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Clinical activity of venetoclax and azacitidine in children with de novo or secondary multiple relapsed/refractory acute myeloid leukemia: a real-world experience

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SHORT TITLE
Venetoclax-Azacitidine in Relapsed/Refractory Pediatric AML/MDS

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UI, AA, SD, BFG wrote the manuscript; AA, SD, UI, AB, MZ, BFG conceptualized and designed the study; LM, NAC, AR, SkT, SD, BFG provided patient data and discussion input; UI, AA analyzed and interpreted data; all authors approved the manuscript.

KEY WORDS:

Venetoclax, azacytidine, relapse, acute myeloid leukemia, pediatrics

DATA SHARING :

The authors agree to share the data

ABSTRACT
Acute myeloid leukemia (AML) remains a major therapeutic challenge, particularly in relapsed or refractory patients, where prognosis is poor. The combination of venetoclax and azacitidine has demonstrated significant efficacy in adults, yet evidence in pediatric populations is still limited and warrants further investigation. We conducted a multi-center retrospective analysis of 38 pediatric patients with relapsed/refractory AML, including patients with de novo and secondary AML, treated with compassionate use ven/aza in four European countries between 2017 and 2023. Patient characteristics, AML-associated genetic alterations, treatment details, clinical responses, and adverse events with ven/aza therapy were analyzed. Among 38 children with relapsed/refractory AML treated with ven/aza, the composite response rate (complete remission or complete remission with incomplete count recovery [CR/CRi]) was 26.3%. Median progression-free survival in responding patients was 22 months, and overall survival for the cohort was 6.1 months. Common ≥grade 3 toxicities included cytopenias (94%) and positive blood cultures (29%). This study demonstrates the real-world efficacy and tolerability of venetoclax and azacitidine in pediatric relapsed/refractory AML. The regimen enabled remission and prolonged survival in select cases, with manageable toxicity and improved outpatient care. Findings support the need for pediatric trials to clarify therapeutic potential and identify predictive biomarkers.

INTRODUCTION

During the past two decades, the overall survival (OS) of children with newly diagnosed acute myeloid leukemia (AML) treated with multi-agent cytarabine- and anthracycline-based chemotherapy regimens has improved to 70-80%, although outcomes vary depending upon specific leukemia-associated genetic characteristics and patients' clinical responses to induction chemotherapy[1-3]. This improvement in OS has largely not been due to the availability of new drugs, but attributed to enhanced utilization of available chemotherapies, refined risk stratification, improved supportive care, and increased allocation to and efficacy of allogeneic hematopoietic stem cell transplantation (HSCT)[4]. In the case of myelodysplastic syndromes (MDS), the progression to AML is an important source of mortality and allogeneic HSCT remains the only potentially curative treatment at this time. Despite substantial advancements in the understanding of MDS biology in recent years[5] patients with high-risk genetic subtypes continuing to experience high rates of chemoresistance and relapse, and have very poor long-term survival. New therapeutic approaches are needed to overcome this obstacle.

Relapse following initial treatment protocols remains a challenge, affecting 25-40% of children with AML. Survival after relapse is influenced by various factors, including time to relapse (<12 months [early] or ≥12 months from initial diagnosis [late]), site(s) of relapse, and somatic leukemia-associated genetic alterations. The reported overall survival (OS) for pediatric AML patients experiencing a first relapse is approximately 40-50%[6]. Outcomes for the approximately 20% of patients experiencing a second relapse or those refractory to second-line treatment are considerably worse, with a 5-year OS of <20%[7, 8]. In cases of early second relapse, almost no patients survive in the long term. Notably, the majority of children with multiply-relapsed/refractory AML historically are not enrolled in clinical trials due to a lack of access to novel targeted therapies and/or geographic restrictions that preclude available trial participation[9].

More effective and less toxic treatment regimens are needed to improve the survival of children with AML, particularly those with relapsed/refractory disease that may be successfully targeted with biologically relevant small-molecule inhibitors or antibody-based or cellular immunotherapies. Several academic institutions, pediatric oncology consortia, and industry-supported early-phase clinical trials with a pediatric-specific investigation of such therapies are being conducted. They are described in several excellent recent reviews[10-12].

Several precision medicine efforts are currently underway to investigate new possible targeted therapies in the relapse-refractory pediatric AML population, such as the revumenib phase I/II study (NCT04065399), which included adults and children with leukemia harboring a *KMT2A* rearrangement or *NPM1* mutation, demonstrated high remission rates and a predictable safety profile in relapsed/refractory *KMT2A*-rearranged acute leukemia[13]

The regimen of venetoclax with azacitidine (or decitabine) was initially investigated in early-phase clinical trials in adult patients with newly diagnosed adult AML who were unfit for intensive induction therapy. Azacitidine plus venetoclax improved CR rates (37% vs. 18%) and median OS (14.7 vs. 9.6 months), becoming the 2021 standard of care for adults unfit for intensive chemotherapy[14, 15]. A Phase II trial (NCT04801797) is comparing it to standard induction, and it is widely used as second-line therapy for relapsed/refractory AML, bridging patients to transplant[16].

