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Short title: HAM plus VEN in R/R AML

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Abstract

Relapsed or refractory acute myeloid leukemia (R/R AML) remains a therapeutic challenge, with standard high-dose cytarabine-based salvage regimens achieving complete remission (CR) rates of around 50%. In the phase 1/2 RELAX trial, adding venetoclax (VEN) to high-dose cytarabine and mitoxantrone (HAM) was considered safe and resulted in CR or CR with incomplete hematologic recovery (CRi) in 75% of patients. Here, we performed a multicenter real-world analysis of 128 R/R AML patients treated with HAM plus VEN (VEN 400 mg days 1-14, cytarabine 1000 mg/m² IV twice daily days 3-5, mitoxantrone 10 mg/m² IV days 5-7) between March 2023 and June 2025. The median age was 60 years (range, 20-74). Patients had a median of 1 prior therapy (range, 1-5), 38% had prior allogeneic hematopoietic cell transplantation (HCT), and 25% VEN exposure. Overall, 69% achieved CR/CRi; response rates were higher in patients with one prior therapy line (78%) and VEN-naïve patients (76%). Sixty-eight percent proceeded to HCT. After a median follow-up of 11.5 months, estimated 1-year RFS and OS rates were 59% and 58%, respectively. In a prognostic classifier derived from pooled real-world and RELAX trial data (183 patients), *TP53* mutation, complex karyotype, ≥2 prior therapy lines, and Eastern Cooperative Oncology Group performance status (ECOG PS) ≥2 predicted inferior outcomes. Patients without these features had a 1-year OS of 73%. HAM plus VEN retains high efficacy and encouraging survival outcomes in a real-world setting, supporting its use as an effective salvage and bridge-to-transplant strategy.

Introduction

Acute myeloid leukemia (AML) that is either relapsed or refractory (R/R) to first line therapy continues to pose a major therapeutic challenge. Despite advances in treatment, the prognosis of these patients remains poor, and standard salvage chemotherapy regimens often yield unsatisfactory outcomes. Cytarabine-based regimens such as HAM (high-dose cytarabine, mitoxantrone), FLAG-IDA (fludarabine, cytarabine, G-CSF, idarubicin), or MEC (mitoxantrone, etoposide, cytarabine) result in complete remission (CR) rates of around 50%, with a median overall survival (OS) rarely exceeding 10 months[1, 2].

The selective BCL-2 inhibitor venetoclax (VEN) has demonstrated substantial activity in AML by inducing apoptosis in leukemic cells, particularly in combination with low-dose cytarabine or hypomethylating agents (HMA)[3, 4]. Adding VEN to HMA has become the standard of care in elderly or medically unfit patients with newly diagnosed AML who are not eligible to receive intensive chemotherapy. This approach has resulted in higher response rates and improved OS compared to HMA alone[3]. Based on these observations, there is a growing interest in integrating VEN with intensive chemotherapy regimens. The combination of VEN with FLAG-IDA was shown to be an effective intensive treatment regimen[5-9]. The phase 1/2 RELAX trial of the Study Alliance Leukemia (SAL) evaluated the feasibility and efficacy of combining the HAM backbone with VEN in patients with R/R AML that were eligible for intensive chemotherapy[10]. HAM plus VEN was found to be feasible, safe and highly effective, with 75% of patients achieving CR/CRi, enabling high rates of transition to allogeneic hematopoietic cell transplantation (HCT). Median OS was not reached, with corresponding 1-year and 2-year survival rates of 65% and 56%, respectively. Safety and efficacy appeared to compare favorably with published results from VEN combinations with FLA(G)-IDA or CPX-351[5-8].

Here, we present a real-world analysis of 128 R/R AML patients treated with HAM plus VEN, made possible by the rapid adoption of this regimen across multiple centers in Germany following completion of the RELAX trial. Our objectives were to evaluate response rate, relapse-free survival (RFS), and OS, and to identify clinical and molecular features associated with benefit from HAM plus VEN through analyses of combined real-world and RELAX datasets.

Methods

Patients and treatment. Adult patients who received at least one cycle of HAM plus VEN, analogous to the RELAX trial regimen[10], in routine clinical practice were included in this

retrospective real-world analysis. Treatment selection was based on the individual treating physician's assessment of patient fitness, no formal implicit or explicit patient selection criteria were applied, and no data were available on patients considered for but ultimately not treated with HAM plus VEN. Eligibility broadly followed the criteria of the RELAX trial, which contributed to the enrollment of a relatively younger and fitter cohort, as HAM-based chemotherapy is generally not administered to elderly or comorbid patients due to concerns regarding treatment-related toxicity. Patients received cytarabine at 1000 mg/m² IV every 12 hours for a total of six doses on days 3-5 and mitoxantrone at 10 mg/m² IV on days 5-7. VEN was initiated on day 1 with a 2-day dose ramp-up and continued daily at a target dose of 400 mg PO for 14 days, with standard dose adjustments for concomitant CYP3A inhibitor use (**Figure 1**). Further details on the RELAX trial (NCT04330820; EudraCT 2018-003025-28) and results are reported elsewhere[10].

Data collection and endpoints. Data were retrieved from the SAL AML registry as well as from electronic medical records of the participating centers. The dataset included demographic information, baseline and AML characteristics at relapse, prior treatment history, details of HAM plus VEN regimen administration, and outcome data. Whenever available, molecular and cytogenetic data were obtained at the time of R/R disease (cytogenetic data, n = 79; molecular data, n = 94), before initiation of HAM plus VEN therapy; otherwise, data from the time of initial diagnosis were used. The co-primary endpoints were the composite CR rate (CR/CRi) in the intention-to-treat population, RFS, and OS. OS was defined as the time from initiation of HAM plus VEN to death from any cause. RFS was defined as the time from CR/CRi after HAM plus VEN to disease relapse or death from any cause. The median time to allogeneic HCT was used as the landmark time point for a landmark analysis of OS, comparing patients who remained alive without allogeneic HCT at the landmark time point with those who proceeded to transplantation following HAM plus VEN.

Prognostic model development. For analysis of response by genetics and development of a prognostic model, data from the real-world cohort were pooled with the RELAX trial cohort. Model development was performed in three stages: (1) variable selection using a random survival forest implemented with the *Boruta* and *randomForestSRC* packages in R; (2) Cox proportional hazards regression with backward selection ($P < 0.20$) to identify the optimal model; and (3) assignment of weighted scores and risk group stratification based on significantly different OS compared with lower scores, resulting in the final prognostic model[11]. A total of 34 clinical and genetic variables were considered as candidate predictors (**Supplementary Table 1**).

Statistical methods. Survival curves were estimated with the Kaplan-Meier method, and group differences were compared using Cox proportional hazards survival regression. A *P* value < 0.05 was considered to indicate statistically significant differences. All statistical analyses were performed using R version 4.5.1.

Ethics statement. The study was conducted in accordance with the Declaration of Helsinki. The SAL AML registry (ClinicalTrials.gov identifier: NCT03188874) was approved by the Ethics Committee of the Technical University of Dresden, Germany (EK 98032010). Data collection and analysis were approved either through patient inclusion in the SAL AML registry or by the local ethics committees of the participating centers.

Results

Patient and disease characteristics. From March 2023, through July 2025, 128 patients with R/R AML, including three initially diagnosed with mixed phenotype acute leukemia (MPAL), received HAM plus VEN in 13 centers in Germany. Baseline characteristics are summarized in **Table 1**. The median age was 60 years (range, 20-74 years), 10 (8%) patients were ≥70 years. Forty-nine (38%) patients were primary refractory to one or two cycles of intensive induction chemotherapy or first line treatment with hypomethylating agents plus VEN, 18 (14%) had refractory relapsed disease, and 61 (48%) had untreated relapse. At initial diagnosis, the majority of patients had *de novo* leukemia (67%), 35 (27%) had leukemia arising from antecedent myelodysplastic neoplasm (MDS) or myelodysplastic/myeloproliferative neoplasm (MDS/MPN), and 7 (5%) had AML post cytotoxic therapy. Patients had received a median of 1 prior treatment lines (range, 1-5), including 48 (38%) patients having undergone a prior allogeneic HCT. Thirty-two patients (25%) had prior exposure to VEN, all in combination with HMA, except for one patient who received VEN with the IDH1 inhibitor ivosidenib and one who received VEN as part of the conditioning regimen for allogeneic HCT. According to ELN 2022 risk stratification, 39 (30%), 28 (22%), and 61 (48%) patients were classified as favorable, intermediate, and adverse risk, respectively. Corresponding numbers for ELN 2024 were 86 (67%), 33 (26%), and 9 (7%). *TP53* mutations were present in 9 (7%) patients and 23 (18%) had AML with complex karyotype (cKT). Twenty-seven (21%) patients had AML with *FLT3* mutation and 22 of these were pretreated with an FLT3 inhibitor (18 with midostaurin and 1 with quizartinib during induction/consolidation, 2 received sorafenib as maintenance post allogeneic HCT, and 5 received gilteritinib as salvage treatment). Compared with the RELAX trial, the real-world cohort included a higher proportion of patients 60 years of age or older (51% vs. 38%), with Eastern Cooperative Oncology Group performance status (ECOG PS) ≥2 (19% vs 9%), AML arising from MDS or MDS/MPN (27% vs 7%), two or more prior lines of

therapy (31% vs 11%), refractory relapse (14% vs 0%), and prior VEN exposure (25% vs 4%) (**Table 1**).

