Original Study

Treatment of Acute Myeloid Leukemias and Myelodisplastic Syndromes Relapsing After Allogeneic Stem Cell Transplantation: An In-Depth Analysis of the GITMO AML/MDS-Relapse Registry Study

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Abstract

AML and MDS relapse is the most frequent cause of allo-SCT failure. This subanalysis of the GITMO AML/MDS relapse study focuses on 647 AML/MDS relapses that were treated with either HMAs-based therapy ($n = 308$) or other treatments ($n = 339$), including intensive chemotherapy, FLT3-inhibitors, and second allo-SCT. The ORR with or without HMA-based salvage treatment was 33% versus 40%, respectively ($P = .006$). The long-term OS and TRM of the two groups were superimposable. Independently from the type of salvage, an advantage in OS was observed when DLI was included ($P < .001$). Relapse within 12 months after SCT, low disease burden at relapse, and the CR status at transplant confirmed their independent strong prognostic impact on both HMA and non-HMA-based group (HR 0.05, 0.44, 0.49 and HR 0.19, 0.32, 0.53, respectively).

Despite the lower ORR observed with HMA-based therapy, the long-term OS was comparable to that observed with other therapies. The immune control of the disease relapse with DLI is of benefit, independently from the salvage therapy

Background: Acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) relapse is the most frequent cause of allogeneic stem cell transplantation (allo-SCT) failure. The utility of post-relapse therapy is controversial due to the high incidence of toxicity and the low efficacy. **Methods:** This sub-analysis of the Gruppo Italiano Trapianto di Midollo Osseo (GITMO) AML/MDS relapse study focuses on 647 AML/MDS relapsing after allo-SCT performed between 2015 and 2021. Following the relapse, these patients were treated with either hypomethylating agents (HMAs)-based therapy ($n = 308$) or other treatments ($n = 339$), including intensive chemotherapy, FLT3-inhibitors, and second allo-SCT. **Results:** HMAs-based therapies were more frequently used in older patients, transplanted not in CR following a reduced-intensity conditioning regimen. The overall response rate (ORR) with or without HMA-based salvage treatment was 33% and 40%, respectively ($P = .006$). The complete remission (CR) rate was 23% and 33% in the two groups, respectively ($P < .001$). The long-term OS and TRM of the two groups were superimposable. Independently from the type of salvage, an advantage in OS was observed when donor lymphocytes infusion (DLI) was included ($P < .001$). Relapse within 12 months after SCT, low disease burden at relapse, and the CR status at transplant confirmed their independent strong prognostic impact on both HMA and non-HMA-based group (HR 0.05, 0.44, and 0.49 and HR 0.19, 0.32, and 0.53, respectively). **Conclusions:** Despite the lower ORR observed with HMA-based therapy, the long-term OS was comparable to that observed with other therapies. The immune control of the disease relapse with DLI is of benefit, independently from the salvage therapy. .

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Introduction

Allogeneic hematopoietic stem cell transplantation (allo-SCT) is a curative option for a large number of high-risk acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) patients; nevertheless, this strategy is ineffective in 30%-70% of the cases due to disease relapse.[1-3] Relapse prevention and its treatment is a current unmet need, with several areas of uncertainty and debate globally regarding optimal management. In this context, increasing attention is deserved to the choice of optimal therapeutic strategy, particularly following the availability of approaches including hypomethylating agents (HMAs) and venetoclax (VEN) and of molecular target drugs (eg, FLT3-ITD and IDH 1-2 inhibitors).[4-9] Moreover, the monitoring of minimal residual disease (MRD) remains crucial for either early immunosuppression withdrawal or maintenance strategy or preemptive therapy.[2,3]

The outcome of AML/MDS relapses after allo-SCT is extremely dismal, with less than 10% of the patients alive and disease-free beyond 1 year following disease recurrence.[1-3] The time from transplant to relapse and the type of relapse (MRD positivity or mixed chimerism vs. hematological relapse with more than 5% of marrow blast cells) are the most important prognostic factors.[1-3,10] Recently, we conducted a registry study within the Gruppo Italiano Trapianto di Midollo Osseo (GITMO), in which 859 AML/MDS patients relapsing after allo-SCT between 2015 and 2021 were collected. Most of these patients (88%) reported a hematological relapse (HIGH disease burden). Although the outcome of these patients is dismal, a treatment approach may be associated with improved outcomes, with the combination of HMAs with or without VEN being the most frequently used therapy.[11] Another bi-centric Italian-French retrospective analysis on the outcome of 134 AML/MDS relapses between 2015 and 2021 recently published[12] reported that a treatment strategy including immunotherapy with DLI in a relapse with LOW disease burden was associated with a 2-year OS of 34% versus 20% for those treated with HIGH disease burden at relapse ($P < .01$). Similarly, Zuanelli Brambilla et al. analyzed 148 single-center AML/MDS relapses after allo-SCT and showed that post-relapse therapy, including either DLI or second allo-SCT, was associated with improved outcomes.[13]

Herein we report an in-depth analysis of the GITMO AML/MDS relapse registry study, with the aim to give a real-life picture of the different strategies adopted in Italy between 2015 and 2021, and to describe the outcome of these patients according to the salvage treatment.

Patients and Methods

The Gruppo Italiano Trapianto di Midollo Osseo (GITMO) AML/MDS relapse study (ClinicalTrials.gov identifier NCT06790680) is a retrospective nationwide analysis of management of relapsed AML/MDS in patients allografted between January 2015 and November 2021 in Italy.[11] Relapses were observed between March 2015 and October 2023. For analysis purposes, patients are divided into two large groups: those treated with HMAs ± VEN ($n = 308$) and those treated with other therapies ($n = 339$). The group of patients who have not received HMAs ± VEN were treated with different strategies, including different intensive chemotherapy regimens (eg, 3 + 7, FLAG-Ida, Mitoxantrone + HD-AraC), and FLT3-inhibitors (alone or in combination with chemotherapy). DLI was used in 243/647 patients (37%), in combination with HMA-based (132/308—43%) or non HMA-based therapies (111/339—33%) according to each center policy. According to the burden of disease, the relapse was defined as LOW in the case of MRD positivity or mixed chimerism and HIGH in the case of hematological relapse with more than 5% of marrow blast cells.

Statistical Analysis

All continuous variables were synthesized as means and SD, except for the follow-up time (median and range) and the time from SCT to relapse (dichotomized on relevant cut-offs). Comparison between patients treated with and without HMAs ± VEN was based on the Kruskal–Wallis test for continuous variables and on the χ^2 test or Fisher's exact test, as appropriate, for categorical variables. The probability of the OS was calculated by the Kaplan–Meier method from the time of relapse treatment, and the log-rank test was used to compare OS between groups. With nonproportional risks, the Fleming–Harrington test was used instead, with the parameters set as $P = 1$ and $\lambda = 0$ to place greater emphasis on differences occurring during the early phase of follow-up.

