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Abstract

Pediatric refractory/relapsed acute myeloid leukemia (r/r-AML), myelodysplastic syndromes with excess blasts (MDS-EB), and therapy-related MDS/AML (t-MDS/AML) remain a clinical challenge due to high rate of treatment failure. Venetoclax plus azacytidine (ven/aza) has transformed adult myeloid neoplasm treatment, but pediatric data are limited and heterogeneous.

This AIEOP retrospective, multicenter study analyzed 50 patients (M/F=1.8/1; median age 11 years) with r/r-AML (n=32), MDS-EB (n=10), or t-MDS/AML (n=8) treated with ven/aza. Responses were defined as complete (CR), partial (PR), or non-response (NR) based on bone marrow (BM) blast evaluation. Adverse events (AEs) were graded per CTCAE v5.0; outcomes were evaluated with Kaplan-Meier and Cox-regression analysis.

Patients received a median of 2 cycles (range, 1-7). Grade ≥3 AEs occurred in 34% of patients. Overall, 30 patients (60%) achieved CR (24 MRD-negative), 9 (18%) PR, and 11 (22%) NR. CR were 58%, 57% and 100% in KMT2A-AML, FLT3-ITD-AML (ven/aza+FLT3-inhibitors) and UBTF-TD myeloid neoplasms, respectively. In 10 MDS-EB, 7 CR and 2 PR were recorded. In 8 t-MDS/AML, we observed 5 CR and 2 PR. Thirty-three patients (66%) underwent hematopoietic cell transplantation (HCT), after a median of 97 days (range: 33-933) from ven/aza start. Median follow-up was 389 days (range 36-1405). Two-year event-free survival (EFS) was 54.5% (CI:46.7-62.3), being 75.8% in transplanted patients, and 77.2% in those achieving CR. CR and HCT were associated with better EFS. Ven/aza shows substantial activity and manageable toxicity in pediatric high-risk myeloid diseases, especially as a bridge to HCT and in specific molecular subgroups, supporting future, prospective, genetically-guided studies.

Introduction

Childhood high-risk myeloid neoplasms, namely relapsed or refractory acute myeloid leukemia (r/r-AML), myelodysplastic syndromes with excess of blasts (MDS-EB) and therapy-related MDS/AML (t-MDS/AML) represent a significant clinical challenge due to their frequent resistance to conventional therapies and high relapse rates[1,2]. Adverse cytogenetic or molecular aberrations, such as complex karyotype, monosomy 7, selected KMT2A- and NUP98-rearrangements, or FLT3-ITD aberrations, strongly impact on outcomes and may guide therapeutic decisions[3,4]. Recently, tandem duplications in UBTF gene (UBTF-TD) have been associated in pediatric and young adult AML with resistance to conventional therapy, MDS features and inferior outcome[5,6]. UBTF-TDs have also been identified in nearly a third of pediatric MDS-EB without germline predisposition, and were found to be associated with worse outcome compared with UBTF-wild type diseases[7,8]. Pediatric r/r-AML, MDS-EB and t-MDS/AML necessitate allogeneic hematopoietic cell transplantation (HCT) as the standard potentially curative treatment. However, the optimal bridging therapy to transplantation remains controversial for pediatric hematologists, considering the poor biologic response to intensive chemotherapy and the substantial risk of pre-transplant treatment-associated toxicity and mortality. The identification and validation of novel therapeutic interventions able to reduce disease burden, while avoiding the toxicity of intensive chemotherapy, represents an unmet need to improve overall outcomes of these conditions[1,2]. The Bcl-2 inhibitor venetoclax has emerged as an effective and safe agent in adult AML, particularly in combination with hypomethylating agents (HMA), such as azacytidine, in patients unfit for receiving intensive cytotoxic therapy[9]. Venetoclax, combined with cytarabine +/- idarubicin, has been tested in a pediatric trial on r/r AML, in which an overall response rate (ORR) of 69% was observed[10]. Another trial tested the use of venetoclax combined with different cytotoxic or HMA in 45 patients with newly diagnosed AML achieving complete response (CR) in 86.7% and minimal residual disease (MRD)-negativity in 44% on day 28 of venetoclax start. Only 12 patients received venetoclax associated to HMA achieving CR in 75% of cases and MRD-negativity in 58.3%[11]. Retrospective studies, including our previous report, described the use of different venetoclax-containing regimens combined with both cytotoxic drugs and HMA[12–20]. Preliminary results on the use of venetoclax plus azacytidine (ven/aza) in children showed highly variable response rates of 16-70%, with 30-60% of patients successfully bridged to HCT[21]. Published retrospective pediatric studies on ven/aza involve heterogenous cohorts predominantly including r/r-AML patients with very few children with MDS-EB or t-MDS/AML[12,15,22–25]. In this nationwide study, we aimed to evaluate ven/aza treatment administered with curative intent as a potential bridge to HCT in pediatric patients with high-risk myeloid neoplasms, including r/r-AML, MDS-EB and t-MDS/AML, focusing on toxicity profile, response rates and clinical outcomes.

Methods

This retrospective multicenter observational study included patients treated across centers of the *Italian Association of Pediatric Hematology and Oncology* (AIEOP) who received ven/aza for high-risk myeloid diseases between January 2022 and June 2025. Nine centers participated. The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethical Committee CE-AVEC (study protocol: PETER, code 656/2024/Oss/AOUBo). Therapy was administered based on individual clinical indication under compassionate off-label use, following local Ethical Committee approval and informed consent from participants and/or legal guardians. Inclusion criteria were (1) age ≤ 20 years at diagnosis; (2) a diagnosis of r/r-AML, MDS-EB or t-MDS/AML according to 2022 International Consensus Classification (ICC)[26]; and (3) having received ≥ 1 ven/aza cycle. Ven/aza cycle was defined as intravenous azacytidine

