Outcomes and genetic dynamics of acute myeloid leukemia at first relapse

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Abstract

Patients with relapsed acute myeloid leukemia (AML) experience dismal outcomes. We performed a comprehensive analysis of patients with relapsed AML to determine the genetic dynamics and factors predicting survival. We analyzed 875 patients with newly diagnosed AML who received intensive treatment or low-intensity treatment. Of these patients, 197 subsequently relapsed. Data were available for 164 of these patients, with a median time from complete remission/complete remission with incomplete blood count recovery to relapse of 6.5 months. Thirty-five of the 164 patients (21%) experienced relapse after allogeneic hematopoietic stem cell transplantation. At relapse, mutations in genes involved in pathway signaling tended to disappear, whereas clonal hematopoiesis-related mutations or *TP53* tended to persist. Patients with normal karyotypes tended to acquire cytogenetic abnormalities at relapse. Patients treated intensively had a higher rate of emergence of *TP53* mutations (16%), compared to patients given low-intensity treatment (1%, *P*=0.009). The overall response rates were 38% and 35% for patients treated with salvage intensive treatment or low-intensity treatment, respectively. Seventeen patients (10%) underwent allogeneic stem cell transplantation after salvage therapy. The median overall survival duration after relapse was 5.3 months, with a 1-year overall survival rate of 17.6%. Complex karyotype (hazard ratio [HR]=2.14, *P*<0.001), a *KMT2A* rearrangement (HR=3.52, *P*=0.011), time in remission <12 months (HR=1.71, *P*=0.011), and an elevated white blood cell count at relapse (HR=2.38, *P*=0.005) were independent risk factors for overall survival duration. More effective frontline and maintenance therapies are warranted to prevent relapsed AML.

Introduction

Acute myeloid leukemia (AML) is an aggressive bone marrow neoplasm that is characterized by recurrent genetic abnormalities and clonal heterogenicity.[1] Patients with AML may be given intensive treatment (IT) or low-intensity therapy (LIT), depending on their age and comorbidities.[2,3] In eligible patients, allogeneic hematopoietic stem cell transplantation (alloSCT) is usually recommended to consolidate remissions after treatment.[2,3] About half of patients aged <60 years will experience a relapse after having achieved a first complete remission (CR1). This incidence is even higher in patients aged >60 years.[3-5] There is not a standard treatment for relapsed AML, although the most accepted strategy is to induce a second complete remission (CR2) and, in eligible patients, consolidate the remission with alloSCT.[4,6] Overall, relapsed AML responds poorly to salvage treatment and portends a dismal outcome.[7,8]

Previously published reports have described the outcomes and identified factors that are predictive of survival in cohorts of patients with relapsed AML.[9-15] Breems and colleagues[9] developed a scoring system using time in remission, cytogenetic findings at diagnosis, age, and a previous transplant (either autologous or alloSCT). They stratified patients into three risk groups with different overall survival (OS) after relapse. Other groups tried to replicate the results of this analysis to identify novel risk factors. Kurosawa and colleagues[11] identified a CR2 and alloSCT after CR2 as favorable prognostic factors. Interestingly, as genetic knowledge regarding AML has expanded, other research groups also identified *FLT3*-internal tandem duplication (ITD) at diagnosis as an adverse prognostic factor in relapsed AML patients[10,12,15,16] In fact, Schlenk and colleagues[15] identified *FLT3*-ITD as an adverse risk factor,

biallelic mutation of *CEBPA* at diagnosis as a favorable risk factor, and an alloSCT after CR2 (as a time-dependent covariate) as a favorable factor. Shimizu and colleagues[14] suggested that the acquisition of cytogenetic abnormalities at relapse could be an adverse risk factor for survival. Previous studies were performed mostly using data obtained at diagnosis. However, it is known that at relapse, AML cells can acquire new genetic lesions and lose some of the genetic abnormalities that were present at diagnosis.[17,18] This is the consequence of intrinsic AML multiclonal biology, together with selective pressure caused by exposure to frontline treatment.[19-22] With the introduction of novel targeted therapies (e.g., FLT3 inhibitors and IDH1/2 inhibitors), it is expected that clones enriched with targetable mutations are less likely to persist at relapse.[23] We performed a comprehensive analysis of patients with relpased AML and available cytogenetic and molecular data at diagnosis and relapse to determine the dynamics of genetic abnormalities and identify factors that are predictive of survival at diagnosis and relapse.

Methods

Patients and response assessment

This was a single-center, retrospective study that included all patients of age 18 or greater who had been diagnosed with AML at The University of Texas MD Anderson Cancer Center from April 2017 through October 2022. The starting date was chosen because an 81-gene next-generation sequencing panel became available at our institution in 2017. Patients received therapy at the same institution, and responses were assessed according to the European LeukemiaNet (ELN) 2022 guidelines.[3] This study was approved by the MD Anderson Institutional Review Board and was conducted in accordance with the Declaration of Helsinki. The overall response rate after frontline therapy was calculated as the proportion of patients achieving either a CR1 or CR1 with incomplete blood count recovery (CRi1). Patients presenting with overt hematologic AML relapse (≥5% blasts in bone marrow, reappearance of blasts in the blood, or the development of extramedullary disease) after a CR or CRi were included in the relapsed AML cohort. The overall response rate at relapse was defined as the sum of patients achieving CR2, CR2 with incomplete blood count recovery (CRi2), or a morphological leukemia-free state.

Genetic assessment

Cytogenetic analysis was performed at diagnosis and relapse using conventional karyotype banding and fluorescence *in situ* hybridization. A mutational analysis was performed at diagnosis and relapse using an 81-gene next-generation sequencing panel as previously described.[24] The sequenced genes are detailed in *Online Supplementary Table S1*. *FLT3*-ITD mutations were detected using a polymerase chain reaction-based DNA analysis. The emergence rate was calculated by dividing the number of patients who acquired the mutation or cytogenetic finding at relapse by the number of patients without that mutation or cytogenetic finding at diagnosis. The clearance rate was calculated by dividing the number of patients clearing the mutation or cytogenetic finding at relapse by the number of patients who had that mutation or cytogenetic finding at diagnosis.

Statistical methods

Baseline characteristics were analyzed using descriptive statistics. A Student *t* test and Mann-Whitney U test were used to compare continuous variables with normal and non-normal distributions, respectively. For categorical variables, the χ^2 and Fisher exact tests were used. To compare characteristics between diagnosis and relapse, a paired-sample approach was used with the McNemar test. The median follow-up time was calculated with a Kaplan-Meier estimate of potential follow-up.[25] The OS duration was calculated from diagnosis to death from any cause. The event-free survival (EFS) duration was calculated from diagnosis to treatment failure, relapse, or death. No response to induction or death during induction was considered as an event at day 1 of treatment. Patients alive but not evaluable for response to treatment were censored at day 1 of treatment. The OS and EFS distributions were estimated with the Kaplan-Meier method and compared with the log-rank test. Univariate and multivariate analyses were performed using Cox proportional hazards regression, and the proportional hazard assumption was checked with Schoenfeld residuals (*Online Supplementary Figures S1, S2*). The 'adjustedCurves' package was used to calculate adjusted survival in the multivariate analysis.[26] All statistical analyses were performed using R statistics version 4.2.2 (R core Team, R Foundation for Statistical Computing, Vienna, Austria).

