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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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 (ven/aza) 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 (R/R) 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 R/R AML treated with ven/aza, the composite response rate (complete remission or complete remission with incomplete count recovery) 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 ven/aza in pediatric R/R 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 continue 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 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 (R/R) 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 R/R 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 R/R pediatric AML population, such as the revumenib phase I/II study (*clinicaltrials gov. Identifier: 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 R/R *KMT2A*-rearranged acute leukemia.[13]

The regimen of venetoclax (ven) with azacitidine (aza) (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. Aza/ven improved complete remission (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 (*clinicaltrials gov. Identifier: NCT04801797*) is comparing it to standard induction, and it is widely used as second-line therapy for R/R AML, bridging patients to transplant.[16]

Currently, 15 clinical trials registered on *clinicaltrials gov.* are evaluating ven/aza 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 (*Online Supplementary Table S1*). While these ongoing studies represent important progress, dedicated pediatric data remain limited[17-21] (*Online Supplementary Table S2*).

Although the use of ven in combination with hypomethylating agents (e.g., aza, decitabine) or low-dose cytarabine is now European Medicines Agency- and Food and Drug Adminstration-approved for adult patients with newly diagnosed AML, access to ven 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 ven/aza therapy in children and adolescents with *de novo* or secondary R/R 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, R/R AML treated with ven/aza 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 number 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, ven/aza 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 aza 75 mg/m^2 was administered intravenously (IV) or subcutaneously once daily on days 1-5 or 1-7 of each 28-day cycle. Ven (provided on a compassionate use basis by AbbVie) was administered orally 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 300-600 mg depending on the body surface area. A 1 to 3-day 'ramp-up' dose escalation of ven 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 ven treatment, cycle lengths were adjusted based on prior hematological toxicity (7-28 days). Ven 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 ven/aza 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 >1x10^9/L and platelet count >100x10^9/L), 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 (e.g., 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 OS data were calculated from available patient data in this study using XLSTAT. EFS was defined as the time between the first day of ven/aza re-induction 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 R/R AML were treated with ven/aza 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 ven/aza 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 ven/aza was 1.5 years (range, 0.1-5.3; interquartile range [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 ven/aza 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 ven/aza 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*-tandem dublication (TD) muta-

Table 1. Summary characteristics of study cohort patients.

Baseline characteristics	N 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)
N of treatment lines before ven/aza, N (%)	
0 or 1 lines	7 (18)
2 lines or more	31 (82)
AlloHSCT before ven/aza, N (%)	22 (58)

AML: acute myeloid leukemia, ven: venetoclax; aza: azacitidine; alloHSCT: allogeneic hematopoietic stem cell transplant.

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

Patient	Type of disease	Disease stage	Previous alloSCT	Cytogenetic alterations	Response	MRD-negative 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*	PR	NA
8	AML	R1	Yes	*KMT2A*::*MLLT10* fusion	CRi	NA
9	AML	R1	Yes	*IDH2* mutation	CR	Yes
10	AML	R1	No	*NUP98* rearrangement	No response	NA
11	AML	R1	Yes	*CBFA2T3*::*GLIS2* fusion	PR	NA
12	AML	R2	Yes	*WT1, RUNX1* and *IDH1* mutations	No response	NA
13	AML	R3	Yes	Other alteration	PR	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* mutations	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	PR	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	PR	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	NA
35	AML	Refractory	No	12p deletion, *STAG2* mutation	CR	NA
36	MDS/AML	Refractory	No	Monosomy 7, *RUNX1* mutation	PR	NA
37	AML	1st line	No	Monosomy 7,*GATA2* mutation	PR	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; ITD: internal tandem dublication; JMML/AML: acute myeloid leukemia derived from juvenile myelomonocytic leukemia; MDS/AML: acute myeloid leukemia derived from myelodysplastic syndrome; MRD: minimal residual disease, negative if <0,1%; NA: not available; PR: partial response (CR not achieved, but >50% reduction in bone marrow blastosis compared with initial blastosis before starting treatment); R: relapse number; t-AML: therapy-related acute myeloid leukemia.

tions, thus carrying a significant relapse risk after HSCT. This patient achieved CR after a single cycle of ven/aza and subsequently underwent allogeneic HSCT, 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 (germline abnormality), severe developmental delay, and was initially treated with one cycle of ven/aza 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 ven/aza 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 threee 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 14 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 PFS in the ten children and adolescents who achieved CR/CRi with ven/aza therapy was 20 months (95% CI: 7.63-39.5). Importantly, five of the ten patients proceeded to subsequent allogeneic HSCT (including 1 patient who achieved MRD negativity before the beginning of conditioning regimen): one as part of first-line therapy with ven/aza, two with primary refractory disease, and two following relapses after the first transplant (Figure 1). At the

