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Outcomes and genetic dynamics of acute myeloid leukemia at first relapse

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Data availability statement

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

Conflict of interest statement

ABat has no conflict of interest to disclose. **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. **ABaz** has no conflict of interest to disclose. **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. **SL** has no conflict of interest to disclose. **KP** has no conflict of interest to disclose. **GT** has no conflict of interest to disclose. **KS** has no conflict of interest to disclose. **NJS** has been a consultant for Takeda Oncology, AstraZeneca, Amgen, Novartis, and Pfizer and 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 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. **GM-B** has no conflict of interest to disclose. **AM** reports support from BioSight, Sanofi, and Astex Pharmaceuticals. **HAA** has no conflict of interest to disclose. **KT** has been a consultant for SymBio Pharmaceuticals and received

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Authorship

ABat: Study conception, data curation, formal data analysis and interpretation, patient care, writing of the manuscript, and review and editing of the manuscript. **ABaz:** Data curation, patient care, and comprehensive review and intensive editing of the manuscript. **TMK:** Study conception, patient care and review and editing of the manuscript. **HK, ND, CDD, GB, KS, NS, MY, GCI, YA, GM-B, AM, HAA, KT, EJ, GG-M:** Patient care and review and editing of the manuscript.**:** Patient care and review and editing of the manuscript. **SL, KP:** Diagnostic analysis and molecular interpretation, and review and editing of the manuscript. **GT:** Diagnostic analysis and cytogenetic interpretation, and review and editing of the manuscript. **SP:** Data provision and curation and review and editing of the manuscript. **FR:** Study conception, patient care, data interpretation, writing of the manuscript, and review and editing of the manuscript.

Abstract

Patients with relapsed acute myeloid leukemia (rAML) experience dismal outcomes. We performed a comprehensive analysis of patients with rAML to determine the genetic dynamics and survival predictive factors. We analyzed 875 patients with newly diagnosed AML who received intensive treatment (IT) or low-intensity treatment (LIT). Of these patients, 197 experienced subsequent rAML. Data was available for 164 patients, with a median time from CR/CRi to relapse of 6.5 months. Thirty-five of the 164 patients (21%) experienced relapse after allogeneic hematopoietic stem cell transplantation (alloSCT). 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 with IT had a higher emergence rate of *TP53* mutations (16%), compared to patients treated with LIT (1%, $P = 0.009$). The overall response rates were 38% and 35% for patients treated with salvage IT or LIT, respectively. Seventeen patients (10%) underwent alloSCT after salvage therapy. The median overall survival (OS) duration after relapse was 5.3 months, with a 1-year OS 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 OS duration. More effective frontline and maintenance therapies are warranted to prevent rAML.

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 undergo intensive treatment (IT) or low-intensity therapy (LIT), depending on their age and comorbidities[2,3]. In eligible patients, an 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 relapsed AML (rAML) after having achieved a first complete remission (CR1). This incidence is even higher in patients aged > 60 years[3–5]. rAML has no standard treatment, although the most accepted strategy is to induce a second CR (CR2) and, in eligible patients, consolidate the remission with an alloSCT[4,6]. Overall, rAML 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 rAML cohorts[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 AML genetic knowledge has expanded, other research groups also identified *FLT3*-ITD at diagnosis as an adverse prognostic factor in rAML 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 are present at diagnosis[17,18]. This is caused by intrinsic AML multiclonal biology, together with selective pressure caused by exposure to frontline treatment[19–22]. With the introduction of novel targeted therapies (i.e. FLT3 or 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 rAML 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. This date was chosen because an 81-gene next-generation sequencing (NGS) panel became available at our institution in 2017. Patients received therapy at the same institution, and responses were assessed according to the European LeukemiaNet 2022 (ELN22) 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 (ORR) after frontline therapy was calculated as the proportion of patients achieving either a first CR (CR1) or first CR with incomplete blood count recovery (CRi1). Patients presenting with overt hematological 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 rAML cohort. The ORR at relapse was defined as the sum of patients achieving CR2, CRi2, and morphologic leukemia-free state (MLFS).

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 (NGS) panel as previously described[24]. The sequenced gens are detailed in **Supplementary Material (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 that acquire 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 patients that had that mutation or cytogenetic finding at diagnosis.

Statistical methods

The baseline characteristics were analyzed using descriptive statistics. Student's 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's 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 overall survival (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 were considered as 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 the Schoenfeld residuals (**Figure S1-2**). 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 **Supplementary material** (**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 the **Supplementary material (Figure S3 and 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% CI 23-28). Most patients (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 (mOS) duration was 16.3 months, with 1- and 2-year OS rates of 58% and 42%, respectively. The median EFS (mEFS) duration was 11.9 months, with 1- and 2-year EFS rates of 50% and 37%. The mOS of patients treated with IT was higher than that of patients treated with LIT (52.6 vs 10.8 months, $P < 0.001$). When comparing groups by age, the mOS was 52.6 months for patients aged < 60 years vs 12.4 months for patients aged ≥ 60 years. The mOS and mEFS were not achieved (NA) and NA, 24.1 and 18 months, and 11.1 and 7.8 months for patients in

the favorable, intermediate, and adverse ELN22 risk groups, respectively (P < 0.001 for both OS and EFS) (**Supplementary material 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 rAML patients analyzed in this study. The baseline characteristics at diagnosis and relapse in the rAML 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, according to the ELN22 classification.

Among patients in the rAML cohort, 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 (HMA) (57 with venetoclax, 70%), and 1 received ivosidenib with venetoclax. Along with frontline treatment, 22 patients (13%) received FLT3 inhibitors, 6 (4%) received IDH1/2 inhibitors, 6 (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 6 (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), with 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) (**Supplementary material Figure S6**).

Cytogenetic and mutation dynamics

We compared the proportion of mutations and cytogenetic findings between the cohort of all patients at diagnosis and the cohort of rAML patients (**Figure 2 and Supplementary material 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 mutation rate differences between diagnosis and relapse were found in *RUNX1* (n = 99 [12%] vs n = 31 [20%], *P* = 0.008), *FLT3*-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 rAML cohort (**Table 1 and Supplementary material 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 vs LIT are detailed in the **Supplementary material (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). 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 rate was significantly higher for *FLT3*-ITD (14/24, 58%), *FLT3*-TKD (11/15, 73%), *NF1* (6/10, 60%) and *KIT* (3/4, 75%), compared to 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 a significantly lower clearance rate. 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%) compared to patients treated with LIT (1/62, 2%) (*P* = 0.009). Patients treated with IT had also 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 and Supplementary material Figure S10)**. Clearance and emergence rates are detailed in the **Supplementary material Table S3**. An additional analysis on patients that received FLT3 inhibitors is detailed in the **Supplementary material (Figure S15 and 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 **(Supplementary material Figures S16-18 and 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 Ven, respectively), and 132 (80%) underwent salvage LIT (n = 68 [41%] and n = 64 [40%], with and without Ven, 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 **Supplementary Material Table S4**. The ORRs of patients treated with IT were 38% (12 of 32) overall and 44% and 35% for patients with and without the addition of venetoclax, respectively (*P* = 0.69). The ORR of patients treated with LIT was 35% (46 of 132). In patients receiving LIT chemotherapy (either low-dose chemotherapy or hypomethylating agents), the ORRs 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%] receiving a second alloSCT).

The median mOS 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 mOS 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 lower mOS than those with > 12 months (4.3 vs 8.1 months [*P* = 0.002]).

The univariate analysis of OS duration is detailed in **Supplementary Material Figure S11-S13**. The multivariate analysis highlighted a white blood cell count (WBC) > 20 x 10^9/L (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]), 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 (**Supplementary material 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 rAML cohort (**Supplementary material Figure S19-20 and Table S7-8**). 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 rAML. 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 WBC > 20 x 10^9/L at relapse) had a markedly shorter mOS duration (3.9 months) than did patients with one or no risk factors (mOS = 7.3 months, *P* < 0.001)**.**

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 rAML predictive factors using clinical and biological data from diagnosis and relapse. Overall, our study showed very poor survival in rAML irrespective of the salvage therapy received and suggests efforts should be directed to improve 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 de novo AML patients[27,28]. However, our cohort was enriched with *TP53* mutations (23%), reflecting a higher incidence of adverse-risk patients referred to our center. This impacted AML risk stratification, in which adverse-risk patients according to the ELN accounted for 56% in this study, which is 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. In 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 rAML had AML with adverse-risk genetics according to ELN 2022 (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 of 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 analyzed the mutation 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 the genes involved in signaling pathways (i.e. *FLT3*, *KIT*, or *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 (i.e. *ASXL1*, *DNMT3A*, *SRSF2*, or *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 chromosome 7 abnormalities emergence 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* or *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 (ORRs 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 (like CBF rearrangements) and the presence of *FLT3*-ITD or a previous alloSCT[9,10]. Our rAML cohort had worse ORR and OS when compared to previously published series of rAML. This may be the result of more effective frontline therapy including high-dose cytarabine based induction in the intensively treated patients, optimized regimens for CBF-AML such as FLAG-GO, use of molecularly targeted agents in the frontline setting, as well as MRD-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, these previous reports included mainly younger patients, most of them treated with intensive chemotherapy. Therefore, our study provides important prognostic data in patients with rAML treated with low intensity therapy, 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 with poor survival[38].

A significant limitation of this study was the substantial heterogenicity 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 first CR 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, rAML 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.

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Tables

Table 1. Baseline characteristics of patients with relapsed AML

	rAML at diagnosis (n = 164)	rAML at relapse (n = 164)	*P* value
Age (years), median (range)	67 (20-94)	67 (21-95)	< 0.001
Male sex, n (%)	86 (52)		-
Race/ethnicity, n (%)			
White	117 (71)		-
Black	19 (12)		
Asian	7 (4)		
Other	21 (13)		
Hemoglobin, median (range) [g/L]	8.9 (7.1-14.3)	9 (5.5-13.7)	0.016
WBC count, median (range) [x 10^9 cells/L]	3 (0.3-81.1)	1.8 (0.2-141.2)	0.545
Neutrophil count, median (range) [x 10^9 cells/L]	0.5 (0-15.1)	0.7 (0-13.5)	0.675
Platelet count, median (range) [x 10^9 cells/L]	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
MECOMr	7 (4)	6 (4)	0.999
KMT2Ar	5 (3)	5 (3)	NA
Complex	51 (31)	55 (34)	< 0.001
ELN 2022, 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)	0.45
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

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 + Ven (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 + Ven (n = 64)	CR: 10 (16) CRi: 14 (22) MLFS: 6 (9) Death: 7 (11) NR: 27 (42)
	Ven (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)

CR: Complete remission, CRi: CR with incomplete hematologic recovery, HMA: Hypomethylating agent, IT: Intensive Therapy, LIT: Low intensity therapy, MLFS: morphologic leukemia-free status, NE: Not evaluable, NR, no response, Ven: Venetoclax,

Figure Legends

Figure 1. Patient disposition. Disposition of the entire cohort of patients with AML. AlloSCT: Allogeneic stem cell transplantation, CR: Complete remission, CRi: CR with incomplete hematologic recovery, MLFS: morphologic leukemia-free status, NE: Not evaluable, NR, no response, PR: Partial response, TRM: Transplant-related mortality

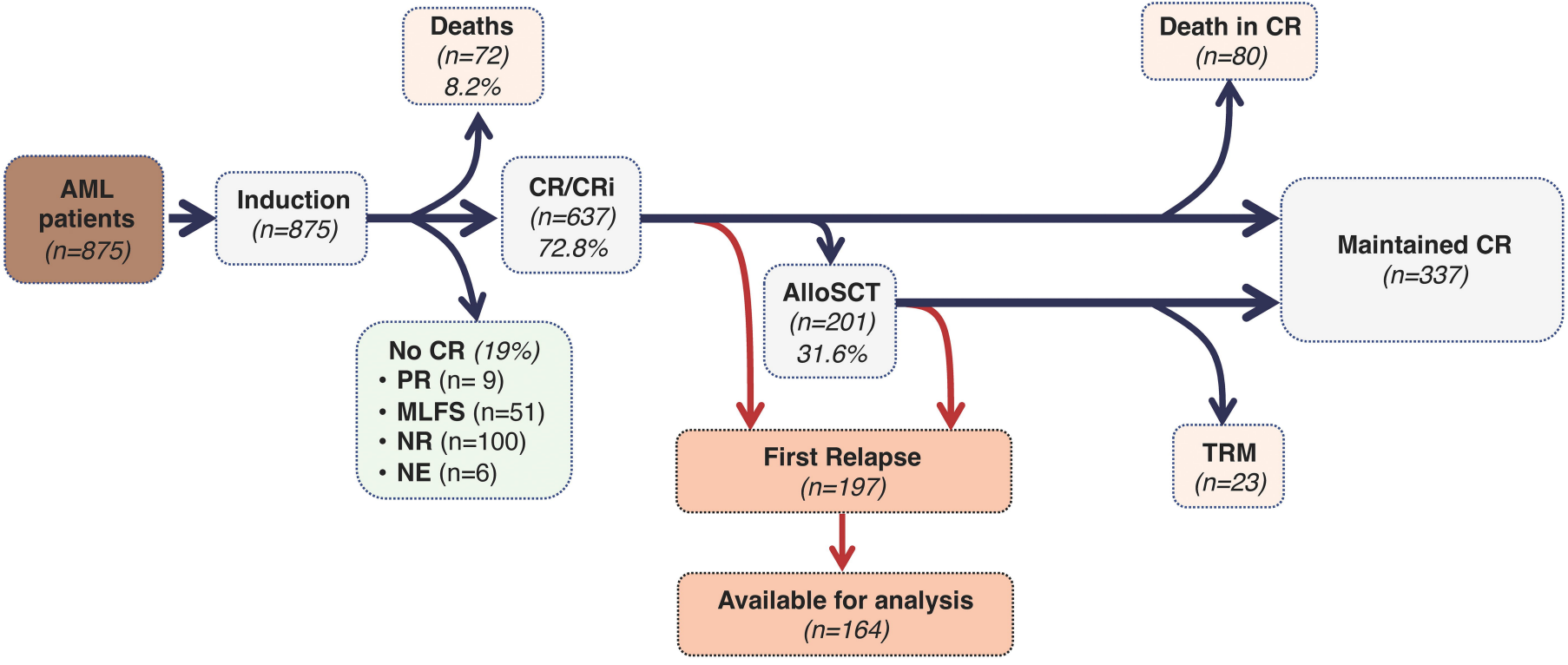
Figure 2. Frequency of mutations and cytogenetic findings. Frequency of mutations and cytogenetic findings in all patients at diagnosis vs rAML at relapse. An asterisk specifies genes with significant proportion changes. Abn: abnormality

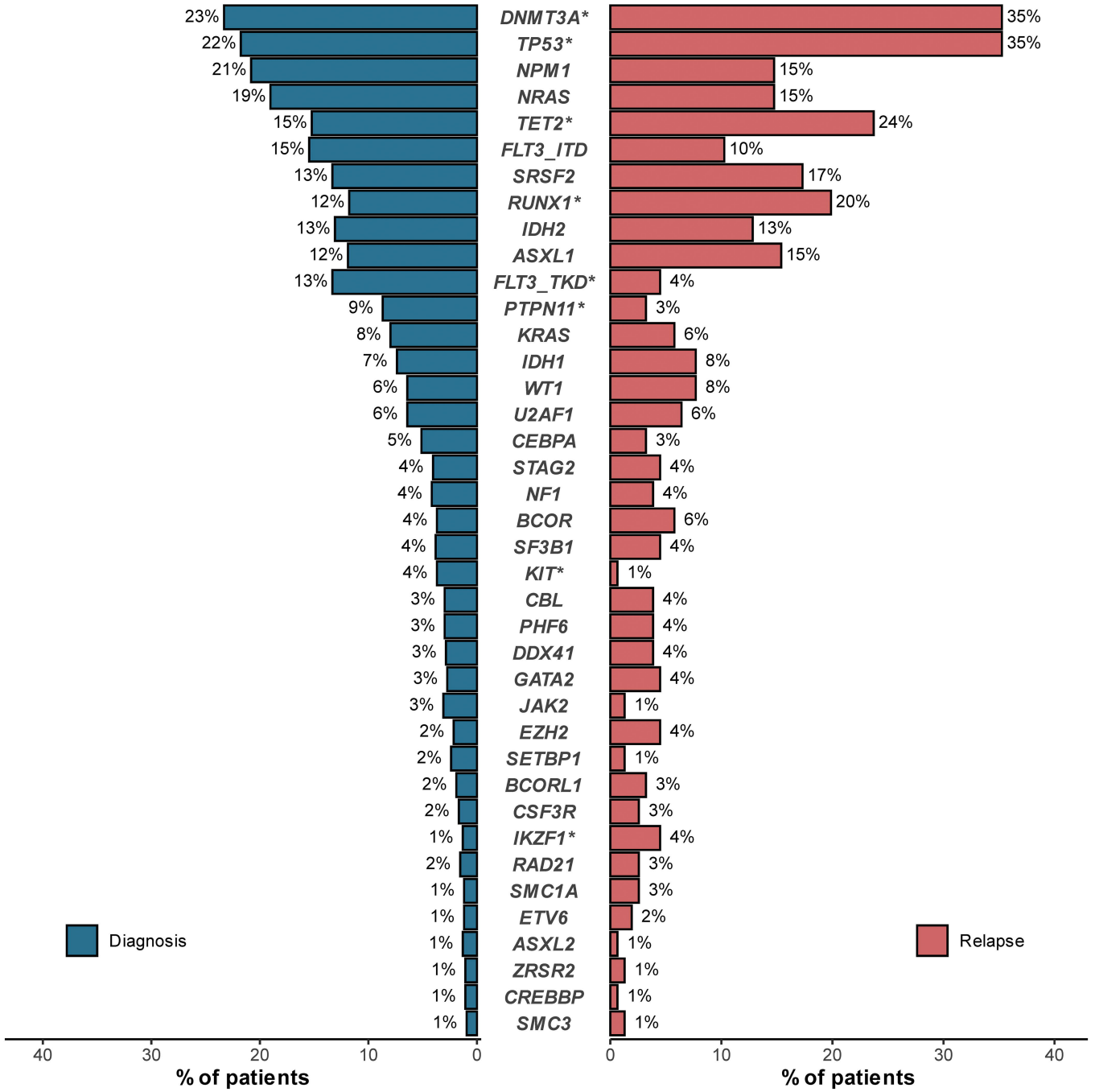
Figure 3. Mutation and cytogenetic dynamics. Genetic dynamics between diagnosis (blue) and relapse (red) in all patients presenting rAML. Only represented the 20 most common mutations. Asterisks highlight statistically significant.

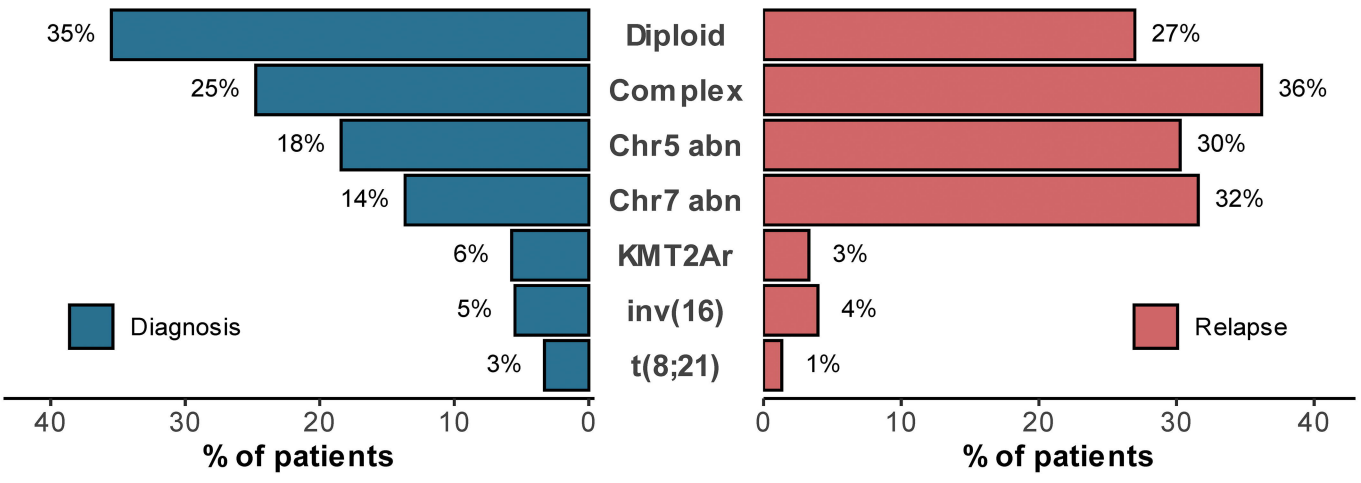
Figure 4. Mutation dynamics by therapy. Dynamics between diagnosis (blue) and relapse (red) in patients treated with intensive therapy (IT) and low intensity therapy (LIT). Asterisks highlight statistically significant between IT and LIT.