Results

Baseline characteristics and outcomes

We analyzed a total of 875 patients who had been diagnosed with AML. The patients' median age was 65 years (range, 18-94 years) and 468 (54%) were male. The patients' baseline characteristics are detailed in *Online Supplementary Table S2*. According to the ELN 2022 classification, 175 (21%), 199 (24%), and 470 (56%) were in the favorable, intermediate, and adverse risk groups, respectively. Mutations of the entire cohort at diagnosis are detailed in *Online Supplementary Figures S3, S4*). Three hundred forty-eight (40%) patients were treated with IT (N=144 with the addition of venetoclax, 41%). Five hundred twenty-seven (60%) patients were treated with LIT (N=379 with the addition of venetoclax, 72%). One hundred one (12%) patients received a concomitant FLT3 inhibitor, 22 (3%) received an IDH1/2

inhibitor, 62 (7%) received gemtuzumab-ozogamicin (GO), and 74 (9%) received an immune checkpoint inhibitor.

The median follow-up time for the entire cohort was 25 months (95% confidence interval [95% CI]: 23-28). Most patients (N=637 [73%]) achieved a CR/CRi, while 166 (19%) did not achieve a CR/CRi and 72 (8%) died before being evaluated for response. AlloSCT in first CR or CRi was performed in 201 patients (32% of all patients achieving a CR/CRi). At the end of the follow-up period, 337 patients were alive and in remission (53% of all patients achieving a CR or CRi) (Figure 1). The median OS duration was 16.3 months, with 1- and 2-year OS rates of 58% and 42%, respectively. The median EFS duration was 11.9 months, with 1- and 2-year EFS rates of 50% and 37%, respectively. The median OS of patients treated with IT was longer than that of patients treated with LIT (52.6 *vs*. 10.8 months, *P*<0.001). When comparing groups by age, the median OS was 52.6 months for patients aged <60 years *vs*. 12.4 months for patients aged ≥60 years. The median OS and median EFS were not achieved and not achieved, 24.1 and 18 months, and 11.1 and 7.8 months for patients in the favorable, intermediate, and adverse ELN 2022 risk groups, respectively (*P*<0.001 for both OS and EFS) (*Online Supplementary Figure S5*).

First relapse

Among all patients analyzed, 197 experienced disease relapse after a CR or CRi (31% of all patients achieving a CR or CRi). Data regarding relapse characteristics and treatment were available for the 164 relapsed AML patients analyzed in this study. The baseline characteristics at diagnosis and relapse in the relapsed AML cohort are detailed in Table 1. The median age at relapse was 67 years (range, 21-95 years), and 84 (51%) were male. At diagnosis, 24 (15%), 26 (14%), and 110 (67%) were classified as favorable, intermediate, and adverse risk, according to the ELN 2022 classification. Among the cohort of relapsed AML patients, 57 (35%) were treated at diagnosis with IT (16 [10%] with venetoclax) and 107 (65%) with LIT. Among patients treated with LIT, 25 (15%) received low-dose chemotherapy (low-dose cytarabine and cladribine, N=9 with venetoclax, 36%), 81 received hypomethylating agents (57 with venetoclax, 70%), and one received ivosidenib with venetoclax. Along with frontline treatment, 22 patients (13%) received FLT3 inhibitors, six (4%) received IDH1/2 inhibitors, six (4%) received GO, and 16 (10%) received an immune checkpoint inhibitor in the setting of a clinical trial. Fifty-one patients (89%) treated with IT achieved a CR1, and six (11%) achieved a CRi; 49 (86%) achieved their best response after the first cycle of treatment. Sixty-five patients (61%) treated with LIT achieved a CR1 and 42 (39%) achieved a CRi; 68 (64%) achieved their best response after the first cycle of treatment. After achieving a CR1, 35 patients (21%) underwent alloSCT (20 after IT, 15 after LIT). The median time from best response to relapse was 6.4 months (range, 0.8-47.8 months), being 7.5 months (range, 0.9-35.3 months) for patients treated with IT and 6.1 (range, 0.8-47.8 months) for those treated with LIT (*P*=0.7) (*Online Supplementary Figure S6*).

Cytogenetic and mutation dynamics

We compared the proportion of mutations and cytogenetic findings between the cohort of all patients at diagnosis

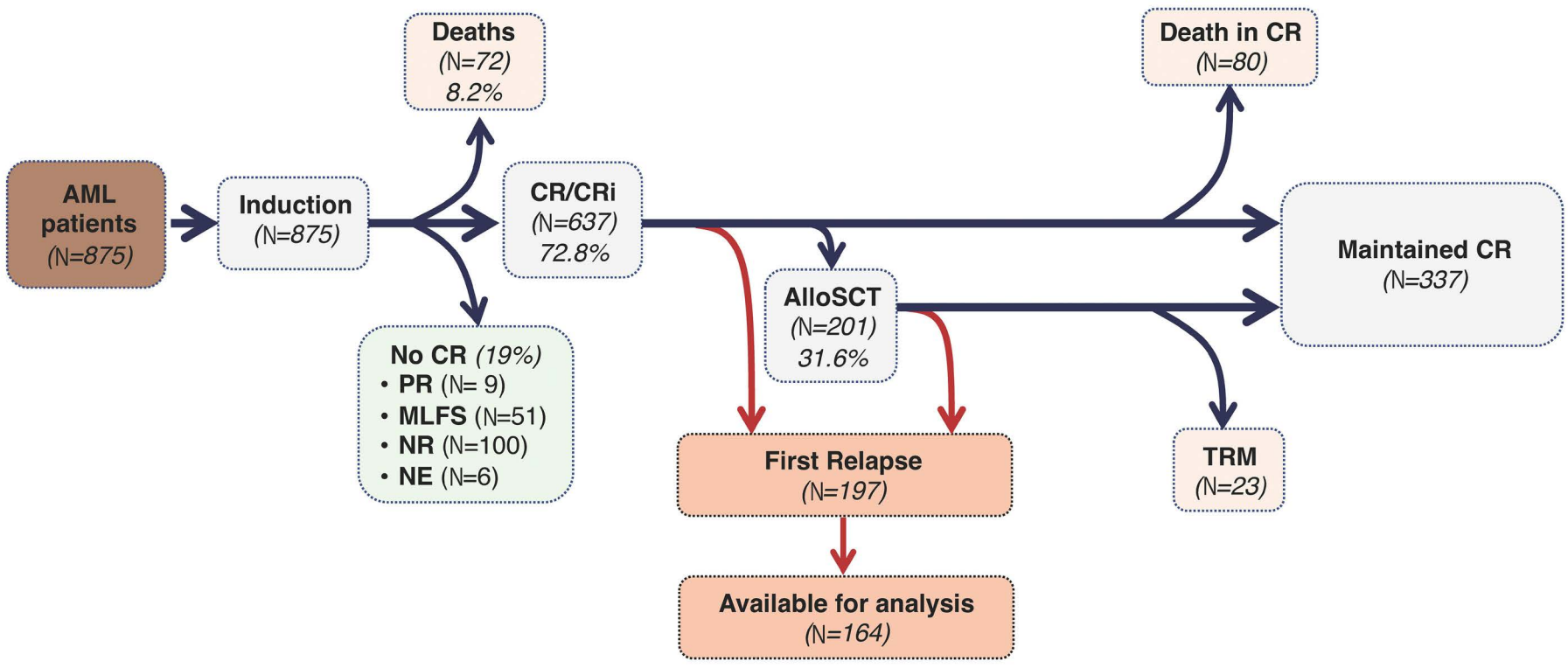


Figure 1. Patients' disposition in the study. Disposition of the entire cohort of patients with acute myeloid leukemia. AML: acute myeloid leukemia; CR: complete remission; PR: partial response; MLFS: morphological leukemia-free status; NR; no response; NE: not evaluable; CRi: CR with incomplete hematologic recovery; AlloSCT: allogeneic stem cell transplantation; TRM: transplant-related mortality.

