%; $P = .08$),

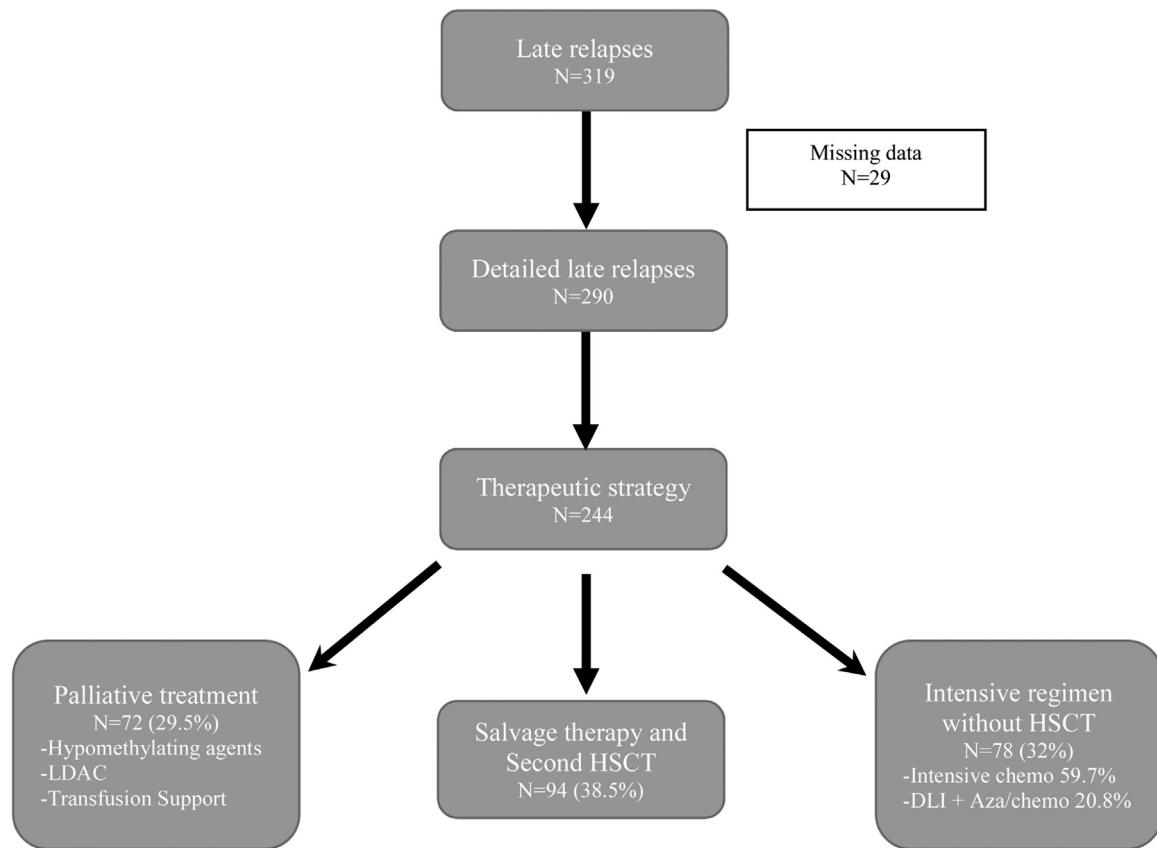


Figure 1. Flow chart of the study. LDAC, low-dose cytarabine; Aza, azacytidine; Chemo, chemotherapy.

whereas no difference emerged with AML patients at LR. MAC was more frequently used for ALL patients (73.5% versus 36.2%; $P < .001$).

Regarding outcomes after LR, mean OS was superior for ALL and was not reached at 40 months, with OS at 2 years after LR at 63.4% for ALL and 40.2% for AML ($P < .0001$) (Supplementary Figure S1).

Factors Associated with LR RFS

Through multivariate analysis, after adjustment on factors (HLA-matching, conditioning), we identified the following factors as associated with shorter LR RFS (Table 4): use of post-transplantation cyclophosphamide (OR, 2.23; 95% CI, 1.21 to 4.14; $P = .01$) and non-achievement of first CR at the time of AHSCT (OR,

Table 1
Acute Leukemia Characteristics

Characteristic	Total Cohort (N = 290)	ALL Patients (N = 50)	AML Patients (N = 240)	P Value
Age, yr, median (IQR)	49 (37.1–59.7)	36.9 (23.6–46.9)	51.6 (39.8–60.8)	<.001*
Sex, n (%)				.59
Male	158 (55.5)	25 (50.0)	133 (55.4)	
Female	132 (45.5)	25 (50.0)	107 (44.6)	
Diagnosis, n (%)				N/A
De novo or secondary AML	225 (77.6)	N/A	225 (93.8)	
Therapy-related AML	15 (5.2)	N/A	15 (6.2)	
Ph ⁺ ALL	11 (3.8)	11 (22.0)	N/A	
Ph ⁻ ALL	39 (13.4)	39 (78.0)	N/A	
Molecular status, n (%)				N/A
NPM1 mutated	25 (10.1)	N/A	25 (12.4)	
FLT3 ITD mutated	47 (19.0)	1 (2.1)	46 (22.9)	
FLT3 TKD mutated	1 (.4)	N/A	1 (.5)	
TP53 mutated	3 (1.2)	0 (0)	3 (1.5)	
N/A	42 (14.5)	3 (6.0)	39 (16.3)	
Extramedullary involvement, n (%)	28 (12.2)	9 (21.4)	19 (10.2)	.08
CNS involvement, n (%)	10 (4.7)	5 (12.5)	5 (2.9)	.03*

N/A indicates not available; CNS, central nervous system.

* $P < .05$.