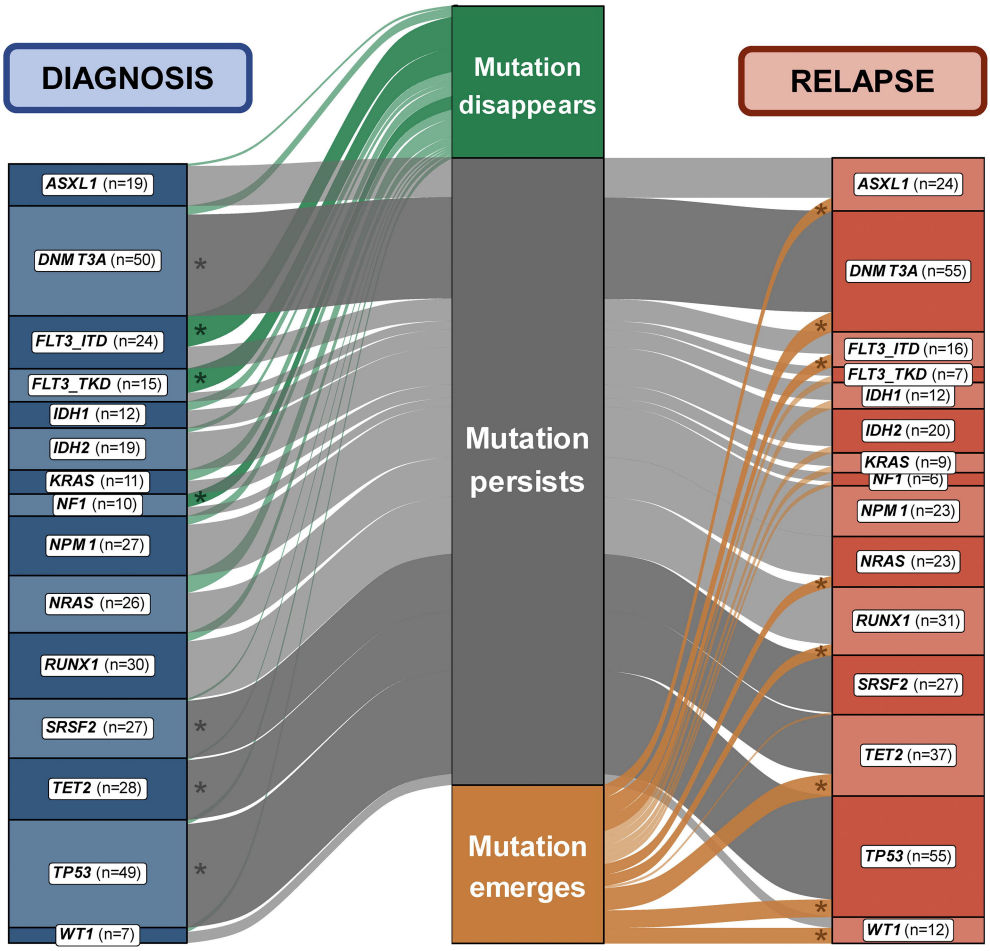
Figure 5. Overall survival after relapse. (A) Overall survival (OS) from relapse. (B) OS from relapse, stratified by the European LeukemiaNet (ELN) 2022 risk classification at diagnosis (Fav: favorable, Int: Intermediate, Adv: Adverse). (C) OS from relapse, stratified by frontline therapy received (IT: intensive therapy, LIT: Low intensity therapy). (D) OS from relapse, stratified by time in remission (TIR) in months.

Figure 6. Multivariate analysis. Multivariate analysis and adjusted median overall survival (OS, months) duration of all patients, patients who received intensive therapy (IT) as frontline therapy, and patients who received low intensity therapy (LIT) as frontline therapy. CG: Cytogenetics, CR: Complete remission, ev: events, HR: Hazard ratio, Rel: Relapse, TIR: Time in remission, WBC: White blood cell count.


AML patients (n=875)
Induction (n=875)
Deaths (n=72) 8.2%
CR/CRi (n=637) 72.8%
No CR (19%)
• PR (n= 9)
• MLFS (n=51)
• NR (n=100)
• NE (n=6)
Death in CR (n=80)
Maintained CR (n=337)
AlloSCT (n=201) 31.6%
First Relapse (n=197)
TRM (n=23)
Available for analysis (n=164)


23% DNMT3A* 35%
22% TP53* 35%
21% NPM1 15%
19% NRAS 15%
15% TET2* 24%
15% FLT3_ITD 10%
13% SRSF2 17%
12% RUNX1* 20%
13% IDH2 13%
12% ASXL1 15%
13% FLT3_TKD* 4%
9% PTPN11* 3%
8% KRAS 6%
7% IDH1 8%
6% WT1 8%
6% U2AF1 6%
5% CEBPA 3%
4% STAG2 4%
4% NF1 4%
4% BCOR 6%
4% SF3B1 4%
4% KIT* 1%
3% CBL 4%
3% PHF6 4%
3% DDX41 4%
3% GATA2 4%
3% JAK2 1%
2% EZH2 4%
2% SETBP1 1%
2% BCORL1 3%
2% CSF3R 3%
1% IKZF1* 4%
2% RAD21 3%
1% SMC1A 3%
1% ETV6 2%
1% ASXL2 1%
1% ZRSR2 1%
1% CREBBP 1%
1% SMC3 1%
Diagnosis
Relapse
40 30 20 10 0
0 10 20 30 40
% of patients
% of patients


35% Diploid 27%
25% Complex 36%
18% Chr5 abn 30%
14% Chr7 abn 32%
6% KMT2Ar 3%
5% inv(16) 4%
3% t(8;21) 1%
Diagnosis
Relapse
40 30 20 10 0
0 10 20 30 40
% of patients
% of patients


DIAGNOSIS
RELAPSE
Mutation disappears
Mutation persists
Mutation emerges
ASXL1 (n=19)
DNMT3A (n=50)
FLT3_ITD (n=24)
FLT3_TKD (n=15)
IDH1 (n=12)
IDH2 (n=19)
KRAS (n=11)
NF1 (n=10)
NPM1 (n=27)
NRAS (n=26)
RUNX1 (n=30)
SRSF2 (n=27)
TET2 (n=28)
TP53 (n=49)
WT1 (n=7)
ASXL1 (n=24)
DNMT3A (n=55)
FLT3_ITD (n=16)
FLT3_TKD (n=7)
IDH1 (n=12)
IDH2 (n=20)
KRAS (n=9)
NF1 (n=6)
NPM1 (n=23)
NRAS (n=23)
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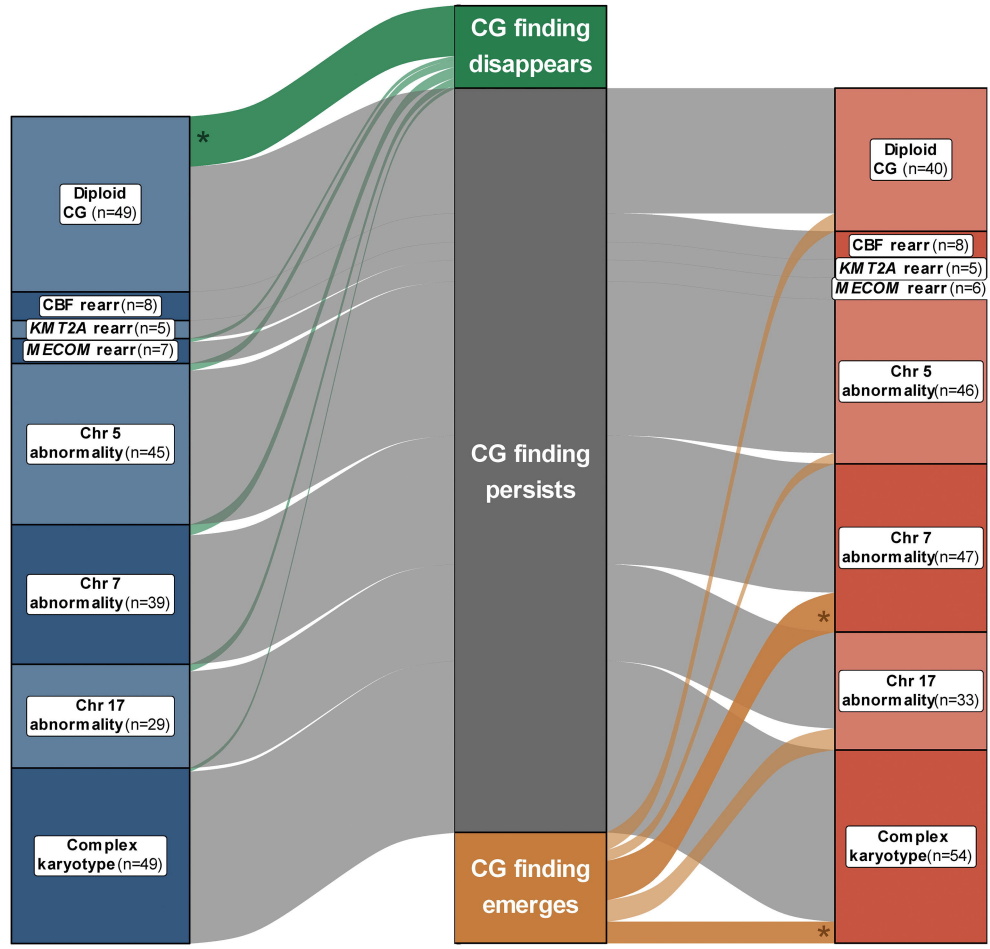
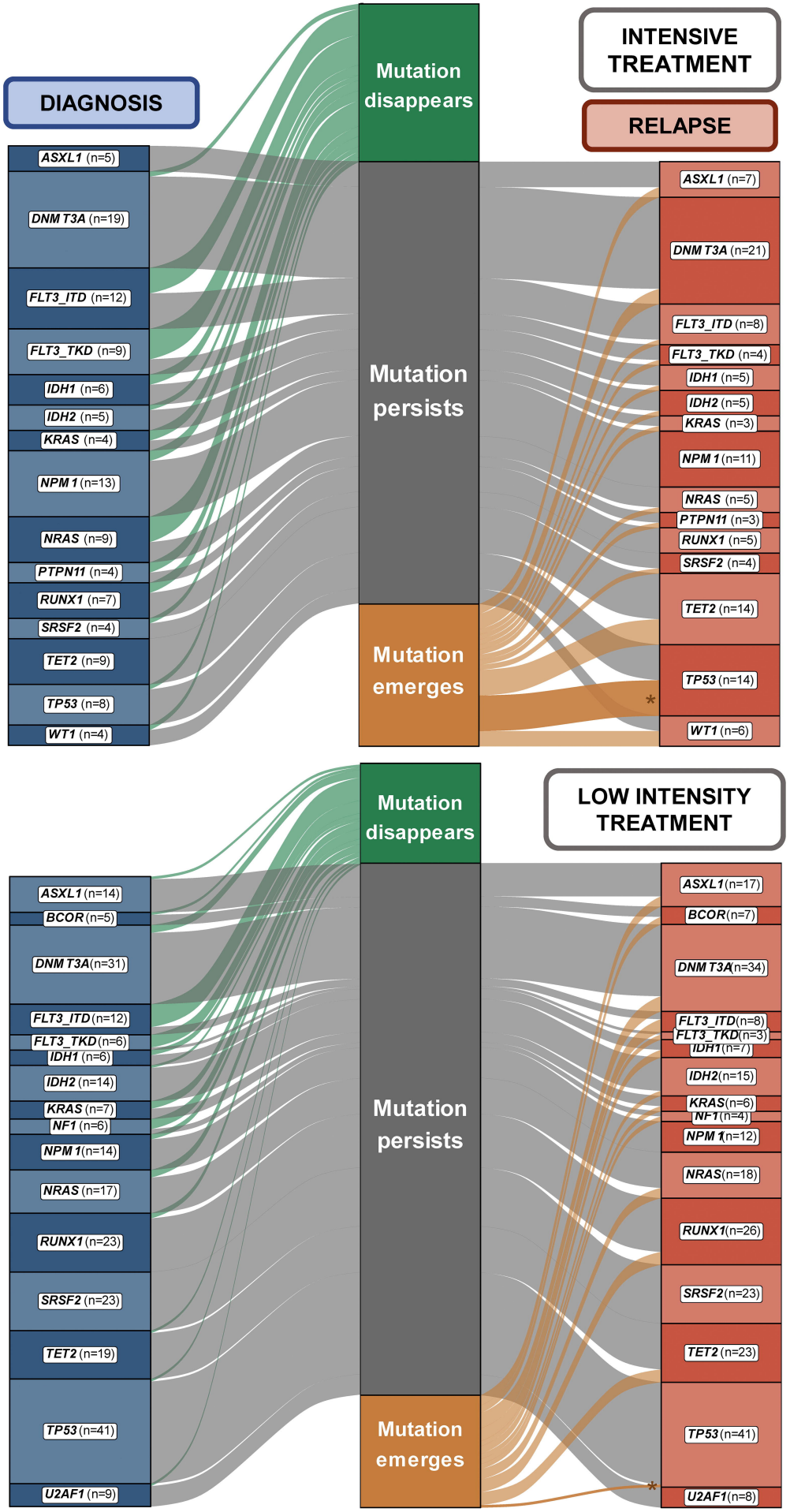
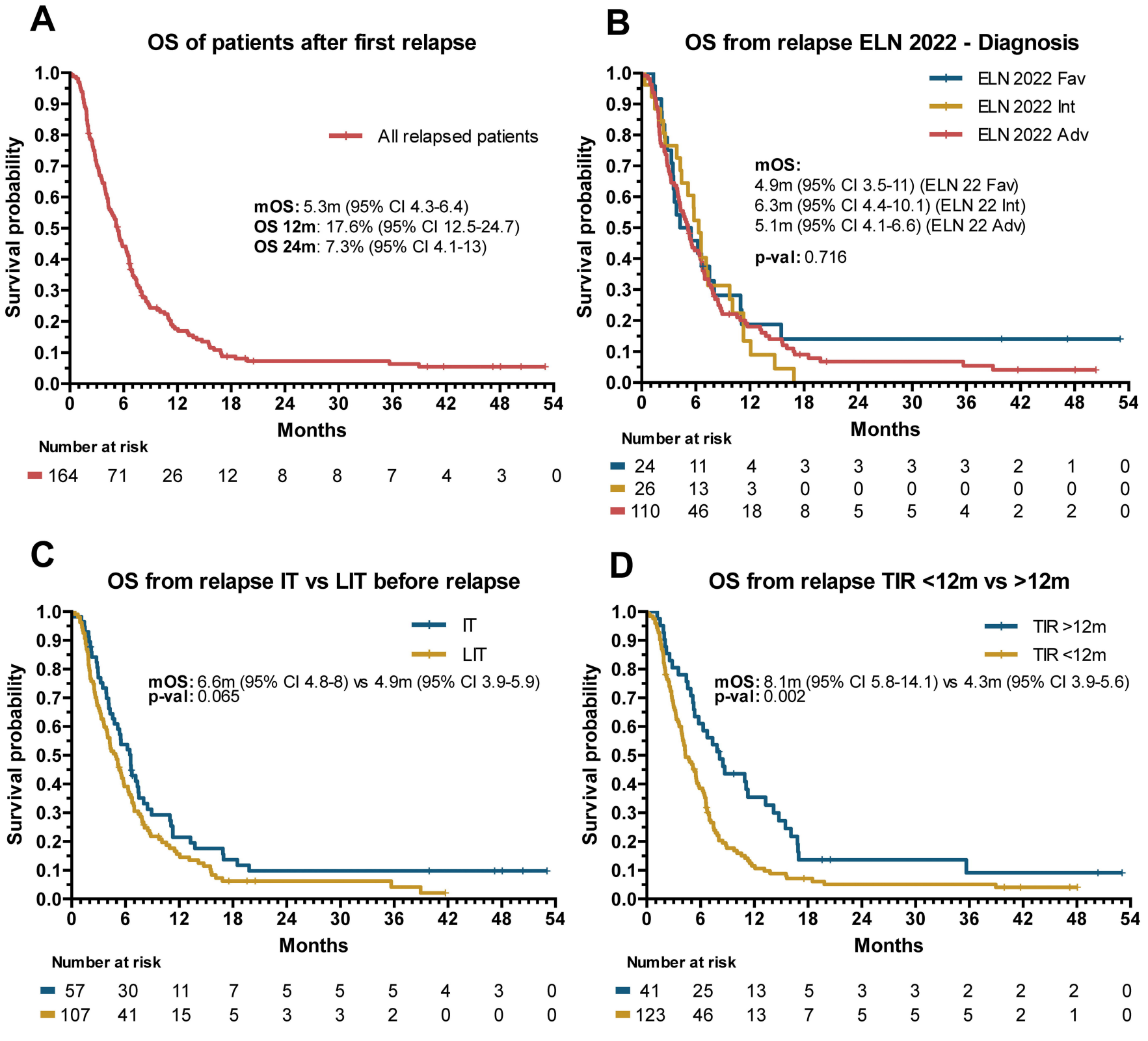
SRSF2 (n=27)
TET2 (n=37)
TP53 (n=55)
WT1 (n=12)


CG finding disappears
CG finding persists
CG finding emerges
Diploid CG (n=49)
CBF rearr (n=8)
KMT2A rearr (n=5)
MECOM rearr (n=7)
Chr 5 abnormality (n=45)
Chr 7 abnormality (n=39)
Chr 17 abnormality (n=29)
Complex karyotype (n=49)
Diploid CG (n=40)
CBF rearr (n=8)
KMT2A rearr (n=5)
MECOM rearr (n=6)
Chr 5 abnormality (n=46)
Chr 7 abnormality (n=47)
Chr 17 abnormality (n=33)
Complex karyotype (n=54)


DIAGNOSIS
INTENSIVE TREATMENT
RELAPSE
Mutation disappears
Mutation persists
Mutation emerges
ASXL1 (n=5)
DNMT3A (n=19)
FLT3_ITD (n=12)
FLT3_TKD (n=9)
IDH1 (n=6)
IDH2 (n=5)
KRAS (n=4)
NPM1 (n=13)
NRAS (n=9)
PTPN11 (n=4)
RUNX1 (n=7)
SRSF2 (n=4)
TET2 (n=9)
TP53 (n=8)
WT1 (n=4)
ASXL1 (n=7)
DNMT3A (n=21)
FLT3_ITD (n=8)
FLT3_TKD (n=4)
IDH1 (n=5)
IDH2 (n=5)
KRAS (n=3)
NPM1 (n=11)
NRAS (n=5)
PTPN11 (n=3)
RUNX1 (n=5)
SRSF2 (n=4)
TET2 (n=14)
TP53 (n=14)
WT1 (n=6)
*
LOW INTENSITY TREATMENT
Mutation disappears
Mutation persists
Mutation emerges
ASXL1 (n=14)
BCOR (n=5)
DNMT3A (n=31)
FLT3_ITD (n=12)
FLT3_TKD (n=6)
IDH1 (n=6)
IDH2 (n=14)
KRAS (n=7)
NF1 (n=6)
NPM1 (n=14)
NRAS (n=17)
RUNX1 (n=23)
SRSF2 (n=23)
TET2 (n=19)
TP53 (n=41)
U2AF1 (n=9)
ASXL1 (n=17)
BCOR (n=7)
DNMT3A (n=34)
FLT3_ITD (n=8)
FLT3_TKD (n=3)
IDH1 (n=7)
IDH2 (n=15)
KRAS (n=6)
NF1 (n=4)
NPM1 (n=12)
NRAS (n=18)
RUNX1 (n=26)
SRSF2 (n=23)
TET2 (n=23)
TP53 (n=41)
U2AF1 (n=8)
*

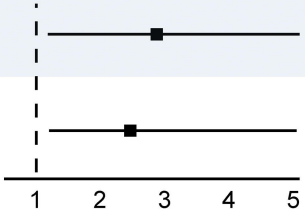

A
OS of patients after first relapse
Survival probability
All relapsed patients
mOS: 5.3m (95% CI 4.3-6.4)
OS 12m: 17.6% (95% CI 12.5-24.7)
OS 24m: 7.3% (95% CI 4.1-13)
Months
Number at risk
164 71 26 12 8 8 7 4 3 0
B
OS from relapse ELN 2022 - Diagnosis
ELN 2022 Fav
ELN 2022 Int
ELN 2022 Adv
mOS:
4.9m (95% CI 3.5-11) (ELN 22 Fav)
6.3m (95% CI 4.4-10.1) (ELN 22 Int)
5.1m (95% CI 4.1-6.6) (ELN 22 Adv)
p-val: 0.716
Survival probability
Months
Number at risk
24 11 4 3 3 3 3 2 1 0
26 13 3 0 0 0 0 0 0 0
110 46 18 8 5 5 4 2 2 0
C
OS from relapse IT vs LIT before relapse
IT
LIT
mOS: 6.6m (95% CI 4.8-8) vs 4.9m (95% CI 3.9-5.9)
p-val: 0.065
Survival probability
Months
Number at risk
57 30 11 7 5 5 5 4 3 0
107 41 15 5 3 3 2 0 0 0
D
OS from relapse TIR <12m vs >12m
TIR >12m
TIR <12m
mOS: 8.1m (95% CI 5.8-14.1) vs 4.3m (95% CI 3.9-5.6)
p-val: 0.002
Survival probability
Months
Number at risk
41 25 13 5 3 3 2 2 2 0
123 46 13 7 5 5 5 2 1 0

