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Running title: AML treated with venetoclax and azacitidine

Real-world clinical outcomes of newly diagnosed AML unfit for intensive chemotherapy treated with venetoclax and azacitidine: does it depend on the number of days of venetoclax administration?

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Until 2020, patients with acute myeloid leukemia (AML) who were not suitable for intensive treatment were mostly limited to symptomatic and palliative care, or to low-intensity regimens including low-dose cytosine-arabinoside (ARA-C) and azacitidine (AZA) monotherapy, which didn't bring much benefit. The situation changed with the arrival of venetoclax, a Bcl-2 inhibitor that causes leukemic cells to rapidly undergo apoptosis. The aim of our retrospective study was to summarize the treatment outcomes of all newly diagnosed patients with AML, unsuitable for intensive chemotherapy, treated with a combination of venetoclax and AZA at the Department of Hematology and Transfusion Medicine, University Hospital in Bratislava from January 1, 2021, to December 31, 2025. A total of 108 patients underwent treatment with a median follow-up of 35.9 months; median age was 69.5 years (47-84 years) and median number of cycles 3 (1-34). Induction mortality rate was 7.4%, and tumor lysis syndrome occurred in 4.5%. The overall response rate, which included the number of complete remissions, complete remissions with incomplete hematopoietic recovery, and morphologically leukemia-free status (CR/CRi/MLFS), was 64%, the median overall survival was 9 months, and disease-free survival was 8 months. As of December 31, 2025, 23 patients are alive and 85 have died. The main cause of death was the progression of the disease. The main contribution of our study is the finding that shortening the duration of venetoclax treatment maintains efficacy. There was no significant difference in remission rate achieved or in overall survival based on the number of days of venetoclax administration (< 14 vs. 14 vs. 21 vs. 28 days). The combination of AZA and venetoclax in AML has proven to be highly effective in inducing complete remission, usually immediately after the first cycle. Unfortunately, remission is not long-lasting, relapses are frequent, and the median overall survival in real-world data is 7.9 to 13.6 months.

Key words: acute myeloid leukemia; azacitidine and venetoclax; clinical characteristics; survival; dose-dependence of venetoclax

For many decades, before introduction of venetoclax into acute myeloid leukemia (AML) therapy, patients considered unfit for intensive chemotherapy were managed supportively. Since 2020 situation has changed dramatically - based on the results of VIALE-A trial-venetoclax (VEN) in

combination with azacitidine (AZA) has become the standard of care for the first line treatment of AML for unfit patients. This trial showed that AZA plus VEN compared to AZA monotherapy induced a significantly higher CR/CRi rate (66.4% vs. 28.3%) with significant improvement in the median OS (14.7 months vs. 9.6 months) [1]. As expected, routine clinical practice and data from real-world studies (RWS) showed poorer outcomes. The median overall survival (OS) for AZA-VEN in RWS ranges from 7.9 to 13.6 months [2-7]. The most significant complication of the AZA-VEN regimen is its myelosuppression that is associated with a higher risk of infectious complications. It remains an open question whether shortening the duration of venetoclax administration-which results in a shorter period of neutropenia during induction and minimizes the risk of infectious complications-will affect the remission rate and overall survival. Only few retrospective studies suggest that shorter VEN cycles may maintain efficacy with enhanced safety [8-10]. The main goal of our study, is to analyze characteristics and outcomes of AML patients treated with AZA-VEN, that have not yet been published by any hematology center in Slovakia. The secondary goal is to analyze whether shortening the duration of venetoclax treatment maintains efficacy while reducing myelotoxicity.

Patients and methods

Study design. This was a single-center retrospective analysis of AML patients treated at the University Hospital in Bratislava, Slovakia. Analysis included all newly diagnosed patients treated with combination AZA-VEN from January 2021 (when venetoclax became available) to December 2025. The diagnosis was established from a bone marrow aspirate based on morphological examination and flow cytometry. All patients had done cytogenetic analysis performed by standard G-banding and molecular testing performed by polymerase chain reaction (PCR). The study was approved by the local Ethics Committee of University Hospital Bratislava (Approval no. EK 1/3/2025). Written informed consent was obtained from all patients.