Therapy administered and response to therapy. All 128 patients received a single course of HAM plus VEN, except for one patient who received a second course as a bridge to allogeneic HCT. VEN was administered for 14 days in 116 (91%) patients and for less than 14 days in 12 (9%) patients, mainly due to infectious complications or inability to tolerate oral intake. Ninety (70%) patients received concomitant azole prophylaxis, with VEN doses reduced to 50-100 mg. The CR/CRi rate was 69% (88/128), with 38 (30%) patients achieving CR and 50 (39%) achieving CRi. Response by subgroups is shown in **Figure 2A**. Patients younger than 60 years achieved a CR/CRi rate of 79%, compared with 59% in those aged 60 years and older. Patients with *de novo* AML achieved a CR/CRi rate of 76%, compared with 57% among those with AML with antecedent MDS or MDS/MPN and 43% among those with AML post cytotoxic therapy. CR/CRi rates were 76% in patients with primary refractory AML, 71% in patients with untreated relapse, and 44% in patients with refractory relapse. CR/CRi rates were 77%, 71%, and 62% among patients with favorable, intermediate, and adverse ELN 2022 risk, respectively, compared with 70%, 70%, and 56% among patients classified according to ELN 2024. Among the 98 (77%) patients who had received one or two prior lines of therapy, as in the RELAX trial, the CR/CRi rate was 77%, compared to 43% in patients who had received three or more prior lines of therapy. Patients with only one prior line of therapy had a CR/CRi rate of 78%, compared with 48% in those who had received two or more lines. Among the 96 (75%) patients without prior VEN exposure, the CR/CRi rate was 76% vs. 47% in those who had previously received VEN. Within this latter group, 30 patients had relapsed after or were refractory to prior HMA plus VEN therapy, of whom 13 (43%) achieved CR/CRi following HAM plus VEN treatment. Among the 17 patients who were refractory to HMA plus VEN immediately prior to HAM plus VEN, 9 (53%) achieved CR/CRi. In total, 87 (68%) patients proceeded to allogeneic HCT. None of the patients received VEN as maintenance therapy after achieving CR/CRi. Nine (7%) patients were not evaluable for response but were included in the intention-to-treat efficacy analysis as treatment failures (no CR/CRi), of which eight died before response assessment and one was not evaluable for response for other reasons.

Safety and tolerability. Treatment-related toxicity data were available for 114 (89%) patients. The most common adverse events were infectious complications, which occurred in 80 patients (70%). Treatment-related organ toxicities were reported in 11 patients (10%) and included cardiac, renal, and hepatic toxicities, as well as gastrointestinal complications. Bleeding complications were reported in 2 patients (2%). Tumor lysis syndrome occurred in 10 patients (9%). The 30-day and 60-day mortality rates were 6% and 12%, respectively.

Survival outcomes. With a median follow-up of 11.5 months (range, 0.3-28.0 months), the estimated 1-year OS rate was 58% (95% confidence interval [CI], 49-69%; **Figure 2B**). In patients with primary refractory AML, the 1-year OS was 66% (95% CI, 52-84%), 62% (95% CI, 49-77%) in those with untreated relapse, and 32% (95% CI, 16-64%) in patients with refractory relapse (**Supplementary Figure S1A**). Patients without prior VEN exposure had superior outcomes, with a 1-year OS rate of 64%, compared with 40% in VEN-exposed patients (HR, 0.43, 95% CI, 0.23-0.78; *P* = 0.005) and 39% in patients with relapse after or refractoriness to prior HMA plus VEN therapy (HR, 0.41, 95% CI, 0.22-0.75; *P* = 0.004; **Supplementary Figure S1B**). Patients who received HAM plus VEN in second line had better OS than those with ≥2 prior lines of therapy (64% vs 46% at 1 year; HR, 0.43, 95% CI, 0.24-0.77, *P* = 0.004; **Supplementary Figure S1C**). This was also evident when comparing patients eligible for the RELAX trial who had received one or two prior lines of therapy with those who had received ≥3 prior lines, with 1-year OS rates of 64% versus 41%, respectively (HR, 0.45;, 95% CI, 0.2-0.81; *P* = 0.008; **Supplementary Figure S1D**). OS did not differ significantly between patients younger than 60 years and those aged 60 or older (61% vs 55%; HR, 0.68, 95% CI, 0.38-1.22, *P* = 0.2; **Supplementary Figure S1E**), but was significantly longer in patients with an ECOG PS of 0-1 compared with those with higher scores (66% vs 34%; HR, 0.37, 95% CI, 0.20-0.67, *P* = 0.001; **Supplementary Figure S1F**). The 1-year OS was 64% (95% CI, 48-84%), 63% (95% CI, 44-88%) and 53% (95% CI, 41-69%) in patients with ELN 2022 favorable, intermediate, and adverse genetic risk, respectively (**Supplementary Figure S1G**). According to ELN 2024, 1-year OS was 68% (95% CI, 58-80%), 55% (95% CI, 38-78%) and 0% in patients with favorable, intermediate, and adverse genetic risk, respectively (**Supplementary Figure S1G**). In the 88 patients who achieved a CR/CRi, the median RFS has not been reached, and the 1-year RFS was 59% (95% CI, 48-72%) (**Figure 2C**). In a subgroup of 27 (21%) patients who remained relapse-free beyond 9 months, 100% had undergone allogeneic HCT, 93% had an ECOG PS of 0-1, 81% had received only one prior line of therapy, and 63% were younger than 60 years. These patients were distributed across all ELN 2022 risk groups (41% favorable, 26% intermediate, and 33% adverse), whereas none were classified as adverse risk according to ELN 2024 (78% favorable and 22% intermediate; **Supplementary Table S2**).

Survival outcomes in patients undergoing allogeneic HCT. Overall, 87 (68%) patients proceeded to allogeneic HCT after a median of 1.8 months (range, 0.4-13.9). Of these, 70 (80%) patients underwent transplantation after achieving CR/CRi, with 67 (77%) in ongoing remission, whereas 17 (20%) patients were transplanted without having achieved CR/CRi. Using the median time to allogeneic HCT as the landmark, 1-year OS following transplantation was 72% (95% CI, 62-83%; **Supplementary Figure S1H**). In contrast, none of the 19 patients who were never transplanted were alive at 1 year.

Response and survival in the combined cohort. We next combined the real-world and RELAX datasets to assess HAM plus VEN efficacy in 183 patients across clinical and molecular subgroups. Predictor variables included molecular and cytogenetic aberrations listed in the ELN 2022 recommendations, as well as additional genes mutated in ≥3 patients in the pooled cohort (*DNMT3A*, *NRAS*, *KRAS*, *IDH1*, *IDH2*; **Figure 3A**). The most frequent mutations involved *NPM1* (27%), *DNMT3A* (24%), *FLT3* (21%), *RUNX1* (19%), *ASXL1* (15%), *IDH2* (14%), *SRSF2* (13%), and *IDH1* (10%), whereas *TP53* mutations were detected in 9% of patients. Complex and monosomal karyotypes were the most common unfavorable cytogenetic abnormalities, observed in 20% and 7% of patients, respectively. Among the 183 patients in the pooled cohort, the overall CR/CRi rate was 71%. CR/CRi rates were 80%, 79%, and 60% across the favorable-, intermediate-, and adverse-risk groups defined by ELN 2022, compared with 73%, 74%, and 40%, respectively, according to ELN 2024. Response rates by individual genetic mutation or chromosomal aberration are summarized in **Figure 3B**. High CR/CRi rates were observed in patients with mutations in *CEBPA* (bZIP, 100%, 95% CI 56-100%), *IDH1* (88%, 95% CI 60-98%), *BCOR* (83%, 95% CI 36-99%), *DNMT3A* (80%, 95% CI 64-90%), or *NPM1* (77%, 95% CI 62-88%). In contrast, CR/CRi rates were lower in *TP53*- (40%, 95% CI, 17-67%) or *SF3B1*-mutated AML (40%, 95% CI, 14-73%) and in AML with abn(17p) (25%, 95% CI, 1-78%) or cKT (45%, 95% CI, 29-63%). In univariable analysis, patients with mutations in *TP53* (OR 0.24, 95% CI, 0.08-0.71), *SF3B1* (OR 0.26, 95% CI, 0.06-0.93), or *ASXL1* (OR 0.39, 95% CI, 0.16-0.94), those with a cKT (OR 0.26, 95% CI, 0.12-0.57; **Figure 4A**), AML post cytotoxic therapy (OR 0.12, 95% CI, 0.03-0.38), antecedent MDS or MDS/MPN (OR 0.3, 95% CI, 0.14-0.65), or refractory disease (OR 0.32, 95% CI, 0.11-0.92; **Figure 4B**) were significantly less likely to achieve CR/CRi with HAM plus VEN. Remission was significantly more likely in patients with only one prior line of therapy (OR 3.89, 95% CI, 1.93-7.96), in those without prior VEN exposure (OR 3.53, 95% CI, 1.64-7.72), and in patients younger than 60 years (OR 2.25, 95% CI, 1.18-4.36; **Figure 4B**). With a median follow-up of 13.7 months (range, 0.3-52.3), median OS was 25.2 months (95% CI, 15.1-NA). The 1-year OS rate was 60% (95% CI, 53-69%).

Prognostic model development. A prognostic model was developed applying a random survival forest on the pooled real-world and RELAX datasets to identify clinical and genetic predictors of poor outcome with HAM plus VEN (**Figure 5**). Thirty-four clinical, cytogenetic, and molecular variables were evaluated (**Supplementary Table S1**, **Supplementary Figure S2**). *TP53* mutation, cKT, ≥2 prior lines of therapy, and ECOG PS ≥2 were associated with inferior OS following HAM plus VEN treatment. A prognostic index was derived from these variables. Patients without any of the identified predictors were classified as favorable risk (score 0, 52% of patients). Patients with any single risk factor or a combination of the predictors cKT, ≥2 prior lines of therapy, and/or ECOG PS ≥2 were classified as intermediate risk (score 1-5, 40%; only

one patient had *TP53* mutation and no additional risk factor). Patients with *TP53*-mutated AML in combination with any of the other predictors were classified as poor risk (score ≥6, 8%). One-year OS was 73%, 56%, and 0%; CR/CRi rates were 85%, 58%, and 36%; 30-day mortality was 3%, 8%, and 15%; and 60-day mortality was 4%, 15%, and 46% in favorable-, intermediate-, and poor-risk groups, respectively.