Multivariate analysis (all patients)

Variable	Total n / Events	HR (90% CI)	p-val	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)

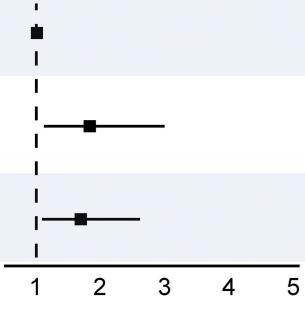
Multivariate analysis (IT patients)

Variable	Total n / Events	HR (90% CI)	p-val	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-val	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)



Supplementary material

Outcomes and genetic dynamics of acute myeloid leukemia in first relapse

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Contents

Tables

Table S1. 81-gene NGS panel

Gene	Exons (codons) tested
ANKRD26(NM_014915)	1 (1-6)
ASXL1 (NM_015338)	11-12 (362-1542)
ASXL2 (NM_018263)	11-12 (381-1436)
BCOR (NM_017745)	2-4 (1-512), 4-6 (514-1080), 7 (1122-1124), 7 (1091-1099), 7-15 (1124-1722)
BCORL1 (NM_021946)	1-6 (1-1261), 6 (1264-1323), 6-11 (1326-1600), 11-12 (1614-1699), 12 (1706-1712)
BRAF (NM_004333)	11 (439-478), 15 (581-620)
BRINP3 (NM_199051)	2-8 (1-471), 8 (475-767)
CALR (NM_004343)	9 (352-418)
CBL (NM_005188)	7-9 (336-477)
CBLB (NM_170662)	7-10 (282-469)
CBLC (NM_012116)	7-9 (336-454), 10 (464-475)
CEBPA (NM_004364)	1 (1-56), 1 (59-91), 1 (96), 1 (128-143), 1 (146-175), 1 (178-201), 1 (249-358)
CREBBP (NM_004380)	2-8 (29-608), 9-10 (615-705), 12-16 (724-1084), 17-31 (1094-1943), 31 (1950-2235), 31 (2238-2443)
CSF3R (NM_156039)	14 (575-622), 17 (681-864)
CUX1 (NM_181552)	2-6 (11-172), 6-9 (174-241), 10-12 (248-359), 13-14 (367-408)
DDX41 (NM_016222)	1-10 (1-366), 11 (369-410), 12-17 (420-623)
DNMT3A (NM_022552)	8-22 (286-862), 23 (866-913)
EED (NM_003797)	1-2 (1-69), 2-8 (71-287), 9-12 (289-442)
ELANE (NM_001972)	1-2 (5-46), 2 (69-75), 4-5 (123-268)
ETNK1 (NM_018638)	3 (228-275)
ETV6 (NM_001987)	1-6 (1-378), 7-8 (385-453)
EZH2 (NM_004456)	2-5 (1-158), 6 (162-205), 7 (209-217), 8-13 (243-512), 14 (516-538), 14-19 (547-732), 20 (752)
FBXW7 (NM_033632)	9-12 (413-708)
FLT3 (NM_004119)	11-17 (437-709), 18-20 (736-847)
GATA1 (NM_002049)	2-3 (1-84)
GATA2 (NM_032638)	2-5 (1-377), 5-6 (379-481)
GFI1 (NM_005263)	2 (2-39)
GNAS (NM_000516)	8 (200-202), 11 (315-324)
HNRNPK (NM_002140)	3-7 (1-96), 7 (101-106), 8-17 (111-465)
HRAS (NM_005343)	2-3 (1-60), 3-4 (87-150)
IDH1 (NM_005896)	4 (132-133)
IDH2 (NM_002168)	4 (125-178)
IKZF1 (NM_006060)	2-8 (1-443), 8 (445-518)
IL2RG (NM_000206)	1-2 (1-45), 2-8 (51-340), 8 (360-370)
IL7R (NM_002185)	5-7 (180-292)
JAK1 (NM_002227)	3-9 (3-445), 10 (453-465), 10-22 (470-1023), 22-24 (1026-1123)
JAK2 (NM_004972)	10 (405-442), 12-14 (505-622), 16 (665-711), 18 (762-802)

JAK3 (NM_000215)	2-23 (1-1069)
KDM6A (NM_021140)	1-19 (1-971), 19-21 (976-1070), 22-29 (1080-1402)
KIT (NM_000222)	8-9 (411-514), 11 (550-592), 17 (788-828)
KMT2A (NM_005933)	2-4 (145-1075), 4-13 (1081-1561), 14-15 (1566-1665), 27 (2178-2195), 27 (2201-2362), 27 (2365-2715), 27 (2721-3216), 27 (3219-3327), 27 (3336-3505), 27 (3521-3582)
KRAS (NM_004985)	2-4 (1-150)
MAP2K1 (NM_002755)	2 (27-90), 3 (98-146)
MPL (NM_005373)	10 (490-522), 12 (552-636)
NF1 (NM_001042492)	2-4 (21-160), 5 (165-190), 6 (201-218), 8-13 (244-468), 13-14 (478-547), 15-17 (568-667), 18 (674-728), 18-22 (746-992), 23-26 (997-1146), 26-30 (1160-1330), 30-31 (1353-1378), 31-34 (1382-1493), 34-35 (1512-1550), 35 (1563-1575), 36-38 (1601-1868), 39 (1870-1884), 39-40 (1886-1947), 40-47 (1952-2322), 47-49 (2325-2438), 50-51 (2441-2491), 51-52 (2494-2555), 53-58 (2580-2840)
NOTCH1 (NM_017617)	26 (1529-1600), 26-28 (1604-1795), 34 (2061-2228), 34 (2234-2274), 34 (2290-2309), 34 (2290-2556), 34 (2061-2228), 34 (2234-2274), 34 (2309-2556)
NPM1 (NM_002520)	11 (283-295)
NRAS (NM_002524)	2-4 (1-150)
PAX5 (NM_016734)	1-10 (1-392)
PHF6 (NM_032458)	2-3 (1-79), 4-10 (81-366)
PIGA (NM_002641)	2 (1-6), 2-5 (15-396), 6 (399-485)
PML (NM_033238)	3 (201-255)
PRPF40B (NM_001031698)	2-19 (2-609), 19-20 (611-658), 20-24 (661-807), 25-26 (815-893)
PTEN (NM_000314)	7-8 (212-340)
PTPN11 (NM_002834)	3-4 (46-125), 7 (253-285), 12 (460-462), 12-13 (465-533)
RAD21 (NM_006265)	2-12 (1-540), 13 (544-560), 14 (569-632)
RARA (NM_000964)	6-7 (211-338)
RUNX1 (NM_001754)	2-9 (1-419), 9 (426-437), 9 (456-474)
SETBP1 (NM_015559)	4 (838-885)
SF1 (NM_004630)	1-2 (1-31), 2 (39-54), 3-12 (57-524), 13 (544-578), 13 (596-600), 13 (605-640)
SF3A1 (NM_005877)	2-7 (22-322), 7-9 (355-424), 9-12 (427-646), 13-16 (651-794)
SF3B1 (NM_012433)	13-16 (574-790)
SH2B3 (NM_005475)	2 (1-40), 2 (43-119), 2 (132-164), 2-6 (233-374), 6-8 (380-576)
SMC1A (NM_006306)	1-25 (1-1234)
SMC3 (NM_005445)	1 (1-5), 2-6 (19-110), 6-11 (113-299), 11-15 (308-477), 15-16 (498-504), 16-17 (507-580), 17-19 (591-706), 20-25 (708-975), 25 (979-1035), 26-29 (1038-1217)
SRSF2 (NM_003016)	1 (1-38), 1 (45-121)
STAG1 (NM_005862)	2 (1-5), 3-5 (10-101), 5-12 (121-392), 13-20 (402-703), 21-22 (724-738), 22-27 (740-953), 27 (955-979), 28 (985-1008), 29-34 (1022-1259)
STAG2 (NM_006603)	2-8 (1-273), 9-16 (287-518), 16 (521-539), 17-22 (547-751), 23-33 (756-1232)
STAT3 (NM_139276)	17 (489-509), 17-22 (521-715)
STAT5A (NM_003152)	3-6 (1-177), 6-7 (181-189), 8-20 (261-795)
STAT5B (NM_012448)	16 (636-673)

SUZ12 (NM_015355)	1 (20-44), 1 (46-84), 2 (92-97), 4-5 (129-169), 7-11 (198-431), 12-16 (468-740)
TERC (NR_001566)	1 (1-36)
TERT (NM_198253)	1-2 (1-172), 2 (258-342), 2 (349-474), 2-4 (477-630), 4-5 (633-692), 6 (711-749), 6-12 (753-954), 12-16 (957-1133)
TET2 (NM_001127208)	3 (1-77), 3 (91-92), 3 (98-815), 3 (829-853), 3-10 (867-1453), 10-11 (1465-2003)
TP53 (NM_000546)	2 (1-25), 4-11 (80-394)
U2AF1 (NM_006758)	2 (15-44), 6 (117-161)
U2AF2 (NM_007279)	1-5 (1-161), 6-12 (163-437), 12 (441-476)
WT1 (NM_024426)	1 (1-5), 1 (7-63), 1-10 (127-518)
ZRSR2 (NM_005089)	1-4 (1-90), 5 (105-130), 6-8 (134-257), 9-11 (260-419), 11 (465-483)

Table S2. Baseline characteristics of all AML patients

	All patients (n = 875)
Age, years	65 (18-94)
Male sex, n (%)	468 (53.5)
Race/ethnicity, n (%)	
White	654 (74.7)
Black	73 (8.3)
Asian	42 (4.8)
Other	72 (8.2)
Unknown/NA	34 (3.9)
WBC	3.4 (0.1-336)
Hgb	8.7 (3.5-14.3)
Platelets	36 (1-1625)
BM blasts	45 (1-97)
Cytogenetics	
Diploid	291 (35.5)
-5/-5q	151 (18.4)
-7/-7q	112 (13.7)
+8	80 (9.8)
inv16/t(16;16)	45 (5.5)
t(8:21)	27 (3.3)
*KMT2A*r	47 (5.7)
*MECOM*r	11 (1.2)
Complex/Monosomal	208 (25.4)
ELN 2022	
Favorable	175 (20.7)
Intermediate	199 (23.6)
Adverse	470 (55.7)
Mutations	
ASXL1	108 (12.8)
BCOR	40 (4.8)
BCORL1	26 (3.1)
DNMT3A	198 (23.5)
EZH2	28 (3.3)
FLT3-ITD	140 (16.6)
IDH1	71 (8.4)
IDH2	119 (14.1)
NPM1	183 (21.8)
PTPN11	82 (9.8)
RUNX1	108 (12.8)
SRSF2	122 (14.5)
TET2	132 (15.7)
TP53	190 (22.6)
U2AF1	62 (7.4)
WT1	61 (7.3)
ZRSR2	17 (2.0)
Treatment	
IT	348 (39.8)
IT + Ven	144 (41.4)*
LIT	527 (60.2)
LIT + Ven	379 (71.9)*

* Percentages calculated by treatment subgroup (IT or LIT).

Table S3. Emergence and clearance rates of mutations and cytogenetic findings

Emergence rate = n of patients with acquired mutation at relapse / n of patients without mutation at diagnosis

Clearance rate = n of patients with acquired mutation at relapse / n of patients without mutation at diagnosis

	All patients		Intensive chemotherapy		Low intensity chemotherapy	
Gene mutation	Emergence rate	Clearance rate	Emergence rate	Clearance rate	Emergence rate	Clearance rate
ASXL1	6/137 (4.4%)	1/19 (5.3%)	2/48 (4.2%)	0/5	4/89 (4.5%)	1/14 (7.1%)
ASXL2	1/156 (0.6%)	0/0	0/53	0/0	1/103 (1%)	0/0
BCOR	3/148 (2%)	2/8 (25%)	0/50	1/3 (33.3%)	3/98 (3.1%)	1/5 (20%)
BCORL1	4/153 (2.6%)	2/3 (66.7%)	0/53	0/0	4/100 (4%)	2/3 (66.7%)
BRAF	0/154	1/2 (50%)	0/53	0/0	0/101	1/2 (50%)
BRINP3	1/155 (0.6%)	0/1	0/53	0/0	1/102 (1%)	0/1
CALR	1/155 (0.6%)	0/1	1/53 (1.9%)	0/0	0/102	0/1
CBL	2/150 (1.3%)	2/6 (33.3%)	1/52 (1.9%)	0/1	1/98 (1%)	2/5 (40%)
CBLC	0/155	1/1 (100%)	0/52	1/1 (100%)	0/103	0/0
CEBPA	1/149 (0.7%)	3/7 (42.9%)	1/50 (2%)	2/3 (66.7%)	0/99	1/4 (25%)
CREBBP	1/156 (0.6%)	0/0	0/53	0/0	1/103 (1%)	0/0
CSF3R	2/154 (1.3%)	0/2	1/52 (1.9%)	0/1	1/102 (1%)	0/1
DDX41	1/150 (0.7%)	1/6 (16.7%)	1/50 (2%)	0/3	0/100	1/3 (33.3%)
DNMT3A	9/106 (8.5%)	4/50 (8%)	3/34 (8.8%)	1/19 (5.3%)	6/72 (8.3%)	3/31 (9.7%)
ETV6	2/155 (1.3%)	0/1	2/53 (3.8%)	0/0	0/102	0/1
EZH2	5/152 (3.3%)	2/4 (50%)	2/52 (3.8%)	1/1 (100%)	3/100 (3%)	1/3 (33.3%)
FBXW7	0/155	1/1 (100%)	0/53	0/0	0/102	1/1 (100%)
FLT3_TKD	3/141 (2.1%)	11/15 (73.3%)	1/44 (2.3%)	6/9 (66.7%)	2/97 (2.1%)	5/6 (83.3%)
FLT3_ITD	6/132 (4.5%)	14/24 (58.3%)	1/41 (2.4%)	5/12 (41.7%)	5/91 (5.5%)	9/12 (75%)
GATA2	4/153 (2.6%)	0/3	1/52 (1.9%)	0/1	3/101 (3%)	0/2
GNAS	1/155 (0.6%)	1/1 (100%)	1/53 (1.9%)	0/0	0/102	1/1 (100%)
IDH1	4/144 (2.8%)	4/12 (33.3%)	1/47 (2.1%)	2/6 (33.3%)	3/97 (3.1%)	2/6 (33.3%)
IDH2	3/137 (2.2%)	2/19 (10.5%)	1/48 (2.1%)	1/5 (20%)	2/89 (2.2%)	1/14 (7.1%)
IKZF1	4/151 (2.6%)	2/5 (40%)	1/51 (2%)	0/2	3/100 (3%)	2/3 (66.7%)
JAK1	1/156 (0.6%)	0/0	0/53	0/0	1/103 (1%)	0/0
JAK2	1/153 (0.7%)	2/3 (66.7%)	0/53	0/0	1/100 (1%)	2/3 (66.7%)
JAK3	1/156 (0.6%)	0/0	0/53	0/0	1/103 (1%)	0/0
KDM6A	2/155 (1.3%)	0/1	2/53 (3.8%)	0/0	0/102	0/1
KIT	0/152	3/4 (75%)	0/49	3/4 (75%)	0/103	0/0
KMT2A	1/155 (0.6%)	0/1	0/53	0/0	1/102 (1%)	0/1
KRAS	3/145 (2.1%)	5/11 (45.5%)	1/49 (2%)	2/4 (50%)	2/96 (2.1%)	3/7 (42.9%)
MPL	1/156 (0.6%)	0/0	0/53	0/0	1/103 (1%)	0/0
NF1	2/146 (1.4%)	6/10 (60%)	0/49	2/4 (50%)	2/97 (2.1%)	4/6 (66.7%)
NOTCH1	0/155	1/1 (100%)	0/53	0/0	0/102	1/1 (100%)
NPM1	0/129	4/27 (14.8%)	0/40	2/13 (15.4%)	0/89	2/14 (14.3%)
NRAS	5/130 (3.8%)	8/26 (30.8%)	1/44 (2.3%)	5/9 (55.6%)	4/86 (4.7%)	3/17 (17.6%)
PHF6	3/150 (2%)	3/6 (50%)	2/51 (3.9%)	0/2	1/99 (1%)	3/4 (75%)
PIGA	0/155	0/1	0/53	0/0	0/102	0/1
PRPF40B	2/155 (1.3%)	0/1	0/53	0/0	2/102 (2%)	0/1
PTPN11	1/149 (0.7%)	3/7 (42.9%)	1/49 (2%)	2/4 (50%)	0/100	1/3 (33.3%)

RAD21	2/154 (1.3%)	0/2	1/51 (2%)	0/2	1/103 (1%)	0/0
RUNX1	5/126 (4%)	4/30 (13.3%)	0/46	2/7 (28.6%)	5/80 (6.2%)	2/23 (8.7%)
SETBP1	0/152	2/4 (50%)	0/53	0/0	0/99	2/4 (50%)
SF3B1	1/150 (0.7%)	0/6	0/51	0/2	1/99 (1%)	0/4
SH2B3	1/155 (0.6%)	0/1	0/52	0/1	1/103 (1%)	0/0
SMC1A	3/155 (1.9%)	0/1	2/53 (3.8%)	0/0	1/102 (1%)	0/1
SMC3	1/155 (0.6%)	0/1	1/52 (1.9%)	0/1	0/103	0/0
SRSF2	1/129 (0.8%)	1/27 (3.7%)	1/49 (2%)	1/4 (25%)	0/80	0/23
STAG1	0/155	1/1 (100%)	0/53	0/0	0/102	1/1 (100%)
STAG2	2/150 (1.3%)	1/6 (16.7%)	2/51 (3.9%)	1/2 (50%)	0/99	0/4
STAT5A	1/155 (0.6%)	0/1	0/53	0/0	1/102 (1%)	0/1
STAT5B	0/155	1/1 (100%)	0/52	1/1 (100%)	0/103	0/0
SUZ12	0/155	1/1 (100%)	0/52	1/1 (100%)	0/103	0/0
TERT	0/155	1/1 (100%)	0/53	0/0	0/102	1/1 (100%)
TET2	10/128 (7.8%)	1/28 (3.6%)	5/44 (11.4%)	0/9	5/84 (6%)	1/19 (5.3%)
TP53	8/107 (7.5%)	2/49 (4.1%)	7/45 (15.6%)	1/8 (12.5%)	1/62 (1.6%)	1/41 (2.4%)
U2AF1	0/145	1/11 (9.1%)	0/51	0/2	0/94	1/9 (11.1%)
U2AF2	0/155	0/1	0/53	0/0	0/102	0/1
WT1	7/149 (4.7%)	2/7 (28.6%)	3/49 (6.1%)	1/4 (25%)	4/100 (4%)	1/3 (33.3%)
ZRSR2	2/154 (1.3%)	2/2 (100%)	1/52 (1.9%)	1/1 (100%)	1/102 (1%)	1/1 (100%)
	All patients		**Intensive chemotherapy**		**Low intensity chemotherapy**	
CG finding	**Emergence rate**	**Clearance rate**	**Emergence rate**	**Clearance rate**	**Emergence rate**	**Clearance rate**
Normal CG	5/100 (5%)	14/49 (28.6%)	4/29 (13.8%)	9/21 (42.9%)	1/71 (1.4%)	5/28 (17.9%)
Complex	6/100 (6%)	2/45 (4.4%)	2/43 (4.7%)	0/7	4/57 (7%)	1/42 (2.4%)
Chr 5 abn	3/104 (2.9%)	2/45 (4.4%)	0/43	0/7	3/61 (4.9%)	2/38 (5.3%)
Chr 7 abn	11/110 (10%)	3/39 (7.7%)	6/46 (13%)	0/4	5/64 (7.8%)	3/35 (8.6%)
Chr 17 abn	6/120 (5%)	2/29 (6.9%)	2/46 (4.3%)	0/4	4/74 (5.4%)	2/25 (8)
t(8;21)	0/147	0/5	0/48	0/2	0/0	0/0
inv(16)	0/143	0/6	0/44	0/6	0/0	0/0
***KMT2A*r**	0/144	0/144	0/48	0/2	0/96	0/3
***MECOM*r**	0/142	1/7 (14.3%)	0/47	1/3 (33.3%)	0/95	0/4
t(6;9)	0/147	0/2	0/49	0/1	0/98	0/1