Currently, 15 clinical trials registered on ClinicalTrials.gov are evaluating venetoclax and azacitidine in hematological malignancies, with most focusing on AML. These trials are largely adult studies that allow pediatric participation, with a few pediatric-specific studies. Many are early-phase trials, exploring combinations with chemotherapy, Menin inhibitors (Ziftomenib, Revuminib), and the CDK9 inhibitor SLS009 to improve outcomes in AML, T-ALL, and MDS. (Supplemental material Table 1). While these ongoing studies represent important progress, dedicated pediatric data remain limited [17-21] (Supplemental material Table 2).

Although the use of venetoclax in combination with hypomethylating agents (*eg*, azacitidine, decitabine) or low-dose cytarabine is now EMA- and FDA-approved for adult patients with newly diagnosed AML, access to venetoclax and other BCL-2 inhibitors for children remains extremely limited to date via early-phase clinical trials and/or off-label usage. Herein, we report real-world multi-institutional retrospective data regarding the therapeutic activity of venetoclax/azacitidine therapy in children and adolescents with de novo or secondary relapsed/refractory AML and propose that formal pediatric-specific investigation of this well-tolerated regimen is warranted.

METHODS

Retrospective analysis of medical records

We performed a retrospective chart review analysis of data from pediatric patients 0 to 19 years of age with de novo or secondary, relapsed or refractory AML treated with venetoclax and azacitidine at pediatric hospitals in France, the Netherlands, Spain, and Israel from January 2017 to December 2023. This study had institutional ethics approvals and was conducted in accordance with the MR004 (Reference methodology of the national data protection commission “Commission Nationale de l’informatique et des libertés” – compliance declaration n°CER-BDX 2023-73) and the Princess Máxima Center Biobank & Data Access Committee (PMCLAB2022.368). Relevant patient characteristics, leukemia-associated somatic cytogenetic and molecular data, venetoclax/azacitidine treatment details, adverse events, and clinical response data were obtained from medical records and anonymized for analysis. Longer-term follow-up data of surviving patients were collected until November 01, 2024.

Chemotherapy dosing

Commercial-supply azacitidine 75mg/m^2 was administered intravenously (IV) or subcutaneously (SC) once daily on days 1-5 or 1-7 of each 28-day cycle. Venetoclax (provided on a compassionate use basis by AbbVie) was administered orally (PO) in tablet or liquid suspension formulation once daily at a body surface area-adjusted adult equivalent dosing of 400 mg/day with dosing ranging from 300-600mg depending on the body surface area. A 1 to 3-day ‘ramp-up’ dose escalation of venetoclax during cycle 1 was used to minimize tumor lysis syndrome (TLS) risk, while subsequent cycles used full dosing for 14-28 days without ramp-up. Following Venetoclax treatment, cycle lengths were adjusted based on prior hematological toxicity (7-28 days). Venetoclax dose reduction of 50-75% was used for patients receiving azole-class antifungal prophylaxis due to known CYP3A inhibition potential[22, 23].

Response assessment

Treatment response to the venetoclax/azacitidine regimen was assessed using bone marrow aspirate morphology and flow cytometry analysis performed between days 28 and 42 of cycle one. Additional disease assessments were not done regularly due to the palliative treatment pathway of some of the patients in our cohort. Complete morphological remission (CR) was defined according to the Cheson criteria[24], as less than 5% bone marrow blasts with hematologic count recovery (absolute neutrophil count (ANC) >1000 cells/µL and platelet count >100,000/µL), CR with incomplete count recovery (CRi) as less than 5% bone marrow blasts with incomplete recovery of at least one cell lineage, and partial response (PR) as persistence of more than 5% blasts in the bone marrow with a reduction of more than 50% compared to the initial blast count at treatment initiation. Measurable residual disease (MRD) was assessed by multi-parameter flow cytometry or genetic alteration/mutation-specific molecular biology assays as described[25]. A line of therapy was defined as a distinct treatment regimen given with curative intent, initiated following non-response or relapse. MRD negativity was defined as an MRD using a flow of <0.1%. Refractory disease was defined as failure to achieve remission following first-line induction therapy for AML, consisting of up to two distinct induction cycles. In cases where post-treatment bone marrow evaluation was not performed (*eg*, due to palliative therapeutic intent), disease progression was inferred from an increase in the absolute number of blasts in the peripheral blood.

Adverse events and therapy-associated toxicity assessment

Adverse events, including their etiology and severity, were documented in accordance with the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. We specifically focused on hematologic, gastrointestinal, and infectious complications, as well as general treatment-related toxicities.

