Venetoclax and azacytidine in childhood primary advanced myelodysplastic syndromes, refractory/relapsed acute myeloid leukemia and therapy-related myeloid neoplasms

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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 the high rate of treatment failure in these diseases. Venetoclax plus azacytidine (ven/aza) has transformed the treatment of myeloid neoplasms in adults, but pediatric data are limited and heterogeneous. This AIEOP retrospective, multicenter study analyzed 50 patients (males:females, 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 blast evaluation. Adverse events were graded as per CTCAE v5.0; outcomes were evaluated with Kaplan-Meier and Cox-regression analysis. Patients received a median of two cycles (range, 1-7) of ven/aza treatment. Grade ≥3 adverse events occurred in 34% of patients. Overall, 30 patients (60%) achieved CR (24 of whom were negative for minimal residual disease), nine (18%) achieved PR, and 11 (22%) had NR. CR rates were 58%, 57% and 100% in *KMT2A*-AML, *FLT3*-ITD-AML (ven/aza+FLT3-inhibitors) and *UBTF*-TD myeloid neoplasms, respectively. Among ten cases of MDS-EB, seven CR and two PR were recorded. Among eight patients with t-MDS/AML, we observed five CR and two PR. Thirty-three patients (66%) underwent hematopoietic cell transplantation (HCT), after a median of 97 days (range, 33-933) from starting ven/aza. The median follow-up was 389 days (range, 36-1,405). Two-year event-free survival was 54.5% (95% confidence interval: 46.7-62.3), being 75.8% in transplanted patients, and 77.2% in those achieving CR. CR and HCT were associated with better event-free survival. 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 blasts (MDS-EB) and therapy-related MDS/AML (t-MDS/AML) represent a significant

clinical challenge because of their frequent resistance to conventional therapies and high rates of relapse.[1,2] Adverse cytogenetic or molecular aberrations, such as complex karyotype, monosomy 7, selected *KMT2A*- and *NUP98*-rearrangements, or *FLT3*-internal tandem duplication (ITD) aberrations, have a strong impact on outcomes and may guide therapeutic decisions.[3,4] Recently, tandem duplications in the *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*-TD 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 cases of *UBTF*-wildtype disease.[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 biological 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, are unmet needs 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, such as azacytidine, in patients unfit to receive intensive cytotoxic therapy.[9] Venetoclax, combined with cytarabine ± idarubicin, has been tested in a pediatric trial in 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 hypomethylating agents in 45 patients with newly diagnosed AML and documented a complete response (CR) in 86.7% and minimal residual disease (MRD)-negativity in 44% on day 28 after starting venetoclax. Only 12 patients received venetoclax associated with hypomethylating agents; 75% of them achieved CR and 58.3% became MRD-negative.[11] Reports of retrospective studies, including our own, described the use of different venetoclax-containing regimens combined with both cytotoxic drugs and hypomethylating agents.[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 heterogeneous 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 indications under compassionate off-label use, following local Ethical Committee approval and informed consent from participants and/or legal guardians. Inclusion criteria were: (i) age ≤20 years at diagnosis; (ii) a diagnosis of r/r-AML, MDS-EB or t-MDS/AML according to the 2022 International Consensus Classification (ICC);[26] and (iii) having received ≥1 ven/aza cycles. A ven/aza cycle was defined as intravenous azacytidine 75 mg/m^2/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: (i) concomitant administration of cytotoxic agents during ven/aza cycles; (ii) ven/aza administered with purely palliative intent; (iii) incomplete/suboptimal venetoclax intake due to poor compliance; and (iv) lack of bone marrow (BM) evaluation. Concomitant antimicrobial prophylaxis, non-cytotoxic targeted therapy (e.g., FLT3-inhibitors) or intrathecal therapy was allowed at the treating physician's discretion. In patients receiving azoles, venetoclax dose was reduced by 50% or 75% according to strong (posaconazole, voriconazole) or moderate (isavuconazole) CYP3A inhibition potential, respectively.[29,30]

Data were collected using an anonymized electronic case report form. Safety was evaluated as the proportion of patients experiencing grade ≥3 adverse events according to Common Terminology Criteria for Adverse Events (CTCAE) v5.0. BM morphological response was assessed according to Cheson criteria as: CR,[31] 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 blast 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, patients were evaluated individually, considering CR as 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 multiparameter flow-cytometry (FCM) or molecular biology assays, when applicable. MRD negativity was defined as <0.1% blasts by FCM and <0.01% by polymerase chain reaction (PCR) (details in *Online Sup-*

plementary Methods).