and the cohort of relapsed AML patients (Figure 2, *Online Supplementary Figure S7*). The most frequent mutations at diagnosis were *DNMT3A* (N=196 [23%]), *TP53* (N=183 [22%]) and *NPM1* (N=175 [21%]). The most frequent mutations at relapse were *DNMT3A* (N=55 [35%], *P*=0.002), *TP53* (N=55 [35%], *P*<0.001) and *TET2* (N=37 [24%] *vs*. N=128 [15%] at diagnosis, *P*=0.01). Other significant differences in mutation rate between diagnosis and relapse were found in *RUNX1*

Table 1. Baseline characteristics of patients with relapsed acute myeloid leukemia.

	rAML at diagnosis, N=164	rAML at relapse, N=164	*P*
Age in years, median (range)	67 (20-94)	67 (21-95)	<0.001
Male sex, N (%)	86 (52)		-
Race/ethnicity, N (%) White Black Asian Other	117 (71) 19 (12) 7 (4) 21 (13)		-
Hemoglobin, g/L, median (range)	8.9 (7.1-14.3)	9 (5.5-13.7)	0.016
WBC count, x10^9 cells/L, median (range)	3 (0.3-81.1)	1.8 (0.2-141.2)	0.545
Neutrophil count, x10^9 cells/L, median (range)	0.5 (0-15.1)	0.7 (0-13.5)	0.675
Platelet count, x10^9 cells/L, median (range)	30 (1-574)	38 (0-271)	0.904
Bone marrow blasts, %, median (range)	45 (2-97)	19 (6-92)	<0.001
Cytogenetics, N (%)			
Normal	55 (34)	41 (23)	0.066
Chr 5q abn/-5	47 (29)	46 (28)	0.999
Chr 7 abn/-7	42 (26)	48 (29)	0.061
Chr 17p abn/-17	31 (19)	33 (20)	0.289
t(8;21)	2 (1)	2 (1)	NA
inv(16)/t(16;16)	6 (4)	6 (4)	NA
t(6;9)	2 (1)	2 (1)	NA
MECOM-r	7 (4)	6 (4)	0.999
KMT2A-r	5 (3)	5 (3)	NA
Complex	51 (31)	55 (34)	<0.001
ELN 2022 group, N (%)			
Favorable	24 (15)	24 (15)	0.752
Intermediate	26 (16)	23 (14)	0.999
Adverse	110 (67)	110 (67)	0.999
Not classifiable	4 (2)	7 (4)	-
Therapy-related, N (%)	37 (23)		-
Mutations, N (%)			
ASXL1	20 (12)	24 (15)	0.131
BCOR	9 (6)	9 (6)	0.999
BCORL1	3 (2)	5 (3)	0.683
DNMT3A	51 (31)	55 (34)	0.267
EZH2	4 (2)	7 (4)	0.450
FLT3-ITD	24 (15)	16 (10)	0.118
IDH1	12 (7)	12 (7)	0.999
IDH2	20 (12)	20 (12)	0.999
NPM1	27 (17)	24 (15)	0.134
PTPN11	8 (5)	5 (3)	0.617
RUNX1	30 (18)	31 (19)	0.999
SRSF2	27 (17)	27 (17)	0.999
TET2	29 (18)	37 (23)	0.016
TP53	52 (32)	55 (34)	0.114
U2AF1	11 (7)	10 (6)	0.999
WT1	9 (6)	12 (7)	0.182
ZRSR2	2 (1)	2 (1)	0.999

rAML: relapsed acute myeloid leukemia; WBC: white blood cells; NA: not applicable; *MECOM*-r: *MECOM*-rearranged; *KMT2A*-r: *KMT2A*-rearranged; ELN: European LeukemiaNet; ITD: internal tandem duplication.

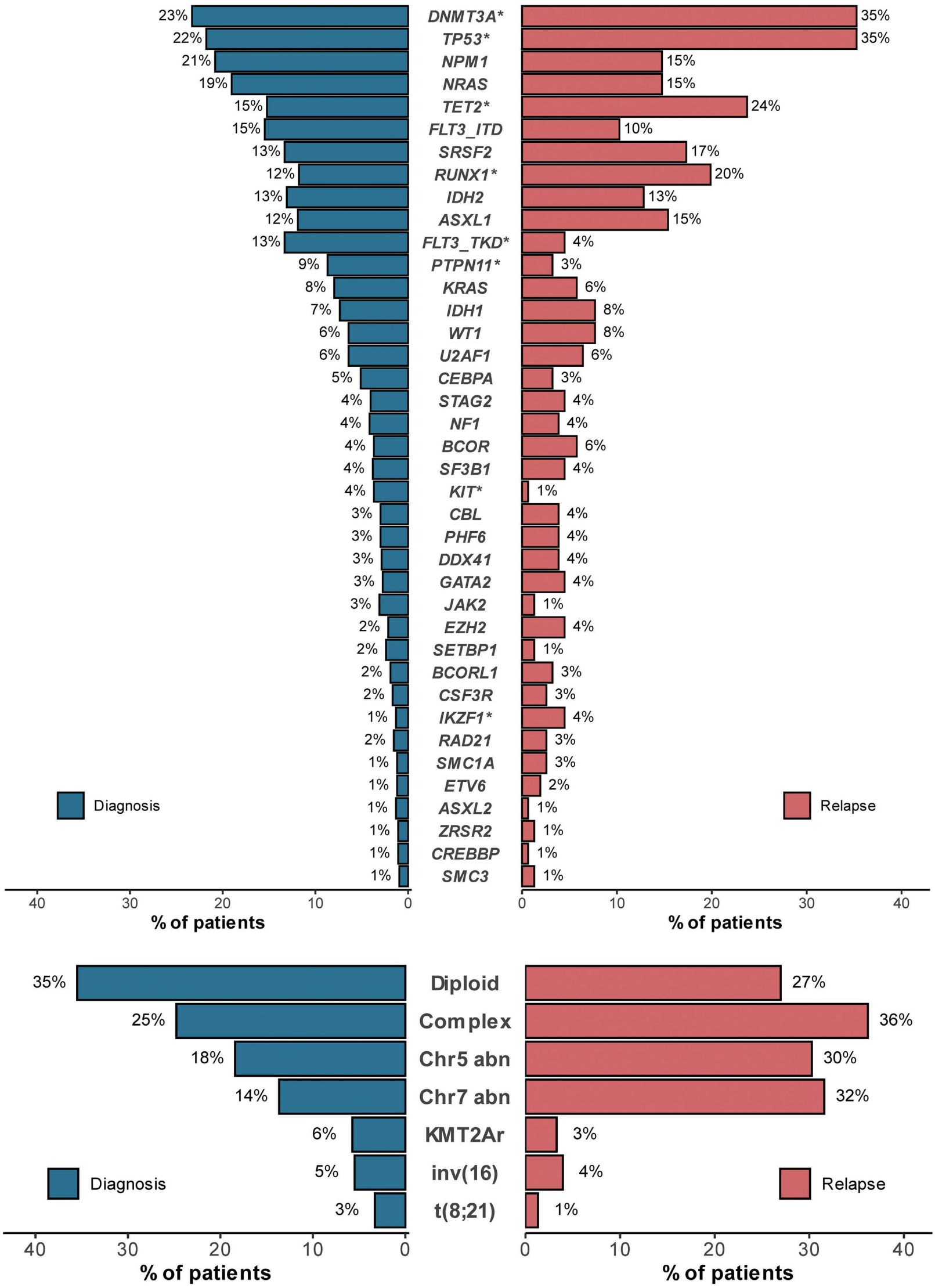


Figure 2. Frequency of mutations and cytogenetic findings in all patients at diagnosis *versus* at relapse. An asterisk specifies genes whose proportions changed significantly. Abn: abnormality. KMT2Ar: KMT2A rearrangement.

