Clinical responses in pediatric patients with relapsed/refractory leukemia treated with azacitidine and venetoclax

Despite optimization and escalation of risk-based chemotherapy regimens, children with relapsed or chemotherapy-refractory acute leukemias, particularly acute myeloid leukemia (AML), remain difficult to cure.[1,2] Traditional intensive cytotoxic chemotherapy salvage regimens require extended inpatient hospitalization due to infectious risk during severe myelosuppression and are accompanied by therapy-related morbidity and deleterious impact upon quality-of-life.[3,4] The combination of the hypomethylating agent azacitidine with venetoclax, an oral selective BH3-mimetic inhibitor of the anti-apoptotic protein B-cell lymphoma 2 (BCL-2), is effective at remission induction in elderly or intensive induction chemotherapy-ineligible adults with previously-untreated AML and is Food and Drug Administration-approved for this indication.[5,6] The recent VENAML phase I clinical trial demonstrated safety and activity of venetoclax combined with idarubicin and/or high-dose cytarabine in children and adolescents/young adults (AYAs) with relapsed refractory AML (*clinicaltrials gov. Identifier: NCT03194932*).[7] However, clinical experience with the azacitidine/venetoclax regimen in children has been limited to small case series.[8,9] Herein, we report clinical characteristics and outcomes of 37 pediatric patients with relapsed/refractory acute leukemias treated at our institution with commercially-available azacitidine/venetoclax therapy.

We analyzed data from patients aged 0-21 years with multiply-relapsed/refractory acute lymphoblastic leukemia (ALL), AML, or mixed-phenotype acute leukemia (MPAL) treated with azacitidine/venetoclax without or with the CD33 antibody-drug conjugate gemtuzumab ozogamicin (GO) at the Children's Hospital of Philadelphia from January 1st, 2018 to March 31st, 2022. Institutional Review Board exemption was obtained for retrospective chart review. Clinical data were abstracted from electronic medical records on baseline patient demographics, leukemia-associated immunophenotyping and genetic characteristics, therapy administration, clinical response, and post-azacitidine/venetoclax outcomes with clinical follow-up through June 30, 2022.

Azacitidine 100 mg/m^2 daily was given intravenously on days 1-5 of each 28-day cycle. Venetoclax was given once daily as oral tablets (swallowed intact or crushed) with 3-day 'ramp-up' dosing during cycle 1 to minimize tumor lysis syndrome (TLS) risk with body surface area-adjusted adult exposure-equivalent dosing (AED) of 200 mg (day 1), 400 mg (day 2), and 800 mg (days 3-28).[10] Subsequent cycles utilized venetoclax at full 800 mg AED for 28 days without ramp-up. Patients receiving concurrent moderate or strong CYP3A inhibitors, including azole-class anti-fungal mediations, received a 50% dose reduction of venetoclax.[10] Some patients with AML treated with azacitidine/venetoclax also received GO 3 mg/m^2/dose during cycle 1 usually given on days 4, 5, or 8.

Of the 37 pediatric patients with relapsed/refractory acute leukemias treated with azacitidine/venetoclax-based therapy during the study period, 27 (73%) had AML, seven (19%) had B-ALL, one (3%) had T-ALL, and two (5%) had MPAL (Table 1). The majority of patients (n=29, 78%) had multiply-relapsed/refractory leukemia. Eight children (22%) had primary chemotherapy-refractory leukemia defined as never achieving bone marrow measurable residual disease (MRD) negativity by flow cytometry at thresholds of <0.01% for ALL and <0.1% for AML. Sex was evenly balanced between female and male patients, and the median age at initial leukemia diagnosis was 8 years. Many children had high-risk cytomolecular genetic alterations, including 11 patients with *KMT2A*-rearranged leukemia (n=3 B-ALL, n=7 AML, n=1 MPAL), four with *CBFA2T3::GLIS2* acute megakaryoblastic leukemia (AMKL), one with *NUP98::KDM5A* AMKL, one with *ETV6::EP300* AML, and one with *FLT3*-ITD AML (Table 2). Of note, four patients with refractory AML treated with azacitidine/venetoclax were initially diagnosed with B-ALL (n=3 *KMT2A*-rearranged, n=1 *TCF3::ZNF384*) and had relapsed with myeloid lineage switch following CD19-directed (n=3) or CD22-directed (n=1) chimeric antigen receptor T-cell immunotherapy (CAR T). Prior to initiation of azacitidine/venetoclax therapy, the median marrow MRD of patients who completed at least one 28-day cycle azacitidine/venetoclax was 10.5% (range, 0.01–91.5%). Three patients with AML and one with MPAL were in MRD-negative/low remission prior to starting azacitidine/venetoclax, which was administered as bridging therapy prior to allogeneic hematopoietic stem cell transplantation (HSCT).