75 mg/m2/day (days 1-7) plus oral venetoclax (tablets or suspension) at weight-adjusted adult-equivalent dosing of 400 mg/day (range, 100-400 mg/day)[27,28] for ≥14 days (days 1-14 up to 1-28). Exclusion criteria were (1) concomitant administration of cytotoxic agents during ven/aza cycles; (2) ven/aza administered with purely palliative intent, (3) incomplete/suboptimal venetoclax intake due to poor compliance; and (4) lack of bone marrow (BM) evaluation. Concomitant antimicrobial prophylaxis, non-cytotoxic targeted therapy (e.g. FLT3-inhibitors) or intrathecal therapy was allowed at treating physician's discretion. In patients receiving azoles, venetoclax dose was reduced by 50 or75% according to strong (posaconazole, voriconazole) or moderate (isavuconazole) CYP3A inhibition potential, respectively [29,30]. Data were collected by an anonymized electronic case report form (eCRF). Safety was evaluated as the proportion of patients experiencing grade ≥3 adverse events (AEs) according to Common Terminology Criteria for Adverse Events (CTCAE) v5.0. BM morphological response was assessed according to Cheson criteria[31] as CR, defined as BM blasts ≤5% with hematologic count recovery, CR with incomplete count recovery (CRi), partial response (PR) defined as 6-20% blasts with >50% decrease from baseline, or non-response (NR), defined as blasts increase, persistence ≥20% or <50% reduction. ORR was calculated as CR+CRi+PR. Extramedullary disease response was evaluated by imaging, biopsy and/or lumbar puncture. Considering the lack of standardized response criteria for pediatric MDS, individual patient evaluation was performed, considering CR as FCM-MRD negativity and PR as stable disease <5% (if blast count <5% at baseline) or >50% blast reduction. Disease burden at ven/aza initiation was categorized as low (BM blasts ≤5%) or high (>5%). MRD was assessed by multi-parameter flow-cytometry or molecular biology assays, when applicable. MRD negativity was defined as <0.1% blasts by flow-cytometry and <0.01% by polymerase chain reaction (PCR) (details in **Supplementary methods**). Clinical outcome was evaluated by overall survival (OS) and event-free survival (EFS), estimated by Kaplan–Meier method. Potential predictors of CR were analyzed by logistic regression, including age, diagnosis, BM blast percentage, HOX-dysregulation-associated molecular lesions (KMT2A rearrangements, NUP98 fusions, NPM1 mutations, UBTF-TD), and FLT3-ITD mutations. Potential predictors of survival included age, diagnosis, HOX-dysregulation, HCT previous to ven/aza treatment, CR achievement and receiving HCT. Odds ratio were reported with 95% confidence interval (CI) and p-values < 0.05 were considered significant. Analyses were performed using SPSS Statistics v26.

Results

Baseline characteristics of patients

Fifty patients were included in the study, with a median age of 11 years (range 1-20) at diagnosis and a male:female ratio of 1.8:1. Patients had either r/r-AML (n = 32), or primary MDS-EB (n = 10) or t-MDS/AML (n = 8). Recurrent molecular abnormalities included KMT2A rearrangements (n = 12), FLT3-ITD mutations (n = 7), UBTF-TD (n = 5) and NUP98 fusions (n = 4). Among relapsed cases (19 AML, 4 MDS-EB, 3 t-MDS/AML), molecular lesions at relapse were consistent with those found at diagnosis. Forty-six patients had received previous cytotoxic therapy lines (median 3 lines, range 1-8). Four patients with newly diagnosed MDS-EB were treatment-*naïve*. Twenty-three patients (46%) had previously received allogenic HCT with a median interval between transplant and ven/aza initiation of 313 days (range, 80-1876). Median BM blast percentage before treatment start was 4% (range 0-92%) with a low and high disease burden present in 30 and 20 patients, respectively. Six patients presented an extramedullary disease (5 isolated extramedullary and one combined with medullary disease). Patient characteristics are summarized in **Table 1**.

Ven/aza cycles

Patients received a median of 2 ven/aza cycles (range, 1-7). Venetoclax was given orally at weight-adjusted adult equivalent dosing of 400 mg/day ranging from 100-400 mg/day, using oral tablets. In case of swallowing difficulties or reduced compliance in younger children, crushed tablets or oral solution provided on compassionate use by the pharmaceutical company were adopted. In forty-five patients, venetoclax dose escalation during the first cycle was adopted on day 1 and 2. Venetoclax was administered for a median of 28 days (range 14-28), the duration of treatment being 14 in 3, 21 in 14 and 28 days in 33 patients, respectively (days 1-14 to 28) based on treating physician's choice. Azacytidine was administered intravenously at 75 mg/m2/day for 7 days (days 1-7). Ten patients received additional agents during ven/aza cycles, including gilteritinib (n = 6), donor-lymphocyte infusion (DLI) (n = 2), gemtuzumab ozogamicin (GO) (n = 1), and luveltamab tazevibulin (STRO-002) (n = 1). Intrathecal therapy with cytarabine at age-adjusted dose was given to 28 patients.

Supportive measures and toxicities

No treatment-related deaths occurred during the study period, and no tumor lysis syndrome was observed, even in the case of high disease burden. Grade ≥3 AEs were recorded in 17 patients (34.0%), including febrile neutropenia (n=7), prolonged neutropenia/pancytopenia (n=7), and fungal infection (n=3), requiring venetoclax interruption in 12 patients (5 for febrile neutropenia, 5 for prolonged neutropenia, and 2 for fungal infection). Three patients discontinued venetoclax after 14 days due to infectious complications (n = 2) or initiation of HCT conditioning (n = 1). Antifungal prophylaxis was administered in 42 patients, including liposomal B amphotericin (n = 21) and azoles (n = 21). Two out of 3 fungal infections occurred in patients on liposomal B amphotericin prophylaxis, while one occurred in a patient without prophylaxis. Antibiotic prophylaxis was given to 27 patients.

Response

Best overall response achieved was CR in 29 patients (58%) [5 morphological CR, 16 FCM- MRD negative, and 8 PCR-MRD-negative], CRi in 1 (2%), PR in 9 (18%), and NR in 11 (22%) patients, respectively, with an ORR of 78%. The clinical course of each patient is summarized in **Figure 1**. Median number of cycles to achieve best response was 1 (range 1-2). Of the 26 patients who received ≥1 cycle, all 15 patients who achieved CR already after cycle 1 maintained their response after cycle 2; among 9 patients with PR after cycle 1, 4 converted to CR, 3 maintained PR, and 2 progressed to NR (**Figure 2A**). In the MDS-EB cohort (n=10), 7 FCM-MRD negative CR, two PR (reduction in BM blasts from 15% to 7% and stable disease <5% blasts), and one NR (progression to AML) were reported (ORR 90.0%). Among 8 patients with t-MDS/AML, 5 CR, 2 PR, and 1 NR were reported (ORR 87.5%). Among the 6 AML patients with extramedullary disease, 3 CR (defined as negativity at PET-FDG/MRI imaging, and biopsy associated with medullary CR) and 3 PR (defined as reduction of extramedullary lesions at PET-FDG/MRI imaging) were reported. Among 12 patients with KMT2A-rearranged AML, 7 achieved CR (FCM or PCR-MRD negative), 2 PR and 3 NR (ORR 75%). Two of these NR cases were rescued with the menin-inhibitor revumenib, achieving remission that was successfully consolidated with HCT. Of 7 patients with FLT3-ITD mutations, 5 received FLT3-inhibitors combined with ven/aza, achieving 4 CR and 1 PR (ORR 71.4%). All 5 patients harboring UBTF-TD achieved cytofluorimetric or molecular CR (ORR 100%). In 5 NUP98-mutated AML, 3 CR, 1 PR and 1 NR were achieved (ORR 80%). A cytofluorimetric CR was also reported in 2 DEK-NUP214 AML (ORR 100%). Of the 2 CBFA2T3-GLIS2 AML, PCR-MRD negativity was achieved in a patient receiving ven/aza associated with the monoclonal antibody directed against FOLR1, STRO-002, while the other patient did not respond. CR and ORR rates in the main genetic subgroups are schematized in **Figure 2B**. No variable reached statistical significance as a predictor of CR. A trend toward higher CR rates was observed in patients with HOX-

