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# 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 (AHSCT) 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.



## 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/evolutive 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),

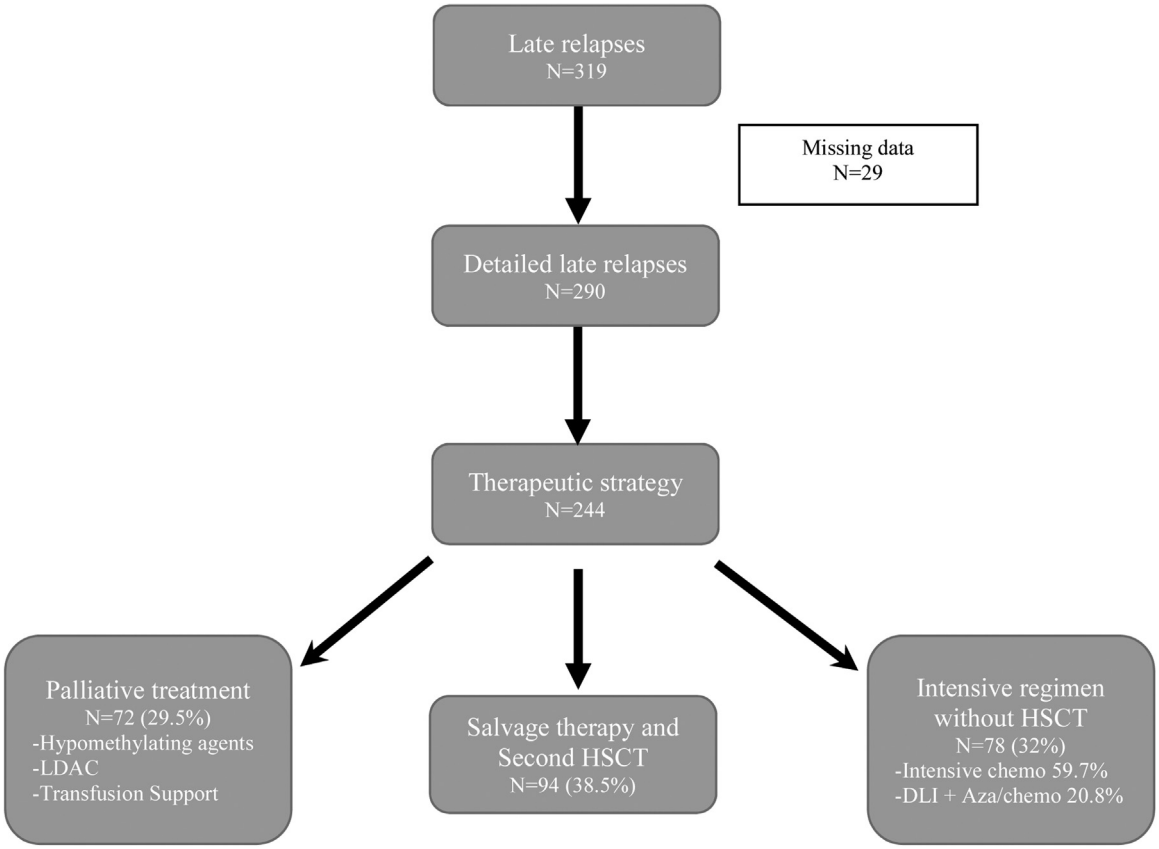


**Figure 1.** Flow chart of the study. LDAC, low-dose cytarabine; Aza, azacytidine; Chemo, chemotherapy.

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].