Clinical outcome was evaluated by overall survival (OS) and event-free survival (EFS), estimated by the 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 prior to ven/aza treatment, CR achievement, and receiving HCT. Odds ratio were reported with a 95% confidence interval (95% CI) and *P* values <0.05 were considered statistically significant. Analyses were performed using SPSS Statistics v26.

Results

Baseline characteristics of the 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), 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 lines of cytotoxic therapy (median 3 lines; range, 1-8). Four patients with newly diagnosed MDS-EB were treatment-naïve. Twenty-three patients (46%) had previously received allogeneic HCT with a median interval between transplant and ven/aza initiation of 313 days (range, 80-1,876). The median BM blast percentage before starting treatment was 4% (range, 0-92%) with low and high disease burden present in 30 and 20 patients, respectively. Six patients had extramedullary disease (5 isolated extramedullary and 1 combined with medullary disease). The patients' characteristics are summarized in Table 1.

Venetoclax and azacytadine cycles

Patients received a median of two ven/aza cycles (range, 1-7). Venetoclax was given orally at weight-adjusted adult equivalent doses 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 administered. In 45 patients, venetoclax dose escalation during the first cycle was adopted on days 1 and 2. Venetoclax was administered for a median of 28 days (range, 14-28), the duration of treatment being 14 in three patients, 21 in 14 and 28 days in 33 patients (days 1 to 14-28) based on the treating physicians' choice. Azacytidine was administered intravenously at a dose of

Table 1. Baseline characteristics of the 50 pediatric patients with acute myeloid leukemia.

Characteristics	Values
Total patients, N	50
Sex, N (%) Male Female	 32 (64) 18 (36)
Diagnosis, N (%) MDS-EB Relapsed AML Refractory AML t-MDS/AML	 10 (20)[a] 19 (38) 13 (26) 8 (16)
Age at diagnosis, years, median (range)	10 (1-20)
Germline variants in myeloid predisposing genes, N	1[b]
Cytogenetic abnormalities, N (%) –7/del(7q) Complex karyotype t(9;11) t(10;11) trisomy 8 t(6;9) t(16;16)	 5 (10) 4 (8) 3 (6) 3 (6) 3 (6) 2 (4) 2 (4)
Molecular abnormalities, N (%) *KMT2A* rearrangements *FLT3*-ITD *NUP98* rearrangements *UBTF*-TD *CBFA2T3::GLIS2* *DEK::NUP214* *NPM1* mutations	 12 (24)[c] 7 (14)[d] 5 (10)[e] 5 (10) 2 (4) 2 (4) 2 (4)
Previous therapies, N (%)	46 (92)[f]
Previous lines of therapies before ven/aza start, median (range)	3 (0-8)
Previous HCT, N (%)	23 (46)
Interval between previous HCT and ven/aza start, days, median (range)	313 (80-1,876)
Age at ven/aza start, years, median (range)	11 (1-21)
BM blasts at ven/aza start, %, median (range) Low (≤5%), N (%) High (>5%), N (%)	4 (0-92) 30 (60) 20 (40)
Extramedullary disease at ven/aza start, N (%)	6 (12)[g]

[a]Of these ten patients, three had MDS-EB1 and seven had MDS-EB2. [b]One *HAX1* mutation/severe congenital neutropenia. [c]*KMT2A* rearrangements included *KMT2A-MLLT10* [t(10;11)] (N=3), *KMT2A-MLLT3* [t(9;11)] (N=3), *KMT2A*-PTD (N=2), *KMT2A-AFDN* [t(6;11)] (N=1), *KMT2A-AFF1* [t(4;11)] (N=1), *KMT2A-ELL* [t(11;19)] (N=1), and *KMT2A-SEPT9* [t(11;17)] (N=1). [d]*FLT3*-ITD associated with *NUP98-NSD1* (N=3), *NPM1* (N=2) and *DEK::NUP214* (N=1). [e]*NUP98* rearrangements included *NUP98-NSD1* (N=3), *NUP98-LNP1* (N=1) and *NUP98-KDMSA* (N=1). [f]Four patients with MDS-EB received ven/aza as the first line of therapy. [g]Five cases of isolated extramedullary disease (1 testis, 2 skin, 2 central nervous system) and one combined (pulmonary + bone marrow). -7/del (7q): monosomy 7 (–7) and deletions of chromosome 7 [del(7q)]; AML: acute myeloid leukemia; BM: bone marrow; HCT: hematopoietic cell transplantation; ITD: internal tandem duplication; MDS-EB: myelodysplastic syndrome with excess blasts; PTD: partial tandem duplication; TD: tandem duplication; t-MDS/AML: therapy-related myelodysplastic syndrome/acute myeloid leukemia; ven/aza: venetoclax and azacytidine.