Patients received up to six cycles of azacitidine/venetoclax during the data collection window (median 2 cycles, range 0-6) and were followed for a median of 4.9 months (range, 0.1-32.6) after initiation of therapy (Table 1; Figure 1A). GO was given with azacitidine/venetoclax in nine of the 27 patients with AML. Thirty-one patients (84%) com-

pleted at least one full cycle of azacitidine/venetoclax, while six children (16%) received fewer than 28 days of therapy. Early discontinuation of azacitidine/venetoclax was due either to severe TLS (n=2) or rapidly progressive disease detected in peripheral blood (n=4). Treatment-free intervals (1 week, n=2; 2 weeks, n=4; ≥3 weeks, n=2) were required for seven patients with AML and one patient with B-ALL between cycles 1 and 2 to facilitate resolution of severe neutropenia or thrombocytopenia. Three patients with cycle 2 delay had concurrent infection (n=1 viral, n=1 bacteria, n=1 unknown etiology), and two others had persistent leukemia. Two patients proceeded to cycle 2 with continued cytopenias. During azacitidine/venetoclax therapy, six patients had acute bacteremia treated successfully with antibiotics, and two patients had biopsy-proven fungal infections.

Response data at first post-azacitidine/venetoclax marrow assessment were available for 29 patients. Median MRD level was 0.5% (range, 0.01-90%), and 14 patients (n=12 AML, n=1 B-ALL, n=1 MPAL) achieved a complete response (CR) with MRD-negative remission (38%, 14 of 37 treated patients). Of the 25 patients with AML who completed cycle 1, 12 (48%) were in MRD-negative remission, including several children with high pretreatment disease burden (Table 2; Figure 1B). Eight of these patients remained in MRD-negative remission with further cycles of azacitidine/venetoclax, while three treated with palliative intent experienced disease progression after cycle 2 (n=2) or cycle 4 (n=1). Children with AML who received azacitidine/venetoclax with GO (n=9) had a similar CR rate (4/9, 44%) to those treated without GO. One patient with B-ALL and one with MPAL also achieved MRD-negative CR (Figure 1C). Of note, six of the 14 azacitidine/venetoclax-induced CR occurred in patients with primary chemorefractory leukemia (n=5 AML, n=1 B-ALL). Three patients with residual leukemia (n=2 AML, n=1 MPAL) after cycle 1 continued azacitidine/venetoclax therapy, and the one with MPAL achieved MRD-negative CR after cycle 2.

Importantly, responses occurred in patients with high-risk leukemia genetics. Eight of 11 patients with relapsed/refractory *KMT2A*-rearranged leukemias (n=2 B-ALL, n=5 AML, n=1 MPAL) completed cycle 1 of azacitidine/venetoclax, and two patients achieved MRD-negative CR (n=1 infant B-ALL, n=1 AML). Patients with ALL-to-AML lineage switch relapse (n=4) had particularly aggressive leukemias with two children unable to complete cycle 1 due to disease progression and the other two experiencing persistent MRD after cycle 1. Strikingly, three of four children with *CBFA2T3::GLIS2* AML achieved MRD-negative remission. Successful remission induction with azacitidine/venetoclax +/- GO was surprisingly HSCT-enabling for 11 patients with AML and one patient with MPAL. HSCT complications in these children included graft-*versus*-host-disease of skin (n=3, grade 2-3 acute), liver (n=1, grade 1 acute), or lung (n=1, grade 3 chronic). Post-transplant death occurred in five of 12 patients (n=3 relapse, n=2 infection in leukemia remission) at a median of 177 days. Two patients with persistent relapsed/refractory B-ALL received subsequent autologous CD19-directed CAR T, and one patient with refractory *KMT2A*-rearranged AML after infant B-ALL lineage switch relapse received allogeneic

Table 1. Demographics, clinical characteristics, and outcomes of the study cohort treated with azacitidine and venetoclax (N=37 patients).