Discussion

We report outcomes from a large, multicenter real-world cohort of patients treated with HAM plus VEN as salvage therapy for R/R AML. Our findings demonstrate that the efficacy and survival outcomes observed in the phase 1/2 RELAX trial are reproducible in routine clinical practice, despite inclusion of a less selected and slightly older patient population with higher rates of prior VEN exposure, secondary AML, and more previous lines of therapy. Numerically, outcomes were somewhat inferior, with a CR/CRi rate of 69% compared with 75% in RELAX, as well as a less favorable survival pattern. However, these differences were smaller than might have been expected given the adverse baseline characteristics. Several factors may account for this observation. First, although no formal selection criteria were applied, patients deemed fit enough to receive HAM plus VEN likely represented a subgroup with sufficient physiological reserve to tolerate intensive salvage therapy, introducing some degree of selection bias even in a real-world setting. Second, the real-world cohort may have benefited from pragmatic treatment adaptations, including more individualized VEN dose modifications based on growing clinical experience, and early supportive care interventions. With a CR/CRi rate of 71% and a median OS of 25.2 months in the combined RELAX and real-world dataset, HAM plus VEN demonstrated encouraging activity and appears to be a viable salvage option for patients with R/R AML. Conventional intensive chemotherapy regimens typically induce remission in around 50% of patients, with median OS rarely exceeding 10 months[1, 2]. VEN-based combinations have expanded therapeutic options in this setting and may offer improved remission rates and enhanced feasibility of bridging to allogeneic HCT. DiNardo *et al.* were the first to combine VEN with fludarabine-containing intensive chemotherapy, namely FLAG-IDA, in patients with R/R AML[5], and the regimen has since been investigated in several real-world studies. Reported outcomes with FLA(G)-IDA-VEN, demonstrate CR/CRi rates between 59% and 75% and 1-year OS rates ranging from 38% to 68%[5-8]. Similarly, Kadia ***et al.*** evaluated VEN in combination with CPX-351 in a cohort comprising 79% ELN 2022 adverse-risk patients, reporting a CR/CRi rate of 39% and a 2-year overall survival of 25%[12]. Against this background, HAM plus VEN represents a fludarabine-free salvage option associated with high remission

rates, substantial transplantation feasibility, and encouraging early survival outcomes, supporting its role as an effective bridge-to-transplant strategy.

Selection of treatment intensity in patients with R/R AML remains challenging but is critical to optimize outcomes. Less intensive HMA plus VEN approaches achieve CR/CRi rates of 43% and a median OS of 8 months in a large meta-analysis of >2,000 patients[13]. In contrast, and consistent with prior studies of VEN combined with intensive chemotherapy[5-8, 12], HAM plus VEN achieved a CR/CRi rate of 71% and a median overall survival of 25.2 months in our pooled analysis. However, direct comparison between approaches is limited by the retrospective nature of the analyses and potential differences in baseline fitness between cohorts. Accordingly, prospective studies are needed to define which patients benefit from higher-intensity regimens and which may be adequately treated with less intensive approaches.

Safety and tolerability of HAM plus VEN in the real-world setting were comparable to the RELAX trial, with most adverse events being infectious complications and a 30-day mortality rate of 6% (vs. 6% in the RELAX trial). However, treatment-related risk with HAM plus VEN was not uniform across patient subgroups. Higher early mortality and inferior survival were observed in patients with impaired clinical fitness (ECOG PS ≥2), more heavily pretreated disease, or adverse biological features. Similar patterns have been reported for VEN in combination with FLA(G)-IDA or other intensive chemotherapies, particularly in *TP53*-mutated AML[14, 15]. To address this heterogeneity, we developed a pragmatic prognostic score incorporating ECOG PS, number of prior therapy lines, *TP53* mutation, and cKT. This model identifies a favorable-risk subgroup of AML patients without *TP53* mutation or cKT, with ECOG PS 0-1 and one prior therapy line, who achieve favorable outcomes following HAM plus VEN. It also defines a high-risk subgroup comprising patients with *TP53*-mutated AML and any additional risk factor, characterized by low response rates and substantial early mortality, for whom alternative, less intensive, or investigational approaches may be more appropriate.

Several limitations should be considered. The retrospective design and absence of a control group preclude direct comparative conclusions. The sample size, while large for a real-world intensive salvage cohort, restricts the precision of subgroup analyses, particularly for rare molecular lesions. Combining real-world and trial data to develop the prognostic model may introduce bias, and independent external validation of the proposed score is needed. Although treatment-related complications and early mortality data were available from the real-world cohort, more granular information on individual toxicities associated with the HAM plus VEN regimen, particularly data on blood count recovery, was not systematically captured. In

addition, the lack of uniformly assessable minimal residual disease data precludes a more detailed assessment of response depth and its association with outcomes.

In conclusion, HAM plus VEN represents an effective salvage regimen for patients with R/R AML, with robust real-world efficacy, high rates of transition to allogeneic HCT, and clinically meaningful survival outcomes. Careful patient selection based on clinical fitness, treatment history and disease biology appear critical to maximize benefit while limiting early toxicity. Future prospective studies will be required to clarify the optimal positioning of VEN-based intensive chemotherapy salvage regimens across distinct clinical and molecular subgroups, and relative to alternative strategies, including allogeneic HCT with sequential conditioning as explored in the ASAP trial[16, 17].

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Table 1. Baseline demographics and disease characteristics

Parameter	Real-world cohort (n = 128)	RELAX trial (n = 55)
Age		
Median (range), years	60 (20-74)	57 (26-74)
≥60 years, n (%)	65 (51)	21 (38)
≥70 years, n (%)	10 (8)	5 (9)
Sex, n (%)		
Female	49 (38)	25 (46)
Male	79 (62)	30 (54)
ECOG PS, n (%)		
0-1	95 (74)	50 (91)
≥2	24 (19)	5 (9)
Missing	9 (7)	0 (0)
AML ontogeny at first diagnosis, n (%)		
De novo	86 (67)	45 (82)
AML with antecedent MDS or MDS/MPN	35 (27)	4 (7)
AML post cytotoxic therapy	7 (5)	6 (11)
Disease status at treatment start, n (%)		
Primary refractory	49 (38)	17 (31)
Refractory relapse	18 (14)	0 (0)
Untreated relapse	61 (48)	38 (69)
WBC, median (range), G/L	2.1 (0.2-82)	2.8 (0.2-30.7)
Blasts in peripheral blood, median (range), %	7 (0-93)	1 (0-95)
Blasts in bone marrow, median (range), %	40 (0-95)	36 (0-99)
Extramedullary disease, n (%)	5 (4)	4 (7)
ELN 2022*, n (%)		
Favorable	39 (30)	10 (18)
Intermediate	28 (22)	20 (36)
Adverse	61 (48)	25 (46)
ELN 2024*, n (%)		
Favorable	86 (67)	33 (60)
Intermediate	33 (26)	16 (29)
Adverse	9 (7)	6 (11)
Previous therapies, n (%)		
Median (range)	1 (1-5)	1 (1-3)
1	88 (69)	49 (89)
≥2	40 (31)	6 (11)
≥3	18 (14)	3 (6)
VEN pretreatment	32 (25)	2 (4)
With HMA	30 (23)	1 (2)
Refractory to HMA/VEN	20 (16)	0 (0)
With other therapies	2 (2)	1 (2)
FLT3 inhibitor	22 (17)	5 (9)
Allogeneic HCT	48 (38)	19 (35)

AML, acute myeloid leukemia; ECOG PS, Eastern Cooperative Oncology Group performance status; ELN, European LeukemiaNet; HCT, hematopoietic cell transplantation; HCT-CI, hematopoietic cell transplantation-specific comorbidity index; HMA, hypomethylating agent; MDS, myelodysplastic neoplasia; MDS/MPN, MDS/myeloproliferative neoplasm; WBC, white blood cells; VEN, venetoclax.
*at initial diagnosis

Figure legends

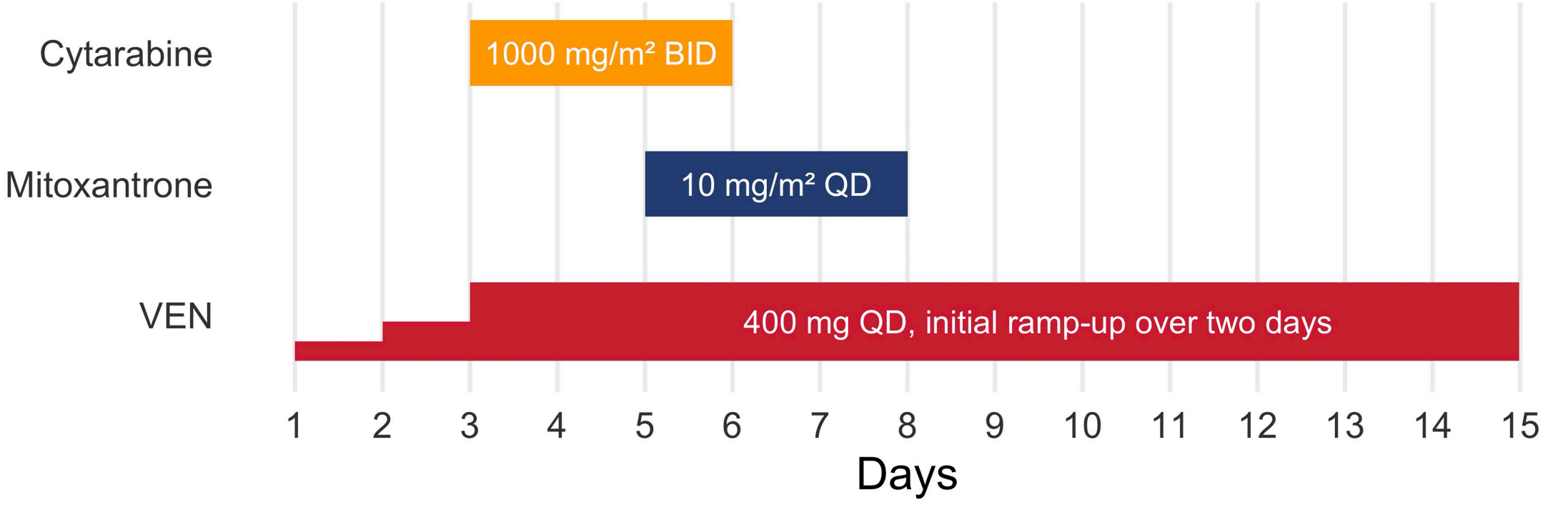
Figure 1. Treatment schematic of the HAM plus VEN salvage regimen. Patients received VEN 400 mg PO QD on days 1-14 (initial 3-day dose ramp-up), cytarabine 1000 mg/m² IV BID on days 3-5, and mitoxantrone 10 mg/m² IV QD on days 5-7. In patients with concomitant use of CYP3A inhibitors, the VEN dose was adjusted