Table 2
AHSCT Characteristics

Characteristic	Total Cohort (N = 290)	ALL Patients (N = 50)	AML Patients (N = 240)	P Value
Previous HSCT, n (%)	29 (10.0)	4 (8.0)	25 (10.4)	.8
Autologous	8 (27.6)	1 (25.0)	7 (28.0)	1.0
Allogeneic	21 (72.4)	3 (75.0)	18 (72.0)	1.0
Disease status at HSCT, n (%):				.29
CR 1	177 (61.7)	31 (62.0)	146 (61.6)	
CR 2	71 (24.7)	14 (28.0)	57 (24.1)	
CR ≥3	5 (1.7)	2 (4.0)	3 (1.3)	
Refractory	34 (11.8)	3 (6.0)	31 (13.1)	
N/A	3 (1)	0 (0)	3 (1.3)	
Undetectable MRD, n (%); N = 137	65 (47.4)	18/39 (46.2)	47/98 (48.0)	1.00
HLA compatibility, n (%)				.34
Identical siblings	140 (49.5)	19 (38.0)	121 (51.7)	
10/10 unrelated donor	81 (28.6)	14 (28.0)	67 (28.6)	
9/10 unrelated donor	47 (16.6)	16 (32.0)	31 (13.2)	
Haploidentical	15 (5.3)	0 (0)	15 (6.4)	
N/A	7 (2.4)	1 (.02)	6 (2.5)	
Stem cell source, n (%)				.001*
Bone marrow	52 (18.0)	19 (38.0)	33 (13.8)	
Peripheral blood	219 (75.8)	28 (56.0)	191 (79.9)	
Cord blood (1 unit)	8 (2.8)	2 (4.0)	6 (2.5)	
Cord blood (2 units)	10 (3.5)	1 (2.0)	9 (3.8)	
N/A	1 (.3)	0 (0)	1 (.04)	
Conditioning, n (%)				
MAC	123 (42.4)	36 (73.5)	87 (36.2)	<.001*
NMA	167 (57.6)	14 (5.8)	153 (63.8)	<.001*
Sequential	25 (8.6)	1 (2.0)	24 (10.0)	.12
TBI	37 (12.8)	28 (56.0)	9 (3.8)	<.001*
GVHD prophylaxis, n (%)				
Post-HSCT cyclophosphamide	30 (10.3)	7 (14.0)	23 (9.6)	.50
Thymoglobulin	117 (40.3)	14 (28.0)	103 (42.9)	.07
Donor age, yr, median (IQR)	37.6 (30.0–52.3)	37.5 (27.0–50.7)	37.2 (27.0–57.3)	1.00

* $P < .05$.

1.20; 95% CI, .74 to 1.94; $P = .02$). cGVHD appeared to be a protective factor with latter LR (OR, .64; 95% CI, .42 to .96; $P = .04$).

Impact of Relapse Prophylaxis Treatment

Maintenance treatment with the aim of preventing relapse after the first AHSCT was performed for 20% of patients. Reasons for this treatment were patients with high-risk cytogenetics or molecular profile (30.2%), with positive MRD before AHSCT (32.6%), therapeutic targets, such as FLT3 ITD, TKD, or IDH1-2 (8.9%), or Bcr-Abl (22.2%). We did not observe any impact on the incidence of LR.

Treatment of LR

Details of the therapeutic strategy were available for 244 patients and are summarized in Table 3.

Response to Chemotherapy

Responses were evaluated in 130 of the 141 patients receiving intensive chemotherapy alone ($n = 127$) or DLI associated with another treatment ($n = 14$). A CR was obtained by 66 patients (50.7%), with an overall response rate (CR + CRi) of 61.5%, and 41 patients (31.5%) were refractory to first-line salvage therapy. Ten patients subsequently responded after a second line of treatment, and 2 more responded to a third line.

We did not find any differences in response by clinical form of relapse or by underlying diagnosis (data not shown).

Second AHSCT

A second AHSCT was performed for 94 patients (38.5%), and details were available for 93 of them (Table 5). CR at AHSCT was obtained for 75.9%. The median age at second AHSCT was 47.5 years (IQR, 33.9 to 56.8 years). In 14% of cases, the same donor was used in the first and second AHSCTs. MAC was provided in 16.1%, and peripheral blood stem cells were the main source of hematopoietic stem cells. aGVHD occurred in 40 patients (43%), including 4 with grade III-IV (10%), and 19 patients (20.4%) developed cGVHD. Post-AHSCT, Epstein-Barr virus and CMV reactivations were observed in 15 patients (22.7%) and 17 patients (25.8%), respectively (Table 6).

Nineteen patients (27.7%) received post-transplantation maintenance. We did not observe any difference in RFS and OS in these patients compared with patients without maintenance.

The mean time of RFS from second AHSCT was 17 months (IQR, 5.9 to 46.3 months), and the median OS after a second AHSCT was 20.4 months (IQR, 7.1 to 49.1 months) (Figure 4A,B). OS at 3 years was 37.2%, and NRM at 2 years was 18.2%.

Table 3
Clinical and Biological Characteristics at LR and Salvage Therapy

Characteristic	Total Cohort (N = 290)	ALL Patients (N = 50)	AML Patients (N = 240)	P Value
Chimerism at LR, n (%)				.41
Donor	58 (37.4)	14 (48.3)	44 (34.9)	
Mixed	58 (37.4)	9 (31.0)	49 (38.9)	
Recipient	39 (25.2)	6 (20.7)	33 (26.2)	
N/A	135 (46.6)	21 (42.0)	114 (47.5)	
Site of LR, n (%)				.50
Bone marrow	182 (72.8)	29 (65.9)	153 (74.3)	
Extramedullary	43 (17.2)	9 (20.5)	34 (16.5)	
Bone marrow and extramedullary	25 (10.0)	6 (13.6)	19 (9.2)	
N/A	40 (13.8)	6 (13.6)	34 (16.5)	
Salvage therapy, n (%)				<.001*
Palliative treatment	64 (26.3)	5 (10.6)	59 (30.1)	
Intensive chemotherapy without HSCT	86 (35.4)	31 (66.0)	55 (28.1)	
Intensive chemotherapy with HSCT	93 (38.3)	11 (23.4)	82 (41.8)	
N/A	47 (16.2)	3 (6.0)	44 (22.4)	
Salvage therapy, n (%)				<.001*
Induction	127 (55.5)	30 (65.2)	97 (53.3)	
Hypomethylating agents	62 (27.1)	1 (2.2)	61 (33.5)	
Monoclonal antibodies	7 (3.1)	6 (13.0)	1 (.5)	
TKIs	6 (2.6)	6 (13.0)	0 (0)	
Target therapies	12 (4.1)	2 (4.3)	10 (5.5)	
DLI associated with other therapy	14 (6.1)	1 (2.2)	13 (7.1)	
N/A	62 (21.4)	4 (8.0)	58 (24.2)	
Response to intensive salvage therapy, n (%)				.20
CR	75 (36.6)	9 (23.1)	66 (39.8)	
CRi	17 (8.3)	5 (12.8)	12 (7.2)	
MRD-positive	23 (11.2)	6 (15.4)	17 (10.2)	
Refractory	90 (43.9)	19 (48.7)	71 (42.8)	
N/A	85 (29.3)	11 (22.0)	74 (30.8)	

* $P < .05$.

