

Clofarabine and intermediate-dose cytarabine after venetoclax failure in relapsed/refractory acute myeloid leukemia: a retrospective single-center experience

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Short Report

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Additional Declarations: No competing interests reported.

Abstract

Background

Resistance to venetoclax (VEN)-based therapies is a therapeutic challenge in acute myeloid leukemia (AML).

Objectives

To evaluate the efficacy of clofarabine in combination with intermediate-dose cytarabine in patients with VEN-resistant AML.

Methods

Two real-world cohorts of relapse/refractory (R/R) adult AML patients: a VEN-failure cohort (n = 9) and a pre-VEN cohort (n = 33). Patients received clofarabine 22.5 mg/m²/day and cytarabine 1 g/m²/day D1-5.

Results

In the VEN-failure cohort 11% of patients achieved partial response (PR), 89% were refractory (ORR 11%). The median OS was 2.0 months. Early mortality occurred in 33% of patients. In the pre-VEN cohort 33% of patients achieved complete remission, 6% PR (ORR 39%). The median OS was 4.0 months. Early mortality was recorded in 3% of patients.

Conclusions

In this limited, retrospective single-center experience, clofarabine plus intermediate-dose cytarabine showed minimal clinical activity and an unfavorable early toxicity profile in VEN-exposed R/R AML patients.

Plain language summary

Limited benefit of clofarabine and cytarabine in patients with acute myeloid leukemia after venetoclax failure

Acute myeloid leukemia (AML) is an aggressive blood cancer. Many patients are treated with venetoclax, but in some cases the disease stops responding to this treatment. When this happens, treatment options are limited and outcomes are often poor.

This study looked at whether a combination of two chemotherapy drugs, clofarabine and cytarabine, could help patients whose AML had come back or did not respond after venetoclax treatment. Researchers reviewed past cases of patients treated at a single hospital in Florence (Italy). They compared two groups: one treated with clofarabine and cytarabine after venetoclax failure, and another treated with the same chemotherapy before venetoclax was widely used.

The results showed that the chemotherapy combination had very limited benefit in patients whose disease had already failed venetoclax. Only 1 out of 9 patients showed some response, and overall survival was short. In contrast, patients treated before the use of venetoclax had better outcomes, with more patients responding to treatment and slightly longer survival. Early deaths were also more frequent in the group previously treated with venetoclax.

In summary, this study suggests that clofarabine and cytarabine may not be effective for patients whose AML no longer responds to venetoclax. These findings highlight the need for new and better treatment options, ideally tested in clinical trials, for this difficult-to-treat group of patients.

Introduction

Venetoclax (VEN) combined with azacitidine (AZA) has been established as the standard of care for elderly or unfit patients with newly diagnosed acute myeloid leukemia (AML), based on the results of the randomized, placebo-controlled, phase III, VIALE-A trial.[1] However, with a median follow-up exceeding 40 months, updated data indicate a continued decline in survival outcomes among patients receiving VEN-AZA in the first-line setting: most patients who initially responded eventually develop treatment resistance over time.[2] Furthermore, VEN-based combinations are widely employed in the relapsed/refractory (R/R) setting, with overall response rates (ORR) ranging from 20 to 60%, however, with generally short duration of response [median overall survival (OS) 5.0-10.7 months] and limited survival benefit unless followed by an allogeneic hematopoietic stem cell transplantation (HSCT).[3]

Resistance to VEN-based regimens, whether primary or acquired, is a growingly relevant issue in AML and represents a major therapeutic challenge.[4] While patients harboring specific molecular alterations may occasionally benefit from targeted therapies (e.g., *FLT3* inhibitors [5], *IDH1/2* inhibitors [6, 7] or menin inhibitors [8]), most VEN-experienced R/R AML patients lack actionable molecular targets and conventional salvage chemotherapy has yielded disappointing results, with ORRs ranging from ~ 20% to 40% - depending on the salvage regimen used and the disease setting. [9, 10] Even more challenging is the setting of disease relapse after allogeneic HSCT, where short-term survival outcomes are particularly poor.

Clofarabine is a rationally designed, second-generation purine nucleoside analogue incorporating the best pharmacokinetic and pharmacodynamic properties of fludarabine and cladribine. Combined with intermediate dose cytarabine and often used in the context of sequential, reduced-intensity conditioning HSCT platform, clofarabine has been explored as a salvage option for R/R AML in the “pre-VEN” era. The rationale of combining clofarabine with cytarabine stems from in vitro evidence of increased intracellular

cytarabine triphosphate concentrations and consequently augmented antileukemic activity of Ara-C on clofarabine-primed leukemic cells. [11]

In a historical multicenter cohort of 47 patients treated with clofarabine and cytarabine as second or subsequent line of therapy, an overall response rate (ORR) of 60.5% was reported, with acceptable toxicity.[12] Interestingly, this and other studies [13], suggested activity of clofarabine combinations regardless of adverse genetics.