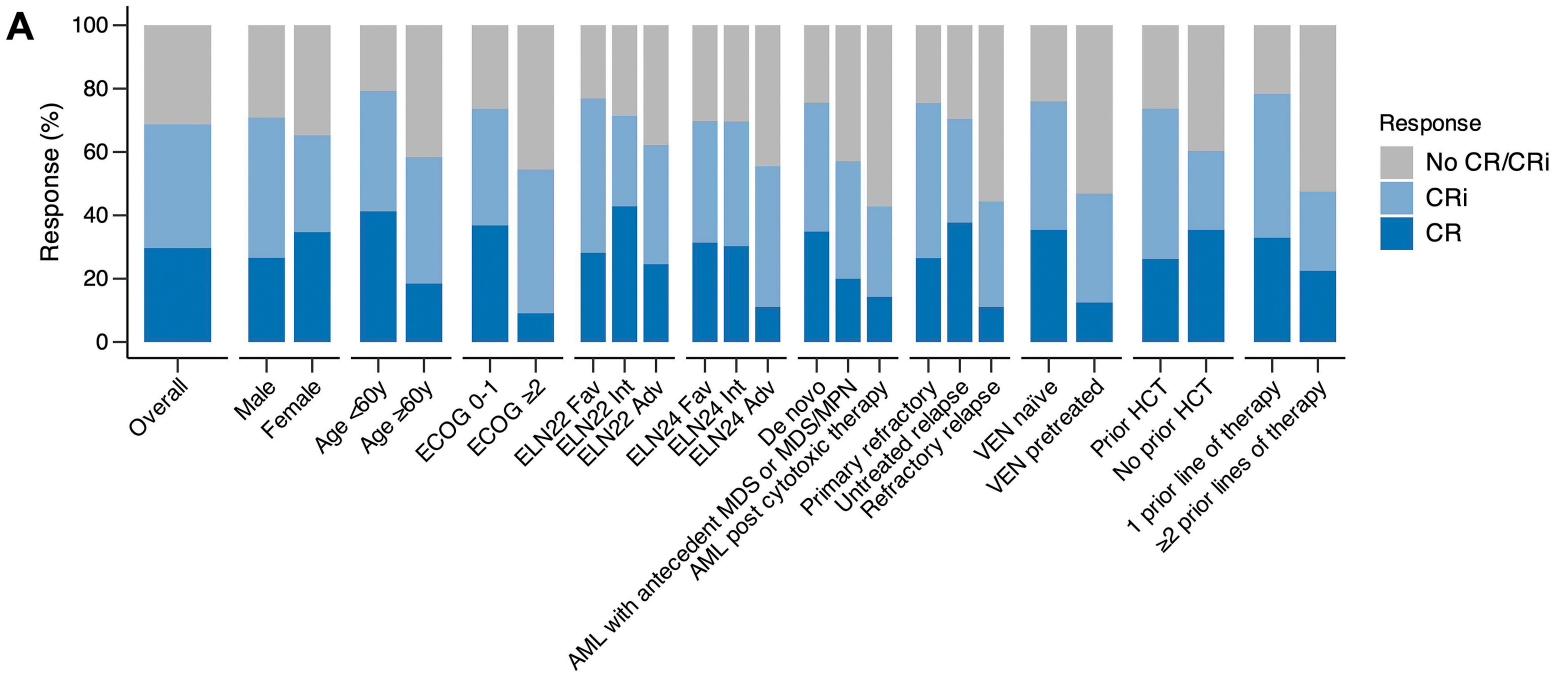
Figure 2. Response and Kaplan-Meier estimates for OS and RFS. (A) CR/CRi rates following HAM plus VEN in the overall cohort and key subgroups.(B-C) Kaplan-Meier estimates of (B) OS and (C) RFS following HAM plus VEN. abnl, abnormal; adv, adverse; AML, acute myeloid leukemia; CR, complete remission; CRi, CR with incomplete count recovery; cKT, complex karyotype (according to ELN 2022 definitions); ECOG, Eastern Cooperative Oncology Group performance status; ELN22, European LeukemiaNet risk classification 2022; ELN24, European LeukemiaNet risk classification 2024; fav, favorable; HCT, allogeneic hematopoietic stem cell transplantation; int, intermediate; MDS, myelodysplastic neoplasia; MPN, myeloproliferative neoplasia; monoKT, monosomal karyotype; VEN, venetoclax; y, years.

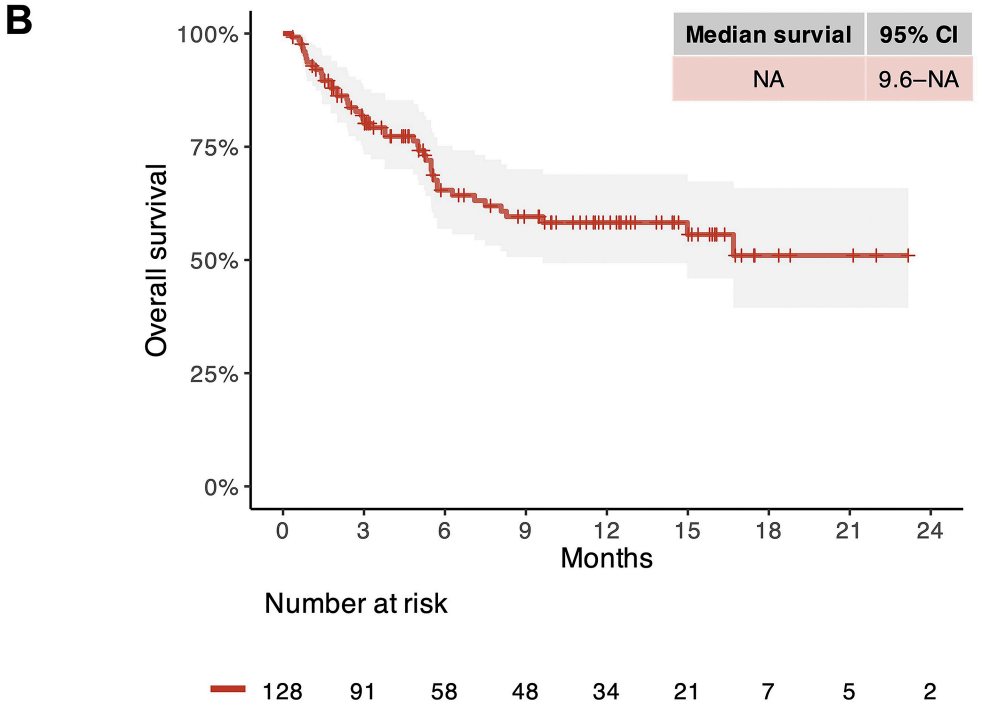
Figure 3. Mutational landscape in the combined real-world and RELAX cohorts. (A) Oncoplot illustrating the genetic landscape of individual AML patients, sorted by mutation and chromosomal aberration frequency. Each column represents one patient. Patients are further sorted by response, with CR/CRi on the left (blue) and non-responders on the right (gray). (B) CR/CRi rates by genetic mutations or chromosomal aberrations. abnl, abnormalities in; AML, acute myeloid leukemia; CR, complete remission; CRi, CR with incomplete count recovery; cKT, complex karyotype (according to ELN 2022 definitions); ELN22, European LeukemiaNet risk classification 2022; ELN24, European LeukemiaNet risk classification 2024; MDS, myelodysplastic neoplasia; MPN, myeloproliferative neoplasia; monoKT, monosomal karyotype; VEN, venetoclax; y, years.

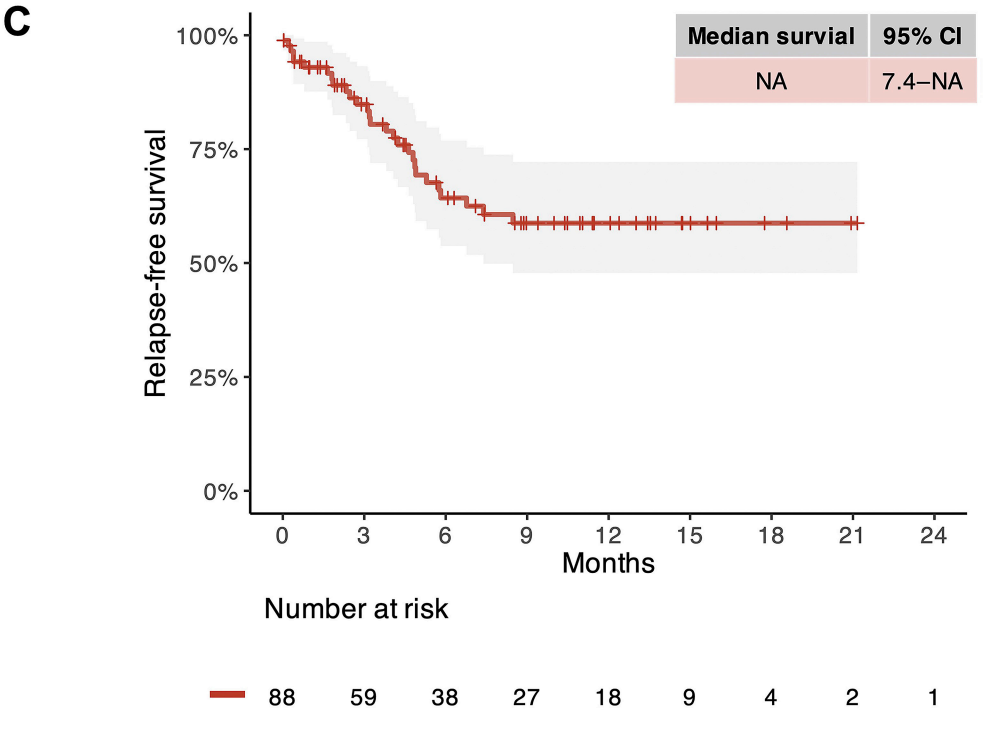
Figure 4. Mutational landscape in the combined real-world and RELAX cohorts. (A) Univariable odds ratios for achieving CR/CRi by (A) mutations or chromosomal aberrations, and (B) key clinical subgroups. Comparators for (B) in descending order: ≥2 lines of therapy, VEN pretreated, age ≥60y, ECOG ≥2, primary refractory, male, no prior HCT, primary refractory, *de novo* AML, and *de novo* AML. abnl, abnormalities in; AML, acute myeloid leukemia; CR, complete remission; CRi, CR with incomplete count recovery; cKT, complex karyotype (according to ELN 2022 definitions); ELN22, European LeukemiaNet risk classification 2022; MDS, myelodysplastic neoplasia; MPN, myeloproliferative neoplasia; monoKT, monosomal karyotype; VEN, venetoclax; y, years.

