

# Cladribine plus cytarabine plus venetoclax in acute myeloid leukemia relapsed or refractory to venetoclax plus hypomethylating agent

Venetoclax (Ven) in combination with a hypomethylating agent (HMA) is the standard first-line treatment for elderly and unfit patients with newly-diagnosed acute myeloid leukemia (AML), after the phase III VIALE-A trial demonstrated superior complete remission (CR) with or without count recovery (CRi) rates and overall survival (OS) with azacitidine-Ven in comparison to azacitidine monotherapy. Despite superior CR/CRi rates observed in the VIALE-A study, primary refractory disease or relapse was documented in 42% of patients receiving azacitidine-Ven.<sup>1</sup> In this regard, we have recently underlined the dismal outcome of patients with newly-diagnosed AML following failure of Ven-HMA therapy, and reported a median survival of ~3 months, which was significantly inferior in the presence of *TP53*, *K/NRAS*, or *ASXL1* mutations. Importantly, in this particular study, only 11 of 71 (15%) patients received subsequent salvage therapy.<sup>2</sup> Similarly, another study of 41 patients with relapsed/refractory AML following frontline Ven-HMA also demonstrated a median OS of 2.3 months, with 24 (59%) patients receiving salvage therapy.<sup>3</sup> The aforementioned studies highlight the dire need for additional salvage regimens to extend survival and ensure optimal transplant eligibility, particularly for patients without targetable mutations. A recent phase II study, with cladribine, low-dose cytarabine (Ara-C) and venetoclax (CAV), alternating with azacitidine, in older patients with newly-diagnosed AML, demonstrated a CR/CRi rate of 93%.<sup>4</sup> In routine practice, CAV is used as salvage therapy in patients with relapsed/refractory AML lacking targetable mutations and ineligible for intensive therapy, although efficacy data with this regimen following Ven-HMA is unknown. Accordingly, we sought to determine the efficacy of CAV as a salvage regimen in AML patients following failure of Ven-HMA, including clinical and molecular predictors associated with treatment response and survival.

Under an institutional review board-approved minimum risk protocol, the Mayo Clinic (MN, AZ, FL) database was searched to identify patients with AML who progressed after treatment with Ven-HMA and subsequently received at least one cycle of CAV, outside of clinical trials, between April 2020 and April 2023. Patients received cladribine 5 mg/m<sup>2</sup> on days 1-5, low-dose Ara-C, 20 mg/m<sup>2</sup> twice daily on days 1-10, and Ven 100-400 mg daily, dose-adjusted based on anti-fungal prophylaxis, on days 1-21, according to treating physician discretion. CAV was administered in the inpatient setting in 17 patients, inpatient followed by outpatient after day 5 in ten, after day 6 in five, after day

7 in four, after day 4 and day 2 in one patient each, and all outpatient in one patient. All patients received anti-bacterial and anti-viral prophylaxis, on the other hand, azole and pneumocystis prophylaxis was administered in 38 (97%) and 21 (54%) of patients, respectively. Bone marrow biopsy, conventional karyotyping, and next-generation sequencing via a 4-, 11-, or 48-gene panel were collected at the discretion of the treating physician, and in most cases occurred at the time of diagnosis. The 2022 European LeukemiaNet (ELN) criteria<sup>5</sup> were applied to define disease risk and response to treatment. Significance testing for covariates associated with response was performed via  $\chi^2$  or Fischer's exact test for categorical variables and Wilcoxon rank-sum test for continuous variables. Follow-up was updated in December 2023 and survival calculated from the time of CAV treatment to last follow-up or death, and group differences were assessed with the log-rank test. JMP Pro 16.0.0 software package, SAS Institute, Cary, NC was used for statistical analysis.