Statistical analysis

Event-free survival (EFS) and overall survival (OS) data were calculated from available patient data in this study using XLSTAT. EFS was defined as the time between the first day of venetoclax/azacitidine reinduction therapy and the occurrence of an event, including death from any cause, relapse, refractory disease, or until the last known date of follow-up. OS was defined as the time between therapy initiation and death from any cause or until the last date of follow-up. The Kaplan-Meier method was utilized for survival estimates, and univariate analyses were performed by logistic regression. The odds ratio (OR) is given with a confidence interval (CI) of 95%. P-values < 0.05 were considered statistically significant. Data were displayed graphically in Excel.

RESULTS

Patient characteristics and therapy details

Thirty-eight pediatric patients with relapsed/refractory AML were treated with venetoclax/azacitidine at our institutions from January 2017 to December 2023. Of these, 31 (81.6%) had a history of *de novo* AML, while seven (18.4%) had secondary AML. Among secondary AML cases, five arose from MDS progression (MDS/AML), one following an allogeneic transplant for JMML (JMML/AML), and one after treatment for acute lymphoblastic leukemia (t-AML). Detailed characteristics of these 38 patients are provided in Table 1. The median ages at original AML diagnosis and venetoclax/azacitidine treatment initiation were 9.2 years (range, 0.01–18.5 years) and 11.9 years (range, 0.11–19.12), respectively. The sex ratio was 1.4 male: female.

The median time from initial diagnosis to initiation of venetoclax/azacitidine was 1.5 years (range, 0.1–5.3) [IQR: 0.1–2.11]. Most patients (n=31, 81.6%) had received two prior multi-chemotherapy agent lines of therapy (median, 2; range, 0–7; IQR, 1–3). The majority (89%) were treated with venetoclax/azacitidine following a relapse of AML: 17 in the first, 14 in the second, and three in the third relapse. Among these, 22 patients (58%) had previously undergone at least one allogeneic transplant in complete first remission (CR1) or CR2. Two patients (5.3%) received venetoclax/azacitidine for primary refractory disease after one line of chemotherapy, while two others received it as first-line therapy due to poor prognostic leukemia-associated genetic factors. The first patient (Patient 34, Table 2) presented with MDS/AML and high-risk *WT1* and *UBTF*-TD mutations, thus carrying a significant relapse risk after HSCT. This patient achieved complete remission after a single cycle of venetoclax/azacitidine and subsequently underwent allogeneic stem cell transplantation, remaining alive and in remission at 17 months of follow-up (Figure 1). The second patient (Patient 37, Table 2) was 18 years of age at diagnosis, with *GATA2*-mutant AML in the setting of constitutional 3q deletion (technically germline mutation), severe developmental delay, and was initially treated with one cycle of venetoclax/azacitidine due to medical comorbidities but did not respond (Figure 1). She was subsequently treated with CPX-351 without remission induction and then transitioned to palliative care due to disease progression.

Patients received a median of two consecutive venetoclax/azacitidine cycles (range, 1-24) [IQR: 1 ; 3] and were followed for a median of 6.6 months (range, 0.2 – 45.6) [IQR: 2.8;16.3] (Table 3). For the 28 patients with available data, the median duration of inpatient hospitalization was 33 days (range, 4-175) [IQR 23; 63], accounting for 51% of the total duration of treatment.

Overall response

Of the 38 children and adolescents in our cohort, 31 underwent bone marrow assessment to evaluate treatment response, while post-treatment bone marrow aspirate was not performed for four patients due to their palliative status, and 3 did not complete the Ven/AZA first cycle due to disease progression or a severe infection. Eight patients achieved CR, and two had CRi, leading to a composite response rate (CRc) of 26.3% within the entire cohort (10/38). Bone marrow MRD was measured for seven of the 10 patients with CR/CRi, and four achieved MRD negativity (<0.1% by flow cytometry). Among responders, the median number of cycles to achieve CRc was 1.5 (range, 1-3) [IQR: 1; 2].

Among the fourteen patients with myelomonocytic AML (FAB M4/M5), nine (64%) showed no response, three achieved CR or CRi, and two were not evaluable for response. (Table 1).

Long-term follow-up

The median OS across the whole cohort was 6.1 months following the start of venetoclax/azacitidine (95% CI: 3.7–10.1). The median progression-free survival in the 10 children and adolescents who achieved CR/CRi with venetoclax/azacitidine therapy was 20 months (95% CI: 7.63, 39.5). Importantly, five out of the 10 patients proceeded to subsequent allogeneic HSCT (including one patient who achieved MRD negativity before the beginning of conditioning regimen) : one as part of first-line therapy with venetoclax and azacitidine, two with primary refractory disease, and two following relapses after the first transplant (Figure 1). At the most recent follow-up, six patients out of the 10 were alive and in continued clinical remission with a median follow-up duration of 28 months after venetoclax/azacitidine (range, 17–46). (Supplemental material Figure 1)

Of interest, three patients continued venetoclax/azacitidine therapy in the long term, receiving 15, 19, and 24 cycles, respectively (Figure 1). One patient (Patient 33, Table 2) in venetoclax/azacitidine-induced CR was initially treated with palliative intent for a third AML relapse after two prior HSCTs. A second patient (Patient 2, Table 2), also with venetoclax/azacitidine-induced CR, was treated at second relapse after the first HSCT. A third patient (Patient 25, Table 2) with second chemo-refractory AML relapse was also treated with venetoclax/azacitidine for life prolongation; despite a lack of CR achievement, this regimen facilitated disease stabilization and good quality of life.