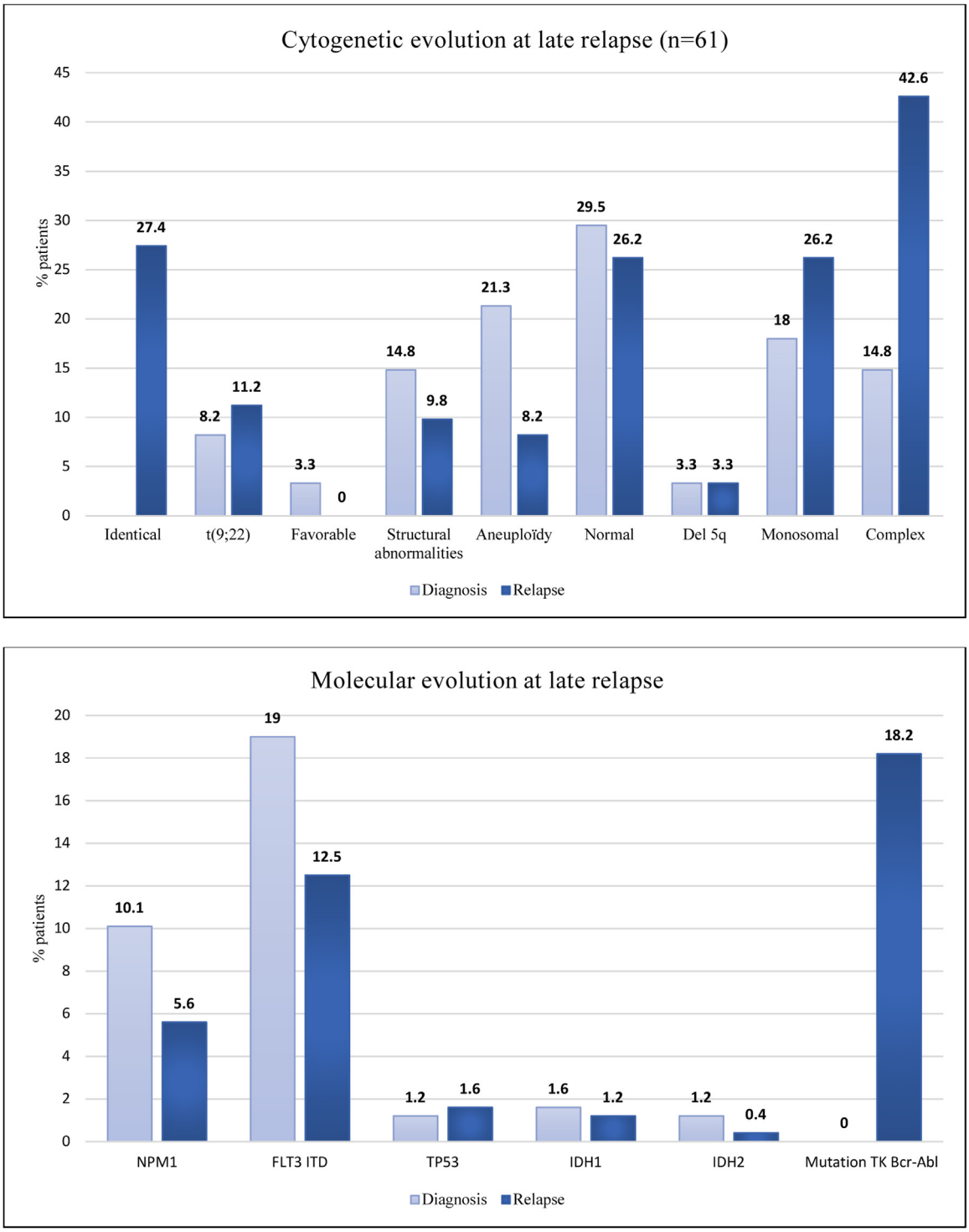


**Figure 2.** Cytogenetics and molecular characteristics at LR. Del, deletion; TK, tyrosine kinase.

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 than 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

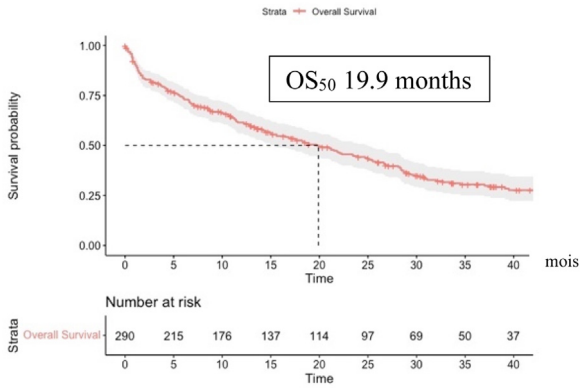


**Figure 3.** OS after LR.

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.