Table S4. Salvage treatments for rAML

Therapy intensity	therapy type	FLT3i	IDHi	Immunotherapy	Other	Treatment
IT	**Chemotherapy IT (n = 23)**	Gilteritinib (n = 2)	Ivosidenib (n = 1)	GO (CD33 ADC) (n = 2)	LY2606368 (CHEK1 inh) (n = 1)	HDAC: 1 (4.3%) Ara-C + VP16: 1 (4.3%) CLIA: 2 (8.7%) CLIA + GO: 3 (13%) CLIA + decitabine: 1 (4.3%) CPX-351 + GO: 4 (17.3%) CPX-351 + ivosidenib: 1 (4.3%) FA + LY2606368: 1 (4.3%) FAI: 1 (4.3%) FAI + gilteritinib: 2 (4.3%) FAI + GO: 1 (4.3%) FA BID: 1 (4.3%) FLAG: 1 (4.3%) FLAG + Ida: 1 (4.3%) FLAG + Ida + GO: 1 (4.3%) Direct HSCT: 1 (4.3%)
	Chemo IT + Ven (n = 9)	-	-	-	-	FLAG+Ida+Ven: 1 (11.1%) CPX-351+Ven: 6 (55.5%) BID FA+Ven: 1 (11.1%) FAI+Ven: 1 (11.1%)
LIT	**LIT (n = 35)**	Gilteritinib (n = 1) Quizartinib (n = 5) Sorafenib (n = 2)	Enasidenib (n = 6) Ivosidenib (n = 1)	GO (CD33 ADC) (n = 1) Avelumab (PD-L1) (n = 1) Magrolimab (CD47) (n = 3) Nivolumab (PD1) (n = 6) Ipilimumab (CTLA-4) (n = 5)	BGB324 (AXL1 inh) (n = 1) DS-3032B (MDM2 inh) (n = 1) Imatinib (n = 1) Palbociclib (CDK4/6 inh) (n = 1) PLX51107 (BET inh) (n = 1)	Aza + quizartinib: 1 (%) Aza + nivolumab + ipilimumab: 5 (16.1%) Aza + nivolumab: 1 (3.2%) Aza + sorafenib + enasidenib: 1 (3.2%) Aza + sorafenib: 1 (3.2%) Aza + enasidenib: 5 (16.1%) Aza + DS-3032B: 1 (3.2%) Aza + ivosidenib + imatinib: 1 (3.2%) Aza + PLX51107: 1 (3.2%) Aza + avelumab + GO: 1 (3.2%) Aza + magrolimab: 3 (9.7%) Dec: 4 (%) Dec + palbociclib: 1 (3.2%) Dec + quizartinib: 3 (9.7%) SGI + Ida: 1 (3.2%) LDAC + Quizartinib: 1 (%) LDAC + BGB324: 1 (%) Clad + LDAC + gilteritinib: 1 (20%) Clad + LDAC: 1 (%) Aspacytarabine: 1 (%)

	LIT + Ven (n = 64)	Gilteritinib (n = 5) Midostaurin (n = 1) Sorafenib (n = 1)	Enasidenib (n = 4) Ivosidenib (n = 1)	GO (CD33 ADC) (n = 2) Avelumab (PD-L1) (n = 2) Magrolimab (CD47) (n = 1) IMGN632 (CD123 ADC) (n = 1)	Trametinib (MEK inh) (n = 1) DS-3032B (MDM2 inh) (n = 1)	Aza + Ven: 7 (%) Aza + Ven + avelumab: 2 (%) Aza + Ven + GO: 1 (2.1%) Aza + Ven + enasidenib: 3 (4.7%) Aza + Ven + magrolimab: 1 (2.1%) Aza + Ven + trametinib: 1 (2.1%) Aza + Ven + IMGN632: 1 (2.1%) Aza + Ven + gilteritinib: 2 (4.2%) Aza + Ven + ivosidenib: 1 (2.1%) Dec + Ven: 18 (%) Dec 10d + Ven: 3 (6.2%) Dec + Ven + Enasid: 1 (2.1%) Dec + Ven + GO: 1 (2.1%) Dec + Ven + gilteritinib: 3 (%) Dec + Ven + sorafenib: 1 (2.1%) Dec + Ven + midostaurin: 1 (2.1%) Clad LDAC + Dec Ven: 1 (6.2%) LDAC + Ven: 3 (%) LDAC + Ven + DS3032b: 1 (6.2%) Clad LDAC + Ven: 10 (62.5%) HHT + Ven: 1 (6.2%) Sapacitabine + Ven: 1 (2.1%)
	Ven (n = 4)	Quizartinib (n = 1)	Ivosidenib (n = 1)	-	APR-246 (TP53mut) (n = 1) CYC065 (CDK inh) (n = 1)	Ivosidenib + Ven: 1 (25%) APR-246 + Ven: 1 (25%) CYC065 + Ven: 1 (25%) Quizartinib + Ven: 1 (25%)

	Other (n = 29)	-	Enasidenib (n = 2) Ivosidenib (n = 2)	AGS62P1 (FLT3 ADC) (n = 1) AMG330 (CD33-CD3) (n = 2) AMV564 (CD33-CD3) (n = 2) MGD006 (CD123-CD3) (n = 1) HU8F4 (PR1) (n = 1) IMGN632 (CD123 ADC) (n = 6) MCLA-117 (CLL1-CD3) (n = 1) Nivolumab (PD1) (n = 2) Ipilimumab (CTLA-4) (n = 2) NKX101 (NK therapy) (n = 1) FT538 (NK therapy) (n = 2)	APTO-253 (cMyc inh) (n = 1) BTX-A51 (CK1a inh) (n = 1) CA4948 (IRAK4 inh) (n = 2) CB-5339 (VCP inh) (n = 1) DS-1594b (Menin inh) (n = 1)	**Enasidenib:** 2 (6.9%) **Ivosidenib:** 2 (6.9%) **Nivolumab + Ipilimumab:** 2 (3.4%) **IMGN632:** 6 (20.7%) **AGS62P1:** 1 (3.4%) **AMG330:** 2 (6.9%) **AMV564:** 2 (6.9%) **APTO-253:** 1 (3.4%) **BTX-A51:** 1 (3.4%) **CA-4948:** 2 (6.9%) **CB-5339:** 1 (3.4%) **DS-1594b:** 1 (3.4%) **NKX101:** 1 (3.4%) **FT538:** 2 (6.9%) **HU8F4:** 1 (3.4%) **MCLA-117:** 1 (3.4%) **MGD006:** 1 (3.4%)

Figures

Figure S1

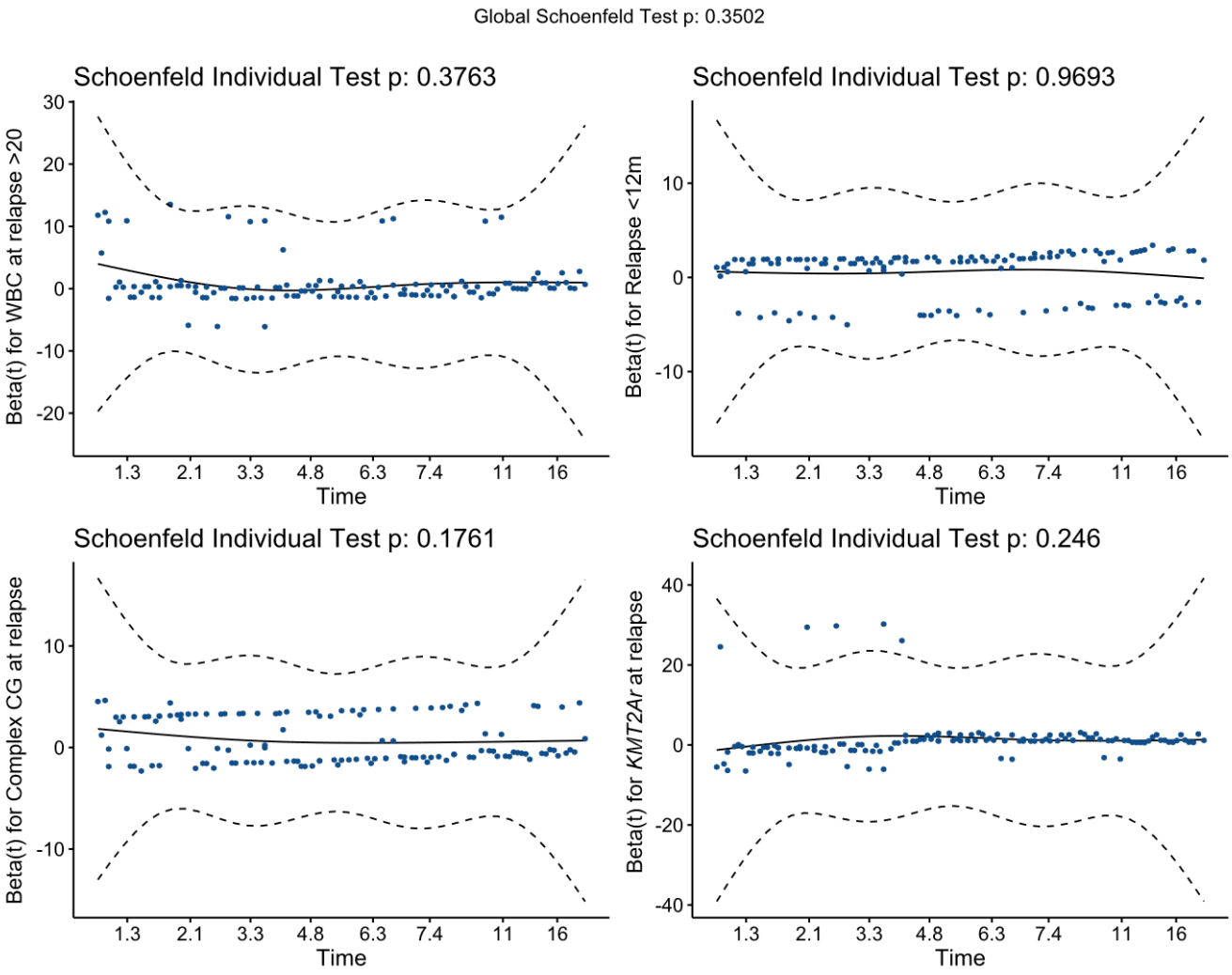

Figure S1. Schoenfeld residuals for variables included in the multivariate analysis for all patients.

Figure S2

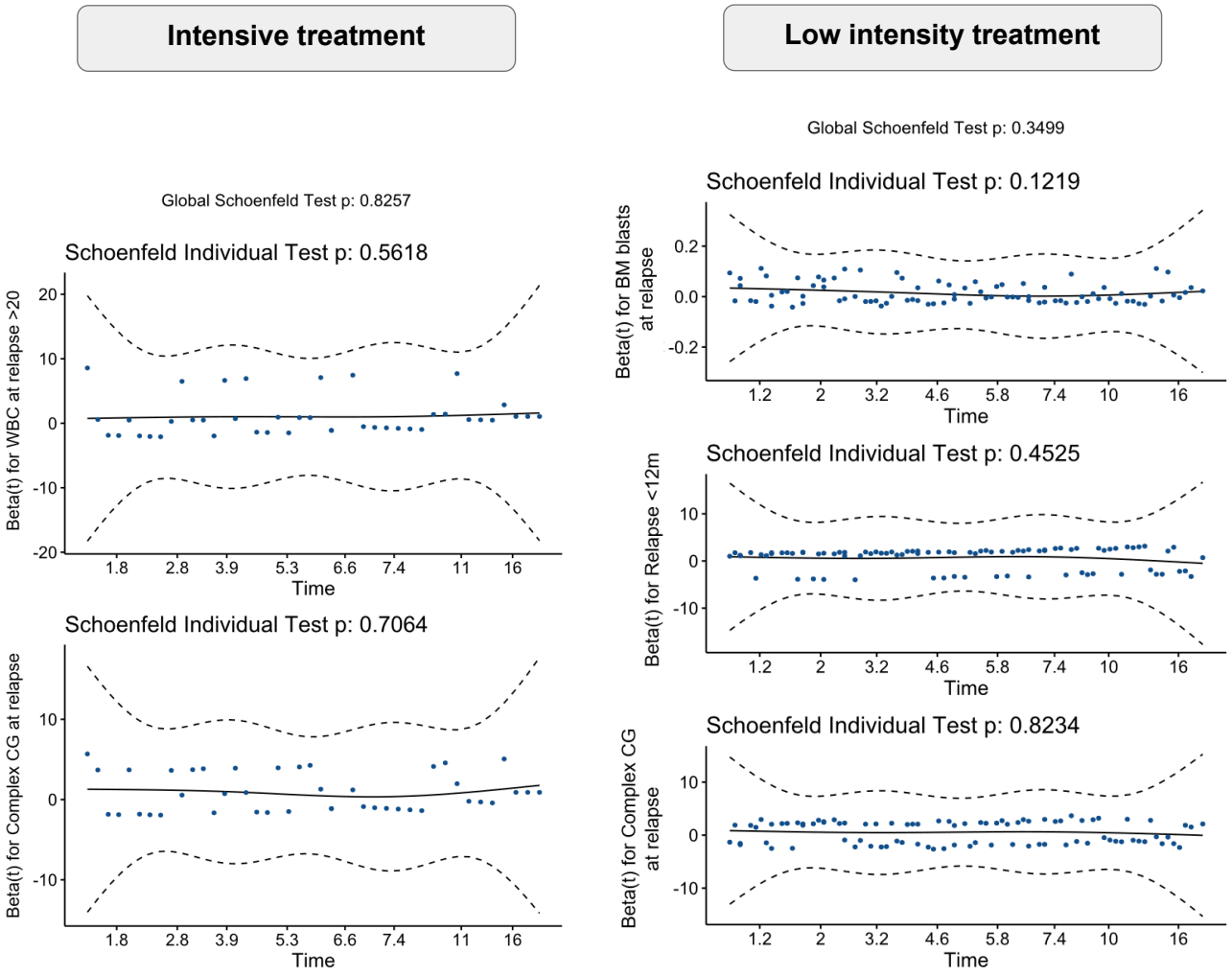

Figure S2. Schoenfeld residuals for variables included in the multivariate analysis for patients treated with intensive and low intensity treatment.

Figure S3

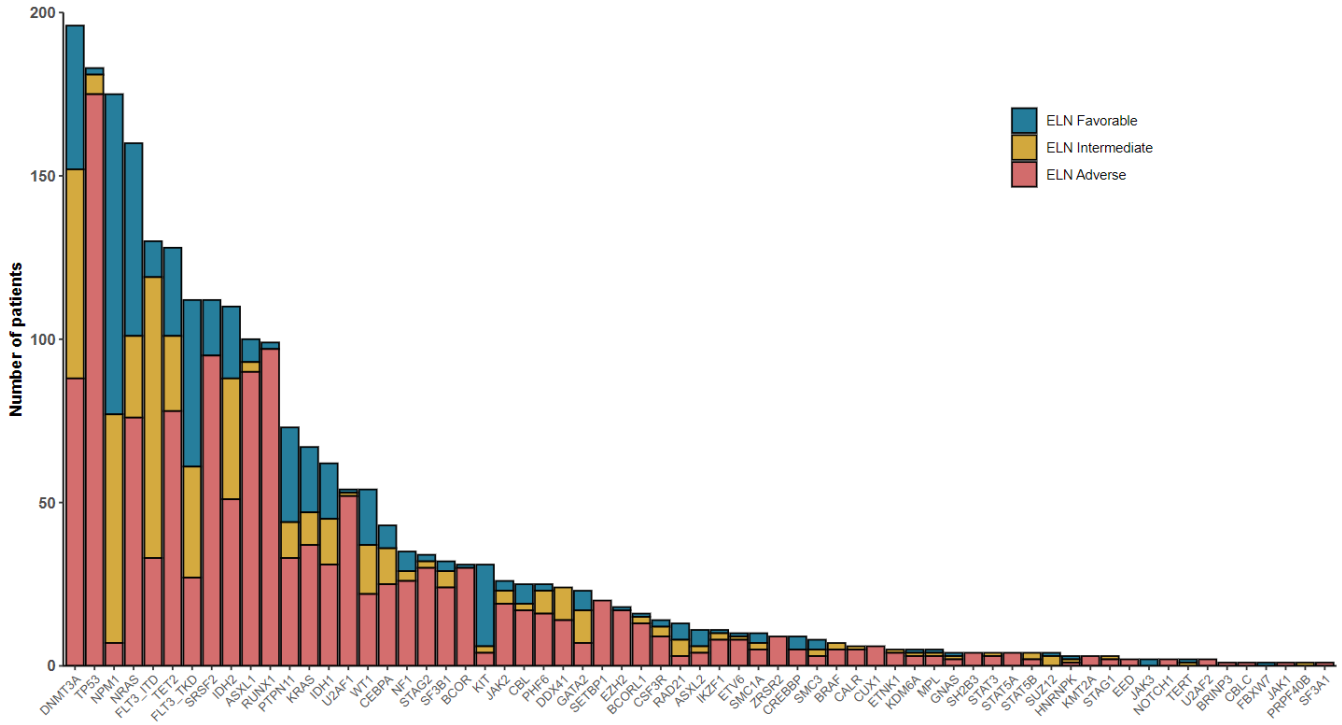

Figure S3. Bar plot describing all mutations detected in all patients at diagnosis, stratified by ELN 2022 risk.

Figure S4

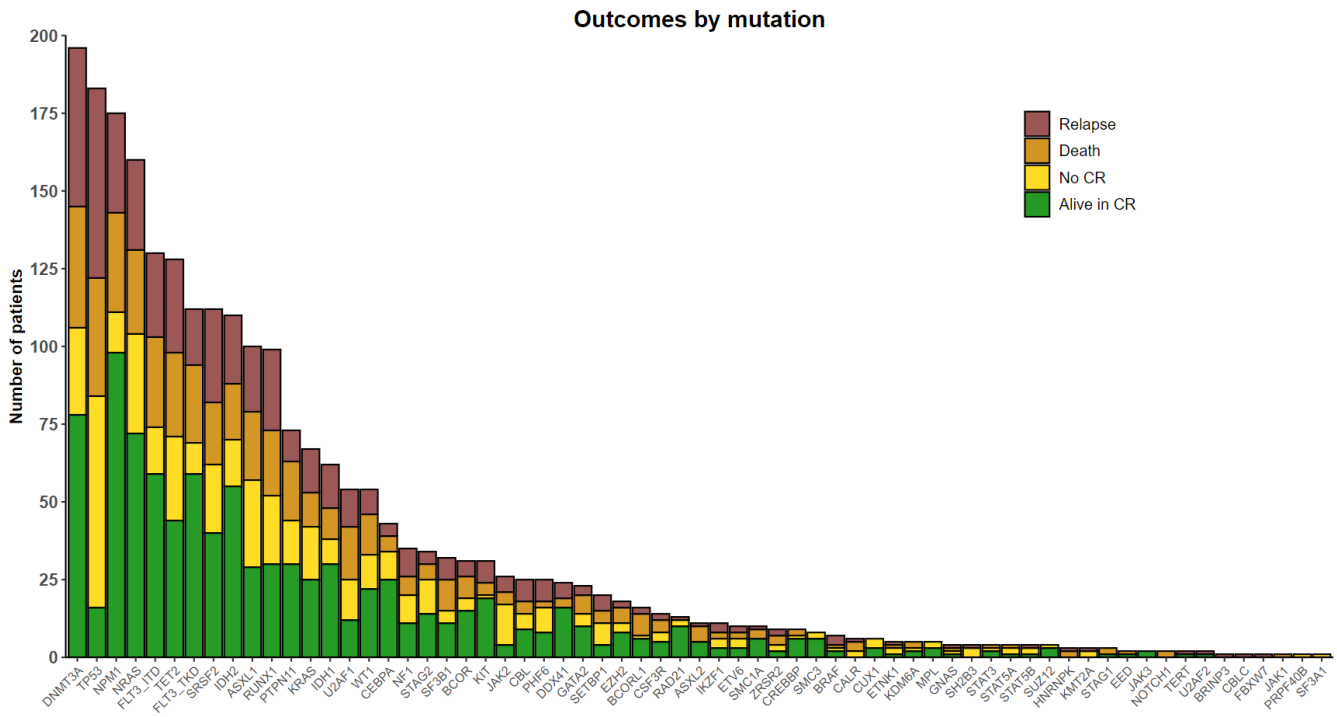

Figure S4. Bar plot describing all mutations detected in all patients at diagnosis, by the outcome of each patient.