75 mg/m^2/day for 7 days (days 1-7). Ten patients received additional agents during ven/aza cycles, including gilteritinib (N=6), donor-lymphocyte infusion (N=2), gemtuzumab ozogamicin (N=1), and luveltamab tazevibulin (STRO-002) (N=1). Intrathecal therapy with cytarabine at age-adjusted doses 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 adverse events 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 because of infectious complications (N=2) or initiation of HCT conditioning (N=1). Antifungal prophylaxis was administered to 42 patients and consisted of liposomal B amphotericin (N=21) and azoles (N=21). Two out of three fungal infections occurred in patients on liposomal B amphotericin prophylaxis, while one occurred in a patient not receiving prophylaxis. Antibiotic prophylaxis was given to 27 patients.

Response

The best overall response achieved was CR in 29 patients (58%) (5 morphological CR, 16 FCM-MRD-negative, and 8 PCR-MRD-negative), CRi in one (2%), PR in nine (18%), and NR in 11 (22%) patients, with an ORR of 78%. The clinical course of each patient is summarized in Figure 1. The median number of cycles to achieve best response was one (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 nine patients with PR after cycle 1, four converted to CR, three maintained PR, and two progressed to NR (Figure 2A). In the MDS-EB cohort (N=10), there were seven 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) for an ORR of 90.0%. Among eight patients with t-MDS/AML, five CR, two PR, and one NR were reported (ORR=87.5%). Among the six AML patients with extramedullary disease, three obtained CR (defined as negativity at PET-FDG/MRI imaging, and biopsy associated with medullary CR) and three had PR (defined as a reduction of extramedullary lesions at PET-FDG/MRI imaging).

Among 12 patients with *KMT2A*-rearranged AML, seven achieved CR (FCM or PCR-MRD negative), two obtained PR and three had NR (ORR=75%). Two of these NR cases were rescued with the menin-inhibitor revumenib, achieving remissions that were successfully consolidated with HCT. Of seven patients with *FLT3*-ITD mutations, five received FLT3-inhibitors combined with ven/aza, achieving four CR and one PR (ORR=71.4%). All five patients harboring *UBTF*-TD achieved cytofluorimetric or molecular CR (ORR=100%). In five cases of *NUP98*-mutated AML, three CR, one PR and one NR were achieved (ORR=80%). A cytofluorimetric CR was also reported in two cases of *DEK::NUP214* AML (ORR=100%). Of the two *CBFA2T3::GLIS2* AML, PCR-MRD negativity was achieved in a patient receiving ven/aza associated with a 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 appeared to have no effect, with comparable CR rates in patients with low and high disease burden (58.1% *vs*. 57.1%, respectively) (Figure 2C).

Outcome and hematopoietic cell transplantation

The median follow-up was 389 days (range, 36-1,405) from ven/aza initiation for the whole cohort. Thirty-three patients (66%) were bridged to myeloablative HCT, at a median of 97 days (range, 33-933) after starting ven/aza (details in *Online Supplementary Table S2*). Among patients bridged to transplantation, the median follow-up was 414 days (range, 39-1,311) 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 (N=3). The median blast percentage at the last BM evaluation before HCT was 0% (range, 0-13%) (details in the *Online Supplementary Material*). The estimated 2-year OS from the start of ven/aza therapy was 57.7% (95% CI: 49.8-65.6) for the whole study population (Figure 3). The 2-year EFS was 54.5% (95% CI: 46.7-62.3) for the whole cohort, being 75.8% (95% CI: 67.7-83.9) for transplanted patients, and 77.2% (95% 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% (95% CI: 78.4-99.4) and 75% (95% CI: 59.7-90.3), respectively (*Online Supplementary Figure S1A*).