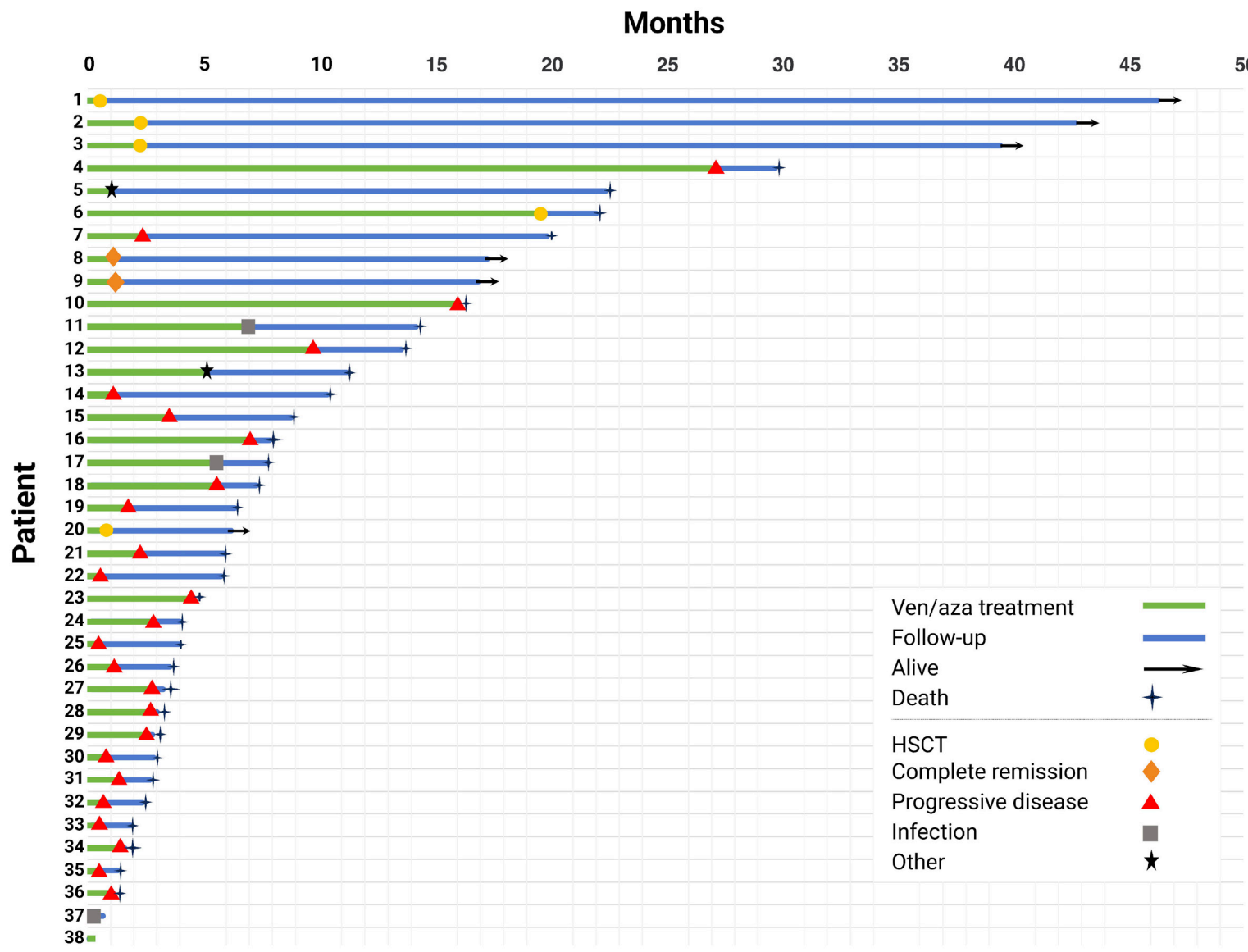

Figure 1. Swimmer plot depicting the clinical course of study patients. HSCT: hematopoietic stem cell transplantation; Other: parent's decision, data not available.

most recent follow-up, six patients of the ten were alive and in continued clinical remission with a median follow-up duration of 28 months after ven/aza (range, 17-46) (*Online Supplementary Figure S1*).