Safety considerations

Venetoclax was administered for a median duration of 27 days per cycle (IQR: 18–28) amongst the 38 patients. Therapy interruption or temporary treatment discontinuation occurred for nine patients due to hematologic and/or infectious toxicities attributed to venetoclax/azacitidine. Importantly, no clinically significant tumor lysis occurred in treated patients[26]. Observed therapy sequelae in the study cohort included gastrointestinal events (19/38 patients, 50%) or general cytopenias (disease or treatment-related, 36/38 patients, 94.7%) (Table 4). Gastrointestinal side effects, primarily nausea, vomiting, and diarrhea, were generally mild to moderate in severity and were resolved with supportive care (grade 1 or 2 in 78% of cases). Febrile neutropenia occurred in 56% of patients, with 11 patients experiencing culture-documented bacterial infections with gram-negative (n=5), gram-positive (n=4) or multi-germ infection (n=2) bacteremia, one of which was fatal. In addition, 3 patients presented with positive fungal infections. Most patients (27/38, 71%) received routine antifungal prophylaxis, usually with liposomal amphotericin B or azoles, during venetoclax/azacitidine therapy due to leukemia-associated severe neutropenia. Despite prophylaxis, fungal infections were documented in four patients.

DISCUSSION

In this study, we report real-world pediatric data regarding the clinical activity and tolerability of venetoclax/azacitidine therapy. We note a 26.3% overall response rate in 38 children and adolescents with relapsed/refractory AML *de novo* or MDS/AML, the majority of whom were multiply relapsed (often after prior HSCT) and/or had high-risk leukemia-associated genetic features. Our observations in this multi-site European case series align well with similar reports of commercial (off-label) venetoclax/azacitidine therapy from pediatric institutions in the United States. In the study by Karol et al. (NCT03236857), 24 of 35 pediatric patients with relapsed/refractory AML received venetoclax plus a hypomethylating agent, yielding an ORR of 21% with no MRD-negative responses. Among 19 patients treated with venetoclax and azacitidine, the ORR was 16%(3/19)[27], lower than in our cohort. A study by Winters and colleagues at the University of Colorado reported morphologic remission induction in six of eight children with relapsed/refractory AML or high-risk MDS, with MRD negativity achieved in the four patients with AML and venetoclax/azacitidine-induced CR[20]. LeBlanc *et al* from Cincinnati Children's hospital reported their experience combining venetoclax with demethylating agents (azacitidine and decitabine) in 27 patients, including 9 newly diagnosed patients. In their cohort the overall remission rate was 67% with a high rate of MRD negativity and subsequent HSCT[21]. Niswander *et al* also recently reported their clinical experience at the Children's Hospital of Philadelphia, treating 37 pediatric patients with multiply relapsed/refractory AML, ALL, or mixed phenotype acute leukemia (MPAL) with venetoclax/azacitidine without or with the anti-CD33 antibody-drug conjugate gemtuzumab ozogamicin (GO). Fourteen patients

(37.8%) achieved MRD-negative complete remission, usually after one cycle of venetoclax/azacitidine, including 12 patients with AML, one with B-ALL, and one with MPAL[19].

Our observed remission rate in pediatric patients with relapsed/refractory de novo or secondary AML is somewhat lower than that of the Niswander study, which also included children with multiple-relapsed/refractory B-ALL or MPAL and used GO for some of the patients. However, GO addition to venetoclax/azacitidine, did not improve response rates in this population compared to venetoclax/azacitidine alone[19]. Our results also differed from those of the Winters, LeBlanc, and Karol's studies, which included patients treated in first or second-line settings, whereas 82% of our patients received venetoclax/azacitidine as third-line therapy or beyond. However, all those studies underscore the importance of consolidating venetoclax/azacitidine-induced remissions with allogeneic HSCT when clinically appropriate[28] and/or the long-term disease stabilization and life prolongation potential in other patients.