Demographics, clinical characteristics and outcomes	
Age at diagnosis (years) Median Range	 8 0.04-21
Sex, N (%) Female Male	 18 (49) 19 (51)
Leukemia subtype of patients who did not complete cycle 1 azacitidine/venetoclax (N=6), N (%) AML ALL (N=3 B-ALL, N=1 T-ALL)	 2 (33) 4 (67)
Leukemia subtype of patients receiving ≥1 cycle (N=31), N (%) AML ALL (N=4 B-ALL) MPAL	 25 (81) 4 (13) 2 (6)
Leukemia status at time of azacitidine/venetoclax, N (%) Primary chemorefractory Multiply-relapsed/refractory	 8 (22) 29 (78)
Completed cycles of azacitidine/venetoclax, N (%) <1 ≥1	 6 (16) 31 (84)
Number of cycles Median Range	 2 0-6
Patients with AML treated with azacitidine/venetoclax + GO, N (%)	9 (36)
Chemotherapy held or delayed	18 (49)
First available response assessment, N (%) End of cycle 1 End of cycle 2 Flow cytometric MRD-negative CR achieved *	 25 (68) 4 (11) 14 (38)
Duration of follow-up (months) Median Range	 4.9 0.1-32.6
Outcome at last follow-up, N (%) Alive Deceased	 12 (32) 25 (68)
Time to death after last azacitidine/venetoclax (months) Median Range	 1.83 0.1-15.37

*0.1% for AML, <0.01% for ALL/MPAL. ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; MPAL: mixed phenotype acute leukemia, GO: gemtuzumab ozogamicin; MRD: measurable residual disease.

Table 2. Patient characteristics, leukemia-associated cytomolecular genetic alterations, and treatment outcomes following azacitidine and venetoclax therapy in study cohort (N=37).

Therapy cycles	Leukemia type	Age at diagnosis years	Sex	Genetic alterations	MRD^
≥ 1	B-ALL	0.5	M	*KMT2A::MLLT1* fusion	+
	B-ALL	0.5	M	*KMT2A::MLLT10* fusion	-
	B-ALL	3	M	*ETV6::RUNX1* fusion, *TP53* mutation	+
	B-ALL	21	F	High hyperdiploidy	+
	AML	17	M	*DNMT3A* and *GATA2* mutations	-
	AMKL	1	M	*CBFA2TA::GLIS2* fusion	-
	AML	16	M	*NRAS* mutation	-
	AML	6	M	*FLT3* TKD and *RUNX1* mutations	-
	AML	14	F	*ELANE* mutation	+
	AML	11	M	*FLT3*-ITD, *RUNX1*, and *WT1* mutations	-
	AMKL	2	M	*NUP98::KDM5A* fusion	+
	AMKL	0.9	F	*CBFA2T3::GLIS2* fusion	-
	AML	15	M	*SET::NUP214* fusion	-
	AML	9	F	*WT1* mutation	+
	AML	8	F	*NRAS* mutation	+
	AML	16	M	Germline *TP53* mutation	-
	AML*	5	F	*TCF3::ZNF384* fusion	NA
	AMKL	1	F	*CBFA2T3::GLIS2* fusion	-
	AML	18	F	*RUNX1* mutation	-
	AML	4	F	*ETV6::EP300* fusion	-
	AML	0.7	F	*FLT3* TKD mutation	+
	AMKL	1	F	*CBFA2T3::GLIS2* fusion	+
	AML	0.7	M	*KMT2A::MLLT10* fusion, *TP53* mutation	-
	AML	2	F	*FLT3*-TKD and *TP53* mutations	+
	AML	13	F	*KMT2A::MLLT1* fusion	+
	AML	19	M	Monosomy 7, *NRAS* mutation	+
	AML*	0.8	M	*KMT2A::MLLT1* fusion	+
	AML	11	M	*KMT2A::MLLT6* fusion	+
	AML	8	F	*KMT2A-SEPT9* fusion, *WT1* mutation	NA
	MPAL	15	F	*KMT2A::AFF1* fusion	+
	MPAL	16	M	*GATA2::ERG* fusion	-
<1	B-ALL	9	F	High hyperdiploidy	NA
	B-ALL	3	M	*KMT2A::MLLT3* fusion	NA
	B-ALL	12	F	Low hypodiploidy	NA
	T-ALL	17	M	*NOTCH, PTPN11*, and *JAK3* mutations	NA
	AML*	0.1	F	*KMT2A::AFF1* fusion, *NRAS* and *KRAS* mutations	NA
	AML*	1	M	*KMT2A::AFF1* fusion	NA

*Initial diagnosis of ALL with lineage switch to AML prior to receipt of azacitidine/venetoclax. ^Measurable residual disease (MRD) at first response assessment defined as <0.1% for AML and <0.01% for ALL/MPAL by flow cytometry analysis. ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; MPAL: mixed phenotype acute leukemia; AMKL: acute megakaryoblastic leukemia; F: female; M: male; NA: not applicable or not assessed (due to disease progression).