(N=99 [12%] *vs*. N=31 [20%], *P*=0.008), *FLT3*-tyrosine kinase domain (TKD) (N=112 [13%] *vs*. N=7 [5%], *P*=0.003), *PTPN11* (N=73 [9%] *vs*. N=5 [3%], *P*=0.03), *IKZF1* (N=11 [1%] *vs*. N=7 [5%], *P*=0.02) and *KIT* (N=31 [4%] *vs*. N=1 [1%], *P*=0.04). The most frequent cytogenetic findings at diagnosis and relapse were a normal karyotype (N=291 [36%] *vs*. N=41 [27%], *P*=0.05), a complex/monosomal karyotype (N=208 [25%] *vs*. N=55 [36%], *P*=0.008), chromosome 5 abnormalities (N=151 [18%] *vs*. N=46 [30%], *P*=0.001) and chromosome 7 abnormalities (N=112 [14%] *vs*. N=48 [32%], *P*<0.001).

We compared cytogenetic and molecular findings at diagnosis and relapse among patients within the relapsed AML cohort (Table 1, *Online Supplementary Figure S8*). The median number of mutations at diagnosis and relapse were 3 (range, 1-12) and 3 (range, 1-14), respectively (*P*=0.07). At diagnosis, *TP53* (N=52 [32%]), *DNMT3A* (N=51 [31%]) and *RUNX1* (N=30 [18%]) were the most frequent mutations, and a normal and complex/monosomal karyotype were present in 55 (34%) and 51 (31%) patients, respectively. Additional comparisons between patients receiving IT and those receiving LIT are detailed in *Online Supplementary Figure S9*. A matched-pairs comparison between diagnosis and relapse showed significant differences in the proportion of *TET2* mutations (N=29 [18%] at diagnosis *vs*. N=37 [23%] at relapse, *P*=0.01) and complex/monosomal karyotype (N=51 [31%] at diagnosis *vs*. N=55 [34%] at relapse, *P*<0.001). In patients treated with IT there were significant differences in the proportion of chromosome 7 abnormalities (N=4 [8%] at diagnosis *vs*. N=10 [20%] at relapse, *P*=0.04). The proportions of core binding factor, t(6;9) and *KMT2A* rearrangements remained unchanged between diagnosis and relapse.

We analyzed the dynamics of mutations and cytogenetic findings between diagnosis and relapse (Figure 3). The clearance rates were significantly higher for *FLT3*-ITD (14/24, 58%), *FLT3*-TKD (11/15, 73%), *NF1* (6/10, 60%) and *KIT* (3/4, 75%), compared to those for all other mutations. Normal karyotype also showed a significantly higher conversion rate (14/49, 29%), indicating that 29% patients with diploid cytogenetics acquired new cytogenetic abnormalities at relapse. On the other hand, *DNMT3A* (4/50, 8%), *SRSF2* (1/27, 4%), *TET2* (1/28, 4%) and *TP53* (2/49, 4%) had significantly lower clearance rates. At relapse, the mutations with a significantly high emergence rate were *ASXL1* (6/137, 4%), *DNMT3A* (9/106, 9%), *EZH2* (5/152, 3%), *FLT3*-ITD (6/132, 5%), *NRAS* (5/130, 4%), *RUNX1* (5/126, 4%), *TET2* (10/128, 8%), *TP53* (8/107, 8%) and *WT1* (7/149, 5%). Chromosome 7 abnormalities (11/110, 10%) and complex karyotype (6/100, 6%) had a significantly higher emergence rate at relapse, compared to other cytogenetic abnormalities. When comparing patients by treatment received, the emergence rate of *TP53* was significantly higher in patients treated with IT (7/45, 16%) than in patients treated with LIT (1/62, 2%) (*P*=0.009). Patients treated with IT also had a significantly higher rate of emergence of a diploid karyotype from previously abnormal cytogenetics (4/29, 14%), compared to patients treated with LIT (1/71, 1%) (*P*=0.02) (Figure 4, *Online Supplementary Figure S10*). Clearance and emergence rates are detailed in *Online Supplementary Table S3*. An additional analysis on patients who received FLT3 inhibitors is provided in *Online Supplementary Figure S15* and *Online Supplementary Table S5*.

In patients with normal karyotype at diagnosis (N=55), the most frequent mutations at diagnosis and relapse were *DNMT3A* (40% and 44%, respectively), *NPM1* (40% and 35%, respectively) and *TET2* (33% and 35%, respectively). At relapse, 64% maintained the normal karyotype, 18% acquired other non-specific cytogenetic abnormalities, 5% acquired chromosome 7 abnormalities, 2% acquired a complex karyotype and 11% did not have a paired karyotype at relapse (*Online Supplementary Figures S16-S18* and *Online Supplementary Table S6*).

Treatment responses and outcomes after relapse

At relapse, 32 (20%) patients underwent salvage IT (N=9 [6%] and N=23 [14%], with and without venetoclax, respectively), and 132 (80%) underwent salvage LIT (N=68 [41%] and N=64 [40%], with and without venetoclax, respectively). Additionally, 18 patients (11%) received FLT3 inhibitors, 18 (11%) received IDH1/2 inhibitors, 12 (7%) received GO, and 33 (20%) received non-GO AML-directed immunotherapy. Salvage treatments are detailed in *Online Supplementary Table S4*. The overall response rates of patients treated with IT were 38% (12 of 32) overall and 44% and 35% for patients with and without additional venetoclax, respectively (*P*=0.69). The overall response rate of patients treated with LIT was 35% (46 of 132). In patients receiving LIT chemotherapy (either low-dose chemotherapy or hypomethylating agents), the overall response rates were 40.4% (40 of 99) overall and 28.6% and 46.9% for patients without and with the addition of venetoclax, respectively (*P*=0.09) (Table 2). Seventeen patients (10%) proceeded to alloSCT (N=7 [41%] received a second alloSCT).

The median OS after relapse in the entire cohort was 5.3 months, with 1- and 2-year OS rates of 18% and 7%, respectively (Figure 5). There were no differences in median OS duration when comparing patients by age at relapse (6.5 *vs*. 5.1 months for patients <60 and ≥60 years old, respectively; *P*=0.11) or type of therapy received at diagnosis (6.6 *vs*. 4.9 months for patients treated with IT and LIT, respectively; *P*=0.065). Patients with a time from CR to relapse <12 months had a shorter median OS than those with a time from CR to relapse >12 months (4.3 *vs*. 8.1 months, respectively; *P*=0.002).

The univariate analysis of OS duration is detailed in *Online Supplementary Figures S11-S13*. The multivariate analysis highlighted a white blood cell count >20x10^9/L (hazard ratio [HR]=2.04, 95% CI: 1.08-3.85; *P*=0.028), time in remission <12 months (HR=1.63, 95% CI: 1.06-2.51; *P*=0.027), adverse cytogenetics (HR=1.81, 95% CI: 1.13-2.9; *P*=0.014),