Figure S5

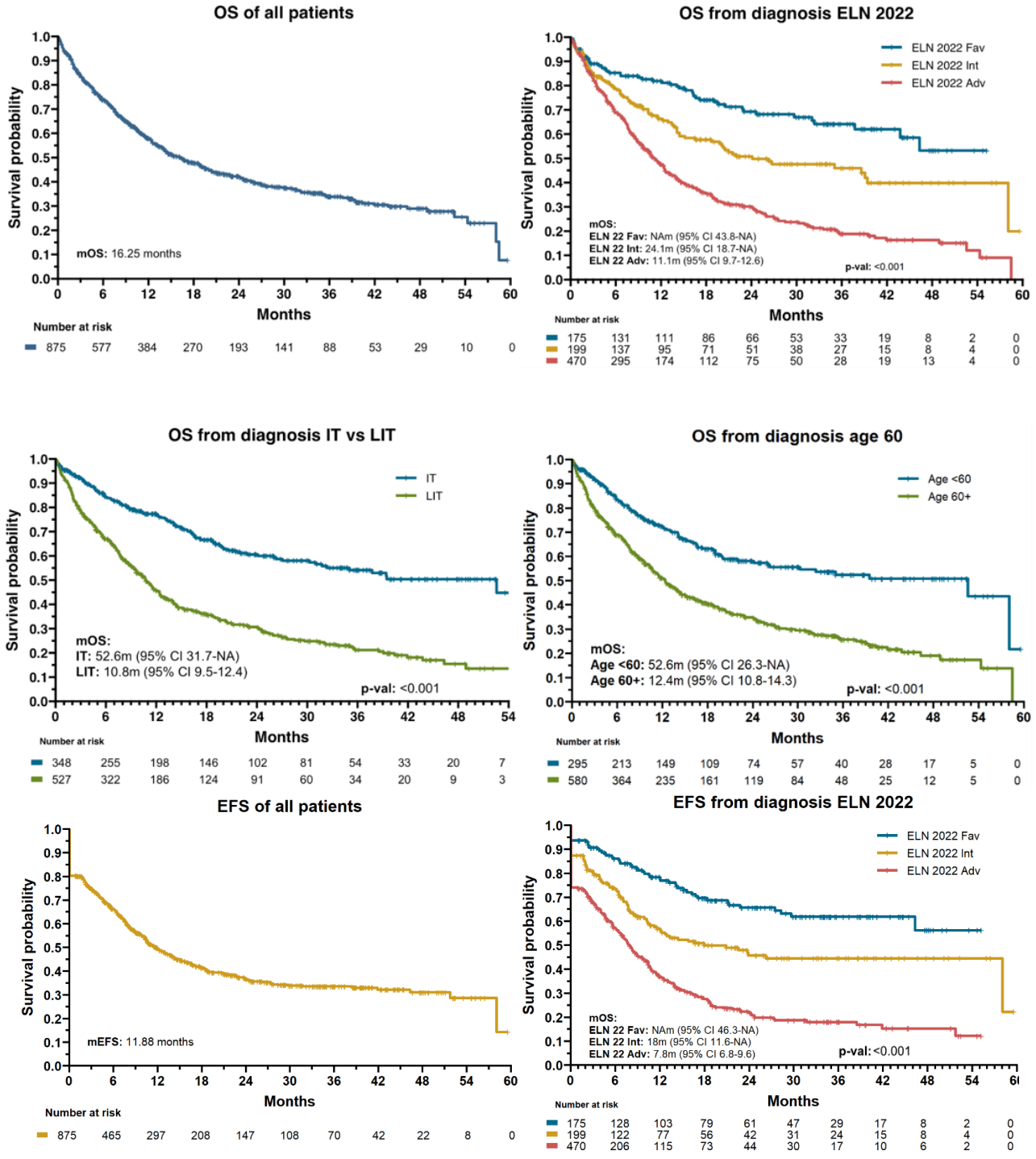

Figure S5. (A) OS of the entire cohort. (B) OS of the entire cohort stratified by the ELN 2022 risk classification. (D) OS of the entire cohort stratified by frontline treatment received. (E) OS of the entire cohort by age. Event-free survival (EFS) of all patients (left) and according to the ELN 2022 classification (right).

Figure S6

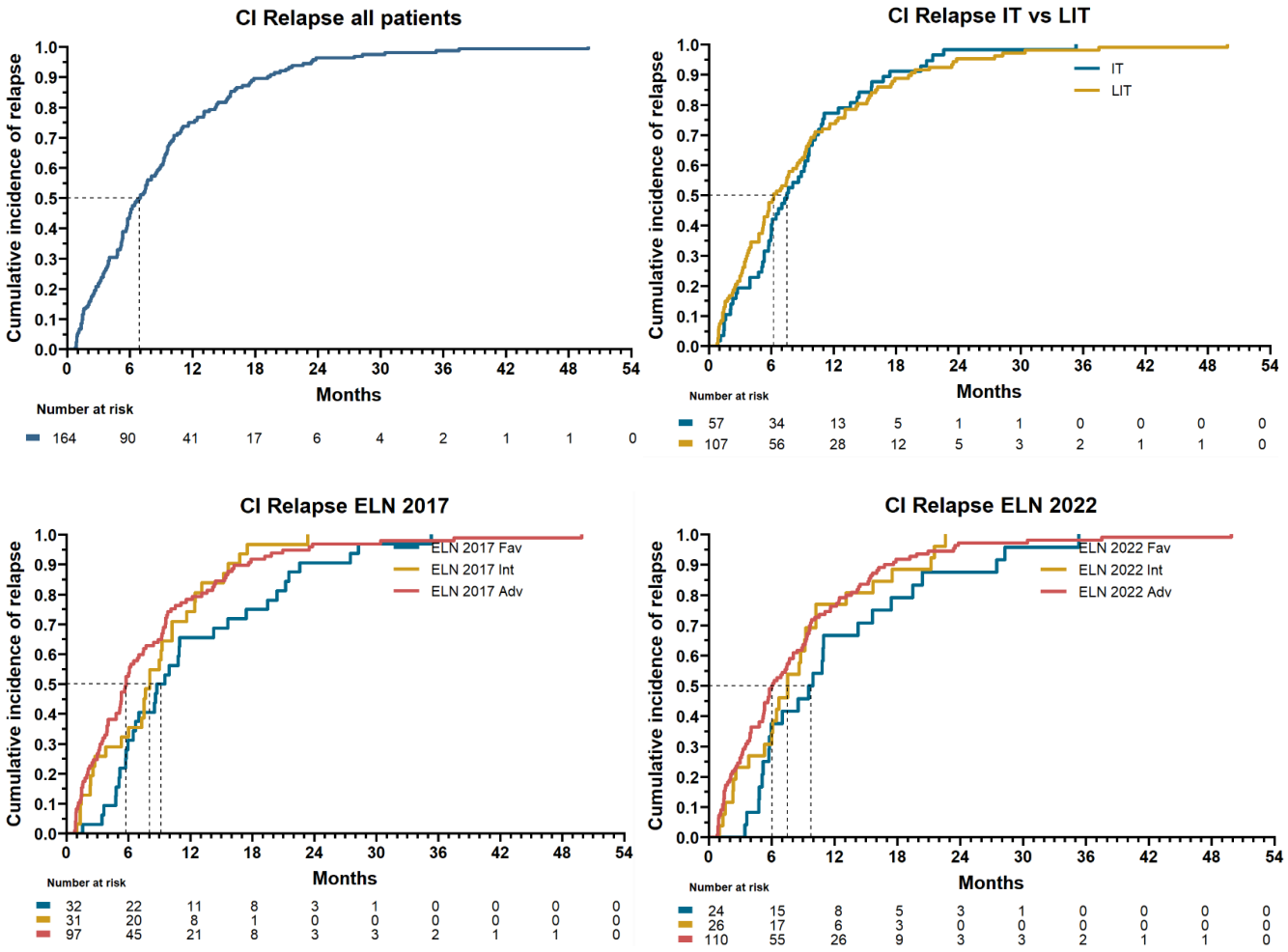

Figure S6. Cumulative incidence of relapse of all rAML patients (upper left), according to therapy received (upper right), according to ELN 2017 (lower left), and according to ELN 2022 (lower right).

Figure S7

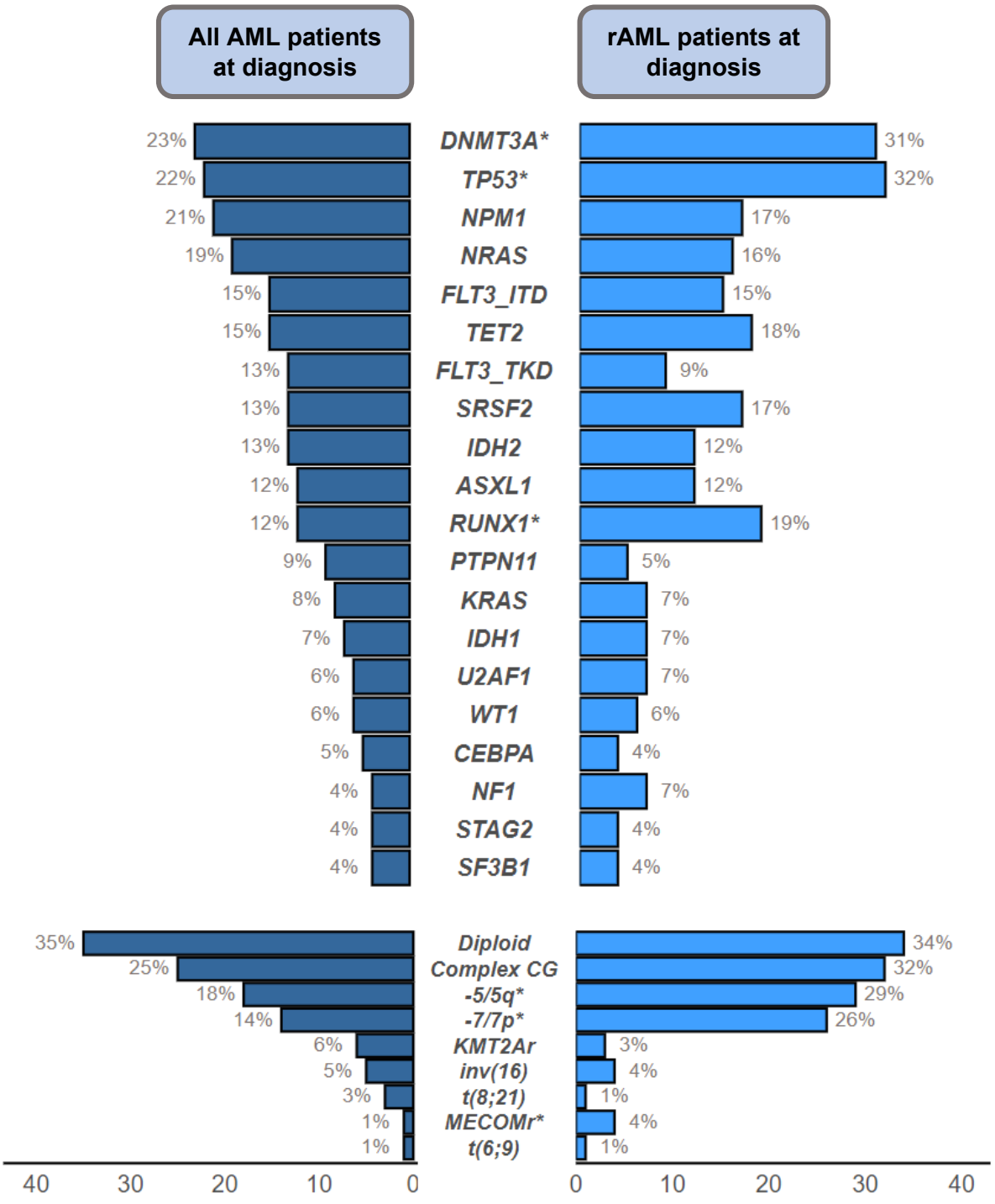

Figure S7. Frequency of mutations and cytogenetic findings in all patients at diagnosis vs rAML patients at diagnosis. An asterisk specifies genes/cytogenetic findings with significant proportion changes.

Figure S8

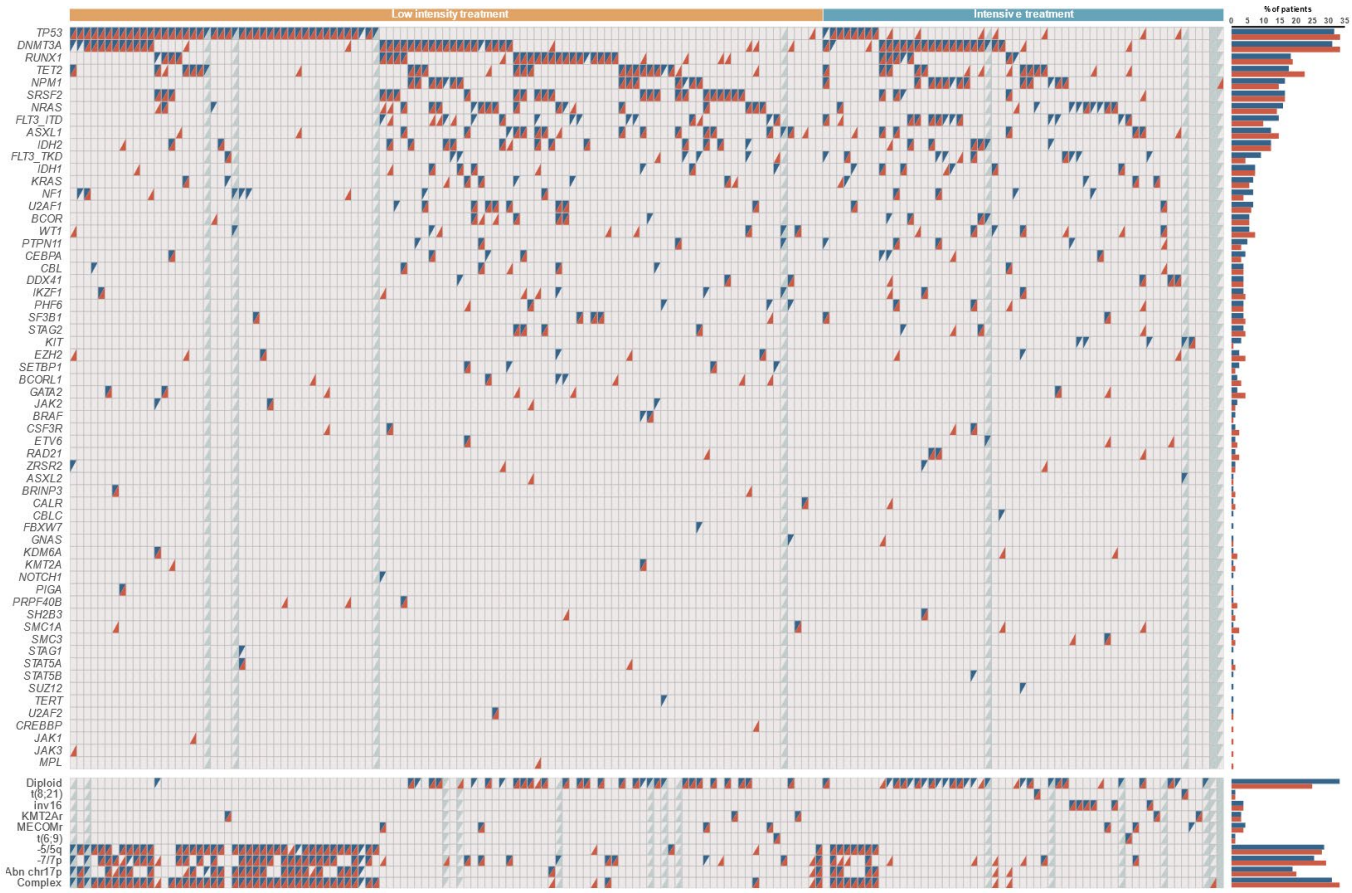

Figure S8. Oncoplot describing mutations at cytogenetic abnormalities at diagnosis (blue) and relapse (red). Patients with no data available are highlighted in grey.

Figure S9

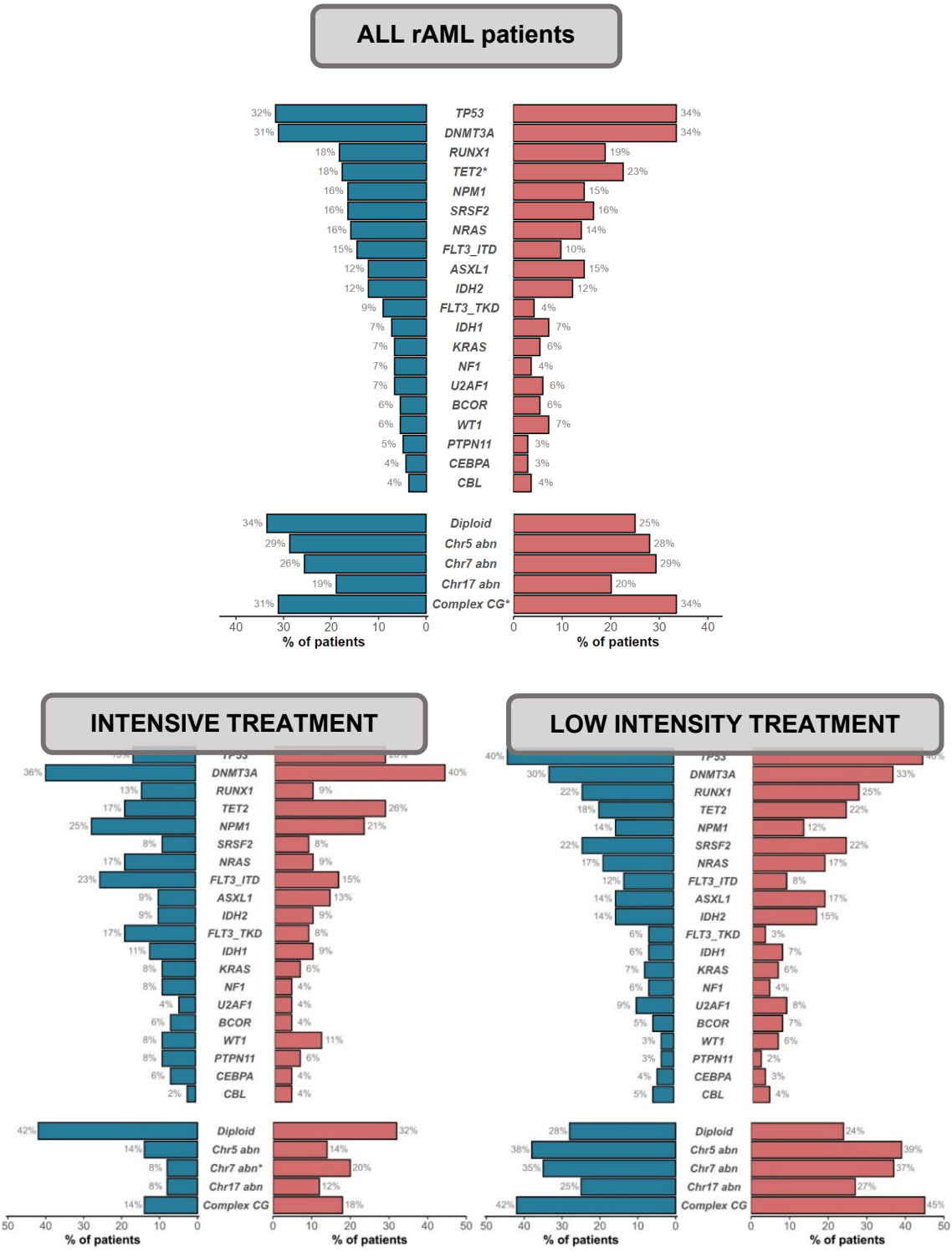

Figure S9. Frequency of mutations and cytogenetic findings at diagnosis and relapse in rAML patients with paired samples. An asterisk specifies genes/cytogenetic findings with significant proportion changes using a paired-sample approach with the McNemar test.