dysregulation and in those with MDS-EB. Disease burden resulted to have no effect, with comparable CR rates in patients with low and high disease burden (58.1% vs 57.1%) (**Figure 2C**).

Outcome and HCT

Median follow-up was 389 days (range, 36 – 1405) from ven/aza initiation for the whole cohort. Thirty-three patients (66%) were bridged to myeloablative HCT, after a median of 97 days (range, 33 – 933) from ven/aza start (details in **Supplementary Table S2**). Among patients bridged to transplant, median follow-up was 414 days (range, 39-1311) from HCT. Nineteen patients were dead at the end of follow-up, and causes of death included disease progression before HCT (n = 12), relapse after HCT (n = 4), and transplant-related mortality (TRM) (n = 3). Median blast percentage at the last BM evaluation before HCT was 0% (range, 0-13%) (details in **Supplementary Material**). The estimated 2-year OS from the start of ven/aza therapy was 57.7% (CI: 49.8-65.6) for the whole study population (**Figure 3**). The 2-year EFS was 54.5% (CI: 46.7-62.3) for the whole cohort, being 75.8% (CI: 67.7-83.9) for transplanted patients, and 77.2% (CI: 68.9-85.5) for patients achieving CR. In patients affected by MDS-EB and t-MDS/AML, 2-year EFS were 88.9% (CI: 78.4 – 99.4) and 75% (CI: 59.7 – 90.3), respectively (**Supplementary Figure S1 – A)**. Relapsed/refractory AML diagnosis (HR 0.313; CI 0.105-0.935; p 0.037), not having received a previous HCT before ven/aza initiation (HR 2.900; CI 1.157-7.266; p 0.023), achieving CR after ven/aza (HR 3.708; CI 1.490-9.226; p 0.005), and being successfully bridged to HCT (HR 0.098; CI 0.035-0.272; p < 0.0001) were significantly associated with better EFS in Cox regression model in univariate analysis. Only achieving CR (HR 0.345; CI 0.130-0.916; p < 0.033) and being bridged to HCT (HR 7.303; CI 2.139-24.935; 0.002) were confirmed to be associated with better outcome in multivariate analysis, as shown in **Supplementary Table S1**. In the 23 patients who received a previous HCT before the start of ven/aza, 2-year EFS was 35.0% (CI: 23.9 – 46.1). Eleven of these patients underwent a second HCT after ven/aza: eight were alive at the end of follow-up, while three died due to disease relapse (**Supplementary Figure S1 - B**).

Discussion

We report the largest nationwide experience on the use of ven/aza in children and adolescents with high-risk myeloid diseases, documenting encouraging clinical activity and patient outcome, especially in light of the dismal prognosis of these disorders. Notably, particularly positive results in terms of EFS were achieved in patients bridged to HCT and in those who achieved CR.

Ven/aza cycles were generally well tolerated, with hematological and infectious toxicities being the most frequent grade ≥3 AEs, and only a minority of patients required early venetoclax interruption. These data confirm and extend previous pediatric findings, demonstrating a more favorable toxicity profile compared with that observed when venetoclax is combined with cytotoxic therapies[10,12]. The ven/aza combination represents an appealing option as a bridge to HCT by reducing toxicities in the immediate pretransplant phase for patients who had received several prior lines of therapy[29]. Our findings on fungal infections also underscore the need to follow a standardized approach for antifungal prophylaxis in children receiving venetoclax-based regimens[32]. Of note, ven/aza was successfully used as a bridge to a second transplant in 11 of 23 heavily pretreated patients who had previously received HCT before starting ven/aza. No TRM associated with these second procedures was observed.

Notably, the observed heterogeneity in venetoclax duration likely reflects the absence of established pediatric standards, as well as real-world adaptations driven by toxicity, treatment combinations, and evolving evidence supporting shorter treatment courses, especially in the early post-HCT phase[25,33]. Regarding the optimal number of ven/aza cycles, we

showed that in the absence of a response, either CR or PR, to the first cycle, a second cycle does not seem to offer any advantage, suggesting to explore therapeutic alternatives, such as CPX-351 that proves to be efficacious in relapsed and secondary myeloid neoplasms[34,35], although further confirmation of its role is needed. Conversely, in the case of CR and PR, a second cycle can be considered, also in light of the conversion from PR to CR we observed in 4 patients. Considering the significantly better outcomes in patients bridged to HCT and that the best response to ven/aza is generally achieved within one to two cycles, transplantation should be consistently pursued as a consolidation strategy, with expedited donor search from treatment initiation and prompt progression to transplant.

We explored the use of ven/aza in primary MDS-EB and t-MDS/AML, two difficult-to-treat conditions without defined optimal management. Establishing the best algorithm for these diseases is of fundamental importance for pediatric hematologists in order to control the disease before HCT and avoid intensive therapy[1]. While percentage of BM blasts at the time of transplantation demonstrated to significantly affect outcome after HCT in adult MDS[36], the role of pre-transplant cytoreductive therapy is a matter of debate in pediatric diseases[1]. Reports from different international consortia showed no advantage in OS in childhood MDS treated with pretransplant cytoreductive therapy[37,38]. Interestingly, a recent study reports that patients who achieved MRD-negative status prior to HCT had a survival benefit[39]. A recent study from the French group suggests that cytoreductive therapy prior to transplantation may be associated with improved OS in the subgroup of childhood MDS with increased blasts. Surprisingly, this improvement was not related to a reduction in the risk of relapse but seems to be associated to lower TRM[40]. In our cohort, the majority of patients present no detectable blasts at the time of HCT, possibly determining the more favorable outcome compared to similar pediatric series reported in literature[25]. Further prospective studies on the role of disease burden pre-HCT are needed, especially in the MDS-EB setting.