Two subgroups' analyses were conducted on patients treated with and without HMAs ± VEN, respectively. A Cox proportional hazards regression model was used to analyze OS as related to clinical variables, and the results were expressed as hazard ratios (HRs) and 95%CIs, with log-rank P values. A score test for the time-varying coefficient = 0 was used to test the proportionality of the PH Cox regression model.[14] In the presence of non- proportional hazards in the OS, the interaction of individual covariates with log of the time was included as a covariate.

The Fine and Gray competing risk regression model was used to model directly the effect of covariates on the incidence of the primary event (either RM or TRM) after accounting for the competing event (TRM or RM, respectively).[15] Consistently with relevant literature, no correction for non-proportional risks was applied.[16,17] For both subgroup analyses, only the variables significantly associated in the univariable analysis were included in multivariable analysis. A two-sided P-value of 0.05 was considered for statistical significance.

Statistical analysis was performed in the R environment (https://cran.r-project.org/).

Results

As reported in Table 1, nearly 90% of the patients were affected by AML, with a mean age at diagnosis and at SCT significantly higher in the group treated with HMAs ± VEN. Interestingly, the HMAs ± VEN group included a higher number of patients who experienced a late relapse after SCT (>6 months; 54.5% vs. 44.2%; $P = .011$) and who were not transplanted in first CR (42.2% vs. 28.3%; $P < .001$). In both groups, more than 80% of the patients had a HIGH disease burden at relapse, meaning evidence of hematological relapse, with circulating blast cells. Donors were MUD or MMUD in approximately 50% of the cases in both groups, and nearly 80% of the patients received PBSC. The intensity of conditioning was higher in patients who subsequently did not receive HMAs ± VEN salvage therapy (78.5% vs. 70.5%; $P = .025$), and DLI was more frequently used following HMAs ± VEN (42.9% vs. 32.7%; $P = .01$). The median time from start of salvage therapy to first DLI was 67 days (range 42-141). The median number of DLI doses administered was 2 (range 1-3). Excluding missing data, the salvage treatment adopted in the non HMA-based group were intensive chemotherapy (mainly fludarabine-based) (144/339; 42.5%), FLT3 inhibitors (76/339; 22.4%), DLI alone (33/339; 9.7%), and second allo-SCT (21/339; 6.2%). Notably, no other patients received second allo-SCT in the whole series.

Supplemental Table 1 reports the distribution of the above clinical and transplant characteristics according to post-relapse therapy (including or not HMAs ± VEN) and the use of DLI (yes vs. no). It is worth noting that the percentage of patients relapsing within 6 months from SCT, as well as the proportion of patients with HIGH disease burden at relapse, was higher in the group treated without HMAs and without DLI.

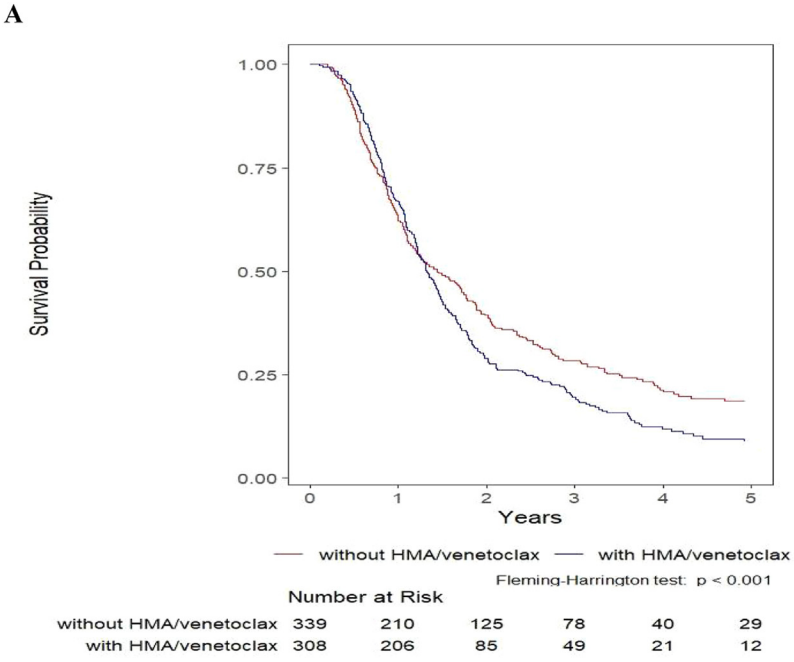
Response rate, OS, and TRM of the 647 patients treated for relapse according to the type of salvage therapy (with/without HMAs ± VEN)

Following salvage therapy with HMAs ± VEN, the overall response rate (ORR; including complete response and partial response) was 33% (102/308). In parallel, the ORR was 40% (136/339) among those patients who did not receive HMAs ± VEN following relapse ($P = .006$). The rate of complete remission (CR) was 23% (72/308) versus 33% (113/339) in the two groups, respectively ($P = .002$).

As reported in Figure 1A, the 1- and 2-year OS was 67% (95% CI 61.7-72.3) and 29% (95% CI 24.3-34.5) in the HMAs group versus 62% (95% CI 57.2-67.5) and 39% (95% CI 34.5-45) in the non-HMAs group, respectively ($P = .351$). Moreover, as reported in Figure 1B, the cumulative incidence of TRM was superimposable comparing patients who received HMAs ± VEN for salvage therapy in respect of those who did not (1- and 2-year TRM 4.5% and 19% vs. 6.8% and 17.4%; $P = .999$). In particular, GVHD following DLI was the cause of death in 14% of the cases in both groups.

Figure 2 reports the OS (Figure 2A) and the TRM (Figure 2B) of the 647 treated patients based on the type of salvage therapy (with or without HMAs ± VEN) and the use of DLI (DLI yes vs. DLI no). Interestingly, the 1- and 2-year OS was 82.6% and 42.7%

Figure 1 Overall survival of the 647 patients according to salvage treatment (with or without HMAs ± VEN) (A) (with HMAs ± VEN: 1-year OS = 67%, 2-year OS = 29%; without HMAs ± VEN: 1-year OS = 62%, 2-year OS = 39%); cumulative incidence of TRM of the 647 patients according to salvage treatment (with or without HMAs ± Venetoclax) (B) (with HMAs ± VEN: 1-year TRM = 4.5%, 2-year TRM = 19%; without HMAs ± VEN: 1-year TRM = 6.8%, 2-year TRM = 17.4%).