Increased efficacy of early treatment with venetoclax/azacitidine has been reported in numerous clinical trials or case series in adults with relapsed/refractory AML[29, 30]. These findings highlight the potential influence of the number of prior therapeutic lines upon the potential efficacy of venetoclax/azacitidine therapy and/or biology of some multiply relapsed/chemorefractory AML subtypes. This observation is in line with the VIALE-A trial for adult patients with *de novo* AML who were unfit to receive chemotherapy[14]. In our study, we also observed a trend towards better response in patients who received venetoclax/azacitidine as first- or second-line therapy compared to later use, although our small patient numbers precluded definitive conclusions.

Although clear biomarkers of venetoclax/azacitidine treatment response versus failure have yet to be identified, emerging data from recent adult AML studies have reported preferential treatment responses in specific genetic subtypes[31]. While the inherent heterogeneity of our real-world cohort and relatively small sample size limited our ability to identify which patients might benefit most from this regimen, we did observe long-term remission in one patient with *IDH2*-mutant AML, consistent with improved response rates and sustained remission in adult patients treated with venetoclax/azacitidine[14, 29, 30, 32]. Conversely, three of our non-responding pediatric patients had AML with *EZH2*, *FLT3*-ITD, *KRAS* or *NRAS*, or *TP53* mutations, all of which were associated with inferior response rates and overall survival with venetoclax/azacitidine in other studies[33, 34]. Ongoing adult and pediatric trials will continue to identify patients most likely to benefit from venetoclax-based therapies and to define the ideal duration of venetoclax exposure per treatment cycle[35]. One is a phase I trial evaluating venetoclax combined with cytotoxic chemotherapy, including azacitidine, in high-risk hematologic malignancies such as MDS/AML (NCT05292664). TP53 mutations are associated with poor responses to venetoclax and azacitidine in adult AML, although they are rare in pediatric AML, limiting the relevance of adult risk classifications. Similarly, myelomonocytic AML subtypes have shown increased resistance to venetoclax-based therapy[36], reflected in our cohort with a 64% non-response rate.

Finally, we also report excellent clinical tolerability of venetoclax/azacitidine therapy in a pediatric population, mainly with multiply relapsed AML. Adverse events seen in our study align with those reported in the pediatric Winters case series and the adult VIALE-A trial. These toxicities were manageable and allowed therapy to be administered in an outpatient setting, which is in accordance with the pediatric Niswander study and is important to consider in palliative care-focused situations. Surprisingly, a few patients within our cohort treated initially with palliative intent achieved CR or long-term stable disease and received venetoclax/azacitidine therapy for more than one year. However, due to the retrospective design of our study, the accuracy and reliability of collected data may have been affected by incomplete clinical documentation or variability in data quality.

Although some long-term responders have been identified, predicting response to the combination remains challenging. ELN criteria for adults do not easily apply to children, and without pediatric-specific biomarkers, their use in earlier treatment lines is limited, given the higher response rates to intensive chemotherapy.

In conclusion, this retrospective multi-center study demonstrates the feasibility, tolerability, and clinical activity of the venetoclax and azacitidine combination in a large cohort of pediatric patients with multiply relapsed or refractory AML, also including patients with secondary AML. The observed response rates, while lower than those reported in select series, likely reflect real-world clinical practice across heterogeneous settings and heavily pretreated populations. Our observations validate results from recent similar pediatric case series and further emphasize the need for pediatric-specific clinical trials, ideally earlier than in third-line therapy. Ongoing early-phase trials evaluating the safety and efficacy of venetoclax/azacitidine in combination with FLT3, IDH1, IDH2, or menin inhibitors in adults with biologically relevant high-risk subtypes of MDS and AML will also surely address existing knowledge gaps in these domains and provide additional rationale for desired clinical investigation, also in children and adolescents.

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Table 1: Summary characteristics of study cohort patients.

Baseline characteristics	Number of patients (N=38)
Sex, N (%)	
Male	22(58%)
Female	16 (42%)
Age at diagnosis, years; median (range)	8.7 (0.01 – 18.5)
Type of AML N (%)	
De novo	31(82%)
Secondary	7 (18%)
FAB classification N (%)	
AML 4/5	14 (37%)
Others	19 (50%)
Not available	5 (13%)
Mutations, N (%)	
IDH1	1 (3%)
IDH2	1 (3%)
FLT3-ITD	4 (11%)
NRAS	3 (8%)
KRAS	2 (5%)
EZH2	2 (5%)
TP53	1 (3%)
Leukemia status at the time of ven/aza, N (%)	
First line	2 (5%)
Primary chemo-refractory	2 (5%)
Relapsed	34 (34%)
Number of treatment lines before ven/aza, N (%)	
0 or 1 lines	7 (18%)
2 lines or more	31 (82%)
AlloSCT before ven/aza N, (%)	22 (58%)

AML - acute myeloid leukemia, ven/aza - venetoclax and azacytidine, AlloSCT - allogeneic stem cell transplant

Table 2: Patient-level data for the study cohort.