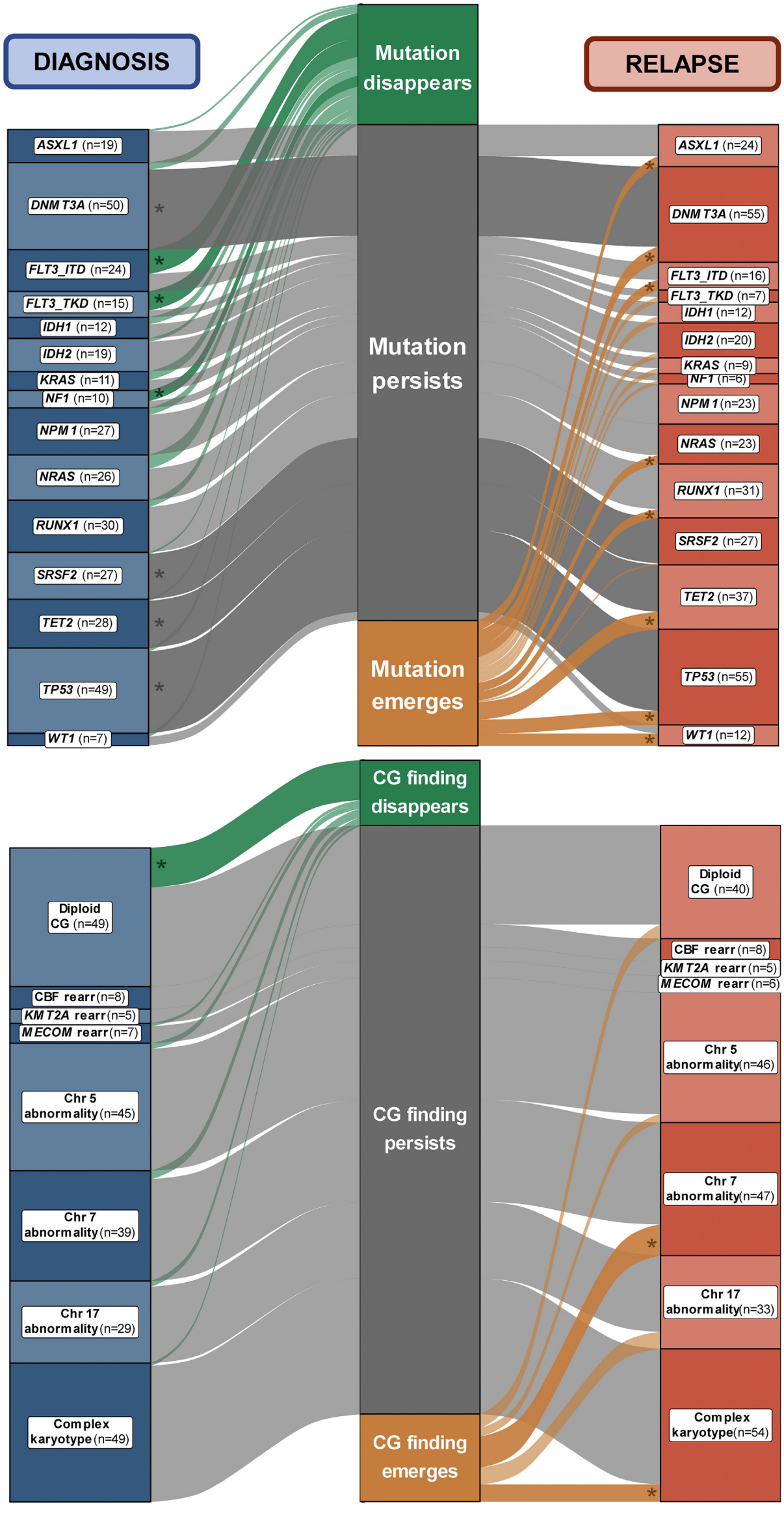


Figure 3. Mutation and cytogenetic dynamics. Genetic dynamics between diagnosis (blue) and relapse (red) in all patients with relapsed acute myeloid leukemia. Only the 20 most common mutations are represented. Asterisks highlight statistically significant changes. CG: cytogenetic.

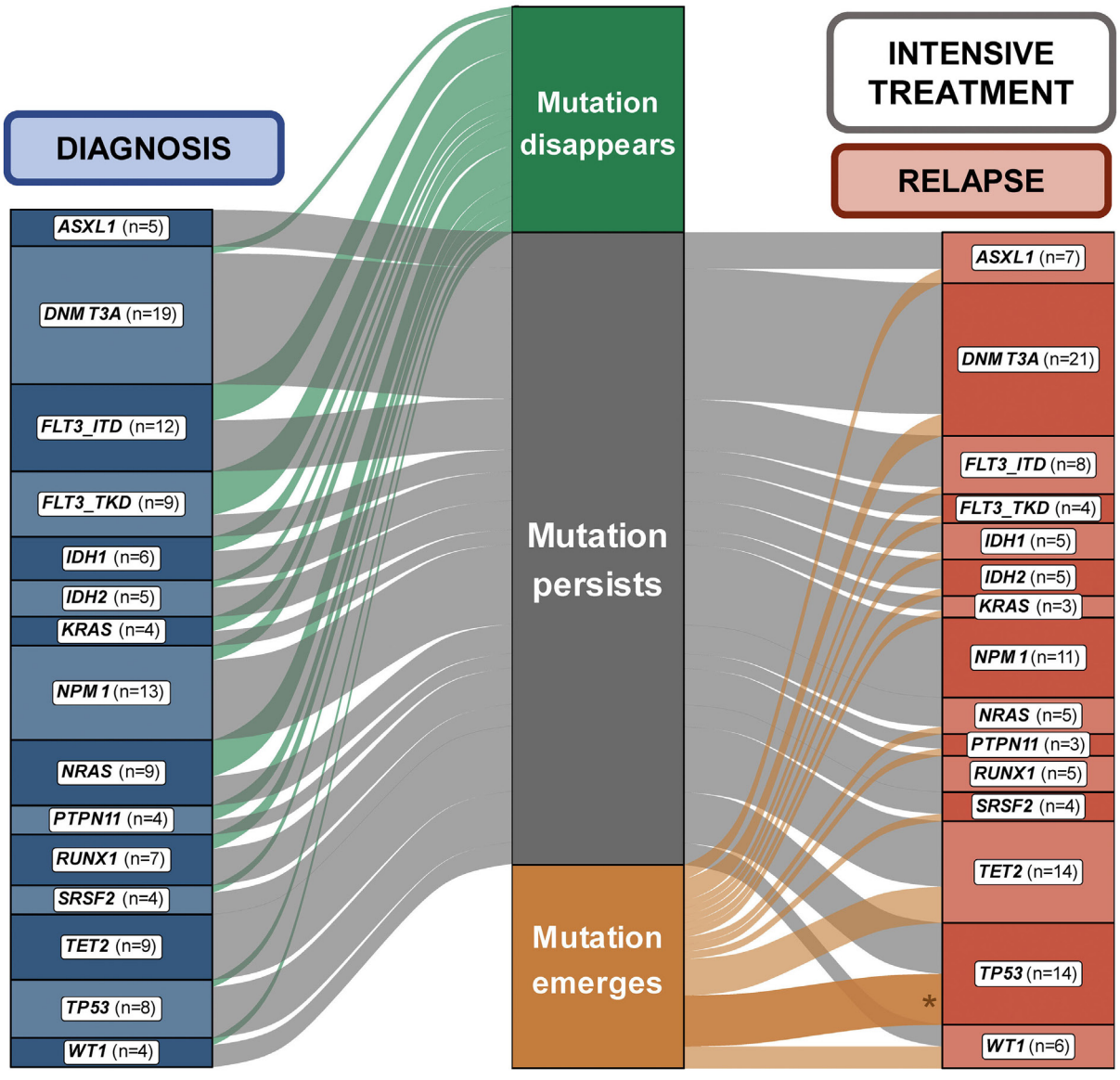


Figure 4. Mutation dynamics by therapy. Mutation dynamics between diagnosis (blue) and relapse (red) in patients with relapsed acute myeloid leukemia treated with intensive therapy and low intensity therapy. Asterisks highlight statistically significant differences between intensive therapy and low intensity therapy.

and *KMT2A* rearrangement (HR=3.74, 95% CI: 1.43-9.78; *P*=0.007) as independent prognostic factors for OS. Adverse cytogenetics was defined as a complex/monosomal karyotype or abnormalities in chromosome 5 or 7 because of their frequent co-occurrence in our cohort (*Online Supplementary Figure S14*). The multivariate analysis of OS in patients who had been previously treated with IT and LIT is detailed in Figure 6.