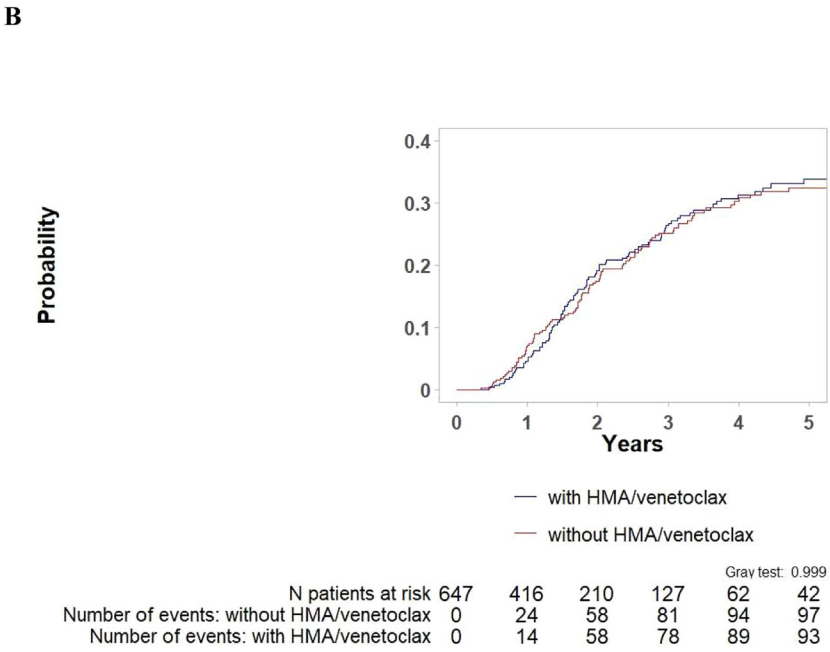
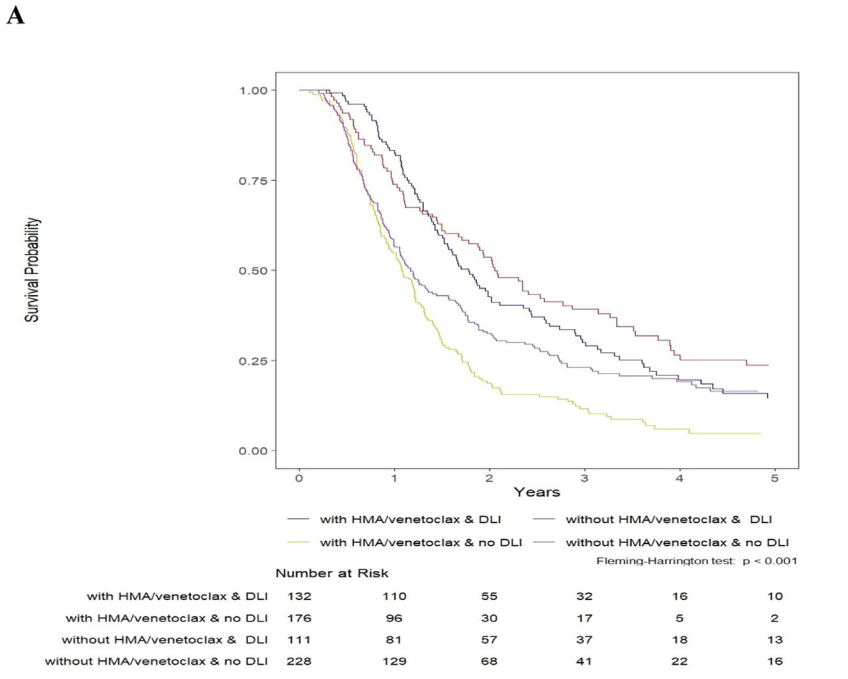


Figure 2 OS of the 647 treated patients according to post-relapse treatment (with or without HMAs ± Venetoclax and DLI yes vs. no) (A) (with HMA/venetoclax—DLI yes: 1-year OS = 82.6%, 2-year OS = 42.7%; with HMA/venetoclax—DLI no: 1-year OS = 54.9%, 2-year OS = 18.7%; without HMA/venetoclax—DLI yes: 1-year OS = 73.8%, 2-year OS = 53.7%, without HMA/venetoclax—DLI no: 1-year OS = 56.4%, 2-year OS = 32.4%); cumulative incidence of TRM of the 647 treated patients according to post-relapse treatment (with or without HMAs ± Venetoclax and DLI yes vs. no) (B) (with HMA/venetoclax—DLI yes: 1-year TRM = 1.5%, 2-year TRM = 19.2%; with HMA/venetoclax—DLI no: 1-year TRM = 6.9%, 2-year TRM = 18.9%; without HMA/venetoclax—DLI yes: 1-year TRM = 7.2%, 2-year TRM = 17.3%; without HMA/venetoclax—DLI no: 1-year TRM = 6.2%, 2-year TRM = 17.1%).



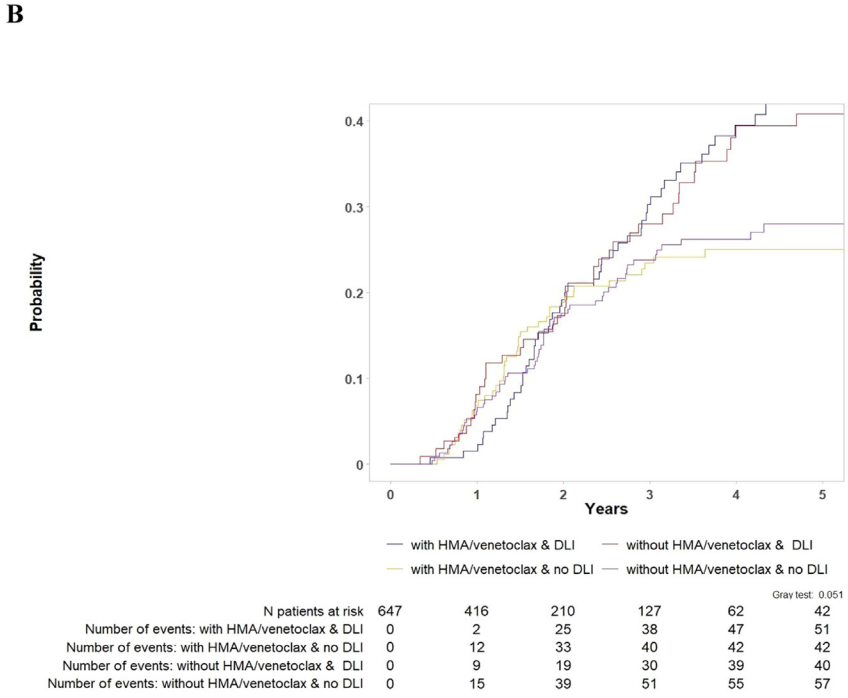


Table 1 Clinical and Transplant Characteristics of the 647 AML/MDS Treated Relapses According to the Type of Salvage Therapy