We confirmed our previously published preliminary results, showing a generally good response and safety profile of venetoclax-containing combinations in MDS-EB and t-MDS/AML[12]. The toxicity profile is of utmost importance in the context of germline predisposition. We reported a dramatic response and a favorable post-HCT outcome in one patient with severe congenital neutropenia (SCN) and CSF3R-mutated MDS-EB, suggesting the potential use of ven/aza also in other conditions (e.g., MDS in GATA-2 deficiency) that demonstrated resistance to conventional chemotherapy and poor outcome. Of note, the absence of standardized pediatric MDS-EB response criteria remains a critical limitation in current clinical practice. This highlights the potential value of using alternative measures, such as post-HCT outcomes, to evaluate treatment efficacy. Interestingly, we observed comparable efficacy in patients with low or high blast count, highlighting the relevance of the biology of disease in determining the response more than the disease burden. In this context, identifying recurrent genetic abnormalities that can help predict response to venetoclax, represents a key consideration[21]. KMT2A-rerranged AML showed satisfactory response rates to ven/aza, confirming previous reports on myeloid and lymphoblastic acute leukemia[12,22]. The two patients who did not responded to ven/aza, achieved remission with revumenib, suggesting a possible benefit of combination strategies with menin inhibitors that are being explored in adult AML[41]. Notably, the combination of venetoclax + azacytidine + revumenib is currently being tested in a phase 1 trial in pediatric AML[42]. NUP98-rearranged leukemias exhibited heterogeneous responses, consistent with the biological diversity of NUP98 fusion partners that may influence venetoclax sensitivity[43]. In our cohort, FLT3-ITD-mutated AML that received ven/aza plus FLT3-inhibitor responded, at least partially, to treatment, confirming preclinical reports on the synergistic effect of these drugs[21]. CBFA2T3:Glis2 AML showed resistance to ven/aza alone, corroborating preclinical experiences showing that dual Bcl-2 family protein inhibition (Bcl-2 plus MCL-1 or BCL-XL) is necessary to treat these diseases[21].

Response in UBTF-TD-associated diseases was one of our most relevant findings, with all UBTF-TD patients achieving deep FCM-MRD negativity and being successfully bridged to HCT. The clinical significance of UBTF-TD in pediatric myeloid diseases has only recently begun to emerge. Poor responses to conventional therapy suggest defining novel therapeutic approaches as bridge to HCT. Initial promising results of menin inhibitor in UBTF-TD AML arise from preclinical studies and one case report[44,45]. Our data provide the basis for further exploring venetoclax-containing combinations in this myeloid subgroup, characterized by a transcriptional expression profile that overlaps with NPM1-mutated AML and by an overexpression of HOX genes, a marker of response to Bcl-2 inhibition[5,8]. Additionally, our group previously reported a distinct methylation profile of patients with pediatric MDS-EB and AML harboring UBTF-TD, providing a rationale to consider UBTF-TD myeloid diseases as a continuum entity driven by a shared genetic lesion, which may benefit from ven/aza treatment[46].

A key strength of this study is that it provides a uniform nationwide experience with centralized analyses and a homogeneous therapeutic approach, minimizing variability and ensuring consistent data quality. The cohort was treated uniformly with curative pre-HCT intent, likely contributing to the more favorable outcomes observed compared to studies including palliative ven/aza regimens[25]. It also delivers first evidences in pediatric MDS-EB and t-MDS/AML, two rare conditions lacking established pre-transplant treatments. Finally, the exclusion of intensive chemotherapy combinations allows a clearer assessment of ven/aza–specific activity and toxicity profile.

Our study is limited by its retrospective design, together with possible selection bias and treatment heterogeneity. Relatively short follow-up may affect the estimation of the cumulative incidence of relapse in these high-risk myeloid diseases. Furthermore, some heterogeneity in treatment duration and concomitant medications may influence the precise interpretation of the results. Finally, the absence of pharmacokinetic analyses should be considered in this pediatric context, owing to the off-label use of the treatment, the age-related physiological changes and frequent drug-drug interactions. Consequently, variability in therapeutic response and the occurrence of toxicities associated with potentially inaccurate dosing cannot be excluded and future prospective pediatric trials incorporating PK/PD analyses are necessary to refine these therapeutic strategies.

Our experience paves the way for future prospective evaluation of ven/aza treatment in pediatric patients with myeloid neoplasms, particularly in MDS-EB and t-MDS/AML. The strong association between HOX gene dysregulation (e.g., KMT2A rearrangements, UBTF-TD) and response to venetoclax observed in our study underscores the need for molecularly driven treatment algorithms. Prospective trials incorporating genomic and transcriptomic biomarkers will be instrumental to optimize therapy selection and refine the role of ven/aza as a bridge to HCT.

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Table 1. Baseline patient characteristics and response rates.

Patients (n = 50)	**n (% or range)**
Sex, n (%)	
- Male	32 (64)
- Female	18 (36)
Diagnosis, n (%)	
- MDS-EB	10 (20) ”
- Relapsed AML	19 (38)
- Refractory AML	13 (26)
- t-MDS/AML	8 (16)
Age at diagnosis, median (range)	10 (1 – 20)
Germline variants in myeloid predisposing genes, n (%)	1 *
Cytogenetic abnormalities, n (%)	
- −7/del(7q)	5 (10)
- Complex karyotype	4 (8)
- t (9;11)	3 (6)
- t (10;11)	3 (6)
- trisomy 8	3 (6)
- t (6;9)	2 (4)
- t (16;16)	2 (4)
Molecular abnormalities, n (%):	
- KMT2A rearrangements	12 (24) ¶
- FLT3-ITD	7 (14) ˆ
- NUP98 rearrangements	5 (10) ~
- UBTF-TD	5 (10)
- *CBFA2T3::GLIS2*	2 (4)
- *DEK::NUP214*	2 (4)
- NPM1 mutations	2 (4)
Previous therapies, n (%)	46 (92) ±
Previous lines of therapies before ven/aza start, median (range)	3 (0-8)
Previous HCT, n (%)	23 (46)
Median interval between previous HCT and ven/aza start (days), median (range)	313 (80 – 1876)
Age at ven/aza start (years), median (range)	11 (1 – 21)
BM blasts (%) at ven/aza start, median (range)	4 (0-92)
- Low (≤5%), n (%)	30 (60)
- High (>5%), n (%)	20 (40)
Extramedullary disease at ven/aza start, n (%)	6 (12) †