We applied previous prognostic classifications to our cohort of patients with relpased AML (*Online Supplementary Figures S19, S20* and *Online Supplementary Tables S7, S8*). The classification by the PETHEMA[10] and GOLEAMS[12] group stratified patients of this study into different prognostic groups with scant survival differences. This study was not intended to provide a validated prognostic score for relapsed AML. However, an exploratory analysis showed that patients with more than one risk factor identified in the multivariate analysis (time in remission <12 months, adverse cytogenetics or *KMT2A* rearrangement at relapse, and a white blood cell count >20x10^9/L at relapse) had a markedly shorter median OS (3.9 months) than that of patients with one or no risk factors (median OS=7.3 months, *P*<0.001).

Table 2. Responses to salvage therapy.

Therapy intensity	Type of therapy	Response, N (%)
IT N=32	Chemotherapy IT, N=23	CR: 2 (9) CRi: 5 (22) MLFS: 1 (4) Death: 6 (26) NR: 9 (39)
	Chemotherapy IT + Venetoclax, N=9	CRi: 3 (33) Death: 1 (11) MLFS: 1 (11) NR: 4 (44)
LIT N=132	LIT – Chemotherapy/ HMA, N=35	CR: 4 (11) CRi: 4 (11) MLFS: 2 (6) Death: 1 (3) NR: 23 (66) NE: 1 (3)
	LIT – Chemotherapy/ HMA + Venetoclax, N=64	CR: 10 (16) CRi: 14 (22) MLFS: 6 (9) Death: 7 (11) NR: 27 (42)
	Venetoclax, N=4	CR: 2 (50) MLFS: 1 (25) Death: 1 (25)
	Other, N=29	CR: 1 (3) CRi: 1 (3) MLFS: 1 (3) Death: 3 (10) NR: 23 (79)

IT: intensive therapy; LIT: low-intensity therapy; CR: complete remission; CRi: CR with incomplete hematologic recovery; MLFS: morphological leukemia-free status; NR: no response; HMA: hypomethylating agent; NE: not evaluable.

Discussion

In this study, we analyzed a large cohort of patients with newly diagnosed and relapsed AML who were treated at our institution, focusing on their clinical and biological characteristics as well as their outcomes according to treatments and genetic abnormalities. This is one of the largest retrospective studies analyzing relapsed AML predictive factors using clinical and biological data from diagnosis and relapse. Overall, our study showed very poor survival in relapsed AML irrespective of the salvage therapy received and suggests efforts should be directed to improving frontline AML treatments to avoid disease relapse. Our cohort of AML patients was generally representative of patients treated at a highly specialized cancer center. As previously reported, the most common mutations in AML are *FLT3*, *NPM1*, and *DNMT3A*, whereas *TP53* accounts for 5%-10% of mutations in patients with *de novo* AML.[27,28] However, our cohort was enriched for *TP53* mutations (23%), reflecting a higher incidence of adverse-risk patients referred to our center. This impacted AML risk stratification, in which, according to the ELN classification, adverse-risk patients accounted for 56% of all patients in this study, higher than previously reported incidences (35%-45%).[29-31] Overall, the response rate was satisfactory (73% of all patients), and more than half of patients were alive and in remission at the end of follow-up. The favorable outcomes with a lower relapse rate of 31% are likely attributed to the prioritization of maximizing initial AML therapy in these patients, namely the use of venetoclax or targeted therapies.

As expected, patients in high-risk categories (those who were older or treated with LIT) had worse OS. For these patients, it is crucial to develop treatments with high anti-leukemic efficacy and an acceptable toxicity profile to avoid both disease relapse and treatment-related death.

Despite the high remission rate, a significant proportion of patients (31%) developed disease relapse, even after high-intensity treatments as well as alloSCT. The majority of the patients with relapsed AML had AML with adverse-risk genetics according to the ELN 2022 classification (67%) at the time of initial diagnosis, although 14% had intermediate-risk and 14% favorable-risk (most with an *NPM1* mutation) AML. The outcomes after relapse were poor irrespective of salvage therapy and the biological characteristics of AML. Disease relapse occurred early after achieving a response (median time from response to relapse, 6.5 months), reinforcing the idea that most relapses occur early. This suggests a need for effective maintenance therapies that may prevent or delay disease relapse.

Changes in the mutational profile as well as chromosomal gains and losses have been previously reported, suggesting that AML subclones evolve after treatment as a result of selective treatment-related pressure.[17,32] In this study, we

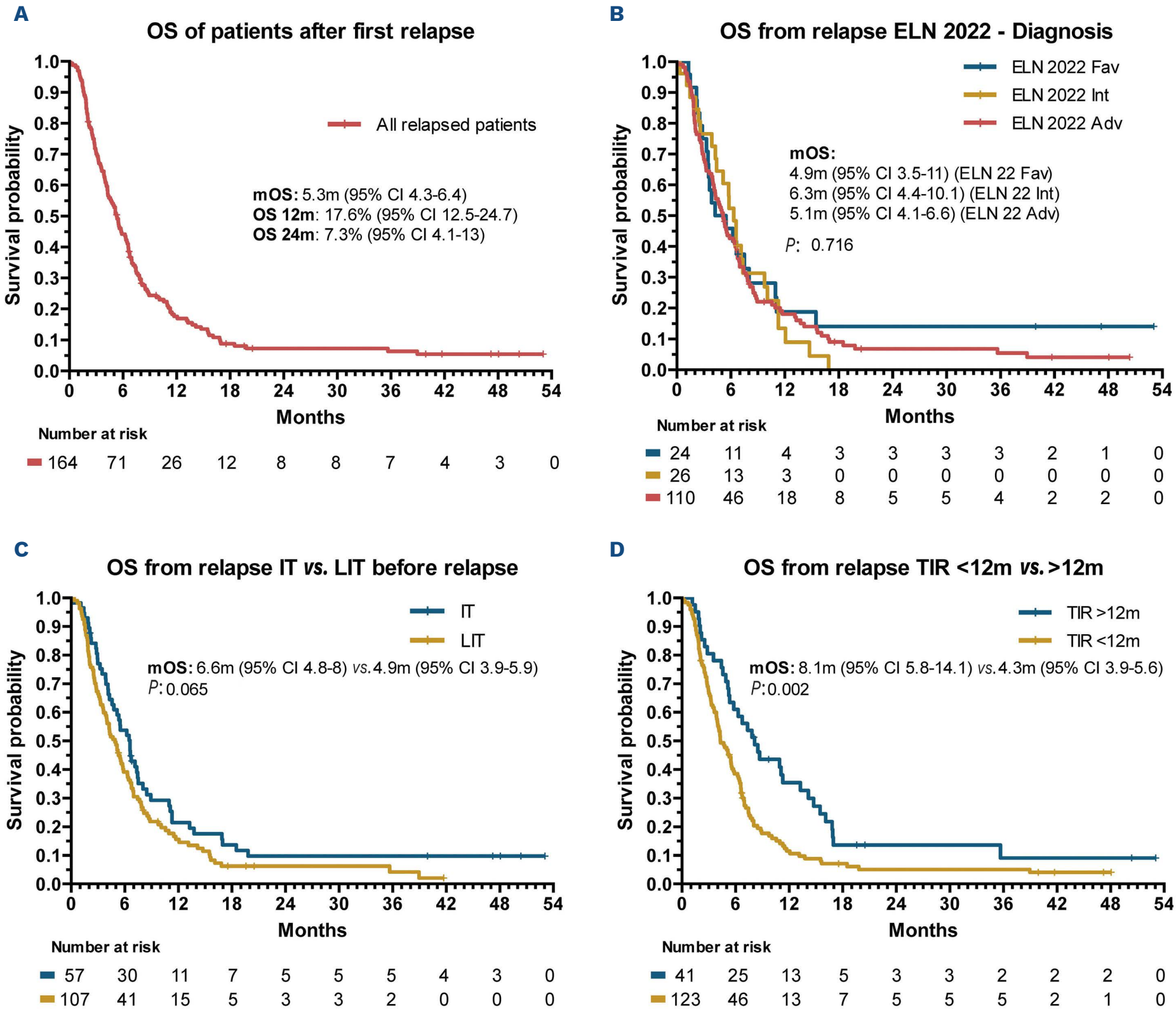
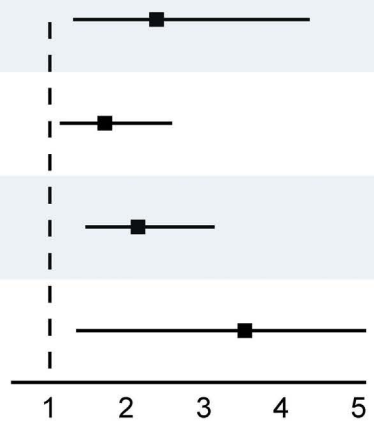