Prognostic Factors	Without HMA/Venetoclax ($N = 339$)	With HMA/Venetoclax ($N = 308$)	*P* Value
Diagnosis			
AML	310 (91.4%)	277 (89.9%)	.599
MDS	29 (8.6%)	31 (10.1%)	
Age at diagnosis			
Mean (SD)	56.3 (12.2)	58.8 (11.8)	**.005**
Age at SCT			
Mean (SD)	50.6 (12.2)	53.6 (11.7)	**.001**
Follow-up from relapse (months)			
Median (min, max)	16.8 (2.47, 104)	15.8 (1.32, 99.0)	.508
Time SCT/relapse (months)			
≤6	189 (55.8%)	140 (45.5%)	**.011**
>6	150 (44.2%)	168 (54.5%)	
Time SCT/relapse (months)			
≤12	258 (76.1%)	228 (74.0%)	.603
>12	81 (23.9%)	80 (26.0%)	
Disease burden at relapse			
High	289 (85.3%)	269 (87.3%)	.512
Low	50 (14.7%)	39 (12.7%)	
Disease status at SCT			
No-CR	96 (28.3%)	130 (42.2%)	**<.001**
CR	243 (71.7%)	178 (57.8%)	
Lines of therapy before SCT			
>1	143 (42.2%)	158 (51.3%)	.175
1	171 (50.4%)	150 (48.7%)	
Missing	25 (7.4%)	0 (0%)	
Donor			
Sibling	107 (31.6%)	80 (26.0%)	.4
MUD/MMUD	165 (48.7%)	158 (51.3%)	
Haploidentical	66 (19.5%)	69 (22.4%)	
UCB	1 (0.3%)	1 (0.3%)	
Stem cells' source			
BM	77 (22.7%)	65 (21.1%)	.863
PBSC	260 (76.7%)	241 (78.2%)	
UCB	2 (0.6%)	2 (0.6%)	
Myeloablative conditioning			
No	73 (21.5%)	91 (29.5%)	**.025**
Yes	266 (78.5%)	217 (70.5%)	
DLI included as salvage therapy			
No	228 (67.3%)	176 (57.1%)	**.010**
Yes	111 (32.7%)	132 (42.9%)	
Type of salvage therapy			
Intensive chemotherapy FLT3 inhibitors DLI alone Second allo-SCT Other Missing	144 (42.5%) 76 (22.4%) 33 (9.7%) 21 (6.2%) 23 (6.8%) 42 (12.4%)	–	**–**

(*continued on next page*)

Table 1 (*continued*)

Prognostic Factors	Without HMA/Venetoclax (*N* = 339)	With HMA/Venetoclax (*N* = 308)	*P* Value
aGVHD (any time after SCT)			
NO	188 (55.5%)	185 (60.1%)	.385
YES	96 (28.3%)	73 (23.7%)	
aGVHD grade 3-4	55 (16.2%)	50 (16.2%)	
GVHD post-DLI			
NO	292 (86.1%)	279 (90.6%)	.485
YES	33 (9.7%)	25 (8.1%)	
Missing	14 (4.1%)	4 (1.3%)	

Abbreviations: aGVHD = acute graft versus host disease; AML = acute myeloid leukemia; BM = bone marrow; cGVHD = chronic graft versus host disease; CR = complete remission; DLI = donor lymphocytes infusion; MAC = myeloablative conditioning; MDS = myelodysplastic syndrome; MMUD = mismatched unrelated donor; MUD = matched unrelated donor; PBSC = peripheral blood stem cells; SCT = stem cell transplantation; UCB = umbilical cord-blood.

Table 2 Univariable and Multivariable Analysis on OS for the 308 Patients Treated With HMAs ± VEN

Variables	Unit of Measure/Category	Mean (SD) or *n* (%)	HR (univariable)			HR (multivariable)		
			HR	95% CI	P Value	HR	95% CI	*P* Value
Age at the baseline	Years	53.6 (11.7)	1.01	0.99-1.00	*P* = .939			
Relapse time[a]	<12 months	228 (74.0)	0.06	0.03-0.13	**P < .001**	0.05	0.02-0.10	**P < 001**
	≥ 12 months	80 (26.0)						
Relapse disease burden	HIGH	269 (87.3)	0.49	0.33-0.74	**P < .001**	0.44	0.29-0.67	**P < .001**
	LOW	39 (12.7)						
Diagnosis at SCT	AML	277 (89.9)	0.9	0.60-1.34	*P* = .596			
	MDS	31 (10.1)						
Disease status at SCT[a]	No-CR	130 (42.2)	0.57	0.44-0.73	**P < .001**	0.49	0.38-0.64	**P < .001**
	CR	178 (57.8)						
Lines therapy	>1	158 (51.3)	0.8	0.63-1.02	*P* = .074			
	1	150 (48.7)						
Donor (vs. Sibling)[a]	Sibling	80 (26.1)						
	MUD/MMUD/UCB	158 (51.5)	0.81	0.61-1.09	*P* = .173			
	Haploidentical	69 (22.5)	0.93	0.65-1.33	*P* = .687			
Stem cells' source (vs. BM)	BM	65 (21.1)						
	PBSC	241 (78.2)	1.17	0.87-1.58	*P* = .298			
	UCB	2 (0.6)	1.88	0.46-7.74	*P* = .381			
Myeloablative conditioning	NO	91 (29.5)	1.20	0.92-1.57	*P* = .178			
	YES	217 (70.5)						
aGVHD (YES vs. NO)	NO	185 (60.1)						
	YES	73 (23.7)	1.1	0.83-1.47	*P* = .505			
	Grade II/IV	50 (16.2)	1.57	1.13-2.19	**P = .008**			
GVHD post-DLI	NO	279 (91.8)	0.49	0.30-0.78	**P = .003**	0.59	0.35-0.98	**P = .041**
	YES	25 (8.2)						
Post-relapse therapy, including DLI[a]	NO	176 (57.1)	0.44	0.33-0.57	**P < .001**	0.43	0.32-0.59	**P < .001**
	YES	132 (42.9)						

p<0.05 is considered statistical significant.
Abbreviations: aGVHD = acute graft versus host disease; AML = acute myeloid leukemia; BM = bone marrow; CR = complete remission; DLI = donor lymphocytes infusion; MDS = myelodysplastic syndrome; MMUD = mismatched unrelated donor; MUD = matched unrelated donor; PBSC = peripheral blood stem cells; SCT = stem cell transplantation; UCB = umbilical cord-blood.

(HMAs ± VEN-DLI yes) versus 54.9% and 18.7% (HMA ± VEN-DLI no) versus 73.8% and 53.7% (without HMA ± VEN-DLI yes) versus 56.4% and 32.4% (without HMA-VEN-DLI no) (Figure 2A; $P < .001$). As reported in Figure 2B, the 1- and 2-year TRM was 1.5% and 19.2% (with HMAs ± VEN-DLI yes) versus 6.9% and 18.9% (with HMA/venetoclax-DLI no) versus 7.2% and 17.3% (without HMA/venetoclax-DLI yes) versus 6.2% and 17.1% (without HMA/venetoclax-DLI no) ($P = .051$). Following the second year, the TRM of patients who received DLI with any salvage therapy was higher than that observed without DLI (at 5 years: HMA-based salvage + DLI and no HMA-based salvage + DLI: 43.4% and 39.5%; HMA-based salvage-no DLI and no HMA-based salvage-no DLI: 25% and 28%; $P = .051$).