A total of 39 AML patients (median age 65 years; range, 22-78; 67% male; 64% *de novo*) relapsed (54%, N=21) or refractory (46%, N=18) to prior chemotherapy, which included Ven-HMA, received CAV (median of 1 cycle; range, 1-5 cycles). A majority (87%, N=34) of these patients had failed or relapsed from Ven-HMA as their most recent line of therapy. Patients had received one (N=16), two (N=14), three (N=5), four (N=2), five (N=1) or nine (N=1) prior therapies, including a median of three cycles of Ven-HMA (range, 1-14 cycles). Nine (23%) patients relapsed following allogeneic hematopoietic stem cell transplant (AHSCT). Median duration of remission in relapsed patients was 7.04 months (range, 1.2-70.1 months). ELN cytogenetic risk at diagnosis included intermediate (46%, N=18), and adverse (54%, N=21). Mutations at diagnosis involved *TP53* in nine (23%), *K/NRAS* in eight (21%), *RUNX1* in seven (18%), *TET2* in six (15%), *ASXL1* in five (13%), *STAG2* in five (13%), and *IDH1/2* in two (5%) patients, one of whom had received ivosidenib. Eleven (28%) patients achieved CR (5%, N=2) or CRi (23%, N=9); median time to response was 1 month and median response duration was 4.4 months. In addition, one patient achieved partial remission (PR) and two patients had bone marrow aplasia not fulfilling criteria for morphologic leukemia-free state (MLFS). Measurable residual disease (MRD) was negative by multiparametric flow cytometry in two of seven (29%) informative CR/CRi cases. CR/CRi rates were higher in females (54% vs. 15%; *P*=0.01), *de novo* versus secondary AML (40% vs. 7%; *P*=0.02), absence versus presence of

adverse karyotype (50% vs. 9%;  $P<0.01$ ), and absence versus presence of *K/NRAS* (35% vs. 0%;  $P=0.01$ ), *ASXL1* (32% vs. 0%;  $P=0.05$ ), and *STAG2* mutations (32% vs. 0%;  $P=0.05$ ) (Table 1). Multivariable analysis confirmed superior response in *de novo* AML ( $P<0.01$ ), absence of adverse karyotype, ( $P<0.01$ ), and absence of *K/NRAS* mutations ( $P=0.02$ ). Notably, CR/CRi rates were not impacted by relapsed versus refractory disease (29% vs. 28%;  $P=0.95$ ), exposure to more than one prior line of therapy (25% vs. 33%;  $P=0.58$ ), prior AHSCT (22% vs. 30%;  $P=0.64$ ), or *TP53* mutations (22% vs. 30%;  $P=0.64$ ) (Table 1). Five (13%) of patients had monocytic leukemia, CR/CRi rates were 40% versus 26% in patients with versus without monocytic leukemia ( $P=0.54$ ). The most common

treatment-emergent adverse event was infection (51%,  $N=20$ ), comprising bacteremia ( $N=11$ ), bacterial pneumonia ( $N=10$ ), and abscesses ( $N=6$ ). Of these infections, 85% were grade 3 or higher, including two fatal episodes of septic shock, involving an *E. faecium* peri-rectal abscess and *P. aeruginosa* pneumonia. None of the patients developed pneumocystis pneumonia. Less frequent toxicities included liver dysfunction (18%,  $N=7$ , of which 3 were grade 3) and tumor-lysis syndrome (2%,  $N=1$ , grade 3).

At a median follow-up of 4.7 months (range, 0.4-17.5 months) from the initiation of CAV, 34 deaths (87%), seven relapses (18%), and seven AHSCT (18%) were documented. Five patients remain alive and are disease-free at the time of this

**Table 1.** Clinical characteristics at time of treatment with cladribine, Ara-C (low-dose), venetoclax for 39 patients with acute myeloid leukemia, relapsed/refractory to venetoclax and hypomethylating agent stratified by achievement of complete response or complete response with incomplete count recovery.