Profiling, stratification and treatment. Since January 2023 mutational profiling was performed by next-generation sequencing (NGS), using 58 genes (oncoReveal Myeloid Panel). Patients were risk-stratified based on the 2022 European LeukemiaNet (ELN) criteria [11]. In addition, we also retrospectively applied ELN 2024 classification, created for patients treated with less intensive therapy, based on 4-gene prognostic model, which includes *TP53*, *FLT3*-ITD, *NRAS*, and *KRAS* mutations [12]. Secondary AML was defined as having had a documented antecedent hematologic neoplasm. Patients with therapy-related AML received prior chemotherapy and/or radiotherapy for another malignancy.

The treatment protocol consisted of azacitidine 75 mg/m^2 subcutaneously days 1-7, plus venetoclax 100 mg on day 1, 200 mg on day 2, then 400 mg daily starting on day 3, for 7, 14, 21 or 28 days- depending on the physician's decision. The decision was made at the start of treatment, we mainly used VEN 21-28 during the first years after the regime was introduced (2021-2022) and VEN ≤ 14 in the later period (2023-2025), especially in frail patients. All patients after the initial ramp-up who had severe neutropenia with an ANC below 0.5 × 10^9/l received antifungal prophylaxis with posaconazole, and the dose of venetoclax was reduced to 100 mg daily. No other anti-infective prophylaxis was administered. Those patients at high risk of tumor lysis syndrome (TLS)-with high white blood count, renal impairment and elevated uric acid-were admitted to hospital for the ramp-up and then discharged if stable, while others were treated entirely on an outpatient basis, unless requiring admission for febrile neutropenia or other complication. Transfusion thresholds were generally 15 × 10^9/l for platelets and hemoglobin 85 g/l for red cells. Remission status was assessed by BM aspirate on day 21-28 of cycle 1. Patients with residual leukemia immediately proceeded to a second identical course; those in a leukemia-free state received G-CSF Filgrastim and after hematologic recovery (ANC above 1.0 ×10^9/l and Plt above 50 × 10^9/l) proceeded to maintenance therapy with AZA-VEN. Dose adjustments during post remission maintenance therapy were made based on hematologic toxicity according to physician judgment. The most common dosage adjustment for recurrent G4 neutropenia/thrombocytopenia lasting ≥ 7 days was shortening VEN administration to 14 days or less, and for severe bone marrow hypocellularity, shortening AZA administration to 5 days. Overall survival (OS) was defined as the number of days from the date of diagnosis to the date of death. Disease-free survival (DFS) was defined as the time from achieving complete remission to relapse or death, whichever occurs first. Responders included patients who achieved complete remissions, complete remissions with incomplete hematopoietic recovery (CR, CRi) and morphologic leukemia-free state (MLFS), non-responders included patients who achieved partial response (PR), and progressive disease (PD) using the revised International Working Group (IWG) response criteria [13].

Statistics. Statistical analyses were performed using the IBM SPSS software (version 26.0). Categorical variables were analyzed and compared by Chi square analysis. Survival determinations were made using the Kaplan-Meier method and compared using the log rank test. Two-sided p-values of less than 0.05 were considered significant.

Results

We treated 108 patients, median age 69.5 years (47-84 years), median follow-up 35.9 months. The patient characteristics are shown in Table 1. Antecedent hematologic neoplasm included 9 patients