CD123-directed CAR T. At last follow-up, 12 patients (32%) were alive, all of whom received at least one cycle of azacitidine/venetoclax (Figure 1A).

To our knowledge, we report the largest retrospective cohort of children and adolescents/young adults with multiply-relapsed/refractory acute leukemias treated with azacitidine/venetoclax to date. Within a heavily-pretreated and/or highly chemorefractory cohort of patients, we describe an encouraging 38% MRD-negative CR rate. Our observations are similar to those of Winters and colleagues, who described MRD-negative CR in three of six (50%) pediatric patients with relapsed/refractory AML treated with azacitidine/venetoclax and a lack of response in one child with ALL-to-AML lineage switch relapse.[8] Our data are also concordant with several retrospective case series of adults with relapsed/refractory AML in whom activity has been reported across numerous leukemia genetic backgrounds, including those with poor-risk cytogenetics.[11-13] We observed remarkable rates of MRD-negative CR with azacitidine/venetoclax therapy in our cohort of children with multiply-relapsed/refractory leukemias, including many with high-risk cytomolecular genetic features such as *CBFA2T3::GLIS2* AMKL. Importantly, our pediatric patients largely received azacitidine/venetoclax therapy in the outpatient oncology clinic with inpatient admission for tumor lysis or myelosuppression monitoring