Choice of Salvage Therapy

We then compared the different treatment strategies after LR. RFS and OS from LR were significantly higher in the subgroup undergoing a second AHSCT after salvage therapy, with a mean RFS of 24.1 months, compared with 6.8 months (IQR, 1.2 to 24 months) after intensive chemotherapy alone and 5.7 months (IQR, .7 to 24.9 months) in the case of palliative treatment (Figure 4C,D). The response to first-line salvage therapy significantly affected survival (Figure 4E).

Finally, we compared RFS between patients receiving immunomodulation by DLI versus those undergoing AHSCT. We observed an advantage for AHSCT, with a median RFS after first-line salvage therapy of 22.6 months (IQR, 14.2 to 58 months) versus 9.6 months (IQR, 13.1 to 22.2 months; $P = .0095$) (Figure 4F).

Prognostic Factors

Survival after LR was associated mainly with the response to salvage therapy and number of lines of treatment (Table 7). Patients who were not in first CR before a second AHSCT and who presented with aGVHD had a worse prognosis. RFS was better when patients presented with cGVHD and benefited from relapse prophylaxis treatment (Table 7).

DISCUSSION

The present study is the first cohort study describing acute leukemia LR after AHSCT. We report 319 acute leukemia LRs,

with an incidence of 4.2%, representing 12.4% of all relapses. Only a few publications have reported any cases of acute leukemia LR not in the context of AHSCT. Studies on relapse after AHSCT concerned only early relapse, occurring within a median of 6 months after AHSCT. Reported survival was poor at 2 years, ranging from 10% to 20% [1,18,19]. The main prognostic factor is the delay from AHSCT to relapse. Here we report a 2-year OS of 44% after LR post-AHSCT. Two main studies reporting long-term follow-up after AHSCT for acute leukemia advised that LR remains a major concern as the leading cause of death [7,8].

Currently there are no clear definitions of LR and VLR. Several studies have defined LR as a relapse occurring more than 2 to 5 years after AHSCT and VLR as occurred more than 5 to 10 years post-AHSCT. We report a frequency of LRs of 4.2%, in accordance with those previous studies [4,5,20–27].

We also report a high frequency of extramedullary relapse (17.2% exclusively; 10% both medullary and extramedullary). Previous studies have reported a frequency of extramedullary relapse ranging from 8% to 41% [28–30]. In a retrospective cohort, extramedullary relapse occurred later than non-extramedullary relapse (13.5 months versus 6.1 months) [19]. Unlike in other studies [28,31], we did not observe a better prognosis associated with isolated extramedullary relapse. Reported risk factors for extramedullary relapse include ALL, hyperleukocytosis at diagnosis, poor cytogenetics, conditioning without TBI, and receipt of cyclosporine [29,32].