Figure 5. Overall survival after relapse. (A) Overall survival (OS) from relapse. (B) OS from relapse, stratified by the European LeukemiaNet 2022 risk classification at diagnosis (favorable, intermediate or adverse). (C) OS from relapse, stratified by frontline therapy received (intensive or low intensity). (D) OS from relapse, stratified by time in remission (>12 months or <12 months). mOS: median overall survival; m: months; 95% CI: 95% confidence interval; ELN 2022: European LeukemiaNet 2022 risk classification; Fav: favorable; Int: intermediate; Adv: adverse; IT: intensive therapy; LIT: low-intensity therapy; TIR: time in remission.

analyzed the mutational and cytogenetic profiles of the disease between diagnosis and relapse in a large cohort of patients with available paired biological data. The paired data analysis only showed a significant increase of *TET2* mutations and complex karyotype at relapse. However, when mutations and cytogenetic findings were analyzed individually, some interesting findings came to light. In line with the results of other studies, we found that some mutations in genes involved in signaling pathways (e.g., *FLT3*, *KIT*, and *NF1*) were often cleared between diagnosis and relapse, most likely representing the elimination of sensitive subclones. This effect could have also been promoted by the addition of specific inhibitors, such as FLT3 inhibitors, used in 12% of patients. Conversely, AML clonal founding mutations or clonal hematopoiesis-associated mutations (e.g., *ASXL1*, *DNMT3A*, *SRSF2*, and *TP53*) were retained at relapse. A normal karyotype was less likely to persist at relapse, likely representing the acquisition of cytogenetic abnormalities by the clones driving the disease relapse. Moreover, the rate of emergence of chromosome

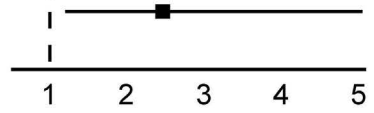
Multivariate analysis (all patients)

Variable	Total n / Events	HR (90% CI)	*P*	Adjusted mOS
WBC > 20x10^9/L	N=93, ev=116 (No) *vs.* N=14, ev=14 (Yes)	2.38 (1.3-4.36)	0.005	6.2 m (No) *vs.* 3.2 m (Yes)
TIR <12m	N=39, ev=33 (No) *vs.* N=105, ev=97 (Yes)	1.71 (1.13-2.58)	0.011	7 m (No) *vs.* 5.3 m (Yes)
AdverseCG	N=93, ev=82 (No) *vs.* N=51, ev=48 (Yes)	2.14 (1.46-3.13)	<0.001	4.2 m (Yes) *vs.* 6.7 m (No)
KMT2Ar	N=139, ev=125 (No) *vs.* N=5, ev=5 (Yes)	3.52 (1.34-9.26)	0.011	5.8 m (No) *vs.* 2.2 m (Yes)



Multivariate analysis (IT patients)

Variable	Total n / Events	HR (90% CI)	*P*	Adjusted mOS
WBC > 20x10^9/L	N=42, ev=35 (No) *vs.* N=7, ev=7 (Yes)	2.87 (1.18-6.98)	0.02	6.7 m (No) *vs.* 3.5 m (Yes)
AdverseCG	N=35, ev=29 (No) *vs.* N=14, ev=13 (Yes)	2.46 (1.2-5.04)	0.014	7.3 m (No) *vs.* 4.1 m (Yes)



Multivariate analysis (LIT patients)

Variable	Total n / Events	HR (90% CI)	*P*	Adjusted mOS
BM Blasts	N=95, ev=88	1.01 (1.01-1.02)	0.002	
TIR <12m	N=27, ev=23 (No) *vs.* N=68, ev=65 (Yes)	1.83 (1.12-2.99)	0.015	7 m (No) *vs.* 5 m (Yes)
AdverseCG	N=46, ev=41 (No) *vs.* N=49, ev=47 (Yes)	1.69 (1.09-2.61)	0.018	6.4 m (No) *vs.* 4.4 m (Yes)

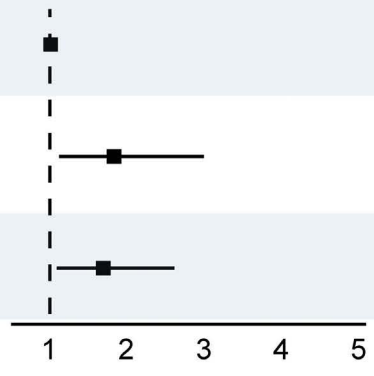


Figure 6. Multivariate analysis. Multivariate analysis and adjusted median overall survival duration of all patients, patients who received intensive therapy as frontline therapy, and patients who received low-intensity therapy as frontline therapy. HR: hazard ratio; 90% CI: 90% confidence interval; mOS: median overall survival; WBC: white blood cell count; ev: events; m: months; TIR: time in remission; CG: cytogenetics; *KMT2Ar*: *KMT2A* rearrangement; IT: intensive therapy; LIT: low-intensity therapy; BM: bone marrow.

7 abnormalities at relapse was higher than that of other cytogenetic abnormalities. We hypothesize that chromosome 7 abnormalities occur as an additional chromosome abnormality, causing a survival advantage in these cells, likely due to the loss of tumor suppressor genes such as *EZH2* and *MLL3*.[33]

The type of treatment at diagnosis impacted the genetic dynamics between diagnosis and relapse. The higher frequency of emergence of *TP53* mutations in patients in the IT group most likely represents the selection of pre-existing AML clones that are intrinsically resistant to conventional chemotherapy.[34,35] More specific studies with deeper approaches, such as single-cell analysis, are needed to provide better knowledge of cytogenetic and molecular dynamics after specific treatments.[36,37]

In this study, responses to salvage treatments were poor (overall response rates of 38% and 35% in patients treated with IT or LIT, respectively). Moreover, survival was poor, and few factors were predictive of a longer survival duration. These results differ from those of previous publications that identified some predictive factors that impact survival, such as favorable cytogenetics (e.g., core-binding factor rearrangements) and the presence of *FLT3*-ITD or a previous alloSCT.[9,10] Our relapsed AML cohort had a worse overall response rate and OS when compared to previously published series of patients with relapsed AML. This may be the result of more effective frontline therapy including high-dose cytarabine-based induction in the intensively treated patients, optimized regimens for core-binding factor-AML such as FLAG-GO (fludarabine, cytarabine, granulocyte colony-stimulating factor plus GO), use of molecularly targeted agents in the frontline setting, as well as minimal residual disease-directed preemptive therapy. This potentially selected for more resistant clones at relapse. Furthermore, we analyzed factors predicting outcome of therapy at relapse, which has not been extensively reported in previously published studies of relapsed patients.[9,10,12,15] Moreover, previous reports described mainly younger patients, most of whom were treated with IT. Therefore, our study provides important prognostic data in patients with relapsed AML treated with LIT, including venetoclax. Adverse cytogenetics at the time of relapse was consistently related to a significantly worse outcome in patients who received IT and LIT, according to univariate and multivariate analyses. Most patients with adverse cytogenetics had complex karyotypes and *TP53* mutation, which is known to be a highly resistant genotype associated with poor survival.[38]

A significant limitation of this study was the substantial heterogeneity in treatments received at diagnosis and relapse, which can influence the external validity of the results. Moreover, the number of patients who underwent alloSCT in CR1 and after relapse was limited. Another limitation is the lack of availability of cytogenetic and mutational data at the time of CR, therefore limiting the interpretation of genetic dynamics. Finally, this cohort comprised patients diagnosed from 2017 to 2022, resulting in a limited median follow-up.