A diagnosis of r/r-AML (hazard ratio [HR]=0.313; 95% CI: 0.105-0.935; *P*=0.037), not having received a previous HCT before ven/aza initiation (HR=2.900; 95% CI: 1.157-7.266; *P*=0.023), achieving CR after ven/aza (HR=3.708; 95% CI: 1.490-9.226; *P*=0.005), and being successfully bridged to HCT (HR=0.098; 95% CI: 0.035-0.272; *P*<0.0001) were significantly associated with better EFS in a Cox regression model in univariate analysis. Only achieving CR (HR=0.345; 95% CI: 0.130-0.916; *P*<0.033) and being bridged to HCT (HR=7.303; 95% CI: 2.139-24.935; *P*=0.002) were confirmed to be associated with better outcome in multivariate analysis, as shown in *Online Supplementary Table S1*. In the 23 patients who received a previous HCT before the start of ven/aza, 2-year EFS was 35.0% (95% 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 (*Online Supplementary Figure S1B*).

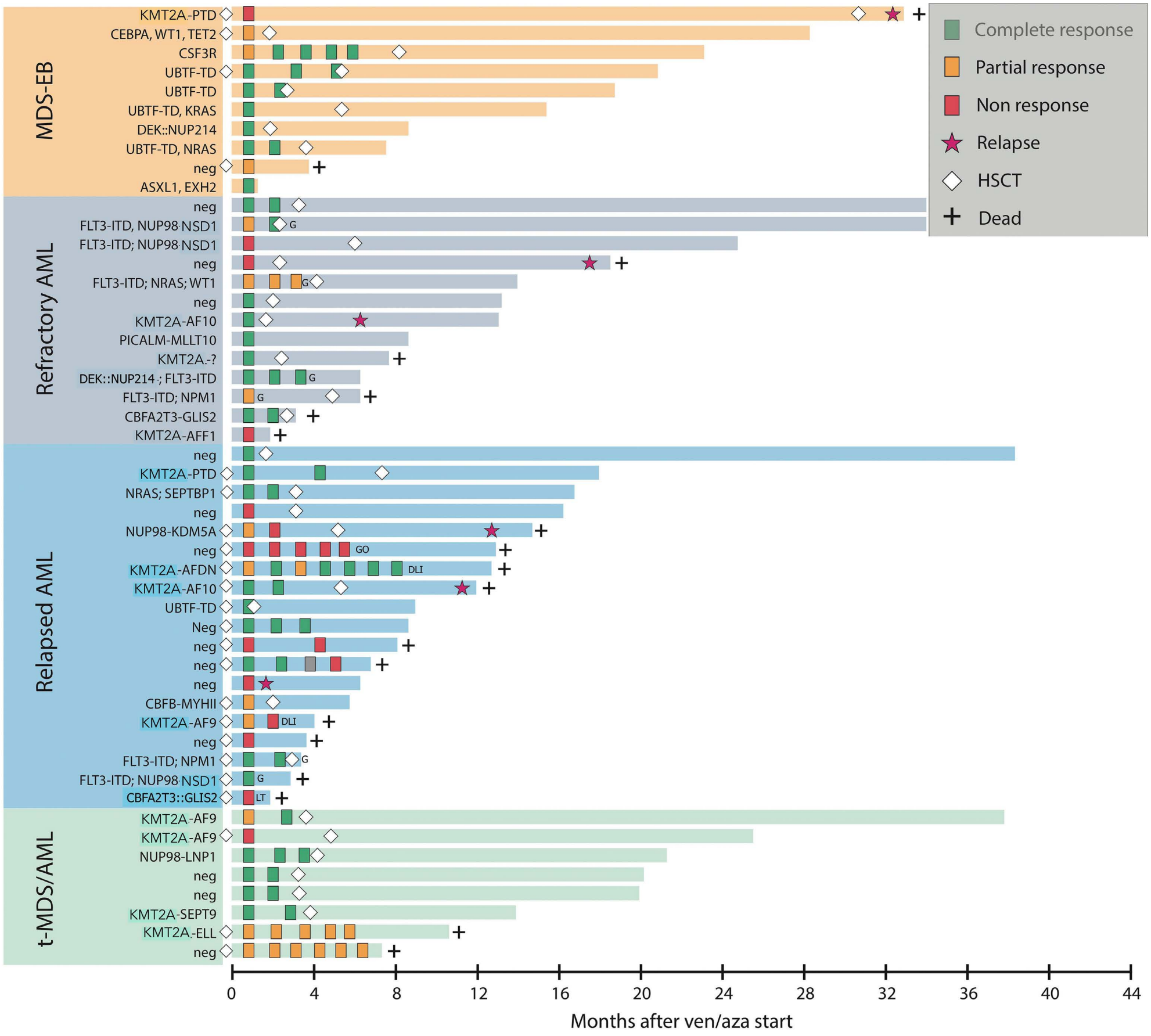

Figure 1. Swimmer plot of patients receiving venetoclax and azacytidine. Patients are divided into four groups according to their diagnosis. Dots represent the disease evaluation and response is specified in the legend. AML: acute myeloid leukemia; BM: bone marrow; HSCT: hematopoietic stem cell transplantation; ITD: internal tandem duplication; MDS-EB: myelodysplastic syndrome with excess of blasts; neg: negative; PTD: partial tandem duplication; TD: tandem duplication; t-MDS/AML, therapy-related myelodysplastic syndrome/acute myeloid leukemia; ven/aza: venetoclax and azacytidine.