” MDS-EB1 (3) and MDS-EB2 (7)

* one HAX1 mutation / severe congenital neutropenia (SCN)

¶ KMT2A rearrangements included KMT2A-MLLT10 [t(10;11)] (3), KMT2A-MLLT3 [t(9;11)] (3), KMT2A-PTD (2) KMT2A-AFDN [t(6;11)] (1), KMT2A-AFF1 [t(4;11)] (1), KMT2A-ELL [t(11;19)] (1) and KMT2A-SEPT9 [t(11;17)] (1)

ˆ FLT3-ITD associated with NUP98-NSD1 (3), NPM1 (2) and *DEK::NUP214* (1)

~ NUP98 rearrangements included NUP98-NSD1 (3), NUP98-LNP1 (1) and NUP98-KDMSA

± Four MDS-EB received ven-aza as the first line of therapy

† 5 isolated extramedullary (one testis, two skin, two CNS) and one combined (pulmonary + BM)

AML: acute myeloid leukemia; BM: bone marrow; HCT: hematopoietic cell transplantation; MDS-EB: myelodysplastic syndrome with excess of blasts; t-MDS/AML, therapy-related MDS/AML; -7/del (7q): monosomy 7 (−7) and deletions of chromosome 7 [del(7q)]; HCT, hematopoietic cell transplantation; ven/aza, venetoclax and azacytidine; BM, bone marrow

Figure 1. Swimmer plot of patients receiving ven/aza. Patients are divided in four groups according to diagnosis. Dots represent the diseases evaluation and response is specified in the legend.

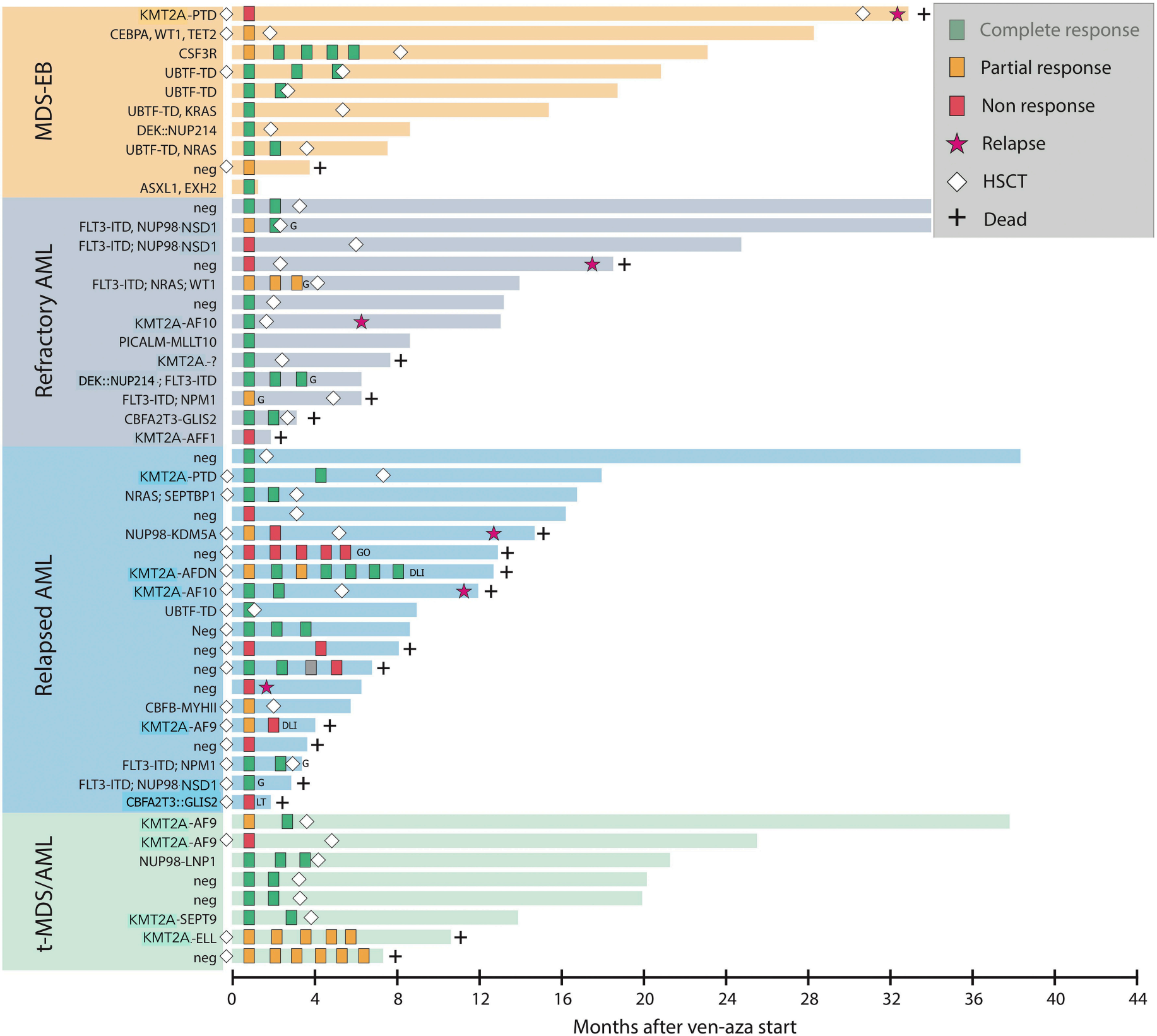
AML: acute myeloid leukemia; BM: bone marrow; HCT: hematopoietic cell transplantation; MDS-EB: myelodysplastic syndrome with excess of blasts; t-MDS/AML, therapy-related MDS/AML; HCT, hematopoietic cell transplantation; ven/aza, venetoclax and azacytidine; BM, bone marrow; ven/aza, venetoclax and azacytidine; G, gilteritinib; DLI, donor-lymphocyte infusion; GO, gemtuzumab ozogamicin; LT, luveltamab tazevibulin (STRO-002)