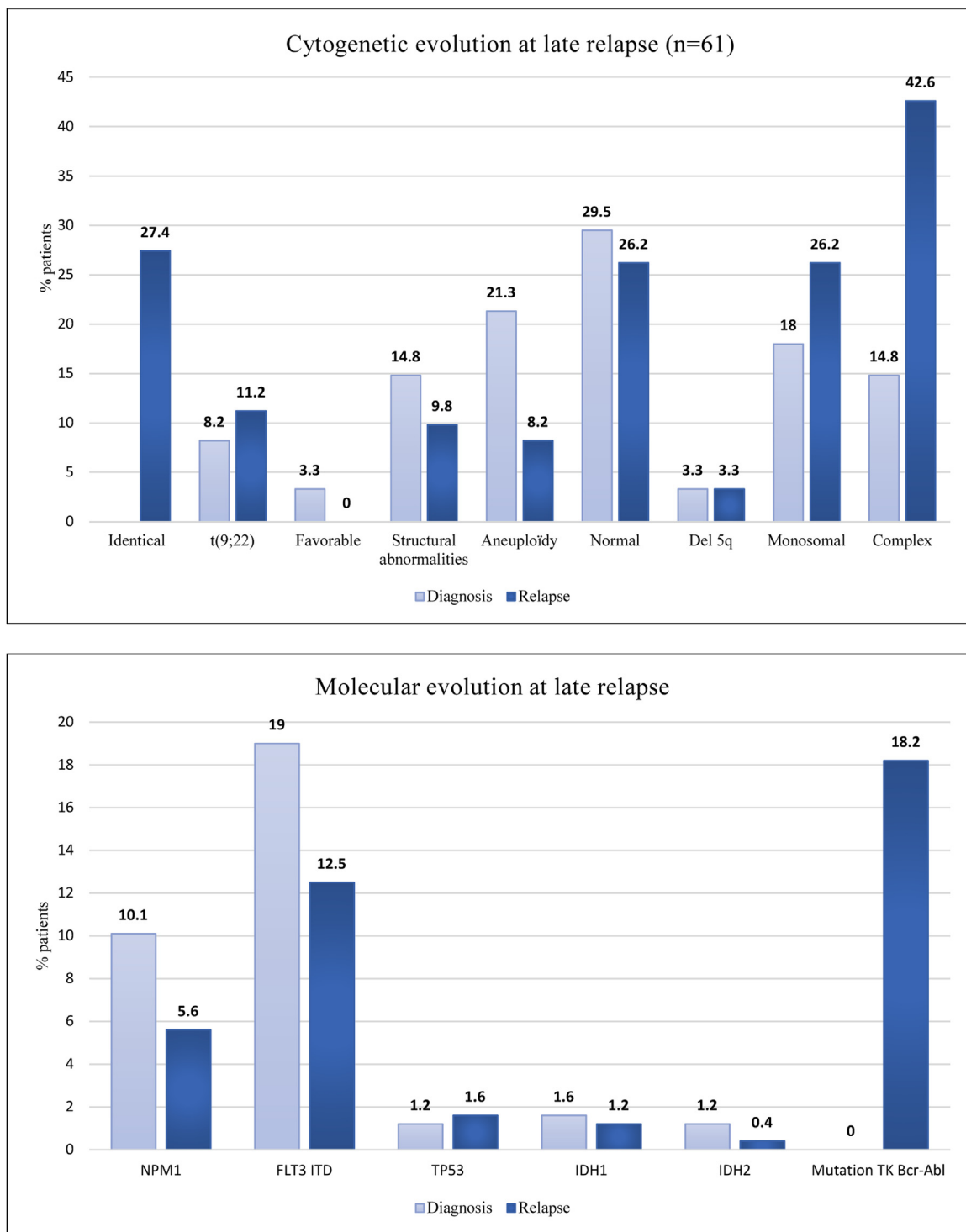


Figure 2. Cytogenetics and molecular characteristics at LR. Del, deletion; TK, tyrosine kinase.

A biological explanation of these extramedullary relapses after AHSCT is the compartmentalization of the graft-versus-leukemia (GVL) effect, with a lesser GVL effect exerted by cytotoxic T cells outside the bone marrow. In line with this hypothesis, we found that one-third of patients had persistent full donor chimerism at LR and 60% had extramedullary disease. Expression by the microenvironment of inhibitors involving the programmed cell death 1/programmed death ligand 1 axis has been described, which

induces T cell anergy, notably in lymph nodes [33]. This may explain the higher frequency of extramedullary relapse after AHSCT than after chemotherapy alone (25% versus 9%).

This raises the question of whether LR acute leukemia derives from the original clones, as suggested in the study by Aldoss et al. [21], or from dormant subclones [22]. Different molecular mechanisms distinguish early relapse and LR; early relapse may underline subclones' chemoresistance with

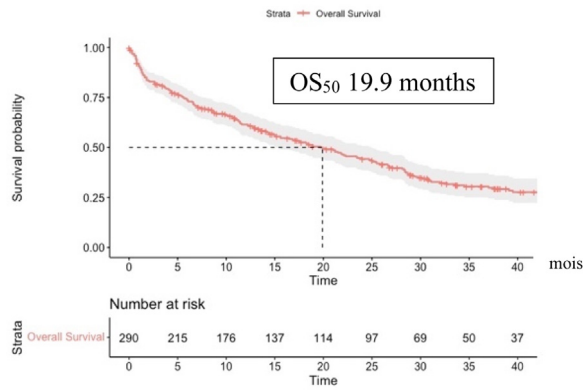


Figure 3. OS after LR.

mutations drive-resistance, whereas LR may be associated with reexpansion of dormancy of leukemic stem cells [34].

We observed 72.6% of clonal evolution at LR. This evolution is well described, ranging from 38.8% to 63% [21–23]. The most frequent abnormality is a complex karyotype, followed by monosomal, aneuploidy, and new clones [26,27]. Regarding molecular evolution, we did not observe a high frequency of *TP53* mutations, which are more closely associated with early relapse and confer a poor prognosis.

This argues for the fact that clonal evolution and genomic instability leading to LR are not driven by *TP53*, as previously shown [35]. Interestingly, we observe a high frequency of loss of *FLT3* ITD in 66.3% of cases. This result is similar to data from the RATIFY study, in which 44% of patients lost *FLT3* ITD under treatment with midostaurin [36]. This model of initiatory and progression mutation of *FLT3* is well described [37,38].

We observed a low frequency of *NPM1* mutation (10.1%), which correlated with the good prognosis of this mutation. Mutations associated with epigenetics and spliceosomes, such as *IDH1* and *IDH2*, seem to be stable [22,35]. Regarding clonal evolution in ALL, we observed the acquisition in the TKD of Bcr-Abl in 16.7% of cases, a well-known mechanism of relapse.

We found that some factors were associated with a delay of LR. In our cohort, the use of cyclophosphamide post-AHSCT to prevent GVHD was associated with earlier relapse, independent of HLA matching (OR, 1.05; 95% CI, .82 to 1.34; $P = .7$). Most studies on post-transplantation cyclophosphamide have a short follow-up (<2 years), not allowing for the collection of long-term data. In a retrospective study, Nagler et al. [17] reported a higher incidence of relapse at 2 years in the context of matched sibling transplantation in patients receiving post-transplantation cyclophosphamide as GVHD prophylaxis (41.1%; 95% CI, 41.6% to 65.5%) compared with those receiving cyclosporine A and methotrexate (21.3%; 95% CI, 13.1% to

Table 4
Multivariate Analysis, LR DFS

Variable	Coefficient (95% CI)	P Value
MRD status at AHSCT	1.07 (.71–1.63)	.74
Disease status at AHSCT	1.31 (1.04–1.64)	.02*
High-risk cytogenetics	1.20 (.74–1.94)	.47
Post-transplantation cyclophosphamide	2.23 (1.21–4.14)	.01*
HLA-matching	1.05 (.82–1.34)	.70
MAC	.96 (.63–1.46)	.84
CMV reactivation	1.02 (.63–1.65)	.93
cGVHD	.64 (.42–.96)	.04*

* $P < .05$.

Table 5
AHSCT after LR (N = 93)

Characteristic	Value
Age, yr, median (IQR)	
Recipient	47.5 (33.9–56.8)
Donor	30.3 (25–39.6)
Disease status at HSCT, n (%)	
CR2	56 (64.4)
CR≥3	10 (11.5)
Refractory	21 (24.1)
N/A	6 (6.5)
Detectable MRD, n (%)	26/40 (65.0)
Conditioning, n (%)	
MAC	15 (16.1)
RIC	78 (83.9)
Sequential	15 (16.1)
GVHD prophylaxis, n (%)	
Post-transplantation cyclophosphamide	22 (23.7)
Antithymocyte globulin	64 (68.8)
Cyclosporine	78 (83.9)
Tacrolimus	8 (8.6)
Mycophenolate mofetil	51 (54.8)
Methotrexate	17 (18.3)
Identical donor, n (%)	13 (14.0)
HLA compatibility, n (%)	
Identical siblings	9 (10.1)
10/10 unrelated donor	48 (53.9)
9/10 unrelated donor	11 (12.4)
Haploidentical	21 (23.6)
N/A	4 (4.3)
Stem cell source, n (%)	
Bone marrow	9 (10.1)
Peripheral blood	77 (86.5)
Cord blood	3 (3.4)
N/A	4 (4.3)

RIC indicates reduced-intensity conditioning.