Figure S10

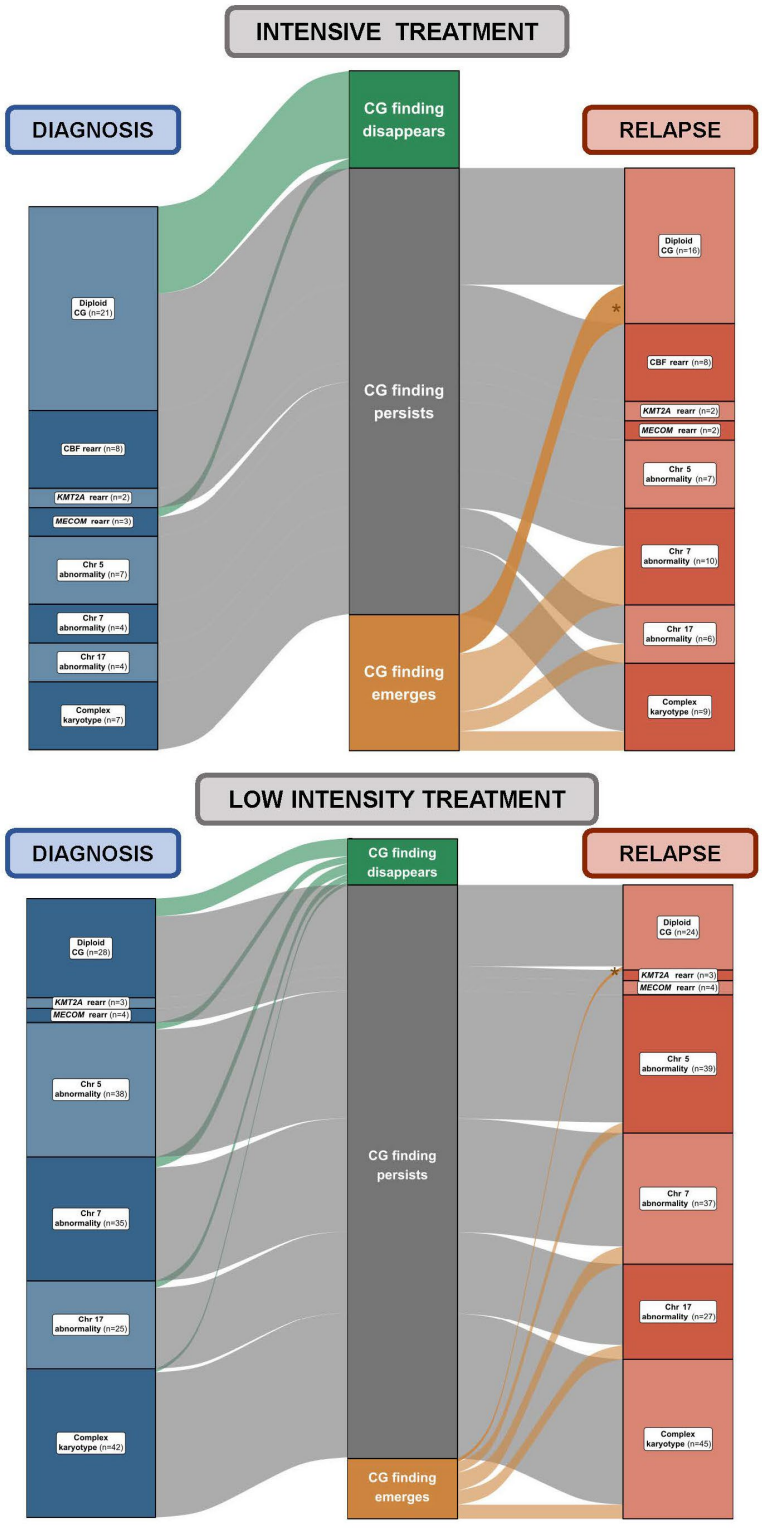

Figure S10. Cytogenetic dynamics from diagnosis to relapse of patients treated with IT (top) or treated with LIT (bottom).

Figure S11

Variable	Total n / Events	HR (90% CI)	p-val	mOS	p-val
Sex (male)	n=78, events=71 (Female) vs n=86, events=78 (Male)	1.01 (0.73-1.4)	0.94	5.5 months (Female) vs 5.1 months (Male)	0.938
Age at relapse (≥60)	n=40, events=35 (No) vs n=124, events=114 (Yes)	1.36 (0.93-1.99)	0.116	6.5 months (No) vs 5.1 months (Yes)	0.113
trAML	n=127, events=115 (No) vs n=37, events=34 (Yes)	1.1 (0.75-1.62)	0.614	5.5 months (No) vs 4.7 months (Yes)	0.613
Prior alloSCT	n=129, events=116 (No) vs n=35, events=33 (Yes)	0.87 (0.59-1.28)	0.484	4.8 months (No) vs 6.6 months (Yes)	0.481
LIT (vs IT)	n=57, events=49 (IT) vs n=107, events=100 (LIT)	1.38 (0.98-1.95)	0.066	6.6 months (IT) vs 4.9 months (LIT)	0.065
Prior Ven	n=81, events=70 (No) vs n=83, events=79 (Yes)	1.16 (0.84-1.6)	0.38	5.6 months (No) vs 4.8 months (Yes)	0.378
Time from CR to Rel (cont)	n=164 events=149	0.98 (0.95-1)	0.055		
<12m from CR to Rel	n=41, events=35 (No) vs n=123, events=114 (Yes)	1.8 (1.22-2.64)	0.003	8.1 months (No) vs 4.3 months (Yes)	0.002
WBC (cont)	n=158 events=143	1.01 (1-1.02)	0.019		
WBC > 20x10^9/L	n=143, events=128 (No) vs n=15, events=15 (Yes)	2.37 (1.38-4.08)	0.002	5.6 months (No) vs 2.8 months (Yes)	0.001
ANC (cont)	n=158 events=143	1.11 (1.03-1.2)	0.005		
Hgb (cont)	n=158 events=143	0.86 (0.77-0.96)	0.008		
Plt (cont)	n=158 events=143	1 (0.99-1)	0.444		
BM blast (cont)	n=157 events=142	1.01 (1-1.01)	0.027		
Diploid CG	n=111, events=101 (No) vs n=41, events=36 (Yes)	0.63 (0.43-0.93)	0.019	4.7 months (No) vs 7 months (Yes)	0.018
Chr5 abn	n=106, events=93 (No) vs n=46, events=44 (Yes)	1.68 (1.17-2.41)	0.005	6.5 months (No) vs 3.8 months (Yes)	0.004
Chr7 abn	n=104, events=92 (No) vs n=48, events=45 (Yes)	1.75 (1.21-2.52)	0.003	6.5 months (No) vs 4.8 months (Yes)	0.003
Chr17 abn	n=119, events=107 (No) vs n=33, events=30 (Yes)	1.33 (0.89-2)	0.169	5.8 months (No) vs 5.1 months (Yes)	0.168
KMT2Ar	n=147, events=132 (No) vs n=5, events=5 (Yes)	3.85 (1.53-9.65)	0.004	5.8 months (No) vs 2.6 months (Yes)	0.002
CBFr	n=144, events=132 (No) vs n=8, events=5 (Yes)	0.53 (0.22-1.31)	0.171	5.5 months (No) vs 7.3 months (Yes)	0.165
Complex CG	n=83, events=71 (No) vs n=69, events=66 (Yes)	1.8 (1.28-2.53)	0.001	6.7 months (No) vs 4.2 months (Yes)	0.001
ASXL1	n=133, events=119 (No) vs n=24, events=24 (Yes)	1.26 (0.81-1.95)	0.313	5.5 months (No) vs 5.2 months (Yes)	0.312
BCOR	n=148, events=134 (No) vs n=9, events=9 (Yes)	1.01 (0.51-1.99)	0.974	5.3 months (No) vs 6.5 months (Yes)	0.973
CBL	n=151, events=137 (No) vs n=6, events=6 (Yes)	1.12 (0.49-2.54)	0.792	5.5 months (No) vs 4.4 months (Yes)	0.787
CEBPA	n=152, events=139 (No) vs n=5, events=4 (Yes)	0.65 (0.24-1.78)	0.405	5.5 months (No) vs 3.1 months (Yes)	0.402
DDX41	n=151, events=138 (No) vs n=6, events=5 (Yes)	0.7 (0.28-1.71)	0.431	5.2 months (No) vs 8.1 months (Yes)	0.431
DNMT3A	n=102, events=94 (No) vs n=55, events=49 (Yes)	0.81 (0.57-1.14)	0.227	4.7 months (No) vs 6.6 months (Yes)	0.226
EZH2	n=150, events=137 (No) vs n=7, events=6 (Yes)	1.03 (0.45-2.35)	0.938	5.3 months (No) vs 7.3 months (Yes)	0.934
FLT3 TKD	n=150, events=136 (No) vs n=7, events=7 (Yes)	1.78 (0.83-3.82)	0.138	5.5 months (No) vs 3.8 months (Yes)	0.137
FLT3 ITD	n=141, events=128 (No) vs n=16, events=15 (Yes)	0.95 (0.55-1.62)	0.84	5.2 months (No) vs 6.5 months (Yes)	0.834
GATA2	n=150, events=137 (No) vs n=7, events=6 (Yes)	0.73 (0.32-1.65)	0.447	5.5 months (No) vs 5.1 months (Yes)	0.443
IDH1	n=145, events=135 (No) vs n=12, events=8 (Yes)	0.57 (0.28-1.16)	0.123	5.2 months (No) vs 7.7 months (Yes)	0.118
IDH2	n=137, events=124 (No) vs n=20, events=19 (Yes)	0.75 (0.46-1.21)	0.24	5.1 months (No) vs 7.3 months (Yes)	0.239
KRAS	n=148, events=135 (No) vs n=9, events=8 (Yes)	1.56 (0.76-3.2)	0.228	5.5 months (No) vs 3.9 months (Yes)	0.221
NPM1	n=133, events=121 (No) vs n=24, events=22 (Yes)	1.06 (0.67-1.68)	0.789	5.2 months (No) vs 5.8 months (Yes)	0.795
NF1	n=151, events=137 (No) vs n=6, events=6 (Yes)	2.5 (1.09-5.75)	0.031	5.5 months (No) vs 3.2 months (Yes)	0.026
NRAS	n=134, events=121 (No) vs n=23, events=22 (Yes)	1.09 (0.69-1.72)	0.709	5.5 months (No) vs 4.9 months (Yes)	0.707
RUNX1	n=126, events=115 (No) vs n=31, events=28 (Yes)	0.83 (0.55-1.26)	0.389	5.1 months (No) vs 6.7 months (Yes)	0.389
SF3B1	n=150, events=136 (No) vs n=7, events=7 (Yes)	0.61 (0.28-1.31)	0.204	5.2 months (No) vs 11.7 months (Yes)	0.199
SRSF2	n=130, events=118 (No) vs n=27, events=25 (Yes)	1.12 (0.72-1.72)	0.617	5.5 months (No) vs 5.3 months (Yes)	0.617
STAG2	n=150, events=136 (No) vs n=7, events=7 (Yes)	1.02 (0.47-2.18)	0.967	5.5 months (No) vs 5.1 months (Yes)	0.968
TET2	n=120, events=108 (No) vs n=37, events=35 (Yes)	1.11 (0.76-1.63)	0.577	5.5 months (No) vs 5.1 months (Yes)	0.574
TP53	n=102, events=91 (No) vs n=55, events=52 (Yes)	1.72 (1.21-2.43)	0.002	6.3 months (No) vs 4.2 months (Yes)	0.002
U2AF1	n=147, events=133 (No) vs n=10, events=10 (Yes)	1.17 (0.61-2.22)	0.642	5.5 months (No) vs 4.8 months (Yes)	0.636
WT1	n=145, events=131 (No) vs n=12, events=12 (Yes)	1.26 (0.7-2.29)	0.439	5.5 months (No) vs 5 months (Yes)	0.439

Figure S11. Univariate analysis for OS in all patients.

Figure S12

Variable	Total n / Events	HR (90% CI)	p-val	mOS	p-val
Sex (male)	n=30, events=26 (Female) vs n=27, events=23 (Male)	0.82 (0.47-1.44)	0.49	6.2 months (Female) vs 6.6 months (Male)	0.482
Age at relapse (≥60)	n=36, events=31 (No) vs n=21, events=18 (Yes)	1.24 (0.69-2.23)	0.477	6.5 months (No) vs 6.6 months (Yes)	0.469
trAML	n=51, events=43 (No) vs n=6, events=6 (Yes)	1.04 (0.44-2.44)	0.932	6.6 months (No) vs 5.9 months (Yes)	0.926
Prior alloSCT	n=37, events=30 (No) vs n=20, events=19 (Yes)	1.01 (0.56-1.82)	0.967	5.1 months (No) vs 6.7 months (Yes)	0.986
Prior Ven	n=41, events=33 (No) vs n=16, events=16 (Yes)	1 (0.55-1.84)	0.987	6.5 months (No) vs 7 months (Yes)	0.987
Time from CR to Rel (cont)	n=57 events=49	0.99 (0.95-1.04)	0.805		
<12m from CR to Rel	n=13, events=11 (No) vs n=44, events=38 (Yes)	1.54 (0.78-3.05)	0.21	11.1 months (No) vs 6.2 months (Yes)	0.206
WBC (cont)	n=54 events=46	1.01 (1-1.02)	0.136		
WBC > 20x10^9/L	n=47, events=39 (No) vs n=7, events=7 (Yes)	2.09 (0.91-4.78)	0.08	6.7 months (No) vs 4.1 months (Yes)	0.073
ANC (cont)	n=54 events=46	1.09 (0.97-1.22)	0.158		
Hgb (cont)	n=54 events=46	0.9 (0.76-1.06)	0.206		
Plt (cont)	n=54 events=46	1 (0.99-1.01)	0.6		
BM blast (cont)	n=53 events=45	1 (0.99-1.02)	0.466		
Diploid CG	n=35, events=29 (No) vs n=16, events=14 (Yes)	0.69 (0.36-1.32)	0.263	6.2 months (No) vs 7.6 months (Yes)	0.26
Chr5 abn	n=44, events=36 (No) vs n=7, events=7 (Yes)	1.9 (0.84-4.31)	0.123	6.6 months (No) vs 3.2 months (Yes)	0.116
Chr7 abn	n=41, events=34 (No) vs n=10, events=9 (Yes)	2.42 (1.12-5.25)	0.025	7.1 months (No) vs 4.2 months (Yes)	0.021
Chr17 abn	n=45, events=38 (No) vs n=6, events=5 (Yes)	1.58 (0.61-4.12)	0.345	6.6 months (No) vs 4.8 months (Yes)	0.339
KMT2Ar	n=49, events=41 (No) vs n=2, events=2 (Yes)	4.15 (0.95-18.25)	0.059	6.6 months (No) vs 3.1 months (Yes)	0.041
CBFr	n=43, events=38 (No) vs n=8, events=5 (Yes)	0.66 (0.26-1.68)	0.381	6.6 months (No) vs 7.3 months (Yes)	0.378
Complex CG	n=36, events=29 (No) vs n=15, events=14 (Yes)	2.19 (1.13-4.24)	0.02	7.1 months (No) vs 4.1 months (Yes)	0.017
ASXL1	n=47, events=40 (No) vs n=7, events=7 (Yes)	1.63 (0.72-3.68)	0.237	6.6 months (No) vs 4.6 months (Yes)	0.229
BCOR	n=52, events=45 (No) vs n=2, events=2 (Yes)	0.81 (0.19-3.35)	0.769	6.2 months (No) vs 9.9 months (Yes)	0.773
CBL	n=52, events=45 (No) vs n=2, events=2 (Yes)	2.43 (0.56-10.44)	0.234	6.6 months (No) vs 4.4 months (Yes)	0.219
CEBPA	n=52, events=46 (No) vs n=2, events=1 (Yes)	0.2 (0.03-1.48)	0.116	6.2 months (No) vs 16.9 months (Yes)	0.082
DDX41	n=50, events=43 (No) vs n=4, events=4 (Yes)	1.08 (0.38-3.06)	0.881	6.2 months (No) vs 7.2 months (Yes)	0.878
DNMT3A	n=33, events=30 (No) vs n=21, events=17 (Yes)	0.67 (0.37-1.22)	0.192	4.1 months (No) vs 6.7 months (Yes)	0.185
EZH2	n=52, events=45 (No) vs n=2, events=2 (Yes)	1.42 (0.34-5.95)	0.63	6.5 months (No) vs 6 months (Yes)	0.627
FLT3 TKD	n=50, events=43 (No) vs n=4, events=4 (Yes)	1.7 (0.6-4.77)	0.315	6.6 months (No) vs 4.5 months (Yes)	0.323
FLT3 ITD	n=46, events=40 (No) vs n=8, events=7 (Yes)	0.78 (0.35-1.75)	0.554	5.5 months (No) vs 6.6 months (Yes)	0.537
GATA2	n=52, events=45 (No) vs n=2, events=2 (Yes)	0.58 (0.14-2.41)	0.453	6.2 months (No) vs 13.3 months (Yes)	0.449
IDH1	n=49, events=45 (No) vs n=5, events=2 (Yes)	0.55 (0.13-2.3)	0.415	6.5 months (No) vs NA months (Yes)	0.41
IDH2	n=49, events=42 (No) vs n=5, events=5 (Yes)	1 (0.39-2.54)	0.995	6.2 months (No) vs 6.6 months (Yes)	0.993
KRAS	n=51, events=44 (No) vs n=3, events=3 (Yes)	4.04 (1.15-14.11)	0.029	6.6 months (No) vs 3 months (Yes)	0.018
NPM1	n=42, events=36 (No) vs n=12, events=11 (Yes)	1.22 (0.61-2.45)	0.574	5.4 months (No) vs 6.6 months (Yes)	0.592
NF1	n=52, events=45 (No) vs n=2, events=2 (Yes)	1.68 (0.4-7.1)	0.478	6.5 months (No) vs 5.6 months (Yes)	0.486
NRAS	n=49, events=42 (No) vs n=5, events=5 (Yes)	1 (0.39-2.58)	1	6.6 months (No) vs 2.8 months (Yes)	0.991
RUNX1	n=49, events=42 (No) vs n=5, events=5 (Yes)	1.83 (0.71-4.7)	0.212	6.6 months (No) vs 4.6 months (Yes)	0.204
SF3B1	n=52, events=45 (No) vs n=2, events=2 (Yes)	0.65 (0.16-2.69)	0.551	6.2 months (No) vs 12.5 months (Yes)	0.551
SRSF2	n=50, events=43 (No) vs n=4, events=4 (Yes)	3.04 (1.03-8.98)	0.044	6.6 months (No) vs 4.3 months (Yes)	0.034
STAG2	n=51, events=44 (No) vs n=3, events=3 (Yes)	0.67 (0.21-2.17)	0.504	6.5 months (No) vs 13.3 months (Yes)	0.505
TET2	n=40, events=35 (No) vs n=14, events=12 (Yes)	0.87 (0.45-1.68)	0.679	5.5 months (No) vs 7.1 months (Yes)	0.686
TP53	n=40, events=34 (No) vs n=14, events=13 (Yes)	1.25 (0.65-2.39)	0.504	6.5 months (No) vs 6.4 months (Yes)	0.495
U2AF1	n=52, events=45 (No) vs n=2, events=2 (Yes)	0.84 (0.2-3.47)	0.806	6.5 months (No) vs 9.3 months (Yes)	0.81
WT1	n=48, events=41 (No) vs n=6, events=6 (Yes)	1.92 (0.8-4.61)	0.147	6.6 months (No) vs 5 months (Yes)	0.139

Figure S12. Univariate analysis for OS in patients receiving IT at diagnosis.