Variables	All patients N=39	Patients in CR/CRi N=11	Patients not in CR/CRi N=28	P/multivariate P
Age in years, median (range)	65 (22-78)	67 (44-78)	63 (22-77)	0.21
>60 years, N (%)	25 (64)	8 (32)	17 (68)	0.7
Male, N (%)	26 (67)	4 (15)	22 (85)	0.01/0.42
AML type, N (%)				
<i>De novo</i>	25 (64)	10 (40)	15 (60)	0.02/<0.01
Secondary or therapy-related	14 (36)	1 (7)	13 (93)	
Hemoglobin g/dL, median (range)	8 (5.4-16.2)	8 (6.5-10.2)	7.9 (5.4-16.2)	0.88
Leukocyte count $\times 10^9/L$ , median (range)	1.9 (0.1-41.9)	2.2 (0.1- 21.5)	1.8 (0.1-41.9)	0.95
Platelet count $\times 10^9/L$ , median (range)	27 (4-553)	35 (9-553)	24 (4-479)	0.46
Circulating blasts %, median (range)	13 (0-94)	5 (0-92)	13 (0-94)	0.94
Bone marrow blasts %, median (range)	20 (5-90)	14 (5-88)	23 (5-90)	0.30
ELN 2022 cytogenetic risk stratification, N (%)				
Intermediate	18 (46)	9 (50)	9 (50)	<0.01/<0.01
Adverse	21 (54)	2 (10)	19 (90)	
Mutations, N (%)				
<i>TP53</i>	9 (23)	2 (22)	7 (78)	0.64
<i>K/NRAS</i>	8 (21)	0 (0)	8 (100)	0.01/0.02
<i>RUNX1</i>	7 (18)	2 (29)	5 (71)	0.98
<i>TET2</i>	6 (15)	3 (50)	3 (50)	0.22
<i>ASXL1</i>	5 (13)	0 (0)	5 (100)	0.05/0.22
<i>STAG2</i>	5 (13)	0 (0)	5 (100)	0.05/0.22
<i>CEPBA</i>	3 (8)	1 (33)	2 (67)	0.84
<i>SF3B1</i>	3 (8)	1 (33)	2 (67)	0.84
<i>U2AF1</i>	3 (8)	1 (33)	2 (67)	0.84
<i>NPM1</i>	2 (5)	2 (100)	0 (0)	0.02/0.47
<i>IDH1/2</i>	2 (5)	1 (50)	1 (50)	0.50
<i>DNMT3A</i>	2 (5)	1 (50)	1 (50)	0.50
<i>SRSF2</i>	2 (5)	0 (0)	2 (100)	0.24
<i>BCOR</i>	2 (5)	1 (50)	1 (50)	0.50
<i>CSF3R</i>	2 (5)	1 (50)	1 (50)	0.50
<i>WT1</i>	2 (5)	2 (100)	0 (0)	0.02/0.47
Number of prior therapies, median (range)	2 (1-9)	2 (1-9)	2 (1-5)	0.51
Prior allogeneic HSCT, N (%)	9 (23)	2 (18)	7 (25)	0.64
Allogeneic HSCT, N (%)	7 (18)	5 (45)	2 (7)	<0.01