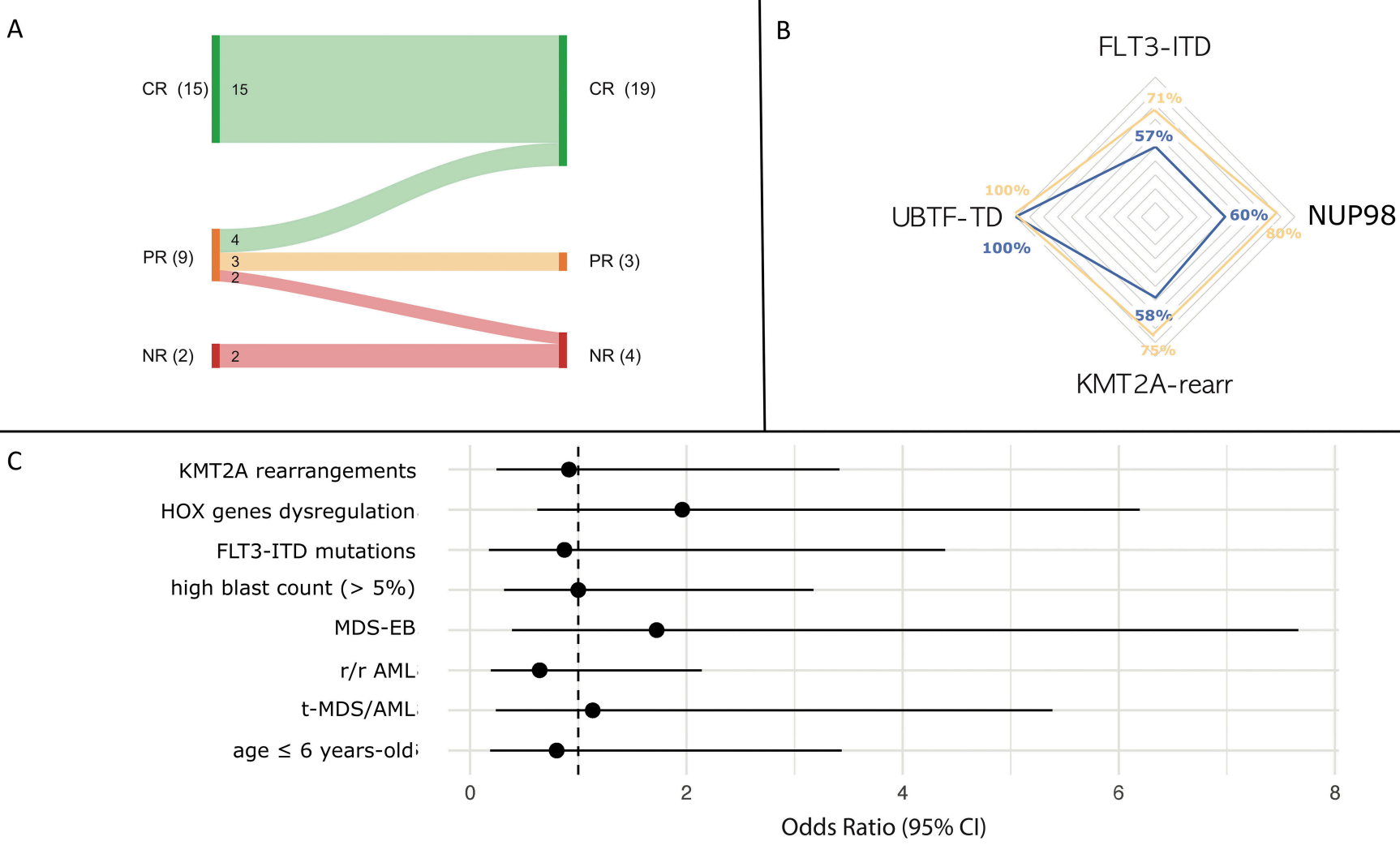
Figure 2. Summary of response in patients receiving ven/aza. (A) response across cycles in patients receiving ≥ 1 ven/aza cycle (B) CR (blue line) and ORR (orange line) in main genetic subgroups and (C) forest plot summarizing factors potentially associated with CR to ven/aza

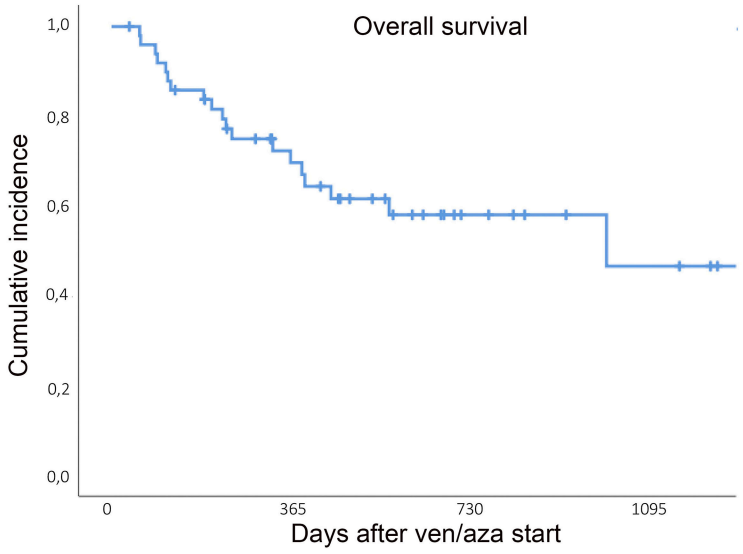
CR, complete response; PR, partial response; NR, no response; AML: acute myeloid leukemia; MDS-EB: myelodysplastic syndrome with excess of blasts; t-MDS/AML, therapy-related MDS/AML; ven/aza, venetoclax and azacytidine;

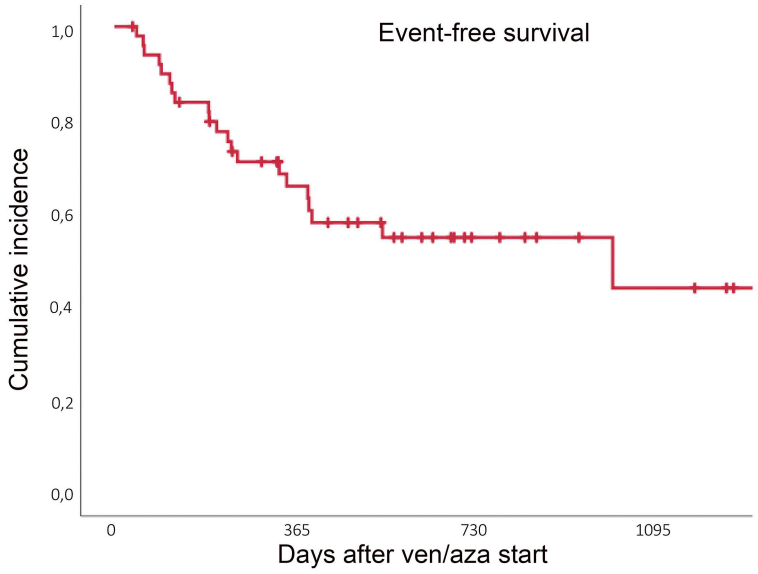
Figure 3. Kaplan-Mayer estimates of survival in patients receiving ven/aza. (A) OS for the entire cohort, (B) EFS for the whole cohort, (C) EFS in patients bridged or not to HCT and (D) EFS in patients achieving or not CR with ven/aza.