In conclusion, recent years have seen improvements in the outcomes of AML patients. However, relapsed AML is still very challenging to treat, with poor outcomes regardless of the type of salvage therapy. We identified demonstrable clonal changes between diagnosis and relapse, emphasizing the importance of performing cytogenetic and molecular testing at relapse. Developing more effective frontline treatments, improving accessibility to alloSCT, as well as maintenance strategies are necessary to reduce rates of disease recurrence.

Disclosures

HK has received research funding from AbbVie, Amgen, Ascentage Pharma, BMS, Daiichi Sankyo, ImmunoGen, Jazz Pharmaceuticals, and Novartis as well as honoraria from AbbVie, Amgen, Amphista Therapeutics, Ascentage Pharma, Astellas Pharma, Biologix, Curis, Ipsen, KAHR, Novartis, Pfizer, Precision Biosciences, Shenzhen TargetRx, and Takeda Oncology. TMK has been a consultant for AbbVie, Agios, BMS, Genentech, Jazz Pharmaceuticals, Novartis, Servier, and PinotBio; has received research funding from AbbVie, BMS, Genentech, Jazz Pharmaceuticals, Pfizer, Cellenkos, Ascentage Pharma, GenFleet Therapeutics, Astellas Pharma, AstraZeneca, Amgen, Cyclacel Pharmaceuticals, Delta-Fly Pharma, Iterion Therapeutics, GlycoMimetics, and Regeneron Pharmaceuticals; and has received honoraria from Astex Pharmaceuticals. ND has received research funding from Astellas Pharma, AbbVie, Genentech, Daiichi Sankyo, Gilead Sciences, ImmunoGen, Pfizer, Bristol Myers Squibb, Trovagene, Servier, Novimmune, Incyte, Hanmi Pharm, Fate Therapeutics, Amgen, Kite Pharma, Novartis, Astex Pharmaceuticals, KAHR, Shattuck, Sobi, GlycoMimetics, and Trillium; has been an advisor for Astellas Pharma, AbbVie, Genentech, Daiichi Sankyo, Novartis, Jazz Pharmaceuticals, Amgen, Servier, Karyopharm Therapeutics, Trovagene, Trillium, Syndax, Gilead Sciences, Pfizer, Bristol Myers Squibb, Kite Pharma, Actinium Pharmaceuticals, Arog Pharmaceuticals, ImmunoGen, Arcellx, and Shattuck; has been a data monitoring committee member for Kartos Therapeutics and Jazz Pharmaceuticals; has been a consultant or board of directors or advisory committee member for Agios, Celgene, Sobi, and STAR Therapeutics; and has received research funding from Karyopham Therapeutics and Newave Pharmaceutical. CDD has been a board of directors or advisory committee member for Genmab, GSK, Kura Oncology, and Notable Labs; has received honoraria from Kura, Astellas Pharma, Bluebird Bio, Bristol Myers Squibb, Foghorn Therapeutics, Immune-Onc Therapeutics, Novartis, Takeda Oncology, Gilead Sciences, and Jazz Pharmaceuticals; is a current holder of stock options for Notable Labs; has been a consultant for AbbVie and Servier; and has received research funding from Servier, Bristol Myers Squibb, Foghorn, Immune-Onc Therapeutics, Loxo Oncology, Astex Pharmaceuticals, Cleave,

and Forma. GB has received research funding from Astex Pharmaceuticals, Ryvu Therapeutics, and PTC Therapeutics; has been a board of directors or advisory committee member for Pacyclex Pharmaceuticals, Novartis, CytomX, and Bio Ascend; and has been a consultant for Catamaran Bio, AbbVie, PPD Development, Protagonist Therapeutics, and Janssen. NJS has been a consultant for Takeda Oncology, AstraZeneca, Amgen, Novartis, and Pfizer and has received research funding from Takeda Oncology, Astellas, and Stemline Therapeutics as well as honoraria from Amgen. MY has received research funding from Daiichi-Sankyo and Pfizer. GCI has been a consultant for Novartis, Kura Oncology, and NuProbe and has received research funding from Celgene, Kura Oncology, Syndax, Merck, Cullinan, and Novartis. YA reports research funding from Jazz Pharmaceuticals, FibroGen, Sun Pharma, BerGenBio, Daiichi-Sankyo/Lilly, and Astex. AM reports support from BioSight, Sanofi, and Astex Pharmaceuticals. KT has been a consultant for SymBio Pharmaceuticals and received honoraria from Mission Bio, Illumina, and Otsuka Pharmaceutical. EJ has received research funding from Amgen, Pfizer, Abbvie, Adaptive Biotechnologies, Astex, Ascentage, and provided consultancy services for Amgen, Pfizer, Abbvie, Takeda, Adaptive Biotechnologies, Astex, Ascentage, Genentech, Novartis, BMS, Jazz Pharmaceuticals, Hikma Pharmaceuticals, and Incyte. GG-M has received research funding from Astex Pharmaceuticals, Novartis, AbbVie, BMS, Genentech, Aprea Therapeutics, Curis, and Gilead Sciences; has been a consultant for Astex Pharmaceuticals, Acceleron Pharma, and BMS; and has received honoraria from Astex Pharmaceuticals, Acceleron Pharma, AbbVie, Novartis, Gilead Sciences, Curis, Genentech, and BMS. FR has received research funding from Amgen, Astex Pharmaceuticals/Taiho Oncology, BMS/Celgene, Syos, AbbVie, Prelude, Xencor, Astellas Pharma, and Biomea Fusion as well as honoraria from Amgen, BMS/Celgene, Syos, AbbVie, and Astellas Pharma; has been a board of directors or advisory committee member for Astex Pharmaceuticals/Taiho Oncology; and has been a consultant for BMS/Celgene, Syos, Novartis, AbbVie, AstraZeneca, and Astellas Pharma. ABat, ABaz, SL, KP, GT, KS, GM-B, HAA, and SP have no conflicts of interest to disclose.

Contributions

ABat conceived the study, curated, analyzed and interpreted data, cared for patients, and wrote, reviewed and edited the manuscript. ABaz curated data, cared for patients and comprehensively reviewed and intensively edited the manuscript. TMK conceived the study, cared for patients, and reviewed and edited the manuscript. HK, ND, CDD, GB, KS, NS, MY, GCI, YA, GM-B, AM, HAA, KT, EJ, and GG-M cared for patients, and reviewed and edited the manuscript. SL and KP performed the diagnostic analysis and molecular interpretation, and reviewed and edited the manuscript. GT performed the diagnostic analysis and cytogenetic interpretation, and reviewed and edited the manuscript. SP provided and curated data and reviewed and edited the manuscript. FR conceived the study, cared for patients, and wrote, reviewed and edited the manuscript.

Data-sharing statement

The data used for this study are not publicly available in order to protect patients' confidentiality. Reasonable requests for deidentified data should be directed to the corresponding author.

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