Univariate and multivariate analysis on post-relapse outcome on the 647 patients treated after relapse, according to the type of salvage therapy (with or without HMAs ± VEN)

In order to potentially identify factors associated with long-term outcome in the two groups, we conducted separate analyses in the 308 and 339 patients treated with or without HMAs ± VEN

Focusing on the 308 patients treated with HMAs ± VEN (Table 2), factors significantly associated with improved OS by multivariable analysis were a time between SCT and relapse ≥12 months (HR 0.05; 95% CI 0.02-0.1; $P < .001$), LOW disease burden at relapse (HR 0.44; 95% CI 0.29-0.67; $P < .001$), being in CR at SCT (HR 0.49; 95% CI 0.38-0.64; $P > .001$), the development of GVHD following DLI (HR 0.59; 95% CI 0.35-0.98; $P = .041$), and DLI included in post-relapse therapy (HR 0.43; 95% CI 0.32-0.59; $P < .001$). Moving to the 339 patients treated with other salvage therapy (Table 3), factors that had a significant impact on OS by multivariable analysis were age at baseline as continuous variable (HR 1.02; 95% CI 1.01-1.03; $P < .001$), an interval SCT-relapse longer than 12 months (HR 0.19; 95% CI 0.07-0.54; $P = .002$), a LOW disease burden at relapse (HR 0.32; 95% CI 0.19-0.54; $P < .001$), being in CR at SCT (HR 0.53; 95% CI 0.40-0.69; $P < .001$), having received a target therapy with FLT3 inhibitor after posttransplant relapse (HR 0.5; 95% CI 0.35-0.71; $P < .001$), and having received a second allo-SCT (HR 0.52; 95% CI 0.29-0.92; $P = .025$).

Moving to RM and TRM and focusing on the 308 patients treated with HMAs ± VEN (Table 4), the following independently affected RM by multivariable analysis: an interval between SCT and relapse > 12 months (HR 0.40; 95% CI 0.29-0.55; $P < .001$), being in CR at SCT (HR 0.57; 95% CI 0.41-0.78; $P < .001$), developing GVHD after DLI (HR 0.37; 95% CI 0.16-0.86; $P < .05$), and inclusion of DLI with salvage therapy (HR 0.52; 95% CI 0.37-0.73; $P < .001$). This latter was also the only independent factor affecting TRM (HR 1.55; 95% CI 1-2.4; $P < .05$). Focusing on the patients who received other salvage therapies (Table 5), the RM was independently influenced by: an interval between SCT and relapse >12 months (HR 0.36; 95% CI 0.24-0.55; $P < .001$), a LOW disease burden at relapse (HR 0.28; 95% CI 0.13-0.59; $P < .01$), being in CR at SCT (HR 0.61; 95% CI 0.42-0.87; $P < .01$), the inclusion of DLI with the salvage therapy (HR 0.60; 95% CI 0.40-0.91; $P < .05$), and the use of second allo-SCT (HR 0.24; 95% CI 0.07-0.74; $P < .05$). In this subset of patients, no factors were independently associated with TRM.

Discussion

The present in-depth analysis on the GITMO AML-relapse study[11] was conducted with the aim to take a real-life picture of the management of these patients, considering separately those who received HMAs ± VEN and those who received other salvage treatments. Indeed, HMA ± VEN was considered for patients with peculiar risk features, such as older age, transplantation not in CR, following reduced-intensity conditioning regimen, and more frequently combined with DLI. Some of these characteristics clearly identify a subset of patients at very high risk. This may partially explain why the CR rate with HMAs ± VEN was lower than that observed with other therapies and that reported by Chiusolo et al. on 91 AML/MDS treated with HMAs and VEN (33% after 2 cycles).[18] Indeed, in our series, VEN was combined with HMAs in 41% of the patients only. Nevertheless, the 2-year OS was perfectly matched with the 2-year OS reported in the paper by Chiusolo et al. (29% vs. 24%). When the outcomes of the two salvage strategies were analyzed, coupled with the use or not of DLI, it was clear that adding immunotherapy improved the OS in both cohorts (Figure 2A). The positive impact of DLI in this population was previously reported by others. In a GITMO study[19] on patients receiving DLI, the 3-year OS was 32%. In one of the multiple reviews published, Schmid et al. reported that the 2-year OS in the setting of relapsed AML treated with DLI ranged from 14% to 25%.[20] The association of DLI plus low-intensity (azacitidine or venetoclax)[20,21] or targeted therapies (sorafenib) showed promising results, increasing the OS rate.[22] In our series, the TRM was comparable irrespective of the adopted salvage strategy (Figure 1B), and the TRM beyond second year was higher in those patients who received DLI as part of salvage therapy (Figure 2B). We can speculate that this effect was linked to GVHD induction. The positive impact of DLI on RM and on OS, counterbalanced by the negative effect on TRM, suggests that it's time to move from unmanipulated DLI to modified DLI.[23,24]

In the HMAs ± VEN cohort, not surprisingly, multivariate analysis showed that the time between transplant and relapse ≥12 months, the CR status at SCT, the LOW burden of disease at relapse, the use of DLI, and the development of GVHD following DLI were independently associated with OS and/or RM (Tables 2 and 4). The positive effect of DLI on outcome may be partially related to the synergistic effect of HMAs with DLI, leading to an enhancement of the tumor-associated antigens expression[25] or of IL-15 production.[22] A long time between transplant and relapse has also been reported as a prognostic factor in several experiences. In a Japanese registry study, relapse before 12 months from transplant was strongly associated with a poor OS; the OS was progressively poorer with shorter time between transplant and disease recurrence,[26] while a relapse time shorter than 6 months was deleterious for survival in other studies.[27,28]

In the no HMAs ± VEN cohort, multivariate analysis confirmed some common factors influencing OS such as late relapse, LOW disease burden at relapse, and CR at allo-SCT (Table 3). These factors, plus DLI and second allo-SCT, were found to impact

Table 3 Univariable and Multivariable Analysis on OS for the 339 Patients Treated Without HMAs ± VEN