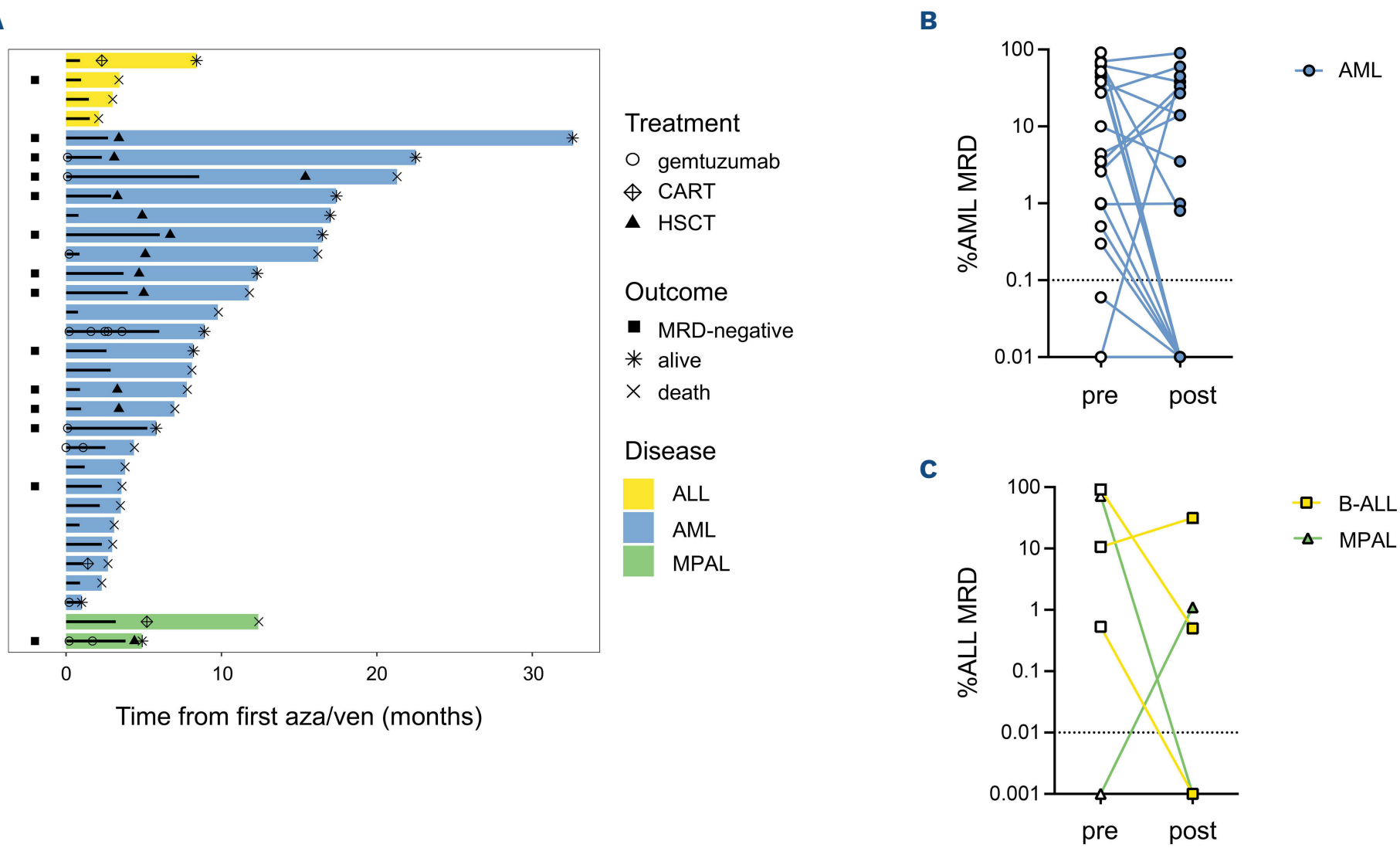

Figure 1. Clinical course and responses of pediatric patients with multiply-relapsed/refractory acute leukemia treated with azacitidine and venetoclax. (A) Swimmer plot depicting the clinical course of each patient who received ≥1 complete cycle of azacitidine and venetoclax (aza/ven; N=31) is displayed. Each colored bar (grouped by leukemia subtype) represents the time after initiation of aza/ven for a single patient. Interior horizontal thick black bars represent aza/ven treatment duration. Squares represent patients with measurable residual disease (MRD)-negative remission at first evaluation (end of cycle 1 or cycle 2). Triangles represent treatment with chimeric antigen receptor T-cell immunotherapy (CAR T), including autologous CD19-directed CAR T (B-cell acute lymphoblastic leukemia [B-ALL]) or allogeneic CD123-directed CAR T (acute myeloid leukemia [AML]). Additional treatment modalities and outcomes are denoted by the symbols in the legend. (B) Clinical response assessment of pediatric patients with relapsed/refractory AML receiving ≥1 cycle of aza/ven. Prior to the initiation of aza/ven, patients had varying AML disease burden in the bone marrow as quantified by flow cytometric analysis of measurable residual disease (MRD). Lines depict response of individual patients from pretreatment (white circles) to first evaluation (blue circles, end of cycle 1 or 2). Many patients achieved MRD-negative remission at a threshold of <0.1% (dotted grey line) at first evaluation, including several patients with high pre-aza/ven treatment disease burden. (C) Clinical response assessment of pediatric patients with relapsed/refractory B-ALL (yellow squares) or mixed phenotype acute leukemia (MPAL) (green triangles) receiving ≥1 cycle of aza/ven as in (B). Lines depict flow cytometric analysis of bone marrow MRD for individual patients from pre-treatment (white symbols) to first evaluation (yellow or green symbols, end of cycle 1 or 2). One patient with B-ALL and 1 patient with MPAL achieved MRD-negative remission at a threshold of <0.01% (dotted grey line). HSCT: hematopoietic stem cell transplantation.

not routinely required, comparable with experience in adult AML.[14] As many children had spent significant time inpatient for receipt of prior frontline or salvage therapies, the ability to receive outpatient chemotherapy anecdotally provided significant quality of life benefit for patients and their families, an area that merits future formal study. Finally, our data suggest that clinical responses of children with relapsed/refractory leukemia, particularly AML, to the azacitidine/venetoclax regimen are rapid and binary with achieved MRD-negative CR typically occurring after cycle 1 therapy or not at all.

Although our study is limited by its retrospective nature, a relatively small number of patients treated at a single institution, and short duration of follow-up, it highlights the robust potential of azacitidine/venetoclax as an effective salvage regimen for pediatric patients with multiply-relapsed and highly-chemorefractory acute leukemias. In our cohort, azacitidine/venetoclax was often initially administered with palliative intent, but was subsequently HSCT-enabling in many patients given their excellent response rates. Given the surprisingly high efficacy in a very chemorefractory population, favorable toxicity rate, and appreciable improvement in quality of life for patients, formal clinical trial investigation of azacitidine/venetoclax-based therapies in children with relapsed or refractory acute leukemias, ideally at an earlier stage of relapse, is warranted.

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Contributions

LMN contributed to study design, performed data collection, analyzed and interpreted data, and wrote the manuscript. PC performed data collection and analyzed and interpreted data. CD analyzed and interpreted data. SKT conceived and directed the study, interpreted data, and wrote and edited the manuscript. All authors reviewed, edited, and approved the final manuscript.

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Data-sharing statement

De-identified data without identifying patient health information are available upon reasonable request from the corresponding author.

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