Patient	Type of disease	Disease stage (R-Relapse number)	Previous SCTs	Cytogenetic alterations	Response	MRD négative if responders
1	AML	*R1*	*No*	Normal c\karyotype	CR	Yes
2	MDS/AML	*R2*	*Yes*	*NUP98* rearrangement	CR	No
3	AML	R1	*Yes*	*FT3* ITD and *WT1* mutations	No response	NA
4	AML	*R2*	*No*	*KMT2A::MLLT10* fusion	Not assessed	NA
5	AML	*R2*	*Yes*	*NUP98::NSD1* fusion, *FLT3* ITD mutation	Not assessed	NA
6	AML	*R2*	*Yes*	*KMT2A::MLLT10* fusion	No response	NA
7	AML	*R1*	*Yes*	Complex karyotype, *WT1* and *NRAS*	Partial response	NA
8	AML	*R1*	*Yes*	*KMT2A::MLLT10* fusion	Cri	No available
9	AML	*R1*	*Yes*	*IDH2* mutation	CR	Yes
10	*AML*	*R1*	*No*	*NUP98* rearrangement	No response	NA
11	AML	*R1*	*Yes*	*CBFA2T3::GLIS2* fusion	Partial response	NA
12	AML	*R2*	*Yes*	*WT1, RUNX1* and *IDH1* mutations	No response	NA
13	AML	*R3*	*Yes*	Other alteration	Partial response	NA
14	t-AML	*R1*	*Yes*	Monosomy 7	No response	NA
15	AML	*R1*	*Yes*	*FLT3* TKD, *RUNX1* and *BCOR* mutations	Not assessed	NA
16	MDS/AML	*R1*	*No*	*FLT3* ITD *and WT1* mutations	No response	NA
17	AML	R1	*No*	*NPM1* and *DNMT3A* mutations	CRi	No
18	AML	*R2*	*Yes*	*KMT2A::MLLT10* fusion	Not assessed	NA
19	AML	*R2*	*Yes*	*KMT2A::MLLT10* fusion	No response	NA
20	AML	*R1*	*No*	Monosomy 7, *FLT3*-ITD, *RUNX1, EZH2* and *BCOR* muta	No response	NA
21	JMML/AML	*R1*	*Yes*	Complex karyotype, *NRAS* and *BCOR* mutations	No response	NA
22	AML	*R1*	*Yes*	*FTL3* ITD	No response	NA
23	AML	R1	*No*	*KMT2A::MLLT9* fusion	CR	No
24	AML	*R1*	*Yes*	Monosomy 7	Partial response	NA
25	AML	*R2*	*No*	*CBFB::MYH11* fusion	No response	NA
26	*AML*	*R2*	*No*	*NRAS* and *KRAS* mutations	No response	NA
27	AML	*R3*	*No*	*NRAS* and *KRAS* mutations	No response	NA
28	MDS/AML	*R1*	*Yes*	Monosomy 7	No response	NA
29	AML	*R2*	*Yes*	*KMT2A::MLLT6* fusion	Partial response	NA
30	AML	*R2*	*Yes*	*CBFA2T3::GLIS2* fusion	CR	Yes
31	AML	*R2*	*Yes*	7q deletion, *RUNX1* mutation	Not assessed	NA
32	AML	*R2*	*No*	Complex karyotype, *KRAS* mutation	No response	NA
33	AML	R3	*Yes*	Other alterations	CR	Yes
34	MDS/AML	*1st line*	*No*	*WT1, UBTF-TD* mutation	CR	No available
35	AML	*Refractory*	*No*	12p deletion, *STAG2* mutation	CR	No available
36	MDS/AML	*Refractory*	*No*	Monosomy 7, *RUNX1* mutation	Partial response	No available
37	AML	*1st line*	*No*	Monosomy 7,*GATA2* mutation	Partial response	NA
38	AML	*R2*	*No*	*KMT2A::MLLT10* fusion, *TP53* and *EZH2* mutations	No response	NA

AML - acute myeloid leukemia; ven/aza - venetoclax and azacytidine; AlloSCT - allogeneic stem cell transplant; CR - complete response; CRi - complete response with incomplete count recovery; JMML/AML - acute myeloid leukemia derived from juvenile myelomonocytic leukaemia; MDS/AML - acute myeloid leukemia derived from myelodysplastic syndrome; MRD - minimal residual disease, negative if <0,1%; PR - partial response (CR not achieved, but > 50% reduction in bone marrow blastosis compared with initial blastosis before starting treatment); t-AML – therapy related acute myeloid leukemia.