Variables	Unit of Measure/Category	Mean (SD) or *n*(%)	HR (Univariable)			HR (Multivariable)		
			HR	95% CI	*P* Value	HR	95% CI	*P* Value
Age at the baseline	Years	50.55 (12.20	1.01	1.00-1.02	**P = .009**	1.02	1.01-1.03	**P < .001**
Relapse time[a]	<12 months	258 (76.1)	0.29	0.01-0.78	**P = .014**	0.19	0.07-0.54	**P = .002**
	≥12 months	81 (23.9)						
Relapse disease burden	High	289 (85.3)	0.34	0.22-0.52	**P < .001**	0.32	0.19-0.54	**P < .001**
	Low	50 (14.7)						
Diagnosis at SCT	AML	310 (91.4)	1.41	0.93-2.12	P = .106			
	MDS	29 (8.6)						
Disease status at SCT[a]	No-CR	96 (28.3)	0.62	0.48-0.80	**P < .001**	0.53	0.40-0.69	**P < .001**
	CR	243 (71.4)						
Lines therapy	>1	143 (45.5)	0.77	0.60-0.99	**P = .045**			
	1	171 (54.5)						
Donor (sibling as reference)[a]	Sibling	107 (31.7)						
	MUD/MMUD/UCB	165 (48.8)	0.92	0.70-1.21	P = .482			
	Haploidentical	66 (19.5)	0.92	0.65-1.30	P = .566			
Stem cells' source (BM as reference)	BM	77 (22.7)						
	PBSC	260 (76.7)	1.26	0.94-1.68	P = .124			
	UCB	2 (0.6)	3.24	0.79-13.31	P = .103			
Myeloablative conditioning	No	73 (21.5)						
	Yes	266 (78.5)	0.76	0.57-1.01	P = .059			
aGVHD (NO as reference)	No	188 (55.5)						
	Yes	96 (28.3)	0.98	0.74-1.30	P = .887			
	Grade II/IV	55 (16.2)	1.42	1.02-1.97	**P = .039**			
GVHD post-DLI	No	292 (89.8)	0.73	0.48-1.12	P = .148			
	Yes	33 (10.1)						
Post-relapse therapy, including DLI[a]	No	228 (67.3)	0.65	0.50-0.84	**P = .001**			
	Yes	111 (32.7)						
Therapy (intensive CHT as reference)	Intensive CHT	144 (42.5)						
	FLT3-inhibitors	76 (22.4)	0.65	0.46-0.90	**P = .009**	0.50	0.35-0.71	**P < .001**
	Second allo-SCT	21 (6.2)	0.51	0.29-0.89	**P = .018**	0.52	0.29-0.92	**P = .025**

p<0.05 is considered statistical significant.
Abbreviations: aGVHD = acute graft versus host disease; AML = acute myeloid leukemia; BM = bone marrow; cGVHD = chronic graft versus host disease; CHT = chemotherapy; CR = complete remission; DLI = donor lymphocytes infusion; MDS = myelodysplastic Syndrome; MMUD = mismatched unrelated donor; MUD = matched unrelated donor; PBSC = peripheral blood stem cells; SCT = stem cell transplantation; UCB = umbilical cord-blood.
[a]Adjusted for the interaction with time to correct for non-proportionality.

significantly on RM (Table 5). Moreover, the analysis suggests that targeted therapy (eg, FLT3 inhibitors) can improve survival (Table 3). Notably, these drugs also show a good safety profile and efficacy in the setting of posttransplant relapse, even if ad hoc studies are lacking.[5] Focusing on the use of second allo-SCT, we observed that only 21/647 cases (3%) received this treatment. This probably reflects the reluctance of clinicians to perform such a procedure, frequently associated with high transplant-related mortality, particularly in the presence of active disease, and with more than 70% of the patients relapsing earlier than 12 months from SCT and approximately 50% earlier than 6 months. Even though a second allo-SCT was rarely used, it maintained its independent positive prognostic impact on OS. Although prospective studies are lacking, data from single-center and registry-based studies report that long-term OS

Table 4 Univariable and Multivariable Analysis on RM and TRM for the 308 Patients Treated With HMAs ± VEN

Variables[§]	RM ($n = 175$)			TRM ($n = 94$)		
	Mean (SD) or *n* (%)	Univariable SHR (95% CI)	Multivariable SHR (95% CI)	Mean (SD) or n(%)	Univariable SHR (95% CI)	Multivariable SHR (95% CI)
Age at the baseline	54.4 (11.0)	1.01 (1.00-1.02)		52.4 (13.0)	0.99 (0.97-1.01)	
Interval SCT-relapse >12 months	34 (19.4)	**0.42 (0.30-0.58)*****	**0.40 (0.29-0.55)*****	25 (26.6)	0.89 (0.58-1.36)	
Disease burden at relapse: LOW	15 (8.6)	**0.53 (0.32-0.90)***		11 (11.7)	0.89 (0.49-1.62)	
Diagnosis at SCT: MDS	19 (10.9)	1.16 (0.72-1.87)		8 (8.5)	0.69 (0.32-1.50)	
Disease status at SCT: CR	90 (51.4)	**0.61 (0.45-0.82)****	**0.57 (0.41-0.78)*****	59 (62.8)	1.28 (0.93-1.27)	
Lines of therapy before SCT: 1	83 (47.4)	0.88 (0.65-1.18)		45 (47.9)	0.95 (0.63-1.43)	
Donor type: MUD/MMUD	89 (50.9)	0.91 (0.68-1.23)		47 (50.5)	0.96 (0.64-1.44)	
Donor type: haploidentical	40 (22.9)	1.01 (0.72-1.43)		23 (24.7)	1.12 (0.70-1.8)	
Stem cells' source: PBSC	140 (80.0)	1.20 (0.83-1.72)		75 (76.6)	0.97 (0.60-1.57)	
Myeloablative conditioning: yes	129 (73.7)	1.23 (0.88-1.72)		63 (67.0)	0.88 (0.56-1.36)	
aGvHD: yes	43 (24.6)	1.06 (0.76-1.48)		22 (23.4)	0.88 (0.54-1.45)	
Grade II/IV aGvHD: yes	33 (18.9)	1.42 (0.96-2.1)		13 (13.8)	0.91 (0.49-1.67)	
GVHD post-DLI: yes	6 (3.4)	**0.27 (0.13-0.59)*****	**0.37 (0.16-0.86)***	13 (14.0)	**2.03 (1.14-3.62)***	
Post relapse therapy including DLI: yes	54 (30.9)	**0.43 (0.31-0.59)*****	**0.52 (0.37-0.73)*****	52 (55.3)	**1.71 (1.13-2.58)***	**1.55 (1.00-2.40)***

Abbreviations: aGVHD = acute graft versus host disease; AML = acute myeloid leukemia; BM = bone marrow; CHT = chemotherapy; CR = complete remission; DLI = donor lymphocytes infusion; MDS = myelodysplastic syndrome; MMUD = mismatched unrelated donor; MUD = matched unrelated donor; PBSC = peripheral blood stem cells; SCT = stem cell transplantation.
[§] Reference categories: HIGH (disease burden), AML (diagnosis at SCT), No-CR (disease status at SCT), >1 (Lines of therapy before SCT), Sibling (Donor type), BM (Stem Cells' source), no (other variables).
*** P<0.05,
** P<0.01,
* P<0.001.

ranged from 19% to 38%, strongly dependent from time to relapse and disease status at second transplant.[29] In general, second transplant performed better than other treatments not including this procedure[23] but the outcome after DLI or second allo-SCT was superimposable,[28,30] even if selection biases claim caution in the interpretation of these data.