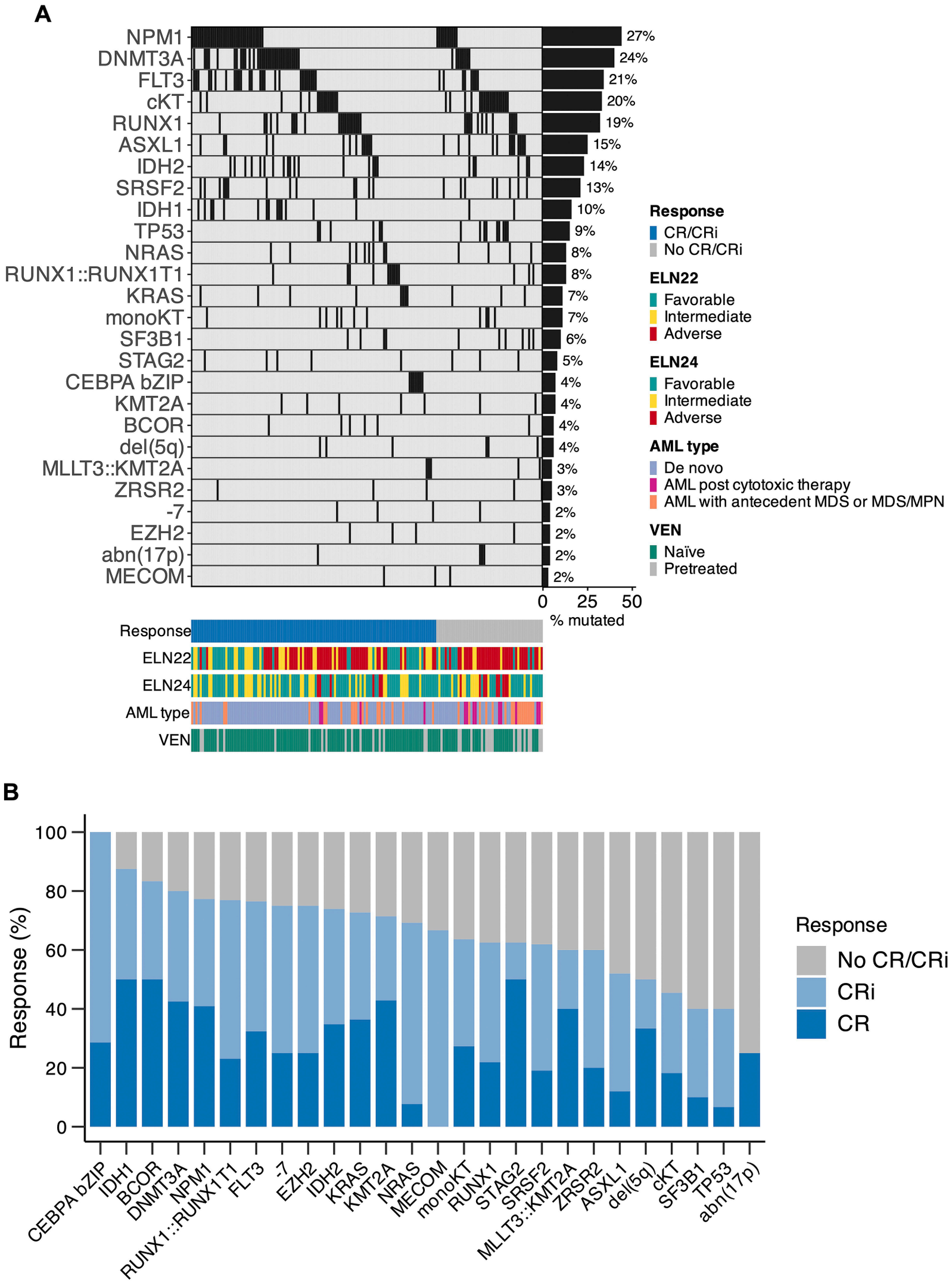
Figure 5. Prognostic score for patient stratification. (A) Prognostic score including key predictors of OS, with points assigned according to hazard ratios. (B) Cohort stratification into favorable (0 points), intermediate (1-5 points), and poor (≥6 points) prognosis groups. (C) Kaplan-Meier estimates of OS by prognostic group. Median survival (months), HR with 95% CI, and *P* values from Cox regression are tabulated. cKT, complex karyotype (according to ELN 2022 definitions); ECOG, Eastern Cooperative Oncology Group performance status.

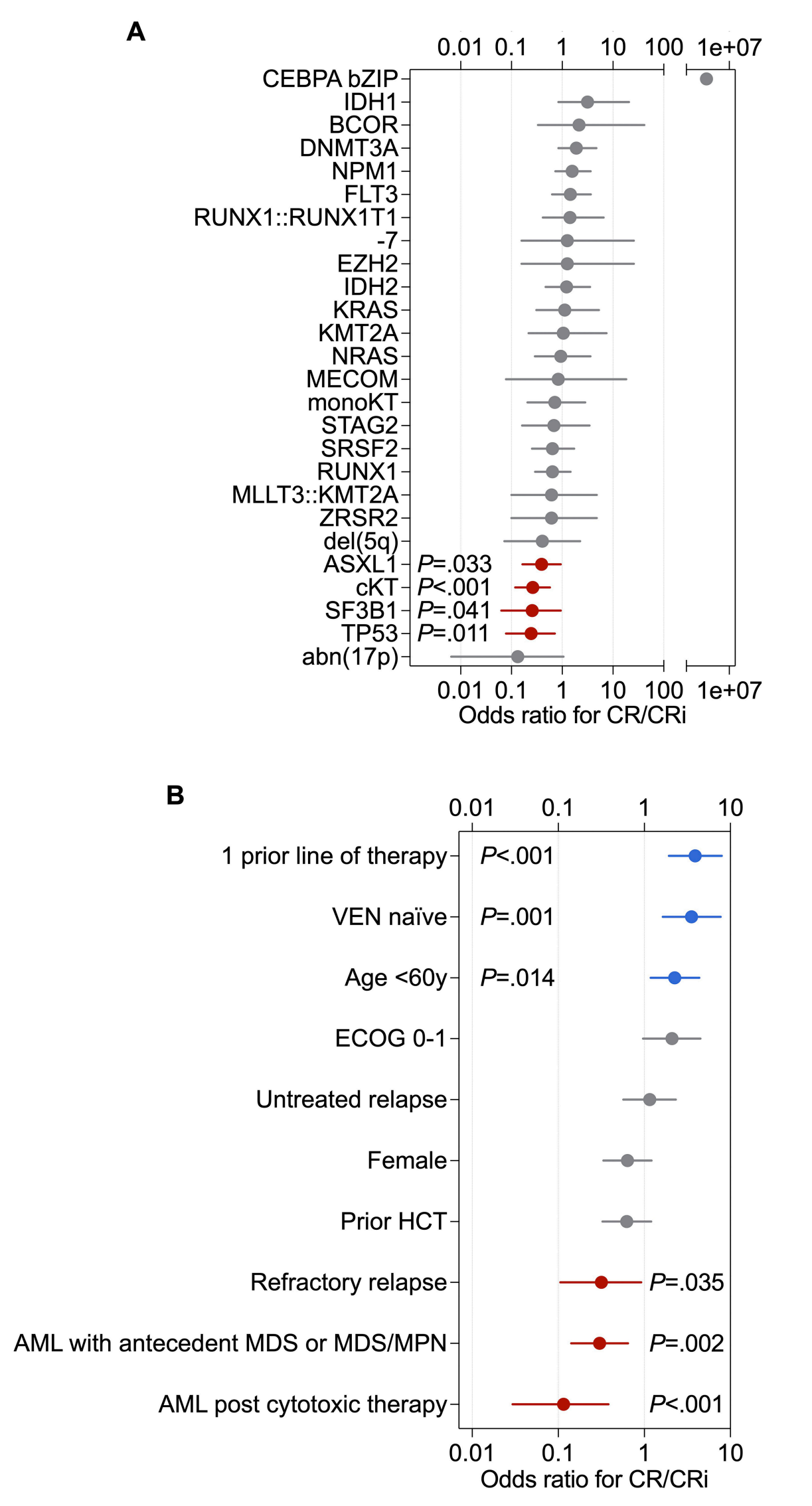

Cytarabine
1000 mg/m² BID
Mitoxantrone
10 mg/m² QD
VEN
400 mg QD, initial ramp-up over two days
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
Days


A
Response (%)
100
80
60
40
20
0
Response
No CR/CRi
CRi
CR
Overall
Male
Female
Age <60y
Age ≥60y
ECOG 0-1
ECOG ≥2
ELN22 Fav
ELN22 Int
ELN22 Adv
ELN24 Fav
ELN24 Int
ELN24 Adv
De novo
AML with antecedent MDS or MDS/MPN
AML post cytotoxic therapy
Primary refractory
Untreated relapse
Refractory relapse
VEN naïve
VEN pretreated
Prior HCT
No prior HCT
1 prior line of therapy
≥2 prior lines of therapy


B
Median survial
95% CI
NA
9.6–NA
Overall survival
100%
75%
50%
25%
0%
0
3
6
9
12
15
18
21
24
Months
Number at risk
128
91
58
48
34
21
7
5
2


C
Median survial
95% CI
NA
7.4–NA
Relapse-free survival
100%
75%
50%
25%
0%
0
3
6
9
12
15
18
21
24
Months
Number at risk
88
59
38
27
18
9
4
2
1