Table 3: Summary of Clinical Course and Outcomes Under Ven/Aza Therapy

Number of cycles, median (range) [IQR]	2 (1-24) [1 ; 3]
Response to treatment N, (%)	
CR	8 (21%)
CRi	2 (8%)
PR	7 (16%)
No response	16 (42%)
Not assessed	5 (13%)
MRD if CR/Cri, N (%)	N = 10
Negative	4 (40%)
Positive	3 (30%)
Not available	3 (30%)
Reason for Ven/Aza discontinuation, N (%)	
Refractory	18 (47%)
Secondary disease progression	9 (24%)
Toxicity	3 (8%)
CR	1 (3%)
AlloSCT	5 (13%)
Other	2 (5%)
Duration of follow up in months, median (range) [IQR]	5.8 (range, 0.2 – 34.5) [IQR: 2.8;10.6]
Status at last follow up, N (%)	
Alive in CR	6 (16%)
Dead	32 (84%)

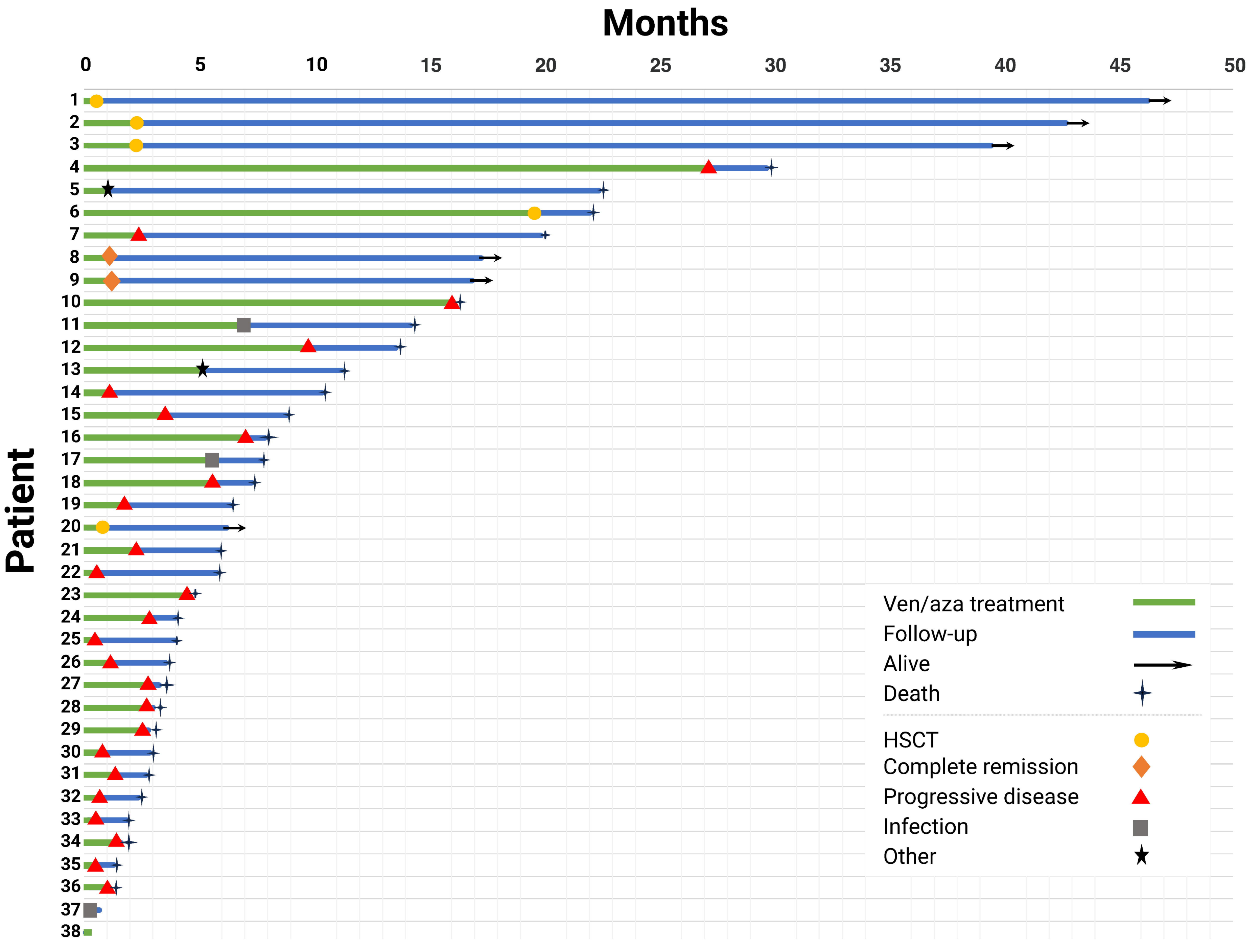
CR - complete response; CRi - complete response with incomplete count recovery,
PR - partial response; MRD - minimal residual disease, negative if < 0.1%; SCT - stem cell transplantation

Table 4: Adverse events observed in study cohort patients.