Table 6
Follow-Up of Second AHSCT after LR (N = 93)

Variable	Value
Chimerism on day 30, n (%)	
Complete donor	71 (91.0)
Mixed	6 (7.7)
Recipient	1 (1.3)
N/A	15 (16.1)
aGVHD, n (%) and maximal grade	40 (43.0)
Grade 1	18 (45.0)
Grade 2	16 (40.0)
Grade 3	3 (7.5)
Grade 4	1 (2.5)
N/A	2 (5.0)
cGVHD, n (%)	19 (20.4)
Relapse prophylaxis, n (%)	18 (19.4)
Type of prophylaxis, (N = 18), n (%)	
TKIs	2 (11.1)
Target therapy	6 (33.3)
Hypomethylating agents	4 (22.2)
DLI associated to other therapy	6 (33.3)
Viral reactivation, n (%)	
EBV	15 (22.7)
CMV	17 (25.8)
N/A	27 (29)

EBV indicates Epstein-Barr virus.

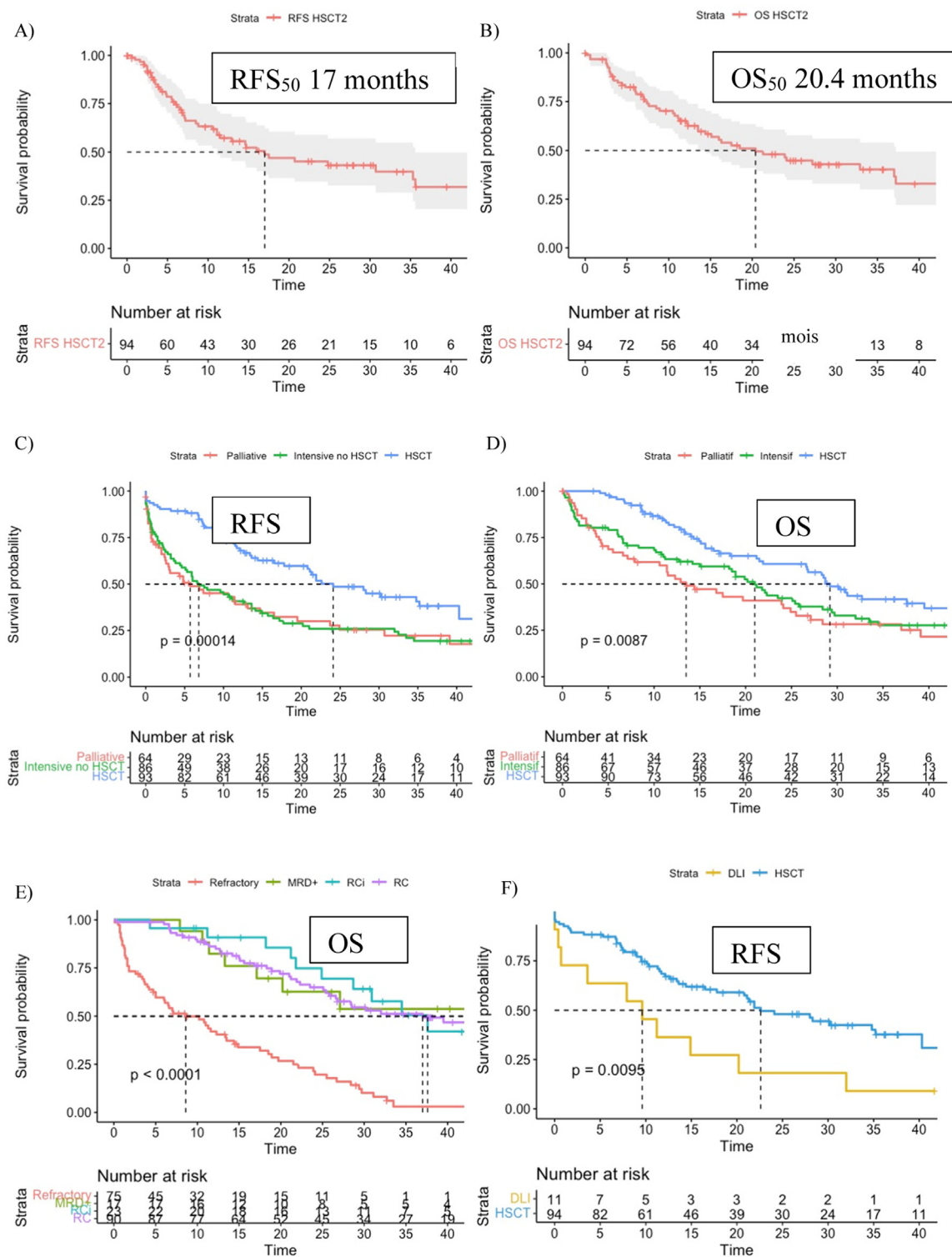


Figure 4. RFS and OS after LR with salvage therapy. (A and B) RFS and OS after LR post-second AHSCT. (C and D) RFS and OS with palliative treatment, intensive salvage therapy alone, and salvage therapy followed by second AHSCT. (E) OS by response to salvage therapy. (F) RFS in patients receiving DLI or second AHSCT.

30.8%). This study suggests a potential negative impact of post-transplantation cyclophosphamide on the GVL effect. After AHSCT with post-transplantation cyclophosphamide, expansion of regulatory T cells is associated with impaired proliferation of functionally natural killer cells [39,40]. Further studies are needed to explore its role in relapse, especially in LR.