Since disease relapse after VEN is driven by biological changes such as reversal of apoptotic dependency on BCL-2 and increased reliance on alternative anti-apoptotic proteins (i.e., MCL-1, BCL-xL), as well as enrichment in adverse-risk molecular profiles (i.e., *TP53*, *RAS/MAPK* gene mutations) [4], it would be of interest to assess whether clofarabine in association with cytarabine may exert meaningful clinical activity in R/R patients after VEN exposure.

Methods

Study design and patients

To address the issue of effectiveness of clofarabine in combination with intermediate-dose cytarabine in patients with VEN-resistant R/R AML, we evaluated, in a real-world setting, the outcomes of patients treated with this regimen after VEN failure. To this purpose, we analyzed two retrospective cohorts of adult patients with R/R AML treated at Careggi University Hospital (Florence, Italy): (i) a “VEN-failure” cohort, including 9 consecutive patients treated between 2017 and 2025 after documented failure of VEN-based therapy; and (ii) a “pre-VEN” cohort, including 33 consecutive patients treated with clofarabine and intermediate dose cytarabine between 2009 and 2019.

Treatment

Both cohorts received the same treatment schedule: clofarabine was administered at a dose of 22.5 mg/m² as a 1-hour intravenous infusion daily for 5 consecutive days, followed by cytarabine at 1 g/m² as a 3-hour intravenous infusion (starting 3 hours after the end of clofarabine infusion) once daily for 5 consecutive days. Antimicrobial prophylaxis was administered as per institutional practice. Early mortality was defined as death within 30 days from treatment start, regardless of cause. Response assessment was conducted in accordance with established criteria.[14, 15]

Statistics

Statistical analyses were conducted using IBM SPSS Statistics, version 31.0.1.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics are presented as counts and percentages for categorical variables and as median for continuous variables. Survival rates were estimated using the Kaplan–Meier method. Given the small sample size of the VEN-failure cohort, formal statistical testing between the two groups was

not performed. Data from the pre-VEN cohort are presented for historical and contextual purposes only and should not be interpreted as a direct statistical comparison

Results

Characteristics and outcome of patients in the “VEN-failure” cohort

The clinical characteristics of patients of the “VEN-failure” cohort (n = 9) are detailed in **Table 1**. At baseline, patients were a median age of 57 years (range 36–65), 5 (55.5%) were females. Two patients (22.2%) had therapy-related AML. Seven patients (77.8%) were classified as ELN 2022 adverse risk category: three patients harbored adverse karyotype [n = 1 complex karyotype; n = 1 inv(3); n = 1 monosomy 7], while the other patients harbored adverse risk gene mutations. One patient (11.1%) was classified as ELN 2022 intermediate risk category, one patient (11.1%) was unclassifiable due to missing NGS data. All patients had previously received VEN in combination with azacitidine and 7 (77.8%) had previously been exposed to high-dose cytarabine. The median number of prior lines of therapy was 2 (range 2–4). Five patients (55.5%) received clofarabine treatment in the setting of disease relapse after HSCT. In terms of clinical efficacy, one patient (11.1%) achieved partial response (PR) and proceeded to HSCT while on active disease, while 8 (88.9%) were refractory, one of whom was bridged to HSCT. The overall response rate (ORR) was 11.1% and CR rate was 0%. At a median follow-up of 2.0 months (95% confidence interval, 0.0 to 4.9), the median event free survival (EFS) was 1.0 months (95% confidence interval, 0.5 to 1.4) and the median OS was 2.0 months (95% confidence interval, 0.0 to 4.9) (**Fig. 1**). No patient in the VEN-failure cohort was alive at the time of last follow-up. When restricting the analysis to patients with adverse-risk disease according to ELN 2022 (n = 7), 1 patient (14.2%) achieved PR, resulting in an ORR rate of 14.2%. Gastrointestinal toxicity was the most frequently reported adverse effect, with nausea, vomiting, diarrhea, and mucositis occurring in most patients, followed in frequency by febrile neutropenia, skin rashes and reversible hepatic abnormalities. Mortality within 30 days occurred in 3 cases (33.3%), mostly due to infectious complications during prolonged cytopenia.