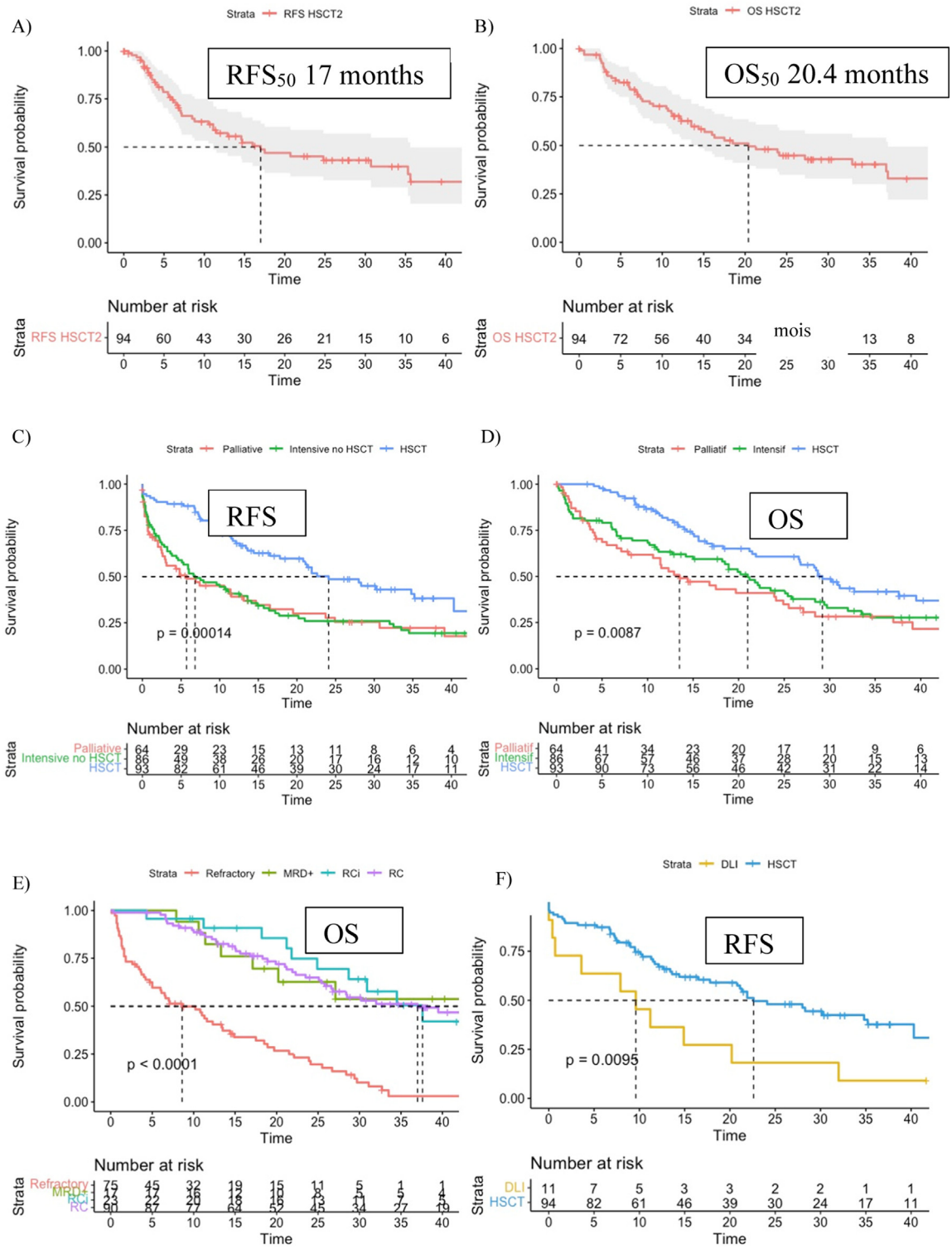


**Figure 4.** RFS and OS after LR with salvage therapy. (A and B) RFS and OS after LR post-second AHSCT. (C and D) RFS and OS with palliative treatment, intensive salvage therapy alone, and salvage therapy followed by second AHSCT. (E) OS by response to salvage therapy. (F) RFS in patients receiving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

| 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 AHSCT. Many studies on early relapse are retrospective; one prospective study showed an advantage of performing AHSCT 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 AHSCT. We found an advantage in OS of performing AHSCT, 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 AHSCT 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 AHSCT [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 AHSCT, low disease burden at relapse, performance status, HLA-matched sibling AHSCT, 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 AHSCT [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 AHSCT, with a 76% decrease in the risk of relapse. However, after the first AHSCT, 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-AHSCT (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-AHSCT 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 AHSCT after LR. Patients who are considered for subsequent AHSCT 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 AHSCT remains the leading cause of death, with short-term survival. This is the first study reporting the outcomes of LR acute leukemia after AHSCT. 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 AHSCT. Our study highlights the importance of maintaining long-term follow-up after AHSCT 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.jtct.2023.02.020.

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