The presence of cGVHD is well known to be associated with a lower incidence of relapse and was associated in our cohort with a longer delay to LR. cGVHD is indirectly correlated to the GVL effect, and 2 other studies have shown a lower risk of acute leukemia relapse when patients develop cGVHD [32,33]. We did not find any association with adverse cytogenetics, as

Table 7
Prognostic Factors after LR and Second AHST

Factor	Coefficient (95% CI)	P Value
Delay of late relapse	.99 (.97–1.01)	.32
Site of relapse	1.04 (.62–1.73)	.89
Response to salvage therapy	.75 (.56–.99)	.049*
Number of lines of salvage therapy	2.32 (1.30–4.16)	.005*
Disease status at second HSCT	1.57 (1.03–2.38)	.035*
Identical donor	1.06 (.33–3.39)	.92
Post-HSCT relapse prophylaxis treatment	.18 (.06–.56)	.003*
aGVHD	2.29 (1.01–5.19)	.047*
cGVHD	.32 (.12–.84)	.021*

* $P < .05$.

was previously reported in a retrospective study with a similar 3-year relapse risk of patients with intermediate-risk and high-risk cytogenetics (8% and 11%, respectively) [27]. Finally, we did not find any association in our Cox models between CMV reactivation and LR.

The overall response rate for all salvage therapies (CR + CRi + MRD-positive) was 56.1%, including 36.6% for CR. For patients receiving an intensive regimen, the CR rate rose to 50.7%. Some cases reporting LRs record a good response to chemotherapy. After obtaining CR, the question is whether to reintroduce the GVL effect through DLI or via a second AHST. Many studies on early relapse are retrospective; one prospective study showed an advantage of performing AHST or DLI only after achievement of CR [41,42].

The combination of an intensive regimen and DLI appears to be superior to the combination of DLI and azacitidine [36,37]. This was confirmed in our cohort (although with a small number of patients), with a 1-year OS of 66.7% ($n = 6$) for the combination of chemotherapy and DLI versus 36% for the combination of azacitidine and DLI ($n = 6$).

Some previous retrospective studies have compared DLI to AHST. We found an advantage in OS of performing AHST, with a median RFS of 22.6 months, compared with 9.6 months with DLI ($P = .0095$). These results are in agreement with another retrospective study in the context of early relapse that showed an advantage for AHST in OS at 1 year (12 months versus 7 months) [2].

We report an RFS and OS at 2 years of 43.4% and 45.6%, respectively. All studies of a second HSCT identified a delay of relapse greater than 6 months to 1 year as the main prognostic factor. In the context of LR, our results are superior compared with RFS and OS for earlier relapse, ranging from 14.6% to 21% and from 20.5% to 25%, respectively [43–49]. We report a NRM of 18.2%, lower than that reported in previous studies of second AHST [50]. This low rate of excess toxicity is probably linked to the delay from first treatment. Besides delayed relapse, other prognostic factors include disease status at second AHST, low disease burden at relapse, performance status, HLA-matched sibling AHST, and MAC [39,40,46,47].

The use of cyclosporine seems to be associated with a lower NRM but a higher incidence of relapse [43,47]. Data on the impacts of aGVHD and TBI on relapse are contradictory; however, as in our study, cGVHD has been associated with a lower rate of relapse after second AHST [46]. Finally, the choice of the same or another donor raises an important question. We did not find any difference between relapse incidence and OS, as in previous studies [44,48,51].

Maintenance treatment as relapse prophylaxis is associated with better outcome after second AHST, with a 76% decrease in the risk of relapse. However, after the first AHST, we did not find any association with outcome for LR. The GVL effect may be more effective in preventing early relapse. Some inhibitors, such as the TKI sorafenib (SORMAIN assay [52]), have shown an advantage for RFS.

Moreover, sorafenib may promote T cell activation through IL-15 production, inducing the GVL effect [53]. The RATIFY assay showed an OS advantage for midostaurin used as maintenance treatment during 1 year post-AHST (51%, versus 44% at 4 years) [50]. In Philadelphia chromosome (Ph)-positive ALL, the use of TKIs is recommended from day +100 to 2 years post-AHST to prevent relapse [54]. The use of prophylactic DLI or maintenance treatment for Ph-negative ALL is currently under study.

Our study has the expected limitations of a retrospective study. The long study period might have led to heterogeneity in patient care and follow-up. For instance, it would be interesting to study the potentially relapse-precipitating effect of the recent use of post-transplantation cyclophosphamide. Our data are in favor of performing a second AHST after LR. Patients who are considered for subsequent AHST should be highly selected.

From a molecular standpoint, the recent development of such techniques as next-generation sequencing (NGS) allow us to better understand the clonal and subclonal architecture of acute leukemia. Recently described mutations include *DDX41*, which is linked to AML and LR [55]. Comparison of molecular samples between diagnosis and LR was possible for only a few of the samples, with limited numbers of mutations. Likewise, chimerism analysis can be performed by multiple techniques of varying sensitivity (eg, short tandem repeat, qPCR, NGS, digital PCR). Consequently, interpretation of chimerism can be difficult through this intracenter and intercenter heterogeneity. Moreover, chimerism value is instantaneous and may be modified through the evolution of leukemia.

Finally, some recently introduced therapeutics, such as the association of venetoclax and azacitidine, have not yet been evaluated. Subgroup analyses were of limited power owing to the small numbers in some groups (eg, relapse prophylaxis treatment, very few LRs), leading to the absence of differences.

CONCLUSION

Relapse after AHST remains the leading cause of death, with short-term survival. This is the first study reporting the outcomes of LR acute leukemia after AHST. In our cohort, LR represented 12.4% of all relapses, with an incidence of 4.2%. Short-term survival was better than that for early relapse, with a median OS after relapse of 19.9 months. LR often presents with extramedullary involvement and is associated with cytogenetic evolution, with the acquisition of complex or monosomal karyotypes. Post-LR survival is closely linked to the possibility of carrying out immunomodulation with a second AHST. Our study highlights the importance of maintaining long-term follow-up after AHST and of evaluating preemptive and maintenance strategies to prevent relapse.

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SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jctc.2023.02.020.