The major limit of our study is that it is a retrospective-registry analysis, including patients with differences in disease biology, treatment eligibility, or time-dependent biases. Moreover, a lot of data has been collected following specific queries, thus limiting the availability of more detailed analysis. This is particularly relevant in the case of the biological characterization of the disease at diagnosis that is not included in this analysis. In the meantime, the multicentric nature of this study and the relatively large number of patients included make the results quite strong and reliable. Eventually, another limitation of the study occurred as therapy-related variables were included in the multivariate analysis as fixed covariates. This approach may introduce immortal time bias, since interventions such as DLI administration or receipt of a second allo-SCT are not baseline characteristics. Patients must survive long enough to receive these treatments, which may artificially inflate their apparent association with overall survival.

In summary, although our findings confirm the overall poor prognosis of AML/MDS patients relapsing after allo-SCT, they also suggest that the combination of DLI with post-relapse therapy may further improve both OS and RM. Novel cellular therapies are currently being developed to reduce DLI-related toxicity while preserving, or even enhancing, their antileukemic potential.[24] While research into the biological mechanisms underlying AML/MDS relapse continues[31] (aiming to identify new therapeutic targets), there is growing consensus on the need to shift away from the traditional *watch-and-wait* approach toward a modern, preemptive treatment strategy. This includes the early use of molecularly targeted agents and DLI at the first evidence of disease relapse.

Clinical Practice Points

- In real life, more than 80% of AML/MDS patients relapsing after allo-SCT are treated at the time of hematological relapse and not in a preemptive setting (eg, minimal residual disease positivity or molecular mixed chimerism).
- HMA–based therapy is used in approximately 50% of the cases, and clinicians' choice is to deserve this treatment to a subgroup of patients with an aggressive disease and at very high risk of complications (eg, older patients, transplanted not in CR).
- Although the ORR and the CR rate observed following HMA-based therapy are lower than that observed with other treatments (including intensive chemotherapy and FLT3 inhibitors), the overall survival and the treatment-related mortality are comparable.

Table 5 Univariable and Multivariable Analysis on RM and TRM for the 339 Patients Treated Without HMAs ± VEN

Variables[§]	RM ($n = 161$)			TRM ($n = 101$)		
	Mean (SD) or *n* (%)	Univariable SHR (95% CI)	Multivariable SHR (95% CI)	Mean (SD) or *n* (%)	Univariable SHR (95% CI)	Multivariable SHR (95% CI)
Age at the baseline	52.0 (12.0)	1.02 (1.00-1.03)		50.6 (11.7)	1.00 (0.99-1.02)	
Interval SCT-relapse >12 months	26 (16.1)	**0.37 (0.24-0.57)*****	**0.36 (0.24-0.55)*****	30 (29.7)	1.17 (0.79-1.73)	
Disease burden at relapse: LOW	11 (6.8)	**0.26 (0.12-0.57)*****	**0.28 (0.13-0.59)****	12 (11.9)	0.64 (0.34-1.21)	
Diagnosis at SCT: MDS	19 (11.8)	1.86 (1.09-3.16)		6 (5.9)	0.72 (0.28-1.85)	
Disease status at SCT: CR	105 (65.2)	**0.58 (0.41-0.82)****	**0.61 (0.42-0.87)****	74 (73.3)	1.15 (0.73-1.83)	
Lines of therapy before SCT: 1	73 (52.1)	0.83 (0.59-1.16)		52 (52.0)	0.89 (0.61-1.33)	
Donor type: MUD/MMUD	79 (49.4)	0.96 (0.68-1.34)		48 (47.5)	0.93 (0.63-1.37)	
Donor type: Haploidentical	38 (23.8)	1.46 (0.98-2.15)		13 (12.9)	0.56 (0.31-1.02)	
Stem Cells' source: PBSC	130 (80.7)	1.39 (0.94-2.06)		71 (70.3)	0.78 (0.51-1.19)	
Myeloablative conditioning: yes	125 (77.6)	0.78 (0.52-1.16)		76 (75.2)	0.85 (0.55-1.32)	
aGvHD: yes	44 (27.3)	0.84 (0.57-1.24)		29 (28.7)	1.10 (0.72-1.68)	
Grade II/IV aGvHD: yes	25 (15.5)	1.02 (0.65-1.59)		22 (21.8)	1.53 (0.95-2.47)	
GVHD post DLI: yes	10 (6.5)	0.57 (0.30-1.09)		14 (14.1)	1.40 (0.82-2.42)	
Post-relapse therapy, including DLI: yes	38 (23.6)	**0.51 (0.34-0.77)****	**0.60 (0.40-0.91)***	41 (40.6)	1.46 (0.98-2.17)	
Therapy: FLT3-inhibitors	30 (18.6)	0.80 (0.53-1.20)		19 (18.8)	0.75 (0.45-1.23)	
Therapy: Second allo-SCT	3 (1.9)	**0.29 (0.09-0.94)***	**0.24 (0.07-0.74)***	11 (10.9)	1.80 (0.90-3.61)	

Abbreviations: aGVHD = acute graft versus host disease; AML = acute myeloid leukemia; BM = bone marrow; cGVHD = chronic graft versus host disease; CR = complete remission; DLI = donor lymphocytes infusion; MDS = myelodysplastic syndrome; MMUD = mismatched unrelated donor; MUD = matched unrelated donor; PBSC = peripheral blood stem cells; SCT = stem cell transplantation; UCB = umbilical cord-blood.

[§] Reference categories: HIGH (disease burden), AML (diagnosis at SCT), No-CR (disease status at SCT), >1 (Lines of therapy before SCT), Sibling (Donor type), BM (Stem Cells' source), no (other variables).

*** $P<0.05$,

** $P<0.01$,

* $P<0.001$.

- The SCT-relapse interval, the disease burden at relapse, and the CR status at SCT confirm their independent strong prognostic impact, irrespective of the salvage therapy.
- An advantage in OS was observed when DLI was included, irrespective of the salvage therapy.
- Only a minority of patients are submitted to second allo-SCT after relapse; this is probably the result of a combination of poor clinical conditions and resistant disease following relapse and salvage therapy.

Data Availability

Data is available upon request.

Disclosures

The authors have stated that they have no conflicts of interest.