Figure S13

Variable	Total n / Events	HR (90% CI)	p-val	mOS	p-val
Sex (male)	n=48, events=45 (Female) vs n=59, events=55 (Male)	1.13 (0.76-1.68)	0.551	5.3 months (Female) vs 4.3 months (Male)	0.549
Age at relapse (≥60)	n=4, events=4 (No) vs n=103, events=96 (Yes)	0.86 (0.31-2.35)	0.764	5.8 months (No) vs 4.9 months (Yes)	0.77
trAML	n=76, events=72 (No) vs n=31, events=28 (Yes)	1.03 (0.66-1.59)	0.902	5 months (No) vs 4.7 months (Yes)	0.9
Prior alloSCT	n=92, events=86 (No) vs n=15, events=14 (Yes)	0.84 (0.48-1.48)	0.548	4.5 months (No) vs 5.8 months (Yes)	0.548
Prior Ven	n=40, events=37 (No) vs n=67, events=63 (Yes)	1.06 (0.7-1.59)	0.788	5.5 months (No) vs 4.2 months (Yes)	0.791
Time from CR to Rel (cont)	n=107 events=100	0.97 (0.95-1)	0.037		
<12m from CR to Rel	n=28, events=24 (No) vs n=79, events=76 (Yes)	1.95 (1.22-3.11)	0.005	8 months (No) vs 4 months (Yes)	0.004
WBC (cont)	n=104 events=97	1.05 (1.02-1.07)	<0.001		
WBC > 20x10^9/L	n=96, events=89 (No) vs n=8, events=8 (Yes)	3.52 (1.68-7.33)	0.001	5.3 months (No) vs 1.5 months (Yes)	<0.001
ANC (cont)	n=104 events=97	1.19 (1.07-1.33)	0.002		
Hgb (cont)	n=104 events=97	0.83 (0.71-0.97)	0.023		
Plt (cont)	n=104 events=97	1 (0.99-1)	0.183		
BM blast (cont)	n=104 events=97	1.01 (1.01-1.02)	0.001		
Diploid CG	n=76, events=72 (No) vs n=25, events=22 (Yes)	0.63 (0.39-1.01)	0.056	4.3 months (No) vs 6.9 months (Yes)	0.054
Chr5 abn	n=62, events=57 (No) vs n=39, events=37 (Yes)	1.48 (0.97-2.24)	0.068	5.8 months (No) vs 4 months (Yes)	0.066
Chr7 abn	n=63, events=58 (No) vs n=38, events=36 (Yes)	1.53 (1-2.35)	0.052	5.2 months (No) vs 4.8 months (Yes)	0.051
Chr17 abn	n=74, events=69 (No) vs n=27, events=25 (Yes)	1.19 (0.75-1.88)	0.463	5.2 months (No) vs 5.1 months (Yes)	0.461
KMT2Ar	n=98, events=91 (No) vs n=3, events=3 (Yes)	3.99 (1.22-12.99)	0.022	5.3 months (No) vs 2.6 months (Yes)	0.013
Complex CG	n=47, events=42 (No) vs n=54, events=52 (Yes)	1.54 (1.02-2.33)	0.039	6.3 months (No) vs 4.3 months (Yes)	0.038
ASXL1	n=86, events=79 (No) vs n=17, events=17 (Yes)	1.09 (0.64-1.84)	0.752	5 months (No) vs 5.3 months (Yes)	0.751
BCOR	n=96, events=89 (No) vs n=7, events=7 (Yes)	1.06 (0.49-2.3)	0.879	5.1 months (No) vs 5.1 months (Yes)	0.879
CBL	n=99, events=92 (No) vs n=4, events=4 (Yes)	0.87 (0.32-2.38)	0.787	5.1 months (No) vs 7.7 months (Yes)	0.79
CEBPA	n=100, events=93 (No) vs n=3, events=3 (Yes)	4.38 (1.33-14.36)	0.015	5.2 months (No) vs 2 months (Yes)	0.008
DDX41	n=101, events=95 (No) vs n=2, events=1 (Yes)	0.33 (0.05-2.35)	0.266	5 months (No) vs 10.9 months (Yes)	0.242
DNMT3A	n=69, events=64 (No) vs n=34, events=32 (Yes)	0.94 (0.61-1.44)	0.785	5 months (No) vs 5.5 months (Yes)	0.787
EZH2	n=98, events=92 (No) vs n=5, events=4 (Yes)	0.9 (0.33-2.45)	0.831	5.1 months (No) vs 8.1 months (Yes)	0.832
FLT3 TKD	n=100, events=93 (No) vs n=3, events=3 (Yes)	2.2 (0.69-7.02)	0.181	5.1 months (No) vs 2.5 months (Yes)	0.171
FLT3 ITD	n=95, events=88 (No) vs n=8, events=8 (Yes)	1.31 (0.63-2.73)	0.463	5.1 months (No) vs 4.9 months (Yes)	0.461
GATA2	n=98, events=92 (No) vs n=5, events=4 (Yes)	0.83 (0.3-2.26)	0.71	5.2 months (No) vs 5 months (Yes)	0.709
IDH1	n=96, events=90 (No) vs n=7, events=6 (Yes)	0.59 (0.26-1.35)	0.21	4.8 months (No) vs 7.7 months (Yes)	0.204
IDH2	n=88, events=82 (No) vs n=15, events=14 (Yes)	0.63 (0.36-1.12)	0.118	4.5 months (No) vs 7.9 months (Yes)	0.115
KRAS	n=97, events=91 (No) vs n=6, events=5 (Yes)	1.13 (0.46-2.81)	0.787	5.1 months (No) vs 4.7 months (Yes)	0.784
NPM1	n=91, events=85 (No) vs n=12, events=11 (Yes)	1.02 (0.54-1.93)	0.941	5.1 months (No) vs 3.9 months (Yes)	0.94
NF1	n=99, events=92 (No) vs n=4, events=4 (Yes)	3.51 (1.24-9.94)	0.018	5.2 months (No) vs 2.5 months (Yes)	0.012
NRAS	n=85, events=79 (No) vs n=18, events=17 (Yes)	1.08 (0.64-1.83)	0.771	5.1 months (No) vs 5 months (Yes)	0.77
RUNX1	n=77, events=73 (No) vs n=26, events=23 (Yes)	0.66 (0.41-1.05)	0.079	4.2 months (No) vs 6.9 months (Yes)	0.076
SF3B1	n=98, events=91 (No) vs n=5, events=5 (Yes)	0.57 (0.23-1.41)	0.225	5 months (No) vs 11.7 months (Yes)	0.218
SRSF2	n=80, events=75 (No) vs n=23, events=21 (Yes)	0.91 (0.56-1.48)	0.7	4.8 months (No) vs 5.5 months (Yes)	0.699
STAG2	n=99, events=92 (No) vs n=4, events=4 (Yes)	1.94 (0.7-5.41)	0.202	5.2 months (No) vs 3.8 months (Yes)	0.198
TET2	n=80, events=73 (No) vs n=23, events=23 (Yes)	1.31 (0.82-2.1)	0.259	5.3 months (No) vs 3.3 months (Yes)	0.255
TP53	n=62, events=57 (No) vs n=41, events=39 (Yes)	1.93 (1.26-2.95)	0.002	6 months (No) vs 3.6 months (Yes)	0.002
U2AF1	n=95, events=88 (No) vs n=8, events=8 (Yes)	1.25 (0.6-2.59)	0.547	5.1 months (No) vs 3.8 months (Yes)	0.544
WT1	n=97, events=90 (No) vs n=6, events=6 (Yes)	1 (0.44-2.29)	1	5.1 months (No) vs 5.1 months (Yes)	0.996

Figure S13. Univariate analysis for OS in patients receiving LIT at diagnosis.

Figure S14

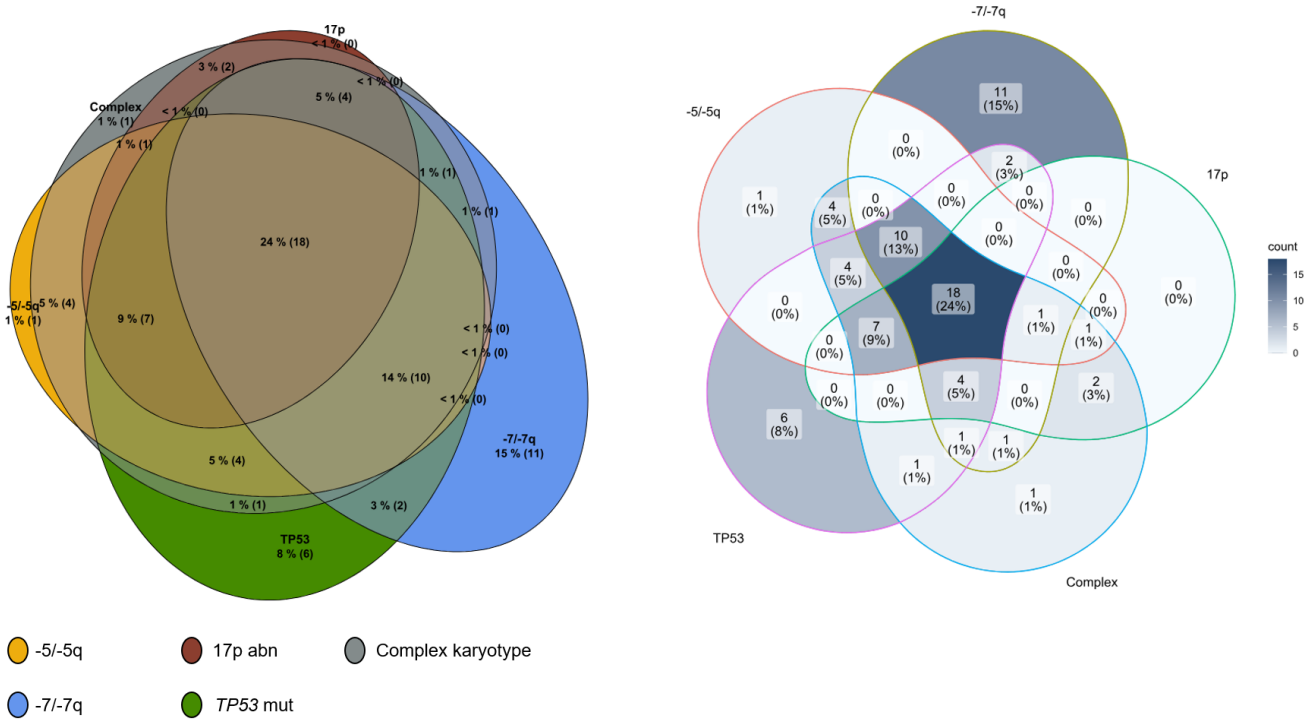

Figure S14. Venn diagram (left) and Euler plot (right) describing cooccurrence of cytogenetic and molecular abnormalities (Chr5, Chr7 and Chr17 abnormalities, complex karyotype, and TP53 mutation). In the Venn diagram, area of interaction is proportional to the number of patients.

Supplementary analysis 1 (SA1)

Patients receiving FLT3 inhibitors

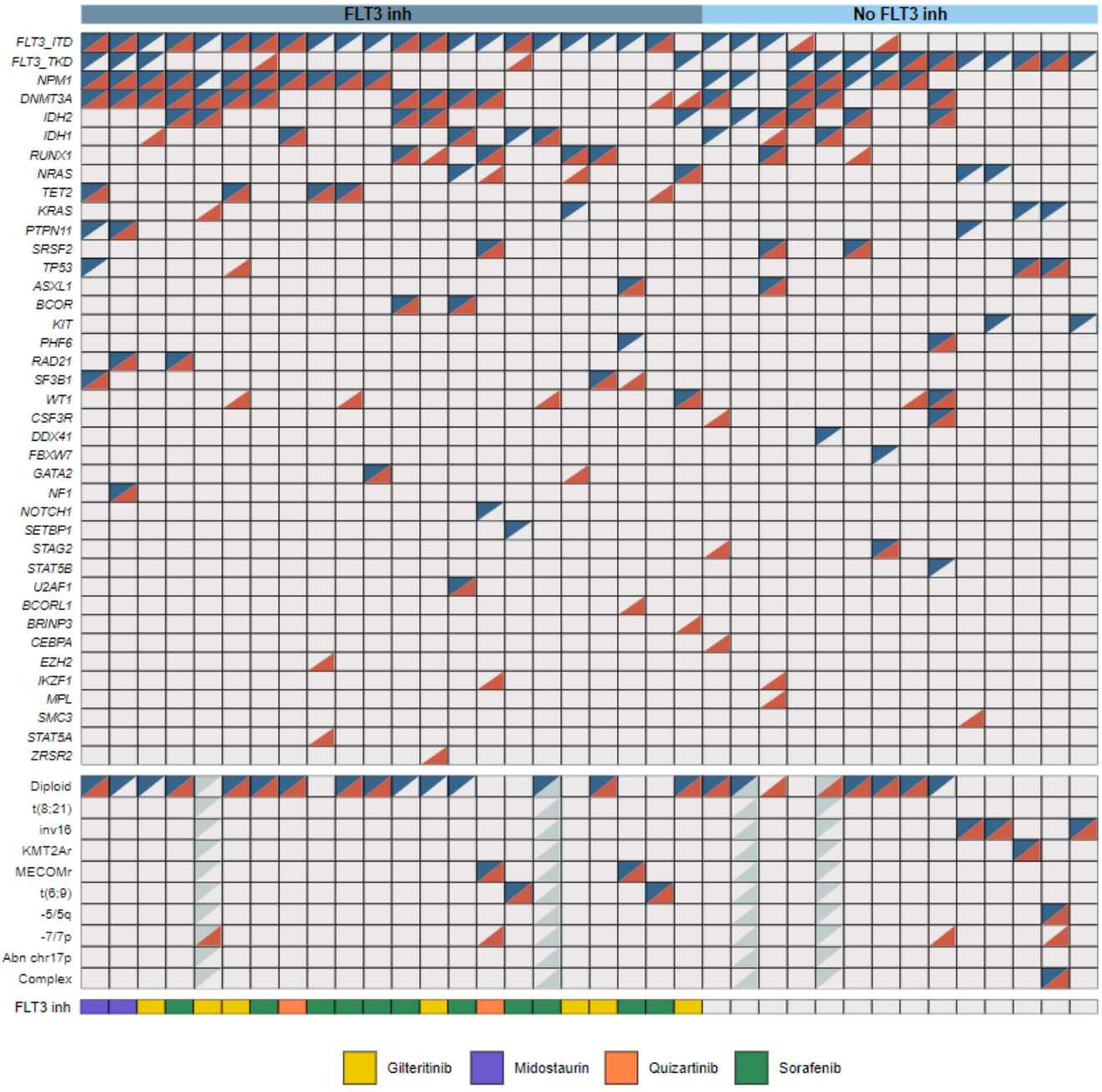

Figure S15. Mutations and cytogenetics at diagnosis and relapse in patients with *FLT3* (ITD and TKD mutations), stratified by therapy (FLT3 inhibitor).

	FLT3 inhibitor (n=22)		No FLT3 inhibitor (n=134)	
Gene	**Emergence rate**	**Clearance rate**	**Emergence rate**	**Clearance rate**
ASXL1	0/21 (0%)	0/1 (0%)	6/116 (5.2%)	1/18 (5.6%)
BCOR	0/20 (0%)	0/2	3/128 (2.3%)	2/6 (33.3%)
BCORL1	1/22 (4.5%)	0/0	3/131 (2.3%)	2/3 (66.7%)
BRINP3	1/22 (4.5%)	0/0	0/133	0/1
DNMT3A	2/11 (18.2%)	0/11	7/95 (7.4%)	4/39 (10.3%)
EZH2	1/22 (4.5%)	0/0	4/130 (3.1%)	2/4 (50%)
FLT3_TKD	2/18 (11.1%)	4/4 (100%)	1/123 (0.8%)	7/11 (63.6%)
FLT3_ITD	0/1	11/21 (52.4%)	6/131 (4.6%)	3/3 (100%)
GATA2	1/21 (4.8%)	0/1	3/132 (2.3%)	0/2
IDH1	1/18 (5.6%)	1/4 (25%)	3/126 (2.4%)	3/8 (37.5%)
IDH2	0/17	1/5 (20%)	3/120 (2.5%)	1/14 (7.1%)
IKZF1	1/22 (4.5%)	0/0	3/129 (2.3%)	2/5 (40%)
KRAS	1/21 (4.8%)	1/1 (100%)	2/124 (1.6%)	4/10 (40%)
NF1	0/21	0/1	2/125 (1.6%)	6/9 (66.7%)
NOTCH1	0/21	1/1 (100%)	0/134	0/0
NPM1	0/11	1/11 (9.1%)	0/118	3/16 (18.8%)
NRAS	2/20 (10%)	1/2 (50%)	3/110 (2.7%)	7/24 (29.2%)
PHF6	0/21	1/1 (100%)	3/129 (2.3%)	2/5 (40%)
PTPN11	0/20	1/2 (50%)	1/129 (0.8%)	2/5 (40%)
RAD21	0/20	0/2	2/134 (1.5%)	0/0
RUNX1	1/18 (5.6%)	0/4	4/108 (3.7%)	4/26 (15.4%)
SETBP1	0/21	1/1 (100%)	0/131	1/3 (33.3%)
SF3B1	1/20 (5%)	0/2	0/130	0/4
SRSF2	0/21	0/1	1/108 (0.9%)	1/26 (3.8%)
STAG1	0/22	0/0	0/133	1/1 (100%)
TET2	1/18 (5.6%)	0/4	9/110 (8.2%)	1/24 (4.2%)
TP53	1/21 (4.8%)	1/1 (100%)	7/86 (8.1%)	1/48 (2.1%)
U2AF1	0/21	0/1	0/124	1/10 (10%)
WT1	3/21 (14.3%)	0/1	4/128 (3.1%)	2/6 (33.3%)
ZRSR2	1/22 (4.5%)	0/0	1/132 (0.8%)	2/2 (100%)

Table S5 Mutations and cytogenetic abnormalities at diagnosis and relapse in patients with *FLT3* (ITD and TKD mutations), stratified by therapy (FLT3 inhibitor).

Supplementary analysis 2 (SA2)

Patients with normal karyotype

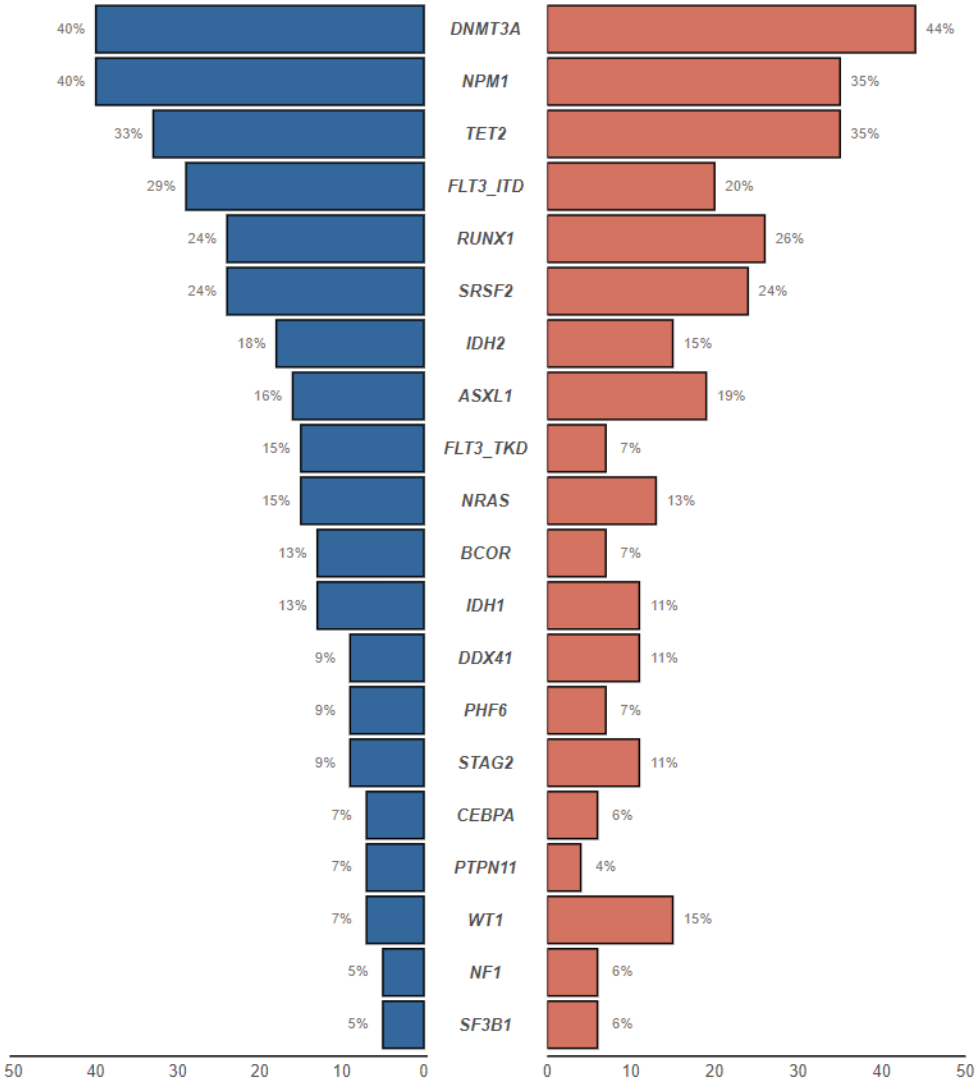

Figure S16. Mutations and cytogenetics at diagnosis (blue) and relapse (red) in rAML patients with *normal karyotype at the moment of diagnosis.*

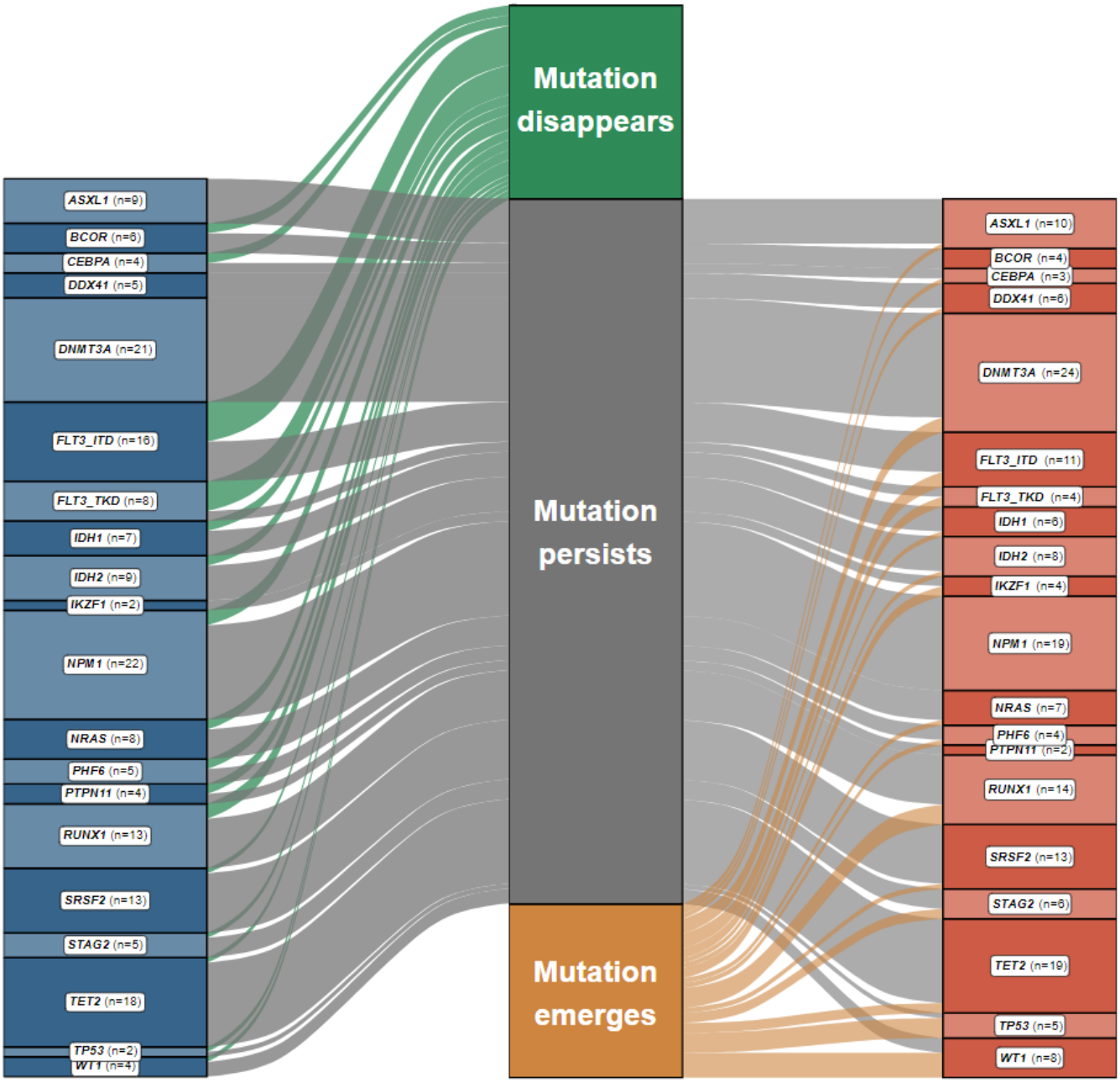

Figure S17. Mutation dynamics between diagnosis and relapse in patients with normal karyotype.