REFERENCES

- Bejanyan N, Weisdorf DJ, Logan BR, et al. Survival of patients with acute myeloid leukemia relapsing after allogeneic hematopoietic cell transplantation: a Center for International Blood and Marrow Transplant Research study. *Biol Blood Marrow Transplant*. 2015;21:454-459.
- Kuhlen M, Willasch AM, Dalle JH, et al. Outcome of relapse after allogeneic HSCT in children with ALL enrolled in the ALL-SCT 2003/2007 trial. *Br J Haematol*. 2018;180:82-89.
- Schmid C, Labopin M, Nagler A, et al. Treatment, risk factors, and outcome of adults with relapsed AML after reduced intensity conditioning for allogeneic stem cell transplantation. *Blood*. 2012;119:1599-1606.
- Giles F, O'Brien S, Cortes J, et al. Outcome of patients with acute myelogenous leukemia after second salvage therapy. *Cancer*. 2005;104:547-554.
- Kantarjian HM, Keating MJ, Walters RS, McCredie KB, Freireich EJ. The characteristics and outcome of patients with late relapse acute myelogenous leukemia. *J Clin Oncol*. 1988;6:232-238.
- Spyridonidis A, Labopin M, Schmid C, et al. Outcomes and prognostic factors of adults with acute lymphoblastic leukemia who relapse after allogeneic hematopoietic cell transplantation. An analysis on behalf of the Acute Leukemia Working Party of EBMT. *Leukemia*. 2012;26:1211-1217.
- Wingard JR, Majhail NS, Brazauskas R, et al. Long-term survival and late deaths after allogeneic hematopoietic cell transplantation. *J Clin Oncol*. 2011;29:2230-2239.
- Socié G, Stone JV, Wingard JR, et al. Long-term survival and late deaths after allogeneic bone marrow transplantation. Late Effects Working Committee of the International Bone Marrow Transplant Registry. *N Engl J Med*. 1999;341:14-21.
- Döhner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood*. 2017;129:424-447.
- Heuser M, Freeman SD, Ossenkoppele GJ, et al. 2021 update on MRD in acute myeloid leukemia: a consensus document from the European LeukemiaNet MRD Working Party. *Blood*. 2021;138:2753-2767.
- van Dongen JJM, van der Velden VHJ, Brüggemann M, Orfao A. Minimal residual disease diagnostics in acute lymphoblastic leukemia: need for sensitive, fast, and standardized technologies. *Blood*. 2015;125:3996-4009.
- Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant*. 1995;15:825-828.
- Jagasia MH, Greinix HT, Arora M, et al. National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: I. The 2014 Diagnosis and Staging Working Group report. *Biol Blood Marrow Transplant*. 2015;21:389-401.e1.
- Thanarajasingam G, Kim HT, Cutler C, et al. Outcome and prognostic factors for patients who relapse after allogeneic hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant*. 2013;19:1713-1718.
- Molina B, Gonzalez Vicent M, Herrero B, et al. Kinetics and risk factors of relapse after allogeneic stem cell transplantation in children with leukemia: a long-term follow-up single-center study. *Biol Blood Marrow Transplant*. 2019;25:100-106.
- Green ML, Leisenring WM, Xie H, et al. CMV reactivation after allogeneic HCT and relapse risk: evidence for early protection in acute myeloid leukemia. *Blood*. 2013;122:1316-1324.
- Nagler A, Labopin M, Dholaria B, et al. Graft-versus-host disease prophylaxis with post-transplantation cyclophosphamide versus cyclosporine A and methotrexate in matched sibling donor transplantation. *Transplant Cell Ther*. 2022;28:86.e1-86.e8.
- Lim ABM, Curley C, Fong CY, et al. Acute myeloid leukaemia relapsing after allogeneic haemopoietic stem cell transplantation: prognostic factors and impact of initial therapy of relapse. *Intern Med J*. 2018;48:276-285.
- Yanada M, Fukuda T, Tanaka M, et al. Long-term results of reduced-intensity conditioning allogeneic hematopoietic cell transplantation for older patients with acute myeloid leukemia: a retrospective analysis of 10-year follow-up data. *Bone Marrow Transplant*. 2020;55:2008-2016.
- Oriol A, Vives S, Hernández-Rivas JM, et al. Outcome after relapse of acute lymphoblastic leukemia in adult patients included in four consecutive risk-adapted trials by the PETHEMA study group. *Haematologica*. 2010;95:589-596.
- Aldoss I, Pillai R, Yang D, et al. Late and very late relapsed acute lymphoblastic leukemia: clinical and molecular features, and treatment outcomes. *Blood Cancer J*. 2021;11:125.
- Yilmaz M, Wang F, Loghavi S, et al. Late relapse in acute myeloid leukemia (AML): clonal evolution or therapy-related leukemia? *Blood Cancer J*. 2019;9:7.
- Verma D, Kantarjian H, Faderl S, et al. Late relapses in acute myeloid leukemia: analysis of characteristics and outcome. *Leuk Lymphoma*. 2010;51:778-782.
- Medeiros BC, Minden MD, Schuh AC, et al. Characteristics and outcomes of acute myelogenous leukemia patients with very late relapse (> 5 years). *Leuk Lymphoma*. 2007;48:65-71.
- Patel A, Agha M, Raptis A, et al. Outcomes of patients with acute myeloid leukemia who relapse after 5 years of complete remission. *Oncol Res*. 2021;28:811-814.
- Rizzari C, Valsecchi MG, Aricò M, et al. Outcome of very late relapse in children with acute lymphoblastic leukemia. *Haematologica*. 2004;89:427-434.
- Ganzel C, Wang XV, Rowe JM, et al. At three years, patients with acute lymphoblastic leukaemia are still at risk for relapse. Results of the international MRC UKALLXII/ECOG E2993 trial. *Br J Haematol*. 2020;191:37-43.
- Harris AC, Kitko CL, Couriel DR, et al. Extramedullary relapse of acute myeloid leukemia following allogeneic hematopoietic stem cell transplantation: incidence, risk factors and outcomes. *Haematologica*. 2013;98:179-184.
- Lee KH, Lee JH, Choi SJ, et al. Bone marrow vs extramedullary relapse of acute leukemia after allogeneic hematopoietic cell transplantation: risk factors and clinical course. *Bone Marrow Transplant*. 2003;32:835-842.
- Clark WB, Strickland SA, Barrett AJ, Savani BN. Extramedullary relapses after allogeneic stem cell transplantation for acute myeloid leukemia and myelodysplastic syndrome. *Haematologica*. 2010;95:860-863.
- Shem-Tov N, Saraceni F, Danylesko I, et al. Isolated extramedullary relapse of acute leukemia after allogeneic stem cell transplantation: different kinetics and better prognosis than systemic relapse. *Biol Blood Marrow Transplant*. 2017;23:1087-1094.
- Ge L, Ye F, Mao X, et al. Extramedullary relapse of acute leukemia after allogeneic hematopoietic stem cell transplantation: different characteristics between acute myelogenous leukemia and acute lymphoblastic leukemia. *Biol Blood Marrow Transplant*. 2014;20:1040-1047.
- Michonneau D, Sagoo P, Breart B, Garcia Z, Celli S, Bousso P. The PD-1 axis enforces an anatomical segregation of CTL activity that creates tumor niches after allogeneic hematopoietic stem cell transplantation. *Immunity*. 2016;44:143-154.
- Spinella JF, Richer C, Cassart P, Ouimet M, Healy J, Sinnett D. Mutational dynamics of early and late relapsed childhood ALL: rapid clonal expansion and long-term dormancy. *Blood Adv*. 2018;2:177-188.
- Quek L, Ferguson P, Metzner M, et al. Mutational analysis of disease relapse in patients allografted for acute myeloid leukemia. *Blood Adv*. 2016;1:193-204.
- Schmalbrock LK, Dolnik A, Cocciardi S, et al. Clonal evolution of acute myeloid leukemia with FLT3-ITD mutation under treatment with midostaurin. *Blood*. 2021;137:3093-3104.
- Bullinger L, Döhner K, Döhner H. Genomics of acute myeloid leukemia diagnosis and pathways. *J Clin Oncol*. 2017;35:934-946.
- Welch JS, Ley TJ, Link DC, et al. The origin and evolution of mutations in acute myeloid leukemia. *Cell*. 2012;150:264-278.
- Russo A, Oliveira G, Berglund S, et al. NK cell recovery after haploidentical HSCT with posttransplant cyclophosphamide: dynamics and clinical implications. *Blood*. 2018;131:247-262.
- Rambaldi B, Kim HT, Reynolds C, et al. Impaired T- and NK-cell reconstitution after haploidentical HCT with posttransplant cyclophosphamide. *Blood Adv*. 2021;5:352-364.
- Schroeder T, Czibere A, Platzbecker U, et al. Azacitidine and donor lymphocyte infusions as first salvage therapy for relapse of AML or MDS after allogeneic stem cell transplantation. *Leukemia*. 2013;27:1229-1235.
- Schmid C, Labopin M, Nagler A, et al. Donor lymphocyte infusion in the treatment of first hematological relapse after allogeneic stem-cell transplantation in adults with acute myeloid leukemia: a retrospective risk factors analysis and comparison with other strategies by the EBMT Acute Leukemia Working Party. *J Clin Oncol*. 2007;25:4938-4945.
- Andreola G, Labopin M, Beelen D, et al. Long-term outcome and prognostic factors of second allogeneic hematopoietic stem cell transplant for acute leukemia in patients with a median follow-up of ≥ 10 years. *Bone Marrow Transplant*. 2015;50:1508-1512.
- Shimoni A, Labopin M, Finke J, et al. Donor selection for a second allogeneic stem cell transplantation in AML patients relapsing after a first transplant: a study of the Acute Leukemia Working Party of EBMT. *Blood Cancer J*. 2019;9:88.
- Savani BN, Mielke S, Reddy N, Goodman S, Jagasia M, Rezvani K. Management of relapse after allo-SCT for AML and the role of second transplantation. *Bone Marrow Transplant*. 2009;44:769-777.
- Vrhovac R, Labopin M, Ciceri F, et al. Second reduced intensity conditioning allogeneic transplant as a rescue strategy for acute leukaemia patients who relapse after an initial RIC allogeneic transplantation: analysis of risk factors and treatment outcomes. *Bone Marrow Transplant*. 2016;51:186-193.
- Bosi A, Laszlo D, Labopin M, et al. Second allogeneic bone marrow transplantation in acute leukemia: results of a survey by the European