CRediT authorship contribution statement

Michele Malagola: Writing – original draft, Data curation, Conceptualization. **Domenica Matranga:** Writing – original draft, Formal analysis. **Luca Castagna:** Writing – original draft, Data curation, Conceptualization. **Daniele Avenoso:** Writing – review & editing. **Vera Radici:** Writing – review & editing. **Mirko Farina:** Writing – review & editing. **Marco Galli:** Writing – review & editing, Data curation. **Eugenia Accorsi Buttini:** Writing – review & editing. **Caterina Alati:** Writing – review & editing. **Simona Bassi:** Writing – review & editing. **Alessandra Biffi:** Writing – review & editing. **Carlo Borghero:** Writing – review & editing. **Alessandro Busca:** Writing – review & editing. **Chiara Nozzoli:** Writing – review & editing. **Angelo Michele Carella:** Writing – review & editing. **Irene Maria Cavattoni:** Writing – review & editing. **Raffaella Cerretti:** Writing – review & editing. **Patrizia Chiusolo:** Writing – review & editing. **Michele Cimminiello:** Writing – review & editing. **Angela Cuoghi:** Writing – review & editing. **Marco De Gobbi:** Writing – review & editing. **Federico Vincenzo:** Writing – review & editing. **Piero Galieni:** Writing – review & editing. **Anna Paola Iori:** Writing – review & editing. **Matteo Parma:** Writing – review & editing. **Francesca Patriarca:** Writing – review & editing. **Vincenzo Pavone:** Writing – review & editing. **Alessandra Picardi:** Writing – review & editing. **Eugenia Piras:** Writing – review & editing. **Nicola Polverelli:** Writing – review & editing. **Lucia Prezioso:** Writing – review & editing. **Benedetta Rambaldi:** Writing – review & editing.

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Supplemental Table

Supplemental Table 1 .

Supplemental Table 1 Clinical and transplant characteristics of the 647 patients according to the type of post-relapse treatment and the inclusion or not of DLI

	With HMA/venetoclax & DLI	With HMA/venetoclax & no DLI	Without HMA/venetoclax & DLI	Without HMA/venetoclax & no DLI	Total	P-value
	(N=132)	(N=176)	(N=111)	(N=228)	(N=647)	
Diagnosis						
AML	124 (93.9%)	153 (86.9%)	103 (92.8%)	207 (90.8%)	587 (90.7%)	0,158
MDS	8 (6.1%)	23 (13.1%)	8 (7.2%)	21 (9.2%)	60 (9.3%)	
Age at diagnosis						
Mean (SD)	58.0 (12.7)	59.5 (11.1)	57.3 (11.7)	55.8 (12.4)	57.5 (12.1)	**0,028**
Age at SCT						
Mean (SD)	52.4 (12.7)	54.4 (10.8)	51.4 (11.9)	50.1 (12.4)	52.0 (12.0)	**0,005**
Follow Up from relapse (months)						
Median [Min, Max]	20.3 [3.85, 99.0]	12.9 [1.32, 73.4]	24.3 [3.39, 98.5]	13.9 [2.47, 104]	16.1 [1.32, 104]	
Time SCT/Relapse (months)						
≤6	51 (38.6%)	89 (50.6%)	59 (53.2%)	130 (57.0%)	329 (50.9%)	**0,009**
>6	81 (61.4%)	87 (49.4%)	52 (46.8%)	98 (43.0%)	318 (49.2%)	
Time SCT/Relapse (months)						
≤12	94 (71.2%)	134 (76.1%)	80 (72.1%)	178 (78.1%)	486 (75.1%)	0,425
>12	38 (28.8%)	42 (23.9%)	31 (27.9%)	50 (21.9%)	161 (24.9%)	
Disease burden at relapse						
HIGH	109 (82.6%)	160 (90.9%)	78 (70.3%)	211 (92.5%)	558 (86.2%)	**<0.001**
LOW	23 (17.4%)	16 (9.1%)	33 (29.7%)	17 (7.5%)	89 (13.8%)	
Disease.status at SCT						
No-CR	55 (41.7%)	75 (42.6%)	33 (29.7%)	63 (27.6%)	226 (34.9%)	**0,003**
CR	77 (58.3%)	101 (57.4%)	78 (70.3%)	165 (72.4%)	421 (65.1%)	
Lines of therapy before SCT						
>1	74 (56.1%)	84 (47.7%)	48 (43.2%)	95 (41.7%)	301 (46.5%)	0,244
1	58 (43.9%)	92 (52.3%)	57 (51.4%)	114 (50.0%)	321 (49.6%)	
Missing	0 (0%)	0 (0%)	6 (5.4%)	19 (8.3%)	25 (3.9%)	
Donor						
Sibling	30 (22.7%)	50 (28.4%)	38 (34.2%)	69 (30.3%)	187 (28.9%)	0,711
MUD/MMUD	73 (55.3%)	85 (48.3%)	53 (47.7%)	112 (49.1%)	323 (49.9%)	
Haploidentical	29 (22.0%)	40 (22.7%)	20 (18.0%)	46 (20.2%)	135 (20.9%)	
UCB	0 (0%)	1 (0.6%)	0 (0%)	1 (0.4%)	2 (0.3%)	
Stem Cells' Source						
BM	35 (26.5%)	30 (17.0%)	23 (20.7%)	54 (23.7%)	142 (21.9%)	0,324
PBSC	97 (73.5%)	144 (81.8%)	88 (79.3%)	172 (75.4%)	501 (77.4%)	
UCB	0 (0%)	2 (1.1%)	0 (0%)	2 (0.9%)	4 (0.6%)	
Myeloablative Conditioning						
NO	37 (28.0%)	54 (30.7%)	23 (20.7%)	50 (21.9%)	164 (25.3%)	0,121
YES	95 (72.0%)	122 (69.3%)	88 (79.3%)	178 (78.1%)	483 (74.7%)	

(*continued on next page*)

Supplemental Table 1 (*continued*)

	With HMA/venetoclax & DLI	With HMA/venetoclax & no DLI	Without HMA/venetoclax & DLI	Without HMA/venetoclax & no DLI	Total	P-value
	(N=132)	(N=176)	(N=111)	(N=228)	(N=647)	
aGVHD (any time after SCT)						
NO	93 (70.5%)	92 (52.3%)	61 (55.0%)	127 (55.7%)	373 (57.7%)	**0,004**
YES	25 (18.9%)	48 (27.3%)	25 (22.5%)	71 (31.1%)	169 (26.1%)	
aGVHD grade 3 – 4	14 (10.6%)	36 (20.5%)	25 (22.5%)	30 (13.2%)	105 (16.2%)	
GVHD post DLI						
NO	103 (78.0%)	176 (100%)	64 (57.7%)	228 (100%)	571 (88.3%)	**<0.001**
YES	25 (18.9%)	0 (0%)	33 (29.7%)	0 (0%)	58 (9.0%)	
Missing	4 (3.0%)	0 (0%)	14 (12.6%)	0 (0%)	18 (2.8%)	

AML=Acute Myeloid Leukemia; MDS=Myelodysplastic Syndrome; SCT=Stem Cell Transplantation; CR=complete remission; MUD=matched unrelated donor; MMUD=mismatched unrelated donor; BM=bone marrow; PBSC=peripheral blood stem cells; UCB=umbilical cord-blood; MAC=myeloablative conditioning; aGVHD=acute graft versus host disease; cGVHD=chronic graft versus host disease.