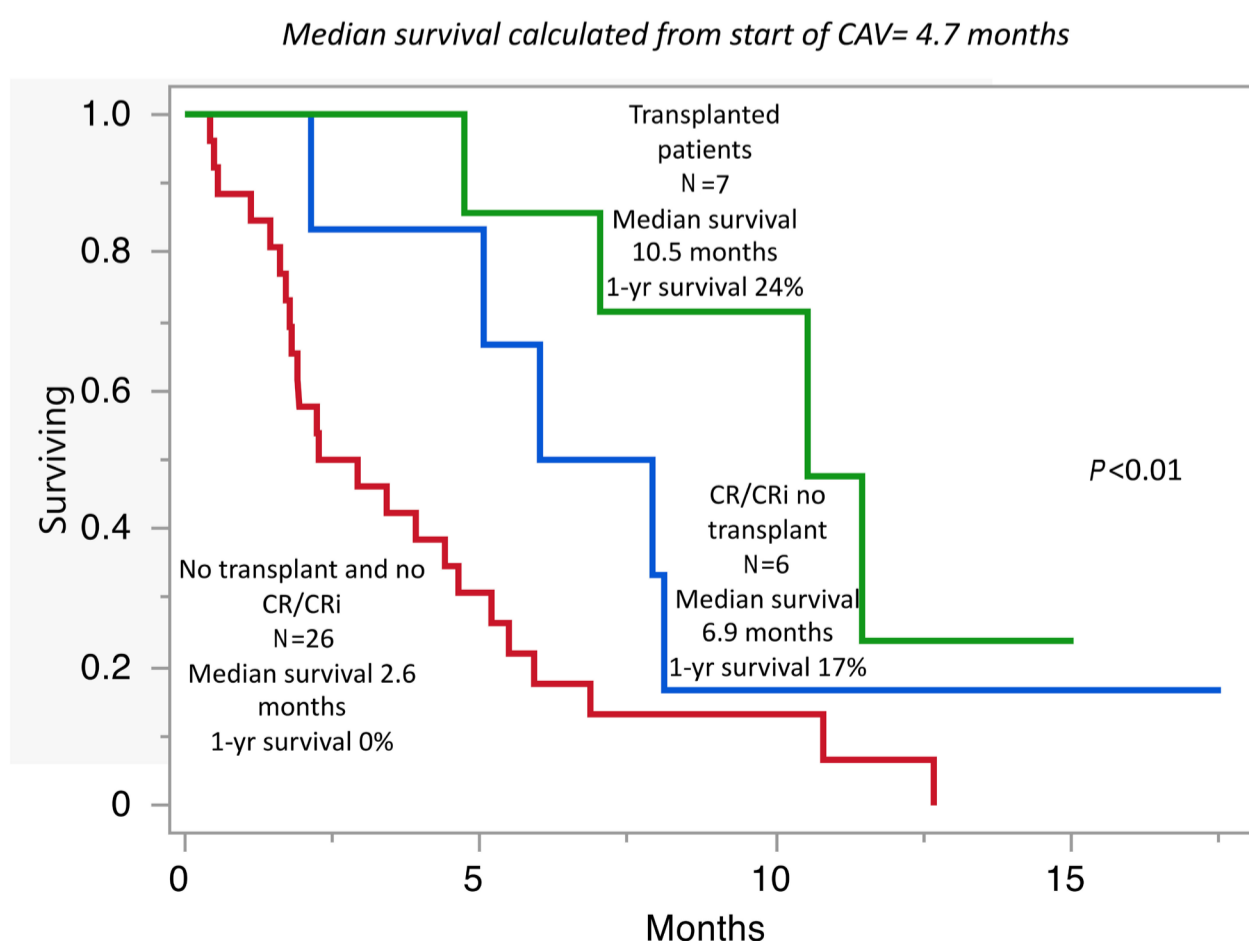
CR: complete response; CRi: complete response with incomplete count recovery; AML: acute myeloid leukemia; ELN: European LeukemiaNet; HSCT: hematopoietic stem cell transplant.

writing, three of whom underwent AHSCT, while one patient each are in CR/CRi and in MLFS for 17.5 and 8.3 months, respectively. Median survival following CAV was 4.7 months, and superior in patients achieving CR/CRi (8.1 vs. 3.2 months;  $P=0.01$ ) and in patients receiving AHSCT (10.5 vs. 3.7 months;  $P=0.01$ ). Figure 1 illustrates survival differences in patients that underwent AHSCT, patients achieving CR/CRi but not transplanted, and patients not achieving CR/CRi, with respective median survival of 10.5, 6.9, and 2.6 months ( $P<0.01$ ). Absence of adverse cytogenetic risk was also associated with superior survival (6 vs. 3.4 months;  $P=0.05$ ). Multivariable analysis confirmed the favorable survival impact of CR/CRi ( $P=0.03$ ) and AHSCT ( $P=0.05$ ); whereas survival impact was not apparent for secondary AML ( $P=0.29$ ), *K/NRAS* ( $P=0.70$ ) or *TP53* ( $P=0.10$ ) mutations. On the other hand, there was a trend towards inferior survival in nine patients with prior AHSCT; median survival 1.9 versus 4.9 months ( $P=0.10$ ). Of the seven patients (18%) receiving AHSCT following CAV, five patients were in CR/CRi before proceeding to transplant, one achieved bone marrow aplasia, and one case was refractory to CAV but achieved a CRi with mitoxantrone + etoposide + cytarabine (MEC) (Table 2).