- Cooperative Group for Blood and Marrow Transplantation. *J Clin Oncol*. 2001;19:3675-3684.
48. Christopheit M, Kuss O, Finke J, et al. Second allograft for hematologic relapse of acute leukemia after first allogeneic stem-cell transplantation from related and unrelated donors: the role of donor change. *J Clin Oncol*. 2013;31:3259-3271.
 49. Hosing C, Saliba RM, Shahjahan M, et al. Disease burden may identify patients more likely to benefit from second allogeneic hematopoietic stem cell transplantation to treat relapsed acute myelogenous leukemia. *Bone Marrow Transplant*. 2005;36:157-162.
 50. Rezvani K, Kanfer EJ, Marin D, et al. EBMT risk score predicts outcome of allogeneic hematopoietic stem cell transplantation in patients who have failed a previous transplantation procedure. *Biol Blood Marrow Transplant*. 2012;18:235-240.
 51. Ortí G, Sanz J, García-Cadenas I, et al. Analysis of relapse after transplantation in acute leukemia: a comparative on second allogeneic hematopoietic cell transplantation and donor lymphocyte infusions. *Exp Hematol*. 2018;62:24–32.
 52. Xuan L, Wang Y, Huang F, et al. Sorafenib maintenance in patients with FLT3-ITD acute myeloid leukaemia undergoing allogeneic haematopoietic stem-cell transplantation: an open-label, multicentre, randomised phase 3 trial. *Lancet Oncol*. 2020;21:1201-1212.
 53. Romero D. Sorafenib prevents AML relapse after allo-HSCT. *Nat Rev Clin Oncol*. 2020;17:591.
 54. Nagler A, Labopin M, Dholaria B, et al. Second allogeneic stem cell transplantation in patients with acute lymphoblastic leukaemia: a study on behalf of the Acute Leukaemia Working Party of the European Society for Blood and Marrow Transplantation. *Br J Haematol*. 2019;186:767-776.
 55. Duployez N, Largeaud L, Duchmann M, et al. Prognostic impact of *DDX41* germline mutations in intensively treated acute myeloid leukemia patients: an ALFA-FILO study. *Blood*. 2022;140:756–768.