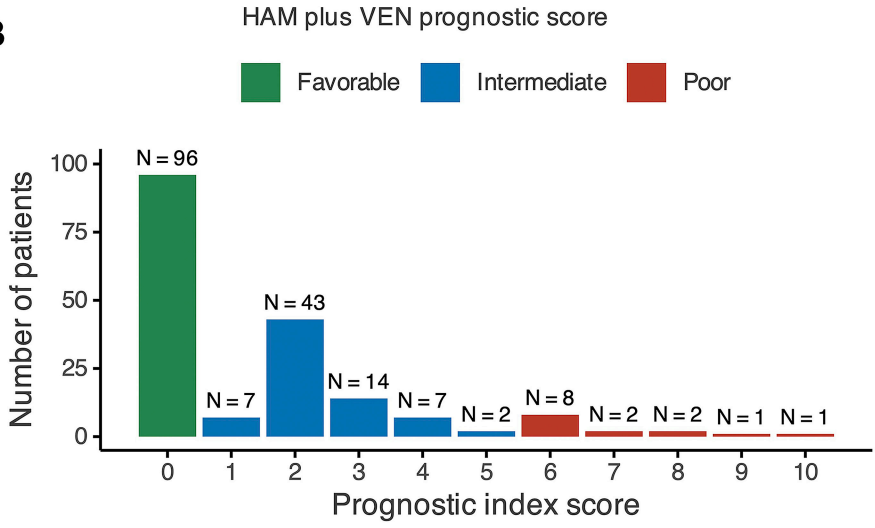

A
NPM1 27%
DNMT3A 24%
FLT3 21%
cKT 20%
RUNX1 19%
ASXL1 15%
IDH2 14%
SRSF2 13%
IDH1 10%
TP53 9%
NRAS 8%
RUNX1::RUNX1T1 8%
KRAS 7%
monoKT 7%
SF3B1 6%
STAG2 5%
CEBPA bZIP 4%
KMT2A 4%
BCOR 4%
del(5q) 4%
MLLT3::KMT2A 3%
ZRSR2 3%
-7 2%
EZH2 2%
abn(17p) 2%
MECOM 2%
0 25 50
% mutated
Response
ELN22
ELN24
AML type
VEN
Response
CR/CRi
No CR/CRi
ELN22
Favorable
Intermediate
Adverse
ELN24
Favorable
Intermediate
Adverse
AML type
De novo
AML post cytotoxic therapy
AML with antecedent MDS or MDS/MPN
VEN
Naïve
Pretreated
B
Response (%)
100
80
60
40
20
0
CEBPA bZIP
IDH1
BCOR
DNMT3A
NPM1
RUNX1::RUNX1T1
FLT3
-7
EZH2
IDH2
KRAS
KMT2A
NRAS
MECOM
monoKT
RUNX1
STAG2
SRSF2
MLLT3::KMT2A
ZRSR2
ASXL1
del(5q)
cKT
SF3B1
TP53
abn(17p)
Response
No CR/CRi
CRi
CR


A
0.01 0.1 1 10 100 1e+07
CEBPA bZIP
IDH1
BCOR
DNMT3A
NPM1
FLT3
RUNX1::RUNX1T1
-7
EZH2
IDH2
KRAS
KMT2A
NRAS
MECOM
monoKT
STAG2
SRSF2
RUNX1
MLLT3::KMT2A
ZRSR2
del(5q)
ASXL1 P=.033
cKT P<.001
SF3B1 P=.041
TP53 P=.011
abn(17p)
0.01 0.1 1 10 100 1e+07
Odds ratio for CR/CRi
B
0.01 0.1 1 10
1 prior line of therapy P<.001
VEN naïve P=.001
Age <60y P=.014
ECOG 0-1
Untreated relapse
Female
Prior HCT
Refractory relapse P=.035
AML with antecedent MDS or MDS/MPN P=.002
AML post cytotoxic therapy P<.001
0.01 0.1 1 10
Odds ratio for CR/CRi

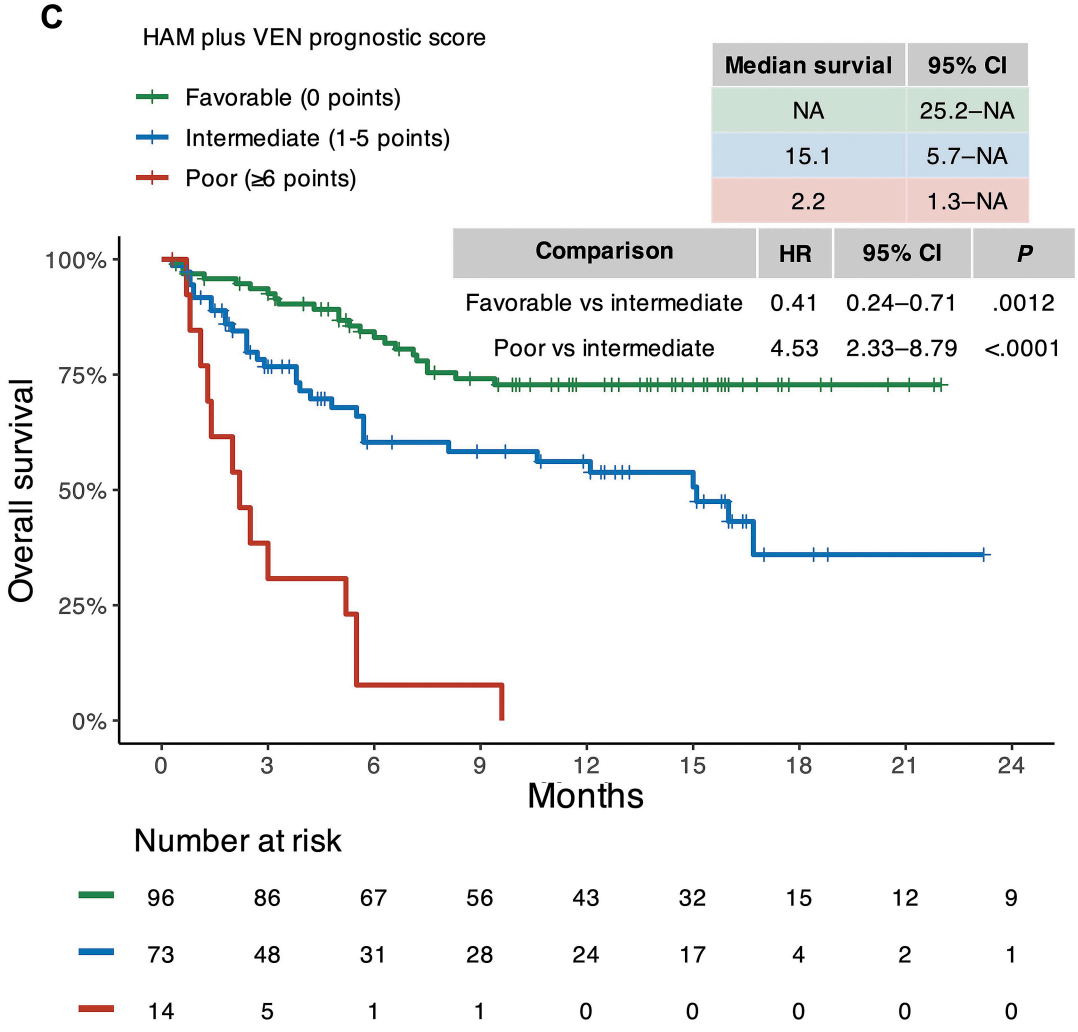
A

Predictor	HR	95% CI	p-value	Points
TP53 mutation	4.79	2.42 - 9.47	<0.001	5
ECOG ≥2	1.92	1.09 - 3.41	0.0248	2
≥2 prior lines of therapy	1.77	1.08 - 2.93	0.0247	2
cKT	1.61	0.9 - 2.88	0.1103	1

B



C



Supplementary Information

Venetoclax combined with high-dose cytarabine and mitoxantrone for R/R AML: outcomes and risk stratification

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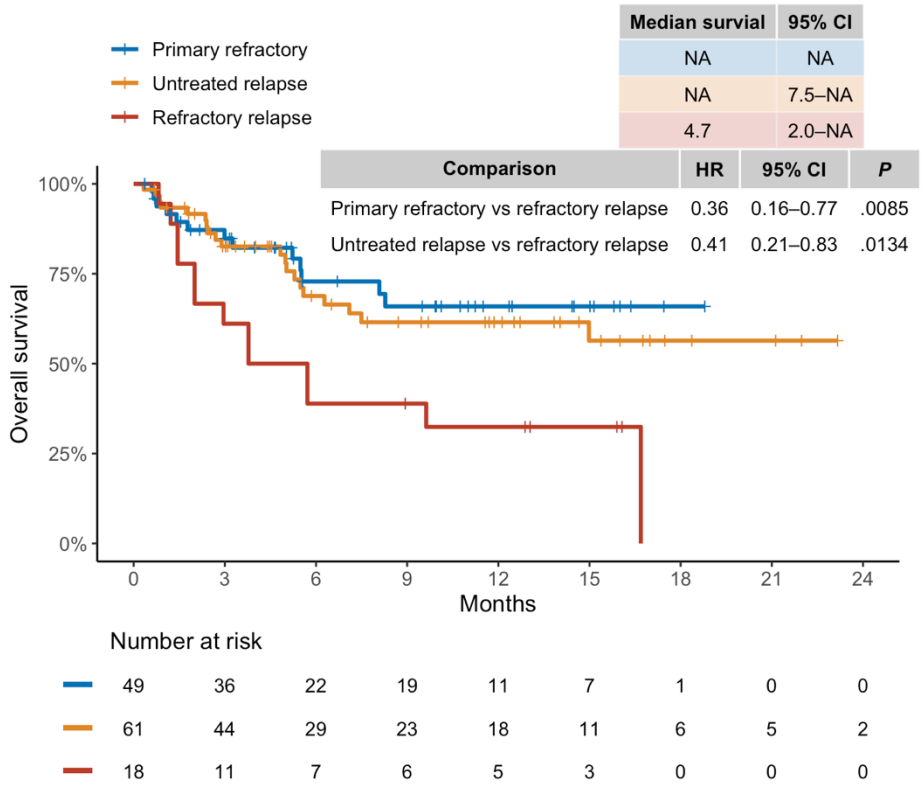
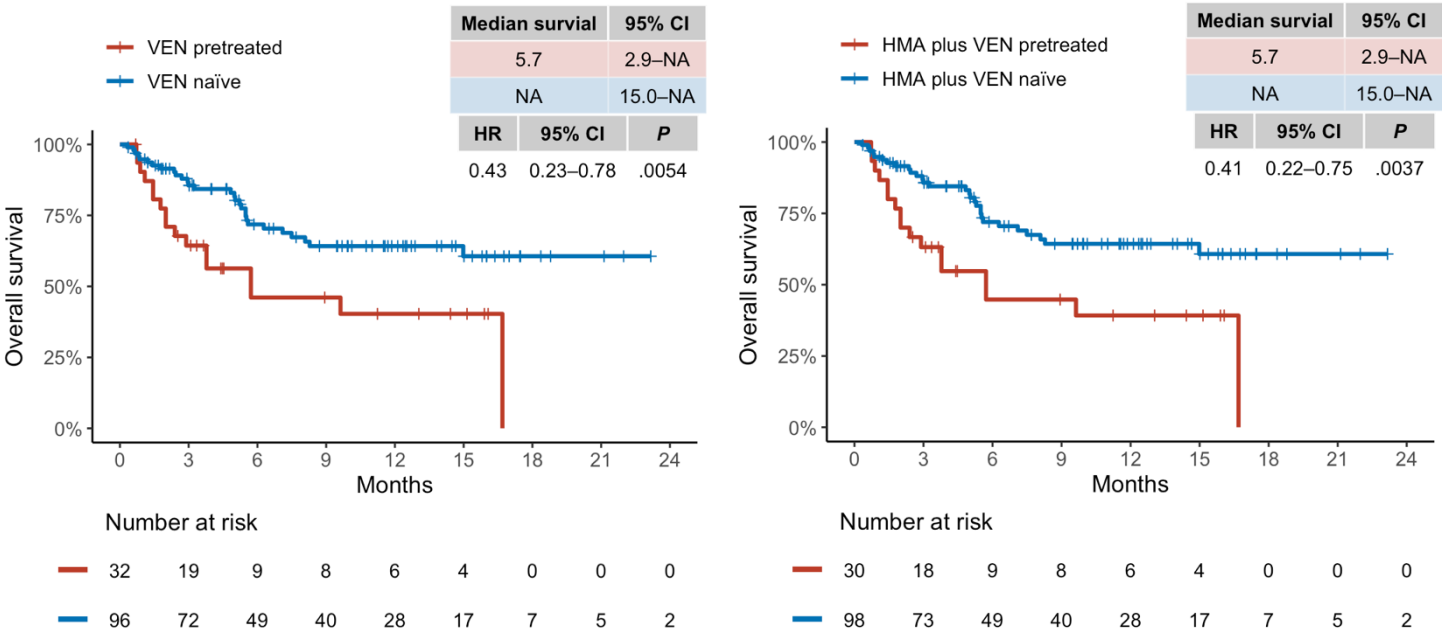
*M.H., L.R. and C.S. contributed equally to this study.