Similar results were obtained when response and survival analysis was restricted to 34 patients who had received Ven-HMA as the most recent line of therapy. CR/CRi was documented in eight (24%) patients, death in 30 (88%), AHSCT in five (15%) and relapse in six (18%) cases; median survival was 4.7 months. Moreover, in 16 patients who received CAV as first salvage therapy, CR/CRi was noted in five (31%) patients, with death in 12 (75%), AHSCT in three (19%) and relapse in two (13%) patients. Median survival was 6.4 months.

The current study demonstrates that the CAV regimen can induce remissions in Ven-HMA- relapsed/refractory AML, albeit at expectedly lower CR/CRi rates in comparison to its efficacy in treatment-naïve AML (CR/CRi rate of 28% vs. 93%).<sup>4</sup> Prior studies examining outcomes of AML patients relapsed/refractory to Ven-HMA reported CR/CRi rates of 21%<sup>3</sup> and 27%<sup>2</sup> following a number of salvage regimens including intensive chemotherapy and *FLT3/IDH1/2* targeted agents. In the current study, only two patients harbored *IDH1/2* mutations, one of whom had previously received ivosidenib. A phase II trial (*clinicaltrials.gov*. Identifier: NCT05190549) investigating the use of CAV in relapsed/refractory AML (N=30) reported CR/CRi rate of 27% and 1-year OS of 60%.<sup>6</sup> In contrast to the present study which featured elderly patients with heavily treated, high-risk disease, the clinical trial enrolled a younger patient population (median age, 39.5 years) with the majority (63%) of patients with ELN 2017 favorable/intermediate risk.

In the current study, *de novo* AML, absence of adverse karyotype, and absence of *K/NRAS* mutation were associated with a higher likelihood of response. In that regard, detailed single-cell DNA sequencing analysis of patient samples pre- and post-treatment with Ven-based regimens, including Ven-HMA and Ven-LDAC, demonstrated frequent acquired mutations in kinase activating pathways, specifically *FLT3* and *RAS*.<sup>7</sup> The frequency of *K/NRAS* mutations in our cohort was 21%, slightly higher than the general prevalence of *K/NRAS* mutations in newly diagnosed AML (10-15%).<sup>8</sup> Thus, *RAS* pathway activating mutations may represent a form of acquired resistance to BCL2 inhibition and predict lower response to salvage therapy. The infectious complications



**Figure 1. Median survival following treatment with cladribine, cytarabine (low-dose), venetoclax in 39 patients with acute myeloid leukemia, relapsed/refractory to venetoclax and hypomethylating agent, stratified by achievement of complete response or complete response with incomplete count recovery and allogeneic transplantation.** CAV: cladribine, cytarabine (low-dose), venetoclax; CR: complete response; CRi: complete response with incomplete count recovery; yr: year.



**Table 2.** Clinical characteristics and survival of seven patients with venetoclax/hypomethylating agent-relapsed/refractory acute myeloid leukemia receiving allogeneic stem cell transplant following salvage treatment with cladribine, cytarabine (low-dose), and venetoclax.

Age in years	Sex	AML type	Prior treatment response	ELN cytogenetic risk	Prior lines of therapy	Response to CAV	MRD	Days from remission to ASHCT	Overall survival in months	Relapse
42	M	<i>De novo</i>	Refractory	Adverse	daunorubicin + cytarabine, CLAG-M, decitabine + venetoclax	Aplasia	Not tested	100	11.5 Dead	Yes
42	M	<i>De novo</i>	Refractory	Adverse	daunorubicin + cytarabine, ziftomenib, decitabine + venetoclax	Refractory	Not tested	34	4.7 Dead	Yes
75	M	Secondary	Refractory	Adverse	decitabine + venetoclax	CRi	Not tested	18	7 Dead	Yes
67	F	<i>De novo</i>	Relapsed	Intermediate	azacitidine + venetoclax, ivosidenib	CRi	Not tested	54	10.5 Dead	No
62	F	<i>De novo</i>	Refractory	Intermediate	decitabine + venetoclax, tagraxofusp	CR	Negative	22	15 Alive	No
65	F	<i>De novo</i>	Relapsed	Intermediate	decitabine + venetoclax	CRi	Positive	16	10.3 Alive	No
73	M	<i>De novo</i>	Refractory	Intermediate	decitabine + venetoclax	CRi	Negative	17	7.5 Alive	No