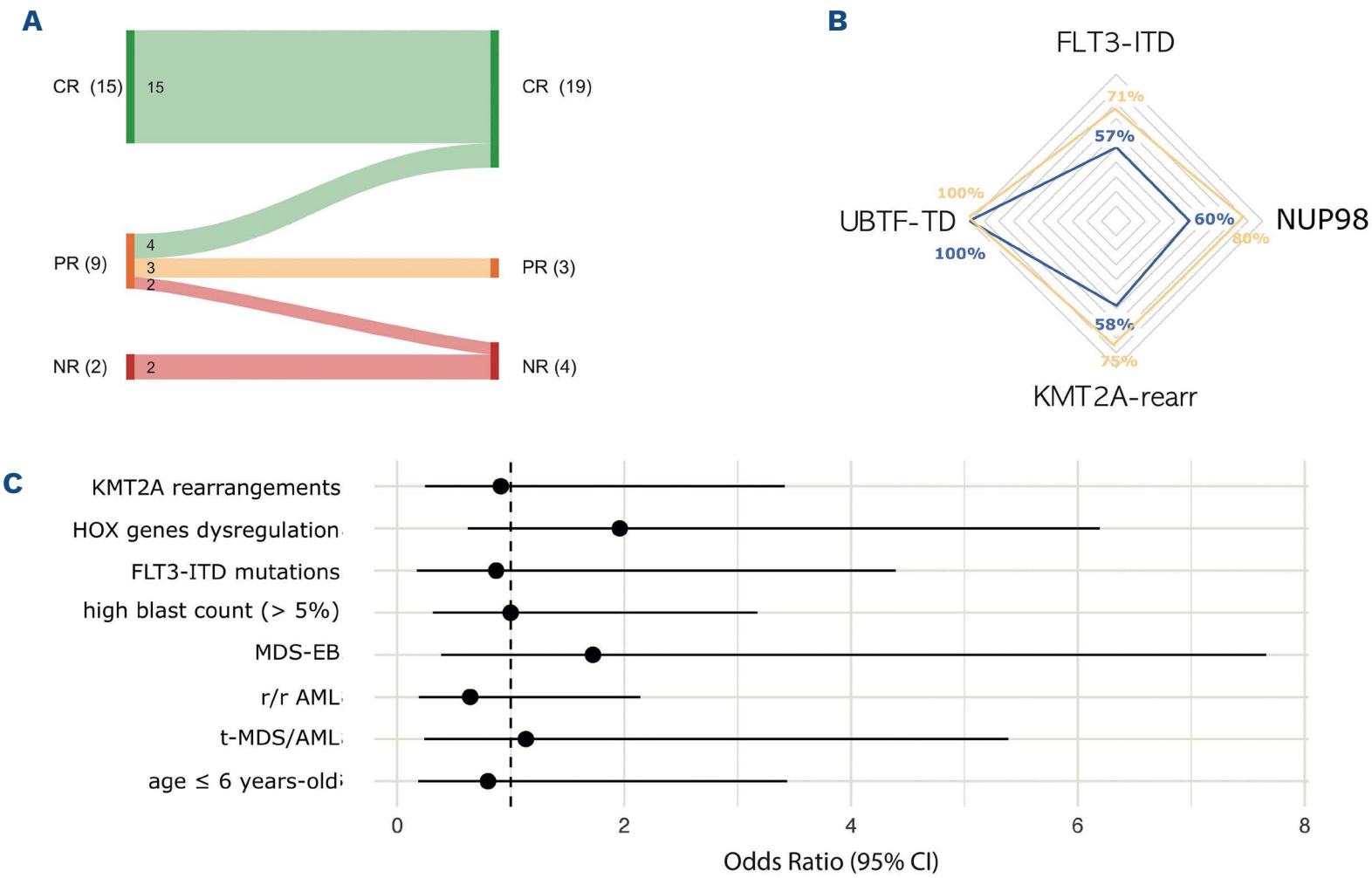

Figure 2. Summary of responses in patients receiving venetoclax and azacytidine. (A) Response across cycles in patients receiving ≥1 cycle of venetoclax and azacytidine. (B) Complete response (blue line) and overall response rate (orange line) in main genetic subgroups. (C) Forest plot summarizing factors potentially associated with a compete response to venetoclax and azacytidine. 95% CI: 95% confidence interval; CR: complete response; ITD: internal tandem duplications; MDS-EB: myelodysplastic syndrome with excess blasts; NR: no response; PR: partial response; rearr: rearrangements; r/r AML: relapsed/refractory acute myeloid leukemia; TD: tandem duplications; t-MDS/AML: therapy-related myelodysplastic syndrome/acute myeloid leukemia; ven/aza: venetoclax and azacytidine.

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 patients' outcome, especially in the 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 hematologic and infectious toxicities being the most frequent grade ≥3 adverse events, 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 have 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 transplant-related mortality associated with these second procedures was observed.

Notably, the observed heterogeneity in duration of venetoclax treatment 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 that it could be useful to explore therapeutic alternatives, such as CPX-351, which has been proven 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 that we observed in four patients. Considering the significantly better outcomes in patients bridged to HCT and that the best