Characteristics and outcome of patients in the “pre-VEN” cohort

The characteristics of patients treated with clofarabine and cytarabine in the “pre-VEN” cohort (n = 33) are outlined in **Table 2**. The pre-VEN cohort partially, but not entirely, overlaps with the multicenter historical cohort described above [12]. At treatment initiation, the median age of the cohort was 54 years (range 23–73), 15 (45.4%) were females. Three patients (9.1%) had therapy-related AML and 3 (9.1%) had secondary AML. According to the ELN 2017 classification, 19 patients (57.6%) were classified as intermediate-risk, 12 (36.4%) were adverse and two (6.0%) favorable. Next-generation sequencing data were unavailable except for two cases at disease relapse. Eleven patients (33.3%) displayed adverse cytogenetic abnormalities, including complex karyotype (n = 6), monosomy 7 (n = 3) and 5q deletion (n = 2). Twenty-nine patients (87.9%) had received prior high-dose cytarabine-based therapy. Three patients

(9.1%) were in relapse after allogeneic HSCT. The median number of previous treatment lines was 1 (range 1–4). Regarding treatment efficacy, 11 patients (33.3%) achieved complete remission (CR), 2 patients (6.1%) achieved PR, whereas 19 patients (57.6%) experienced refractory disease. One patient died from infectious complications before efficacy was evaluated. This translated into an ORR of 39.4%. Of the 11 patients experiencing CR and for whom measurable residual disease (MRD) data were available (n = 9, 81.8%), 3 obtained MRD negativity (33.3%). Ten of the responding patients were able to proceed to allogeneic HSCT (76.9%). At a median follow-up of 87.0 months (95% confidence interval, 37.0 to 101.0 months), the median EFS was 1.0 months (95% confidence interval, 0.1 to 1.8) and the median OS was 4.0 months (95% confidence interval, 0.6 to 7.3) (**Fig. 1**). When analyzing the group of patients with adverse risk disease according to ELN 2017 (n = 12), 3 patients (25.0%) achieved CR and 1 patient (8.3%) PR, with a resulting ORR 33.3%. Overall, the toxicity profile appears similar to that observed in the VEN-failure cohort, although a lower number of grade 3–4 events were reported. Early mortality within 30 days was recorded in 1 patient (3.0%), due to treatment-related septic shock.

Discussion

Taken together, these real-world data suggests that while clofarabine combined with intermediate dose of cytarabine exhibited some moderate activity in the pre-VEN era (ORR 39.4%, CR 33.3%, mOS 4 months), it conversely provided minimal to no benefit in VEN-exposed R/R AML patients (ORR 11.1%, CR 0%, mOS 2 months), with an unfavorable safety profile and a limited potential for HSCT bridging. Although this comparison was limited by its retrospective and non-simultaneous nature, both cohorts received the same treatment schedule and were managed at the same Institution, allowing a consistent assessment of treatment efficacy across different therapeutic eras. We acknowledge that the inferior outcomes observed in the VEN-failure cohort may reflect not only prior venetoclax exposure but also several confounding factors, including heterogeneous baseline genetic risk, time to relapse, number of prior therapy lines, proportion of post-HSCT relapses, and era of supportive care. These same factors, particularly the higher rate of post-HSCT relapse and the more extensive prior treatment history, may also have contributed to the higher treatment-related mortality observed in the VEN-failure cohort. Furthermore, the limited sample size and the disparity in follow-up duration make comparisons of survival outcomes very difficult.

Conclusion

Notwithstanding the limitations, our findings suggest that clofarabine may not be effective in overcoming VEN resistance and does not provide a meaningful clinical benefit as a salvage option in this setting. Priority should be given to promoting the early inclusion of R/R patients in clinical trials after VEN failure.

Declarations

Ethics approval and consent to participate:

the study was approved by the local institutional review board (IRB: project MYNERVA, approval 14560). Written informed consent was obtained from study patients in accordance with the Declaration of Helsinki.

Consent for publication:

Not applicable. This manuscript does not contain any individual person's data in any form. All data are presented in an aggregate form.

Trial registration number/date

Not applicable

Competing interests

F.M.: Speaker bureau Blueprint, Novartis, Abbvie, Servier, GSK, Astellas; F.C., G.C., A.P., J.C., G.C, C.L., M.P. and B.S. none; P.G.: Speaker bureau AbbVie, GSK, AOP, and Novartis; A.M.V.: Speaker bureau AOP, Blueprint, BMS, GSK, Incyte Novartis.