Supplementary Figure S1. Survival outcomes with HAM plus VEN across patient subgroups.

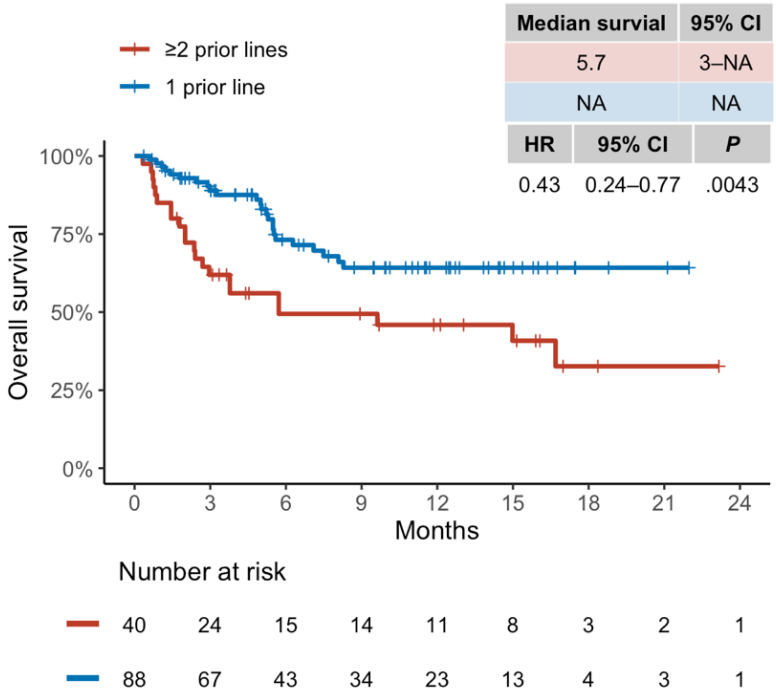
(A) Kaplan-Meier estimates of OS following HAM plus VEN treatment in patients with relapsed refractory, untreated relapsed, and primary refractory disease. NA, not applicable.



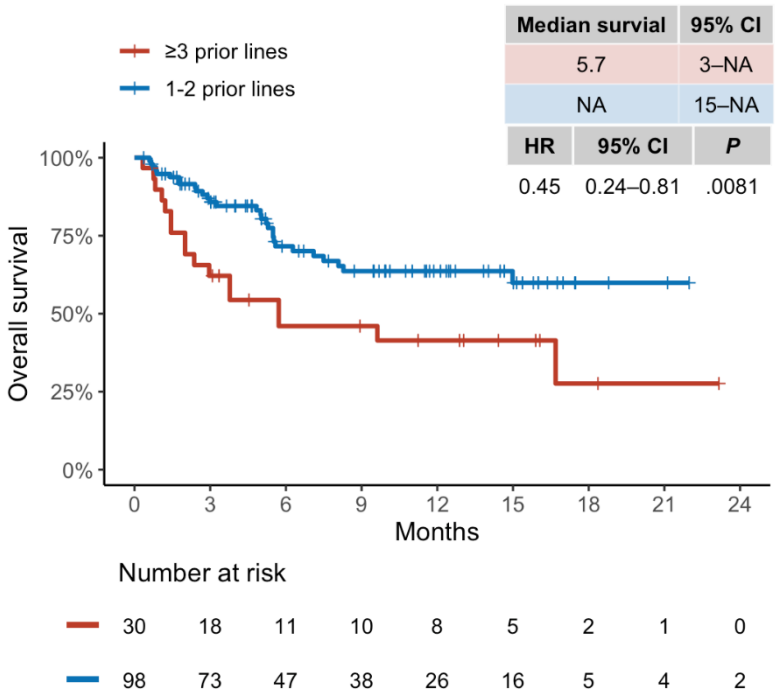
(B) Kaplan-Meier estimates of OS following HAM plus VEN treatment in VEN- naïve and VEN-pretreated patients (left), and HMA plus VEN naïve and pretreated patients (right). NA, not applicable.



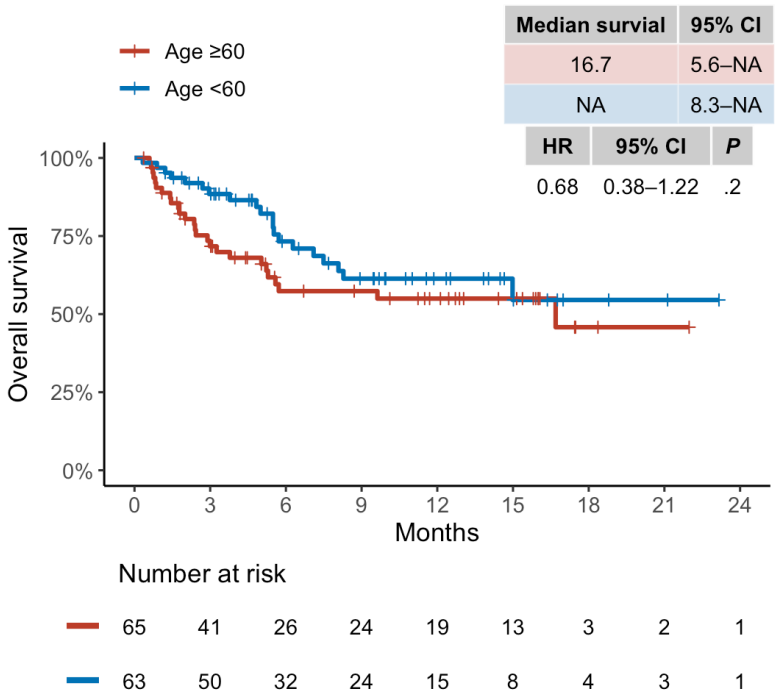
(C) Kaplan-Meier estimates of OS following HAM plus VEN treatment in patients with 1 and ≥2 prior lines of treatment. NA, not applicable.



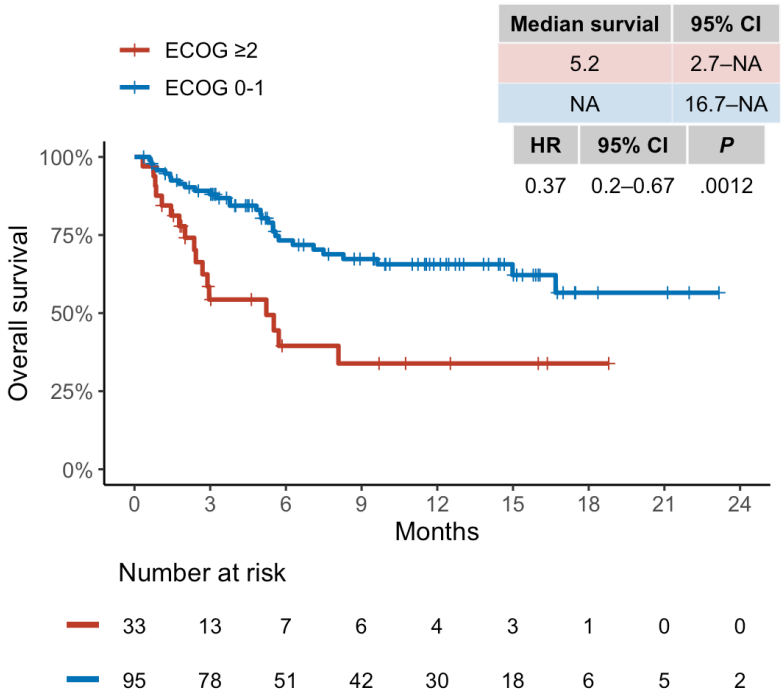
(D) Kaplan-Meier estimates of OS following HAM plus VEN treatment in patients with 1-2 and ≥3 prior lines of treatment. NA, not applicable.