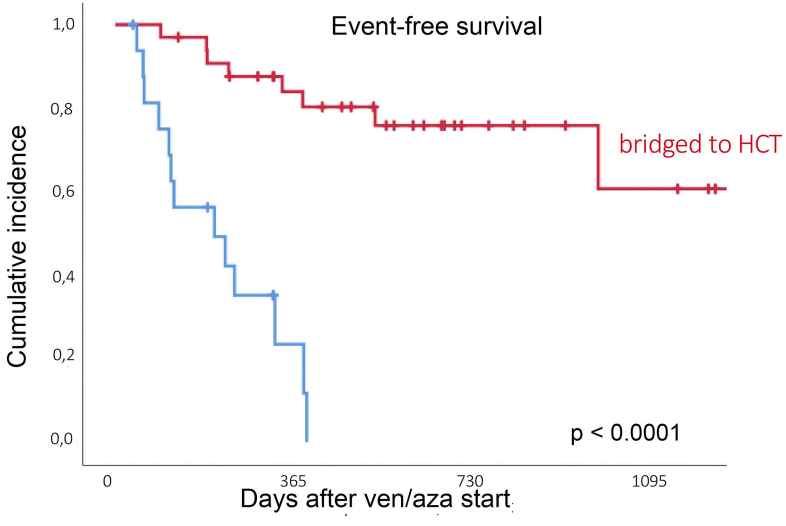
OS, overall survival; EFS, event-free survival; HCT, hematopoietic cell transplantation; ven/aza, venetoclax and azacytidine; CR, complete response

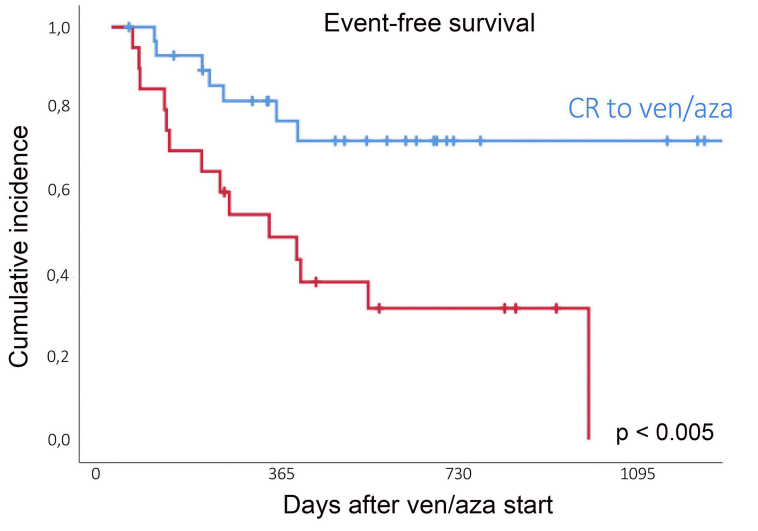

MDS-EB
Refractory AML
Relapsed AML
t-MDS/AML
KMT2A-PTD
CEBPA, WT1, TET2
CSF3R
UBTF-TD
UBTF-TD
UBTF-TD, KRAS
DEK::NUP214
UBTF-TD, NRAS
neg
ASXL1, EXH2
neg
FLT3-ITD, NUP98-NSD1
FLT3-ITD; NUP98-NSD1
neg
FLT3-ITD; NRAS; WT1
neg
KMT2A-AF10
PICALM-MLLT10
KMT2A-?
DEK::NUP214 ; FLT3-ITD
FLT3-ITD; NPM1
CBFA2T3-GLIS2
KMT2A-AFF1
neg
KMT2A-PTD
NRAS; SEPTBP1
neg
NUP98-KDM5A
neg
KMT2A-AFDN
KMT2A-AF10
UBTF-TD
Neg
neg
neg
neg
CBFB-MYHII
KMT2A-AF9
neg
FLT3-ITD; NPM1
FLT3-ITD; NUP98-NSD1
CBFA2T3::GLIS2
KMT2A-AF9
KMT2A-AF9
NUP98-LNP1
neg
neg
KMT2A-SEPT9
KMT2A-ELL
neg
G
G
G
G
GO
DLI
DLI
G
G
LT
Complete response
Partial response
Non response
Relapse
HSCT
Dead
0
4
8
12
16
20
24
28
32
36
40
44
Months after ven-aza start


A
CR (15)
15
CR (19)
PR (9)
4
3
2
PR (3)
NR (2)
2
NR (4)
B
FLT3-ITD
71%
57%
100%
UBTF-TD
60%
NUP98
80%
100%
58%
75%
KMT2A-rearr
C
KMT2A rearrangements
HOX genes dysregulation
FLT3-ITD mutations
high blast count (> 5%)
MDS-EB
r/r AML
t-MDS/AML
age ≤ 6 years-old
0
2
4
6
8
Odds Ratio (95% CI)


Overall survival
Cumulative incidence
1,0
0,8
0,6
0,4
0,2
0,0
0
365
730
1095
Days after ven/aza start


Event-free survival
Cumulative incidence
1,0
0,8
0,6
0,4
0,2
0,0
0
365
730
1095
Days after ven/aza start


Event-free survival
bridged to HCT
p < 0.0001
Cumulative incidence
1,0
0,8
0,6
0,4
0,2
0,0
0
365
730
1095
Days after ven/aza start


Event-free survival
CR to ven/aza
p < 0.005
Cumulative incidence
1,0
0,8
0,6
0,4
0,2
0,0
0
365
730
1095
Days after ven/aza start