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Author Contribution

GC: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing - original draft. FM: Conceptualization, Supervision, Writing - original draft, Writing - review & editing. PG, AMV: Supervision, Writing - review & editing. MP, BS: Data curation, Investigation, Methodology, Supervision, Writing - review & editing. FC, PA, JC, CL, GC, AM: Data curation, Investigation, Methodology. All authors have read and agreed to the published version of the manuscript.

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Data Availability

The data that support the findings of this study are available on request from the corresponding author.

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Figures

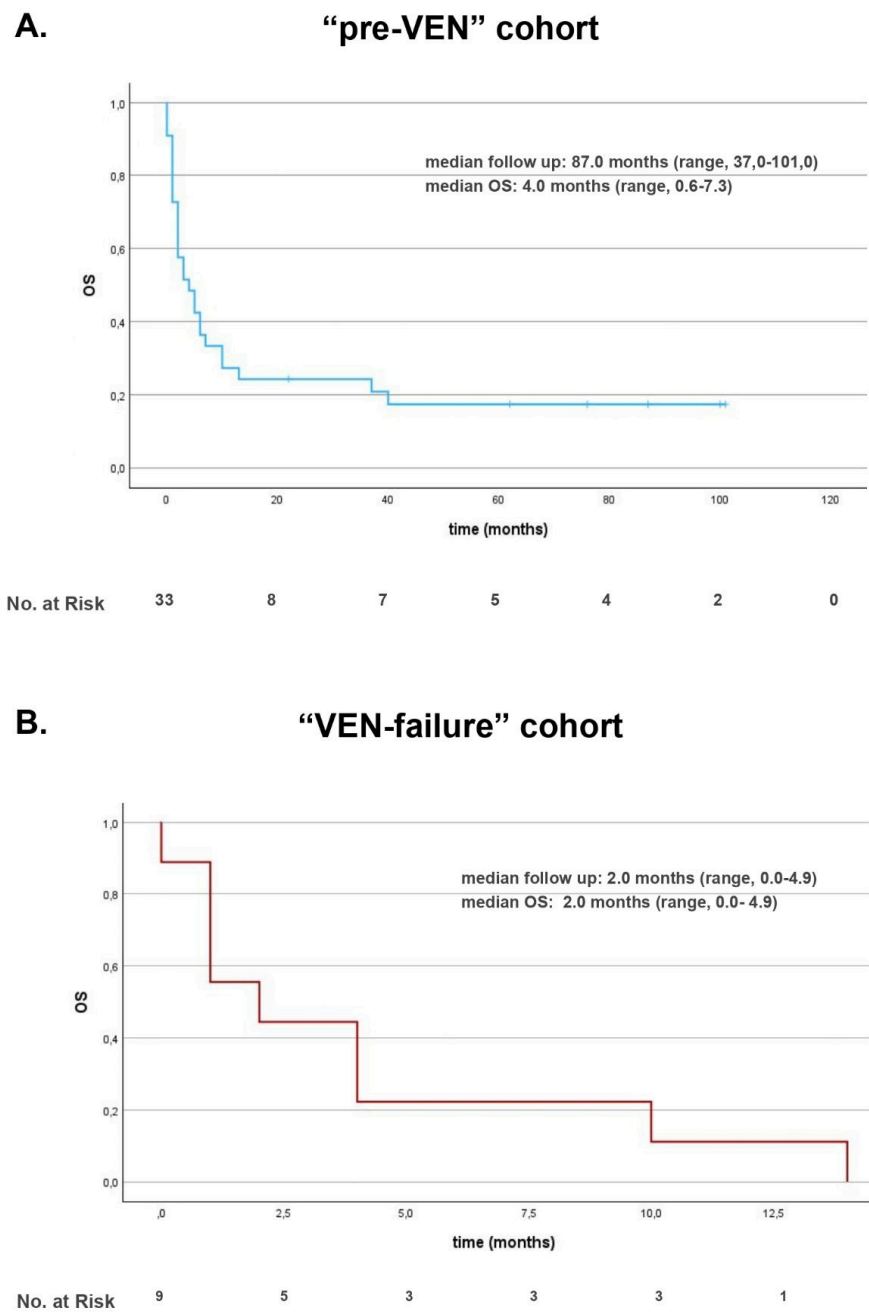


Figure 1. Outcome of patient of the “pre-VEN” cohort and the “VEN-failure” cohort: panel A: overall survival (OS) of patients of the “pre-VEN” cohort; panel B: OS of patients in of the “VEN failure” cohort

Figure 1

Legend not included with this version.

Supplementary Files

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- [floatimage2.jpeg](#)