AML: acute myeloid leukemia; ELN: European LeukemiaNet; CAV: cladribine, cytarabine (low-dose), venetoclax; CLAG-M: cladribine, cytarabine, granulocyte colony stimulating factor, mitoxantrone; CR: complete response; CRi: complete response with incomplete count recovery; MRD: measurable residual disease; M: male, F: female; ASHCT: allogeneic hematopoietic stem cell transplant.

observed in our study were comparable to those previously reported in the phase 2 study of CAV alternating with azacitidine in newly-diagnosed AML.<sup>4</sup>

Survival following CAV was marginally superior at 4.7 months than previously reported survival rates in AML patients following Ven-HMA failure not receiving salvage therapy (median OS ~ 3 months), although there are likely confounders, such as selection of relatively fit patients to receive additional lines of therapy, which preclude comparison. Notably, survival was superior in patients achieving CR/CRi following CAV, particularly in patients bridged to AHSCT. Our findings suggest the CAV regimen, while associated with a high risk of infectious complications, offers a therapeutic option for patients without targeted treatment options after failure of Ven-HMA and has salvage value as a bridge to AHSCT. The current study underlines efficacy of the CAV regimen in the setting of Ven-HMA failure, suggesting that all such cases may not be due to Ven resistance. On the other hand, in the instance of treatment resistance, the use of Ven has unveiled a new type of leukemia stem cell designated as monocytic leukemia stem cell which is resistant to Ven-azacitidine and addition of cladribine to the Ven-azacitidine regimen has been shown to eradicate these stem cells in both *in vitro* and *in vivo* preclinical models.<sup>9</sup> Next steps include incorporation of cladribine to Ven-HMA in the front-line setting with the goal to improve remission rates and reduce relapses.

## Authors

Nickolas Steinauer,<sup>1</sup> Kristen McCullough,<sup>1</sup> Aref Al-Kali,<sup>1</sup> Hassan B. Alkhateeb,<sup>1</sup> Kebede H. Begna,<sup>1</sup> Abhishek A. Mangaonkar,<sup>1</sup> Antoine N. Saliba,<sup>1</sup> Mehrdad Torghabeh,<sup>1</sup> Mark R Litzow,<sup>1</sup> William J. Hogan,<sup>1</sup> Mithun Shah,<sup>1</sup> Mrinal M. Patnaik,<sup>1</sup> Animesh Pardhanani,<sup>1</sup> Talha Badar,<sup>2</sup> Hemant Murthy,<sup>2</sup> James Foran,<sup>2</sup> Cecilia Arana Yi,<sup>3</sup> Ayalew Tefferi<sup>1</sup> and Naseema Gangat<sup>1</sup>

<sup>1</sup>Division of Hematology, Mayo Clinic, Rochester, MN; <sup>2</sup>Division of Hematology, Mayo Clinic, Jacksonville, FL and <sup>3</sup>Division of Hematology, Mayo Clinic, Scottsdale, AZ, USA

Correspondence:

N. GANGAT - gangat.naseema@mayo.edu

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### Disclosures

No conflicts of interest to disclose.

### Contributions

NS, KM, and NG, designed the study, collected data, performed analysis and co-wrote the paper. AA, HA, KHB, AM, AS, MH, MRL, WH, MS, MMP, AP, TB, HM, JF, CA, and AT contributed patients. All

authors reviewed and approved the final draft of the paper.

### Data-sharing statement

Please email the corresponding author.

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