Supplementary methods

BM trephine material of MDS-EB and t-MDS/AML were centrally reviewed at the Department of Pathology of Bambino Gesù Children's Hospital in Rome. Immunophenotypic and molecular analysis were centrally performed at the Pediatric Onco-Haematology Laboratory of the University of Padua. Immunophenotyping was performed according to WHO criteria, using a direct immunofluorescence technique with 8-12 colour combinations[1,2]. For genetic characterization, RNA was extracted from all samples at diagnosis from mononuclear cells isolated by density gradient. Reverse transcription polymerase chain reaction (RT-PCR) was performed to amplify target sequences. Amplification products were analyzed by agarose gel electrophoresis and/or capillary electrophoresis using the 3500 Dx Genetic Analyzer, and/or sequencing[3–7]. All DNA samples of patients were screened for the presence of *UBTF*-TD by polymerase chain reaction (PCR) with specific primers previously validated to detect the presence of tandem duplications (*UBTF* forward (5'FAM): CTGGGCTCCCTGGCAGCAC; *UBTF* reverse: GCCCGCCAAGGGGAAGAGG). PCR products were diluted and analyzed by capillary electrophoresis on a 3500Dx Genetic Analyzer using GeneMapper software. A wild-type peak at 270±1 bp was expected, while additional peaks indicated UBTF-TD. The allelic ratio was calculated as the ratio of mutant to wild-type peak areas. Positive, negative, and non-template controls were included to ensure assay specificity and exclude contamination. Further details were previously reported[8].

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Supplementary results _ *disease burden prior to HCT*

Among the 33 patients successfully bridged to HCT, 26 (78.8%) reached the transplant with 0% BM blasts. In this subgroup, only 1 relapse was observed (alongside 3 deaths due to transplant-related mortality - TRM). In contrast, among the 7 patients with morphologically detectable disease (range 1-13% blasts), 3 relapses occurred. Focusing on the MDS-EB and t-MDS/AML subgroup (n=14), 11 patients had 0% blasts at HCT and one of these patients relapsed after transplant, while three patients were transplanted with morphological disease and none experienced relapses.

Supplementary Table S1. Univariate and multivariate analysis of factors associated to 1-year event-free survival (EFS)

	1-y EFS			
	Univariate		**Multivariate**	
Risk factors	**HR (95% CI)**	**p**	**HR (95% CI)**	**p**
Sex	0.535 (0.227-1.261)	0.153		
r/r AML diagnosis	0.313 (0.105-0.935)	**0.037** *	2.354 (0.751-7.379)	0.142
MDS-EB diagnosis	2.641 (0.614-11.356)	0.192		
t-MDS/AML	2.518 (0.585-10.839)	0.215		
HOX dysregulation	0.942 (0.399-2.224)	0.892		
KMT2 rearrangements	0.560 (0.231-1.355)	0.198		
No HCT previous to ven/aza	2.900 (1.157-7.266)	**0.023** *	1.079 (0.323-3.605)	0.902
CR achieved	3.708 (1.490-9.226)	**0.005** *	0.345 (0.130-0.916)	**0.033**
Bridged to HCT	0.098 (0.035-0.272)	**<0.0001**	7.303 (2.139-24.935)	**0.002**

EFS, event-free survival; HR, hazard ratio; CI, confidence interval; r/r AML, relapsed/refractory acute myeloid leukemia; MDS-EB, myelodysplastic syndromes with excess of blasts; t-MDS/AML, therapy-related MDS or AML; CR, complete response; HCT, hematopoietic cell transplantation

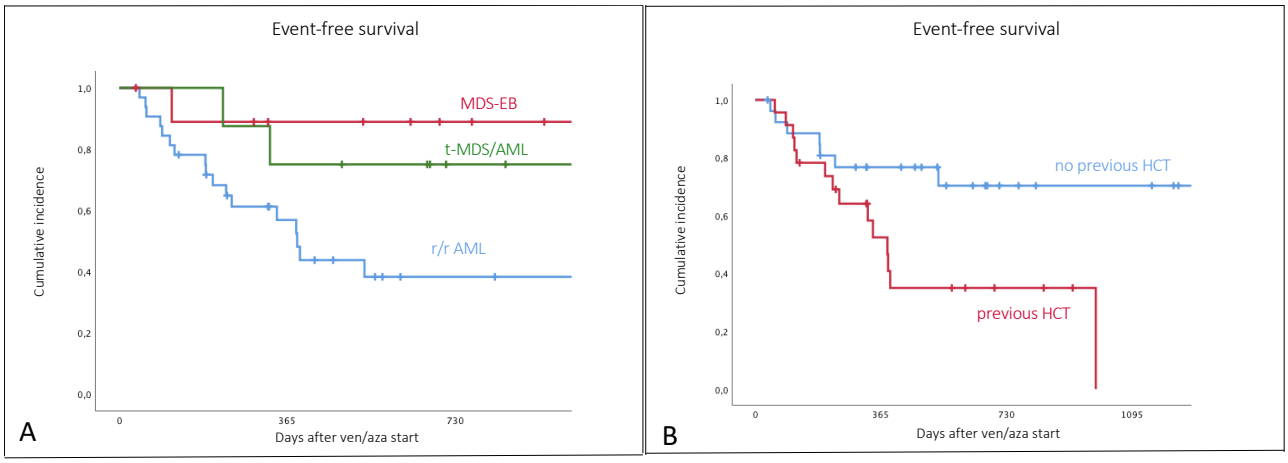
* diagnosis of r/r AML, previous HCT status and CR achievement were not associated with EFS when analysis was restricted to patients bridged to HCT in Cox regression analysis

Supplementary Table S2. Transplant characteristics in patients undergoing hematopoietic cell transplantation (HCT) after ven/aza

Patients (n = 33)	**n (% or range)**
Conditioning, n (%)	
- Bu-based	- 17
- Treo-based	- 6
- TBI-based	- 10
Donor, n (%)	
- MSD	- 9
- MUD	- 12
- UCB	- 1
- haploidentical	- 11
Interval between ven/aza start and HCT (days), median (range)	97 (range, 33 – 933)
BM blast percentage before HCT, median (range)	0 (0 – 13%)*

Bu, busulfan; Treo, treosulfan; TBI, total-body irradiation; MSD, matched sibling donor; MUD, matched unrelated donor; UCB, umbilical cord blood

Supplementary Figure S1. Kaplan-Mayer estimates of EFS in patients receiving ven/aza. (A) EFS according to diagnosis and (B) according to previous transplantation status (B).



EFS, event-free survival; r/r AML: relapsed/refractory acute myeloid leukemia; MDS-EB: myelodysplastic syndrome with excess of blasts; t-MDS/AML, therapy-related MDS/AML; HCT, hematopoietic cell transplantation