(8%) with myelodysplastic neoplasms (MDS), all treated with AZA (median number of submitted cycles was 7), 7 patients (6%) with MDS/MPN overlap syndrome and 4 patients (4%) with myeloproliferative neoplasms (MPN). 16 patients underwent aloHSCT. Per ELN 2022 criteria, 14 patients (13%) had favorable, 47 (43,5%) intermediate, and 47 (43,5%) adverse risk diseases. Using ELN 2024 classification based on 4-gene prognostic model, 76 patients (70%) had higher benefit, 15 (14%) intermediate benefit, and 17 (16%) lower benefit disease. NGS mutation profile was performed to 51 patients. The most common mutations detected at baseline included NPM1 (14%), ASXL1 (10%), SRSF2 (10%), RUNX1 (10%), FLT3-ITD (10%) and others listed in Figure 1. Before starting treatment with AZA-VEN, cytoreduction with hydroxyurea was necessary in 30 patients (28%), Ara-C 500 mg IV given in 2 doses a12 hours were administered to 4 patients, and leukapheresis was performed in 3 patients. Laboratory tumor lysis syndrome occurred in 4.5% of cases. Induction mortality within 30 days of treatment initiation was observed in 8 patients (7.4%). They died from infectious complications (5×), treatment toxicity (2×), and CNS hemorrhage (1×). From 01/2021 to 04/2023 we recorded 7 deaths, from 05/2023 to 12/2025: 1 death. The first cycle of treatment was administered on an outpatient setting in 33% of patients, while the remaining 67% were hospitalized. The median number of cycles administered was 3. The overall response rate (CR/CRi/MLFS) was 64%, while the partial or no response rate was 36%. The therapeutic response occurred immediately after the first cycle in up to 80% of cases. Seven patients have maintained complete remission for more than three years. The median overall survival for the entire patient group was 9 months, and disease-free survival was 8 months (Figure 2). If the patient did not achieve remission during treatment, the median survival was only 4 months. CR/CRi rates were similar in patients receiving VEN for <14 (n=32, 56%) versus 14 (n=41; 56%) versus 21 (n=20; 60%) versus 28 days (n=13; 77%; p=42). In addition, the median OS according to the number of days of venetoclax administration < 14 days, 14 days, 21 days, and 28 days was also not statistically significant 8, 11, 8, and 9 months, respectively (p=0.884; Figure 3). In 2 patients the number of VEN days was unknown. Using the ELN 2022 risk classification median OS among the favorable, intermediate, and adverse risk groups were not reached, 11 and 8 months (p=0.005). However, upon using the 4-gene prognostic model, according ELN 2024 classification median OS among the higher, intermediate, and lower benefit risk groups were 11, 11 and 7 months (p=0.013; Figure 4). Seventeen patients with TP53 mutation, 11 of whom had also complex karyotypes, responded less well to treatment, with an ORR of 41%, and all but one relapsed. Their median overall survival was 7 months. The median overall survival of patients with baseline leukocytosis above 50×10^9/l and below 50×10^9/l was not significantly different, 10 vs. 9 months. Of the 16 patients who underwent alloHSCT, 5 patients are still alive and 11 died, 5 of them from early relapse and 6 from transplant-

related mortality (acute intestinal GVHD-2×, graft failure, intracranial hemorrhage, post-transplant lymphoproliferative disorder-PTLD and sepsis). The median overall survival of patients who underwent alloHSCT vs. those who did not undergo alloHSCT was not significant, 10 months vs. 9 months (p=0.558) (Figure 3). Patients with extramedullary disease survived for a median of 4 months. Of the total group of 108 patients, 23 are alive and 85 have died. The causes of death were progressive disease (78%), infections (8%), transplant related mortality (7%) and other causes (7%) that are listed in Table 2. Nine patients died in complete remission.

Discussion

The median overall survival result for our cohort, which is 9 months, is comparable to RWS, where it ranges from 7.9 to 13.6 months. These real-world results are significantly worse than those reported in the Viale A registry study, which showed a median survival of 14.7 months. One explanation may be that studies from routine clinical practice also included patients who were already receiving hypomethylation therapy for a previous MDS, secondary AML after MPN, MDS/MPN, and AML with extramedullary involvement, who were excluded from the Viale A study.

The AZA-VEN regimen is safe when we learn how to use it correctly. The risk of TLS is low if we start treatment when the white blood cell count is less than 25 × 10^9/l and we take all preventive measures-adequate hydration, allopurinol, and gradual ramp-up of venetoclax (100 mg - 200 mg - 400 mg) [14]. The most serious complications of treatment result from long-term cytopenia. In particular, secondary AML after MDS, which we treated with venetoclax for 28 days, reconstituted the blood count very slowly and was subject to serious infectious complications. Between August 2020 and April 2023, we had seven deaths. Subsequently, when we began to modify the number of days of venetoclax administration to 21 or 14 days, the number of deaths decreased. Between May 2023 and December 2025, we recorded one death.