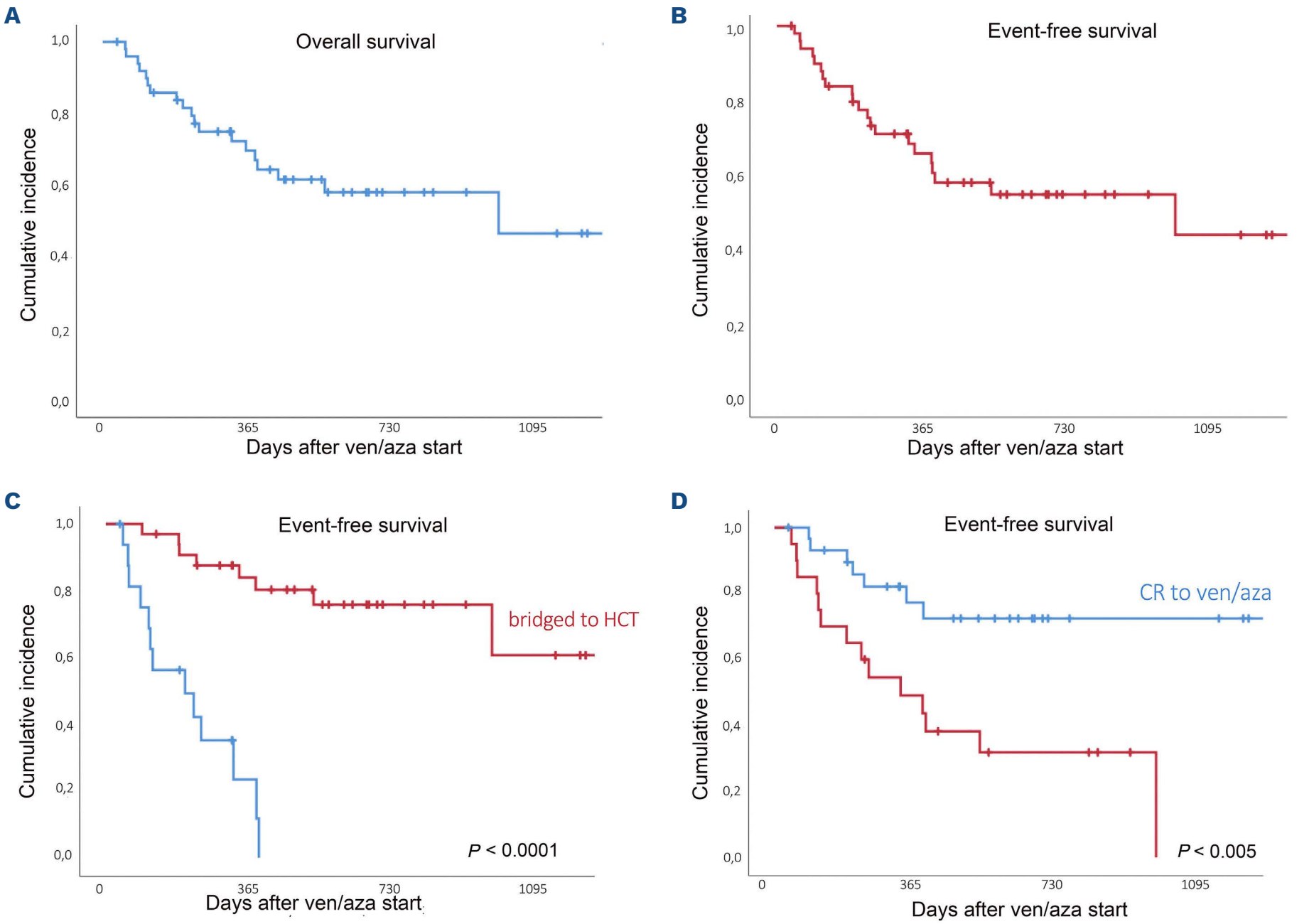

Figure 3. Kaplan-Meier estimates of survival in patients receiving venetoclax and azacytidine. (A) Overall survival of the entire cohort. (B) Event-free survival of the whole cohort, (C) Event-free survival in patients bridged or not to hematopoietic cell transplantation.. (D) Event-free survival in patients achieving or not a complete response to venetoclax and azacytidine. CR: complete response; HCT: hematopoietic cell transplantation; ven/aza: venetoclax and azacytidine.

response to ven/aza is generally achieved within one to two cycles, transplantation should be consistently pursued as a consolidation strategy, with an expedited search for a donor from treatment initiation and prompt progression to the 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 was found to affect outcome significantly 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 showed that patients who achieved MRD-negative status prior to HCT had a survival benefit.[39] A recent study by 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 with lower transplant-related mortality.[40] In our cohort, the majority of patients did not have detectable blasts at the time of HCT, possibly determining their more favorable outcomes compared to those of similar pediatric series reported in the literature.[25] Further prospective studies on the role of disease burden prior to 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 and *CS-*

F3R-mutated MDS-EB, suggesting the potential use of ven/aza also in other conditions (e.g., MDS in GATA-2 deficiency) which show 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, more than the disease burden, in determining the response. In this context, identifying recurrent genetic abnormalities that can help predict response to venetoclax is a key concern.