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

Safety considerations

Ven 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 ven/aza. 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, three patients presented with positive fungal infections. Most patients (27/38, 71%) received routine antifungal prophylaxis, usually with liposomal amphotericin B or azoles, during ven/aza 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 ven/aza therapy. We note a 26.3% overall response rate in 38 children and adolescents with R/R 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) ven/aza therapy from pediatric institutions in the US. In the study by Karol *et al.* (*clinicaltrials gov. Identifier: NCT03236857*), 24 of 35 pediatric patients with R/R AML received ven plus a hypomethylating agent, yielding an ORR of 21% with no MRD-negative responses. Among 19 patients treated with ven/aza, 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

Table 3. Summary of clinical course and outcomes under venetoclax/azacitidine therapy.

Clincal course and outcomes	
N 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)
AlloHSCT	5 (13)
Other	2 (5)
Duration of follow up in months, median (range) [IQR]	5.8 (0.2-34.5) [2.8-10.6]
Status at last follow up, N (%)	
Alive in CR	6 (16)
Dead	32 (84)

Aza: azacitidine; CR: complete response; CRi: complete response with incomplete count recovery; IQR; interquartile range; PR: partial response; MRD: minimal residual disease, negative if <0.1%; alloHSCT: allogeneic hematopoietic stem cell transplantation; ven: venetoclax.

Table 4. Adverse events observed in study cohort patients.

Adverse event	Any grade N of 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.

six of eight children with R/R AML or high-risk MDS, with MRD negativity achieved in the four patients with AML and ven/aza-induced CR.[20] LeBlanc *et al*. from Cincinnati Children's hospital reported their experience combining ven with demethylating agents (aza and decitabine) in 27 patients, including nine 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 R/R AML, ALL, or mixed phenotype acute leukemia (MPAL) with ven/aza 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 ven/aza, including 12 patients with AML, one with B-ALL, and one with MPAL.[19]

Our observed remission rate in pediatric patients with R/R *de novo* or secondary AML is somewhat lower than that of the Niswander study, which also included children with multible R/R B-ALL or MPAL and used GO for some of the patients. However, GO addition to ven/aza, did not improve response rates in this population compared to ven/aza 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 ven/aza as third-line therapy or beyond. However, all those studies underscore the importance of consolidating ven/aza-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 ven/aza has been reported in numerous clinical trials or case series in adults with R/R AML.[29,30] These findings highlight the potential influence of the number of prior therapeutic lines upon the potential efficacy of ven/aza 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 ven/aza as first- or second-line therapy compared to later use, although our small patient numbers precluded definitive conclusions.

Although clear biomarkers of ven/aza 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 ven/aza.[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 OS with ven/aza in other studies.[33,34] Ongoing adult and pediatric trials will continue to identify patients most likely to benefit from ven-based therapies and to define the ideal duration of ven exposure per treatment cycle.[35] One is a phase I trial evaluating ven combined with cytotoxic chemotherapy, including aza, in high-risk hematologic malignancies such as MDS/AML (*clinicaltrials gov. Identifier: NCT05292664*). *TP53* mutations are associated with poor responses to ven and aza 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 ven-based therapy,[36] reflected in our cohort with a 64% non-response rate.

Finally, we also report excellent clinical tolerability of ven/aza therapy in a pediatric population, mainly with multiple 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 ven/aza therapy for more than 1 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. European LeukemiaNet 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 ven/aza combination in a large cohort of pediatric patients with multiply R/R 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 ven/aza 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.

Disclosures

AB served on advisory boards for and received honoraria and/or travel support from Amgen, Astra-Zeneca, Janssen, Jazz Pharmaceuticals, Novartis, Sanofi, Servier, and Wugen; and received research funding from Shire/Sevier. SD served on advisory boards for and received honoraria and/or travel support from Clinigen Health, Jazz Pharmaceuticals, Servier and Takeda. AR served on advisory boards for EUSA Pharma and Sanofi; and received expenses for congress attendance from EUSA Pharma and Roche. PV discloses speakers fees from Servier. SKT receives research funding from Incyte Corporation and Kura Oncology; serves/d on scientific advisory boards for Aleta Biotherapeutics, AstraZeneca, Jazz Pharmaceuticals, Kura Oncology, Syndax Pharmaceuticals, and Wugen, Inc.; and has received travel support from Amgen and Jazz Pharmaceuticals (all for unrelated studies). MZ received institutional funding for clinical trials from Pfizer, Daiichi Sankyo, Jazz Pharma, Takeda, Abbvie and Kura Oncology; and has a consultant role for Janssen, Syndax, BMS, Incyte, Sutro, Kestrel, Beigene, and Sanofi.

Contributions

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

Data-sharing statement

The authors agree to share the data.

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