Adverse event	Any grade/N patients with available data (%)	Grade ≥3 (n)
Neutropenia	33/37 (89%)	32/37 (86%)
Anemia	34/36 (94%)	26/36 (72%)
Thrombocytopenia	34/37 (92%)	31/37 (84%)
Febrile neutropenia	20/36 (56%)	20/36 (56%)
Digestive toxicity	18/36 (50%)	4/36 (11%)
Fatigue	15/32 (47%)	2/32 (6%)
Tumor lysis syndrome	2/37 (5%)	0/37 (0%)
Bacteremia	11/38 (29%)	1/38 (29%)
Gram-negative bacillus	5/11	
Gram-positive cocci	4/11	
Multi germ infection	2/11	
Fungal infection	3/38 (8%)	0/38 (0%)

CTCAE - Common Terminology Criteria for Adverse Events v5.0

Figure 1: Swimmer plot depicting the clinical course of study patients.
Legend: HSCT - hematopoietic stem cell transplantation; Other – Parent's decision, data not available



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Ven/aza treatment
Follow-up
Alive
Death
HSCT
Complete remission
Progressive disease
Infection
Other

Clinical activity of venetoclax and azacitidine in children with de novo or secondary multiple relapsed/refractory acute myeloid leukemia: a real-world experience

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SUPPLEMENTARY DATA

Supplementary Table 1. Active and recently completed clinical trials of venetoclax and azacitidine-based therapies, including pediatric patients with acute leukemias.

Num	NCT Number	Study status	Phase	Disease type	Age	Venetoclax regimen and dose	Azacitidine regimen and dose	Funded By
1	NCT03194932	Completed	I	Acute Myeloid Leukemia	2 Years to 20 Years	for 21 days	for 7 days	academic
2	NCT03941964	Completed	III	Acute Myeloid Leukemia	12 Years and older	OD buiding up in 3 days for 28 days - 400mg	75mg/m2 OD for 7 days	Industry
3	NCT04161885	Recruiting	III	Acute Myeloid Leukemia	12 Years and older	OD for 28 days	OD for 5 days	Industry
4	NCT04588922	Recruiting	I/II	all Hematologic Malignancies	12 Years and older	NA	NA	Industry
5	NCT04904237	Recruiting	II	Acute Myeloid Leukemia	16 Years to 65 Years	NA	NA	academic
6	NCT05659992	Recruiting	I	Acute Myeloid Leukemia	14 Years to 75 Years	OD buiding up in 3 days for 14 days - 400mg	75mg/m2 OD for 7 days	academic
7	NCT05823714	Recruiting	II	Acute Myeloid Leukemia/MDS	Age 8 to 65 years	200mg/day for 7days	75mg/ m^2/day for 7days.	academic
8	NCT05955261	Recruiting	II	Acute Myeloid Leukemia	29 Days to 21 Years	NA	NA	Academia|Industry
9	NCT06068621	Recruiting	II	Acute Myeloid Leukemia	14 Years to 75 Years	400 mg/day, days 1 to 14	75 mg/m2/day, days 1 to 7	academic
10	NCT06177067	Recruiting	I	Acute Myeloid Leukemia	1 Year to 30 Years	NA	NA	Academia|Industry
11	NCT04161885	Recruiting	III	Acute Myeloid Leukemia	12 Years and older	OD for 28 days	OD for 5 days	Industry
12	NCT05292664	Not yet recruiting	I	Relapsed/Refractory Acute Leukemia	2 Years to 21 Years	NA	NA	Academia|Industry
13	NCT05317403	Not yet recruiting	I	Acute Myeloid Leukemia	1 Year to 25 Years	OD buiding up in 3 days for 14 days - 400mg	75mg/m2 OD for 5 days	academic
14	NCT06397027	Not yet recruiting	I	Relapsed/Refractory Acute Leukemia	2 Years to 21 Years	NA	NA	Academia|Industry

Supplementary Table 2: Other studies reporting venetoclax-based therapies in children with relapsed/refractory AML or MDS.

Publication	Total Patients Treated with Ven/Aza	Response to Treatment (% out of total)	Number of Patients Continuing to HSCT (% out of CR/CRi)
Trabal et al. 30(Cancers 2023)	17	**CR:** 7 (41%) **CRi:** 1 (6%)	4 patients (44%)
Winters et al. 21(Pediatric Blood & Cancer, 2020)	8	**CR/CRi:** 6 patients (66%)	4 patients (66%)
Niswander et al. 22(Hematologica, 2023)	19	**CR with MRD negativity:** 9 patients (47%)	7 patients (78%) 1 continue to HSCT with MRD +
Masetti et al.31 (Blood Advances, 2023)	22 (VEN/DEC)	**CR:** 7 patients (36.8%)	4 patients (57%) 1 continue to HSCT with PR
Total	**65**	**CR/CRi: 31 patients (46%)**	**19 patients (61%)**

CR - complete remission, CRi - complete remission with incomplete blood count recovery, HSCT - hematopoietic stem cell transplant, NA - not available.

Supplementary Figure 1: Overall survival for the entire cohort (n=38) since Day 1 of Ven/Aza