[21] *KMT2A*-rearranged 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 respond to ven/aza achieved remission with revumenib, suggesting a possible benefit of combination strategies with menin inhibitors, which are being explored in adult AML.[41] Notably, the combination of ven/aza + revumenib is currently being tested in a phase I trial in pediatric AML.[42] *NUP98*-rearranged leukemias exhibited heterogeneous responses, consistent with the biological diversity of *NUP98* fusion partners which may influence sensitivity to venetoclax.[43] In our cohort, patients with *FLT3*-ITD-mutated AML who received ven/aza plus an 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 the need to define novel therapeutic approaches as a bridge to HCT. Initial promising results of menin inhibition in *UBTF*-TD AML came 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 another 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 those in studies including palliative ven/aza regimens.[25] It also delivers the first evidence 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, given 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 pharmacokinetic and pharmacodynamic 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 those with MDS-EB or 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.

Disclosures

No conflicts of interest to disclose.

Contributions

FB, RM, and FL designed the study. MP and BB performed the laboratory analyses. PM, MB, KG, VP, GP, DL, EM, FV, GB, MT, FS, ES, and EM collected the patients' data. DL and FB designed the figures and performed the analysis. FPT, RM, and FL critically reviewed the manuscript.

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Data-sharing statement

The complete dataset of this study is available at 10.5281/zenodo.17621498

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