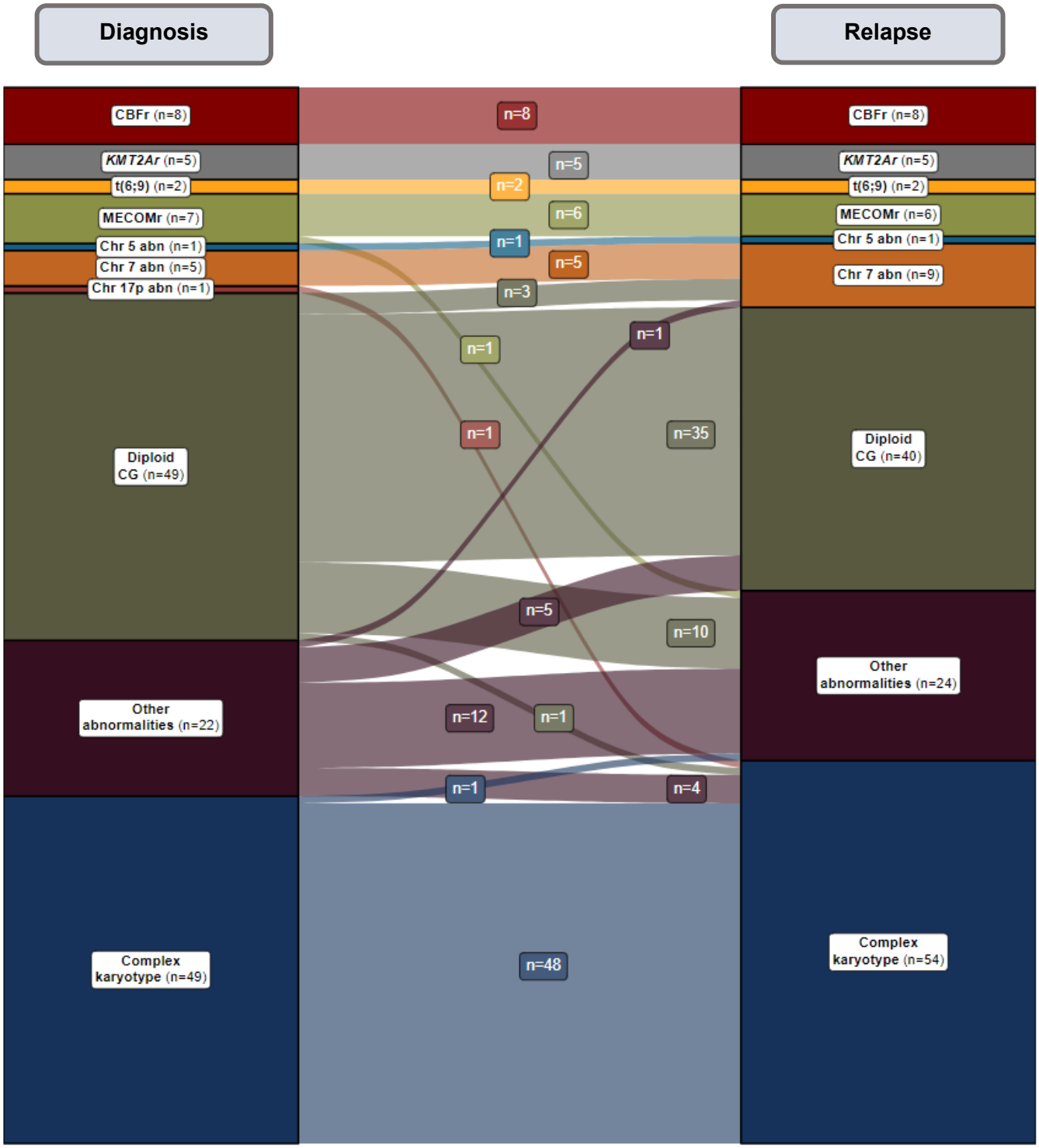

Figure S18. Flow of all patients according to their cytogenetic classification at diagnosis and at relapse. Numbers in the labels represent the number of patients.

ID	CG diagnosis	CG Relapse
9	46,XY[20]	46,XY[18]
20	46,XY[20]	46,XY[20]
24	46,XY,t(4;15)(q21;q21)[1]/46,XY[19]	46,XY[20]
26	46,XY[20]	45,XY,der(5;17)(p10;q10),add(7)(q22),del(12)(p12)[6]/ 44,XY,der(5;17)(p10;q10),-7[14]
34	46,XX[20]	46,XX[20]
35	46,XY[20]	46,XY[20]
37	46,XX[18]	46,XX[20]
38	46,XX[20]	46,XX[20]
43	47,XX,+mar[1]/46,XX[19]	NA
48	46,XX[20]	46,XX[20]
49	46,XY[20]	47,XY,+6[3]/48,idem,+8[2]/46~50,idem,+8,+13[cp3]/ 46,XY[12]
51	46,XY[20]	46,XY[20]
52	46,XY[20]	46,XY[20]
57	46,XY[20]	46,XY[20]
61	46,XX[20]	41,X,-X,-4,add(11)(q24),add(16)(q24)[1]/46,XX[19]
63	46,XX[20]	46,XX[20]
65	46,XY[20]	46,XY[20]
67	46,XY[20]	46,XY[20]
75	46,XX[20]	46,XY[20]
77	46,XX[19]	47,XX,+8[1]/46,XX[19]
78	46,XX,del(7)(p15)[1]/46,XX[19]	46,XX,inv(1)(p13q44)[1]/46,XX,t(1;18)(q22;p11.3)[1]/ 46,XX,-13,+mar[1]/46,XX[15]//46,XY[2]
80	46,XY[20]	46,XY,dup(1)(q21q32)[13]/46,XY,der(6)t(1;6)(q21;p23)[1]/ 46,XY[6]
83	46,XY[20]	46,XY[11]
89	46,XX,add(2)(q21),add(19)(p13.3)[1]/ 46,XX[19]	46,XX[19]/46,XY[1]
90	46,XY[20]	46,XX[20]
92	46,XX[20]	46,XX,t(9;20)(p22;p13)[2]/46,XX,add(9)(p24),-18[1]/46,XX[5]//46,XY[12]
93	46,XX[20]	50,XX,+6,+8,+8,+8[17]/46,XX[3]
94	46,XY[20]	46,XY[20]
95	46,XY[20]	46,XY[20]
97	46,XX[3]	46,XX[20]
98	46,XY[20]	46,XY,del(1)(q21q22)[5]/46,Y,del(X)(q24q26)[1]/ 46,XY[14]
101	46,XX[20]	46,XX,t(4;17)(q12;q25)[15]/46,XX[5]
104	46,XY[20]	46,XY[20]
109	46,XX[20]	46,XX[20]
110	46,XY[20]	47,XY,+8,add(17)(p13)[12]/46,XY[8]
111	46,XY[20]	46,XY,t(6;22)(q25;q11.2)[20]
112	47,XY,+mar[2]/46,XX[18]	46,XY[20]
113	47,XY,+mar[1]/46,XY[19]	46,XY[20]
115	46,XX[20]	45,XX,-7[14]/45,XX,der(7;17)(q10;q10)[2]// 46,XY,inv(9)(p12q13)[6]
117	46,XY[20]	46,XY[20]
123	46,XY[20]	47,XY,+1[1]/46,XY[19]
125	47,XY,+11[1]/46,XY[19]	46,XY[20]
126	46,XY[20]	NA

129	48,XY,+12,+14[1]/46,XY[19]	NA
133	46,XX[20]	46,XX[20]
134	46,XY[20]	48,XY,+8,+21[3]/49,idem,+13[1]/46,XY[16]
136	46,XX[20]	47,XX,+4[17]/46,XX,del(7)(q22)[2]/46,XX[1]
137	46,XY[20]	46,XY[20]
140	46,XX[20]	46,XX[20]
141	46,XX[20]	NA
144	42,XY,-6,-7,-7,+8,add(15)(q24), add(19)(q13.3),-21,-21[1]/46,XY[19]	NA
145	47,XX,+mar[1]/46,XX[19]	47,XX,+11[9]/46,XX,del(7)(q22)[8]/46,XX[3]
150	47,XY,del(2)(p12),-3,+2mar[1]/ 45,XY,der(14;21)(q10;q10)[1]/46,XY[18]	46,XY[20]
161	46,XX[20]	46,XX,del(12)(p13p12)[9]/46,XX[11]
162	46,XY[20]	NA

Table S6. Karyotypes at diagnosis and relapse in patients with normal karyotype. Non-class defining abnormalities found in only one metaphase were not considered clonal.

Supplementary analysis 3 (SA3)

Prognostic scores for rAML

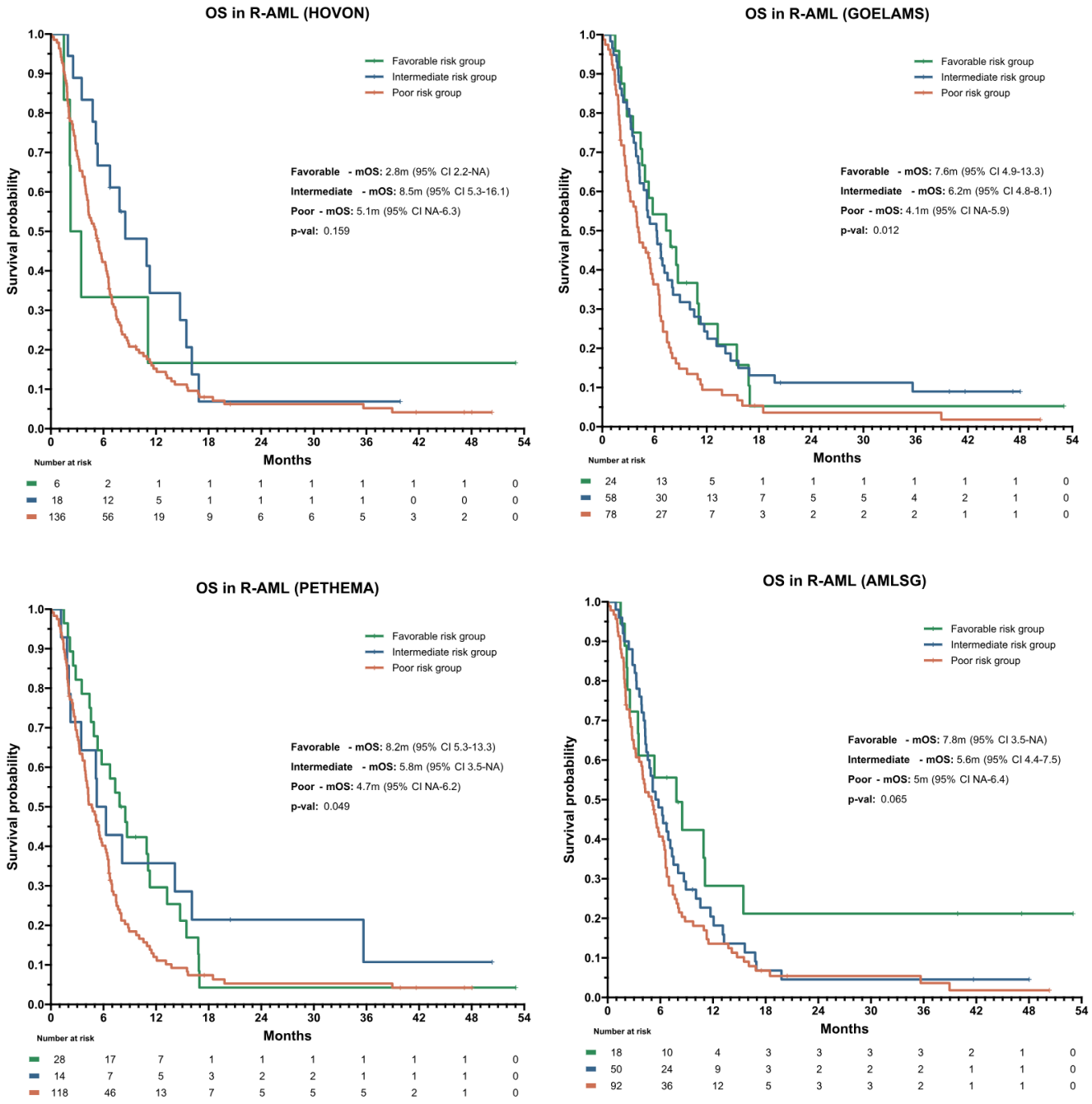

Figure S19. OS according to different prognostic scores. Upper left: HOVON (Breems et al, J Clin Oncol 2005). Upper right: GOELAMS (Chevallier et al, Leukemia 2011). Lower left: PETHEMA (Bergua et al, Br J Haematol 2016). Lower right: AMLSG (Schlenk et al, Leukemia 2017).

	Breems et al J Clin Oncol 2005	Chevallier et al Leukemia 2011	Bergua et al Br J Haematol 2016	Schlenk et al Leukemia 2017	Van der Maas NG et al ASH 2023
Relapse-free interval	Yes (6 - 18m)	Yes (12m)	Yes (12m)	Yes (6 - 18m)	Yes (12m)
Cytogenetics	CBF vs other	Adverse vs non-adverse	Inv16 vs Int vs High risk + t(8;21)	CBF (fav)	*MLL*, Complex karyotype (unfav)
Age	Yes (35 – 45yo)	No	Yes (60yo)	Yes	Yes (60yo)
Previous SCT	Yes (unfav)	No	Yes (AutoSCT unfav, alloSCT no effect)	Yes (unfav)	Yes (unfav)
WBC at diagnosis	No	No	No	No	Yes (>10K)
***FLT3*-ITD**	NA	Yes (unfav)	Yes (unfav)	Yes (unfav)	Yes (unfav)
***TP53* mut**	NA	NA	NA	No	Yes
***CEBPA* mut**	NA	NA	NA	Yes (fav)	No

Table S7. Summary of the different prognostic scores for rAML with the variables included in each classification.

	HOVON			GOLEAMS			PETHEMA			AMLSG		
	n	mOS (months)	1-yr OS	n	mOS (months)	1-yr OS	n	mOS (months)	1-yr OS	n	mOS (months)	1-yr OS
Low risk	6	2.8	17%	24	7.6	26%	28	8.2	30%	18	7.8	28%
Intermediate risk	18	8.5	34%	58	6.2	24%	14	5.8	36%	50	5.6	20%
High risk	136	5.1	15%	78	4.1	9%	118	4.7	12%	92	5	13%
P value (log-rank)	0.159			0.012			0.049			0.065		
Harrell's C-index	0.535			0.575			0.554			0.553		

Table S8. Overall survival, log-rank test and Harrel's C-index for each prognostic score evaluated.

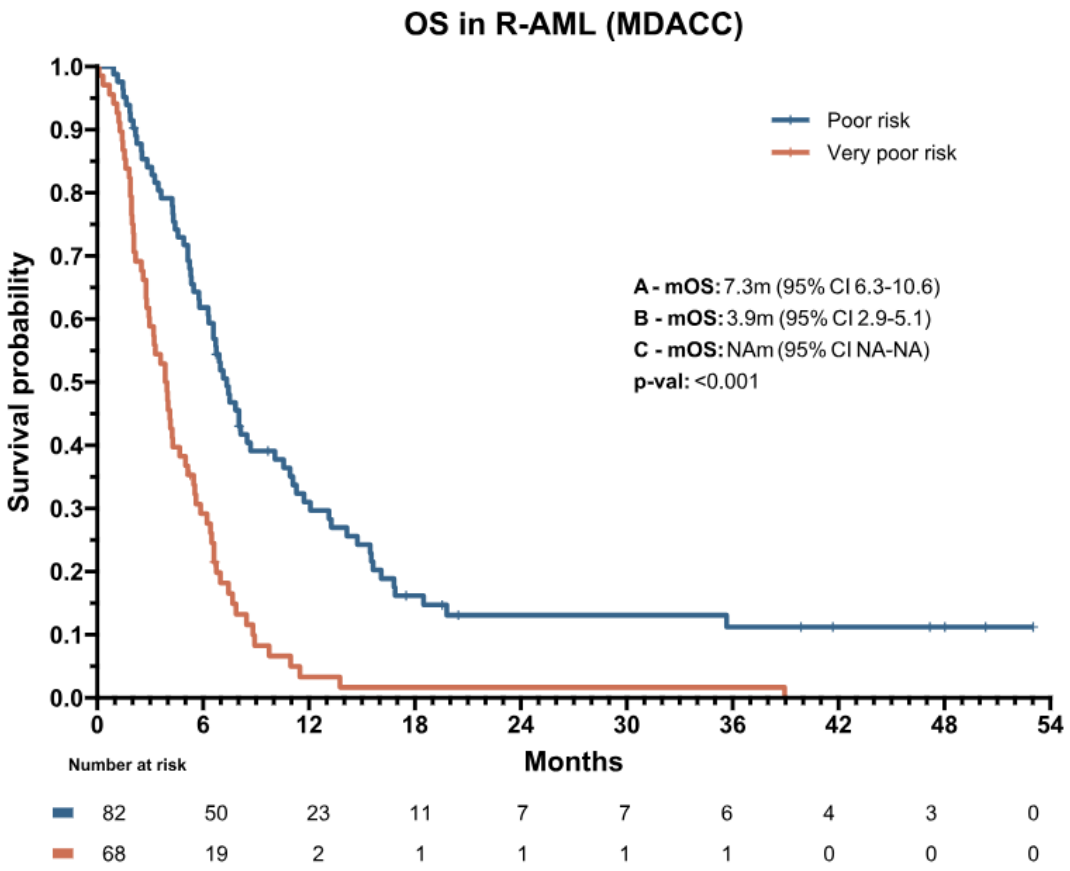

Figure S20. OS according to an exploratory analysis looking at risk groups defined the multivariate analysis. Patients with no or one risk factor (poor risk) vs patients with two or more risk factors (very poor risk). Risk factors are defined by time in remission < 12 months, adverse cytogenetics or *KMT2A* rearrangement at relapse, and a WBC > 20 x 10^9/L at relapse