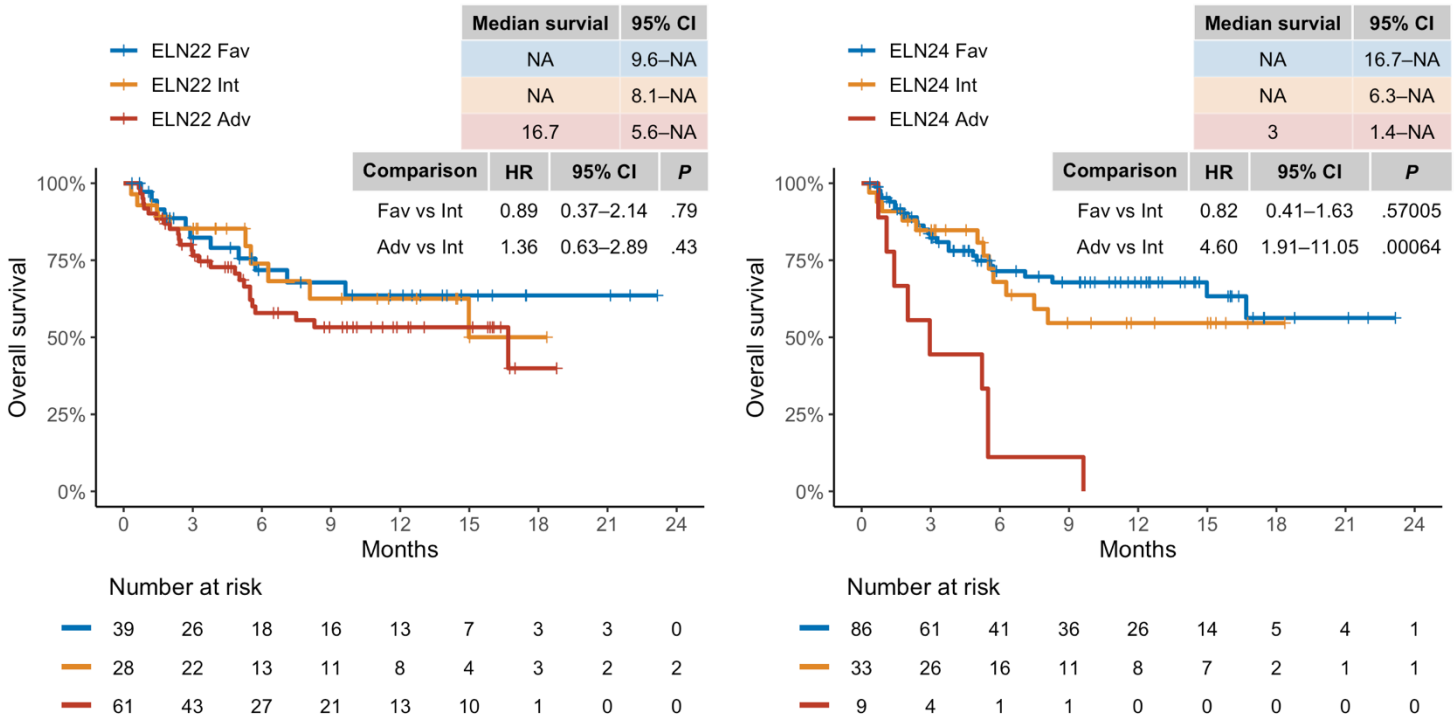
(E) Kaplan-Meier estimates of OS following HAM plus VEN treatment in patients <60 and ≥60 years of age. NA, not applicable.



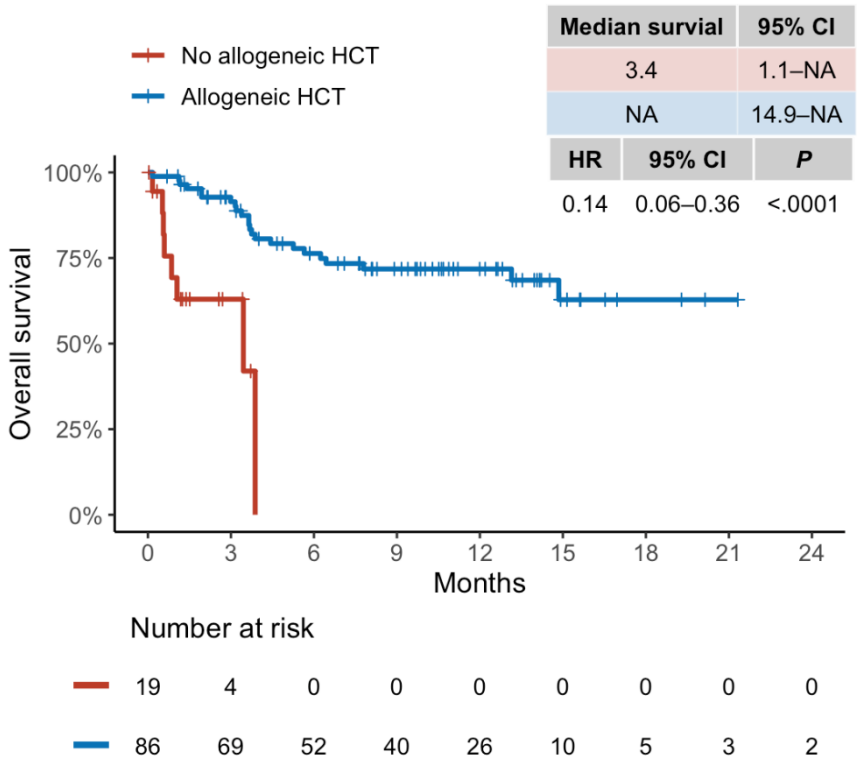
(F) Kaplan-Meier estimates of OS following HAM plus VEN treatment in patients with an ECOG PS of 0-1 and ≥2. NA, not applicable.



(G) Kaplan-Meier estimates of OS following HAM plus VEN treatment in patients with favorable, intermediate and adverse risk according to ELN 2022 (left) and ELN 2024 (right) risk stratifications. NA, not applicable.

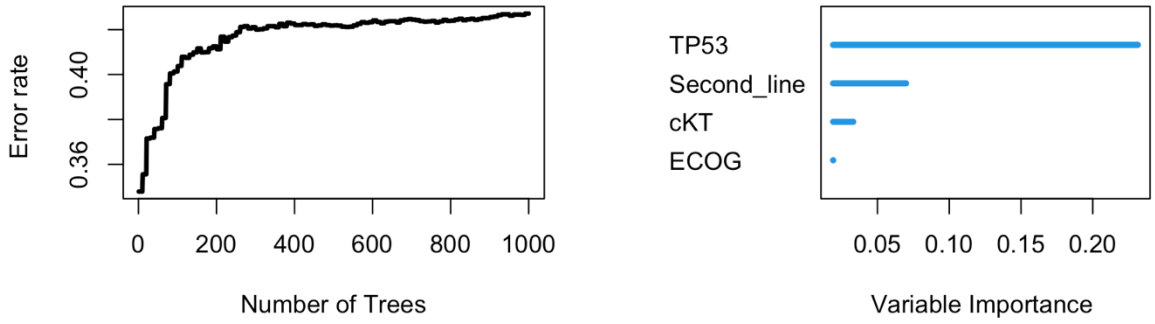


(H) Landmark analysis of OS comparing patients alive without allogeneic HCT at the landmark (median time to HCT) with those who underwent transplantation after HAM plus VEN.



Supplementary Figure S2. Random forest model.

A total of 34 variables (Supplementary Table S1) were assessed for their association with OS using Boruta variable selection with a *P* value threshold of 0.2. Variables confirmed by Boruta were subsequently included in a random forest survival model. Four variables with relevant variable importance and a significant impact on OS were identified.



Supplementary Table S1

Variables included in Boruta-based variable selection for OS ($P < 0.2$).

Variable	Comparison
Sex	Male or female
Age	<60 or ≥60 years of age
ECOG	0-1 vs. ≥2
Disease status	Primary refractory vs. refractory relapse vs. Untreated relapse
Prior HCT	Y/N
Prior lines of treatment	1 vs ≥2
AML type	*De novo* vs. AML with antecedent MDS or MDS/MPN vs. AML post cytotoxic therapy
VEN pretreatment	Naïve vs. pretreated
NPM1 mutation	Y/N (n = 44)
RUNX1::RUNX1T1 fusion	Y/N (n = 13)
FLT3 mutation	Y/N (n = 34)
CEBPA bZIP in frame mutation	Y/N (n = 7)
MLLT3::KMT2A fusion	Y/N (n = 5)
DNMT3A mutation	Y/N (n = 40)
IDH1 mutation	Y/N (n = 16)
IDH2 mutation	Y/N (n = 23)
BCOR mutation	Y/N (n = 6)
EZH2 mutation	Y/N (n = 4)
SF3B1 mutation	Y/N (n = 10)
SRSF2 mutation	Y/N (n = 21)
STAG2 mutation	Y/N (n = 8)
ZRSR2 mutation	Y/N (n = 5)
ASXL1 mutation	Y/N (n = 25)
TP53 mutation	Y/N (n = 15)
RUNX1 mutation	Y/N (n = 32)
KRAS mutation	Y/N (n = 11)
NRAS mutation	Y/N (n = 13)
cKT	Y/N (n = 33)
KMT2A rearrangement	Y/N (n = 7)
MECOM rearrangement	Y/N (n = 3)
del(5q)	Y/N (n = 6)
-7	Y/N (n = 4)
abn(17p)	Y/N (n = 4)
monoKT	Y/N (n = 11)

Supplementary Table S2

Characteristics of patients with CR/CRi following HAM plus VEN who remained relapse-free after 9 months

Parameter	Relapse-free after 9 months (n = 27)
Response rate and post remission treatment	
CR	15 (56)
CRi	12 (44)
Allogeneic HCT	27 (100)
Age	
Median (range), years	56 (20-73)
≥60 years, n (%)	10 (37)
≥70 years, n (%)	2 (7)
Sex, n (%)	
Female	10 (37)
Male	17 (63)
ECOG PS, n (%)	
0-1	25 (93)
≥2	1 (4)
Missing	1 (4)
AML ontogeny at first diagnosis, n (%)	
De novo	19 (70)
AML with antecedent MDS or MDS/MPN	1 (4)
AML post cytotoxic therapy	7 (26)
Disease status at treatment start, n (%)	
Primary refractory	12 (44)
Refractory relapse	1 (4)
Untreated relapse	14 (52)
WBC, median (range), G/L	2.3 (0.2-32)
Blasts in peripheral blood, median (range), %	14 (0-82)
Blasts in bone marrow, median (range), %	49 (0-95)
Extramedullary disease, n (%)	0 (0)
ELN 2022*, n (%)	
Favorable	11 (41)
Intermediate	7 (26)
Adverse	9 (33)
ELN 2024*, n (%)	
Favorable	21 (78)
Intermediate	6 (22)
Adverse	0 (0)
Previous therapies, n (%)	
Median (range)	1 (1-3)
1	22 (81)
≥2	4 (15)
≥3	1 (4)
VEN pretreatment	3 (11)
With HMA	3 (11)
Refractory to HMA/VEN	3 (11)
With other therapies	0 (0)
FLT3 inhibitor	5 (19)
Allogeneic HCT	8 (30)

AML, acute myeloid leukemia; ECOG PS, Eastern Cooperative Oncology Group performance status; ELN, European LeukemiaNet; HCT, hematopoietic cell transplantation; HCT-CI, hematopoietic cell transplantation-specific comorbidity index; HMA, hypomethylating agent; MDS, myelodysplastic neoplasia; MDS/MPN, MDS/myeloproliferative neoplasm; WBC, white blood cells; VEN, venetoclax. *at initial diagnosis