It is known that such a shortened regimen shortens the duration of cytopenia, but it is questionable whether it doesn't lead to poorer responses to treatment and poorer overall survival. The greatest benefit of our work is the finding that despite the high number of shortened regimens-68% of patients received venetoclax for 14 days or less-the number of overall responses was comparable to the Viale A study, 64% vs. 66.4%. An equally important finding was that patients receiving 14 days of venetoclax or less had a comparable median overall survival to patients receiving 21 and 28 days of venetoclax. The data suggest that the regimen with fewer days of venetoclax administration is equally effective and less myelosuppressive with a lower risk of infectious complications than the full 28-day protocol regimen. We assume that shortening the administration of venetoclax doesn't

affect its effectiveness because the anti-leukemic effect with blast clearance happens very quickly. The most important is the time-dependent synergy between AZA and VEN during the first 7 days of cycle, when AZA primes the cells for apoptosis and VEN blocks BCL-2, which triggers MOMP (mitochondrial outer membrane permeabilization), leading to apoptosis.

The ELN 2022 classification is not suitable for AML patients receiving non-intensive treatment, as no significant differences in survival were observed between the groups. Mutations that worsen the prognosis in intensive chemotherapy treatment (e.g., myelodysplasia-related mutations such as ASXL1, RUNX1, SRSF2) do not have the same adverse prognostic significance in AZA-VEN treatment [15-16]. Consequently, the ELN 2024 classification was developed based on 4 genes: TP53, FLT3-ITD, NRAS, and KRAS. Our study confirmed that both classifications have prognostic significance; this was likely due to the fact that NGS did not become the standard of care until January 2023, and until then, patients with aforementioned MR genes remained undiagnosed and were classified as intermediate risk rather than adverse risk.

The AZA-VEN regimen is not considered curative. Nevertheless, our cohort includes seven patients who have been in complete remission for more than three years. The question of when it is safe to discontinue treatment and whether treatment can actually lead to a cure in a very small portion of patients remains unanswered. Unfortunately, most patients die from refractory or relapsed AML, so the possibility of allogeneic hematopoietic stem cell transplantation must always be considered. In some patients, 15% in our cohort, performance status improves after achieving remission to such an extent that they are able to undergo this treatment modality. HSCT did not bring to our 16 patients any benefit in terms of overall survival, which can be explained by the patients' high-risk genetic profile- especially by the presence of TP53 mutation and four HSCT were salvage-outside CR. Patients with TP53 mutation, which often occurs in conjunction with complex karyotype, represent a major medical problem. The number of achieved remissions is the lowest compared to other mutations, and remission has very short duration [17-18]. In our cohort only 5 months. The only chance for longer-term overall survival is HSCT, but this also helps only a very small percentage of patients.

Acknowledgements: This study has limitations that must be acknowledged. The non-randomized allocation of treatment duration with venetoclax introduces potential selection bias, particularly as frailer patients were more likely to receive shorter therapeutic cycles, which may have confounded outcome assessments. Prospective validation is warranted to refine risk-adapted dosing strategies.
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Figure Legends

Figure 1. Frequency of genetic aberrations.

Figure 2. Overall survival of all patients (N=108) and Disease free-survival of the CR/CRi patients (N=65).

Figure 3. Overall survival: According to venetoclax dosage and HSCT performed.

Figure 4. Overall survival: According to ELN 2022 and ELN 2024 risk classification.

Table 1. Patient characteristics.

Total	**108**
Age, median (range), years	69.5 (47-84)
male/female	53/55
ELN 2017/2022 risk category	
favorable	14 (13%)
intermediate	47 (43.5%)
adverse	47 (43.5%)
ELN 2024 classification	
higher benefit disease	76 (70%)
intermediate benefit disease	15 (14%)
lower benefit disease	17 (16%)
Prior HMA for MDS	9 (8%)
Secondary AML	20 (18%)
Therapy-related	4 (4%)
Underwent aloHSCT	16 (15%)
Extramedullary disease	7 (7%)

327 **Table 2.** Causes of death.

Refractory disease	**29**
Relapse	**37**
Infection	7
TRM	6
Toxicity	3
CNS hemorrhage	1
Pulmonary embolism	1
Unknown	1

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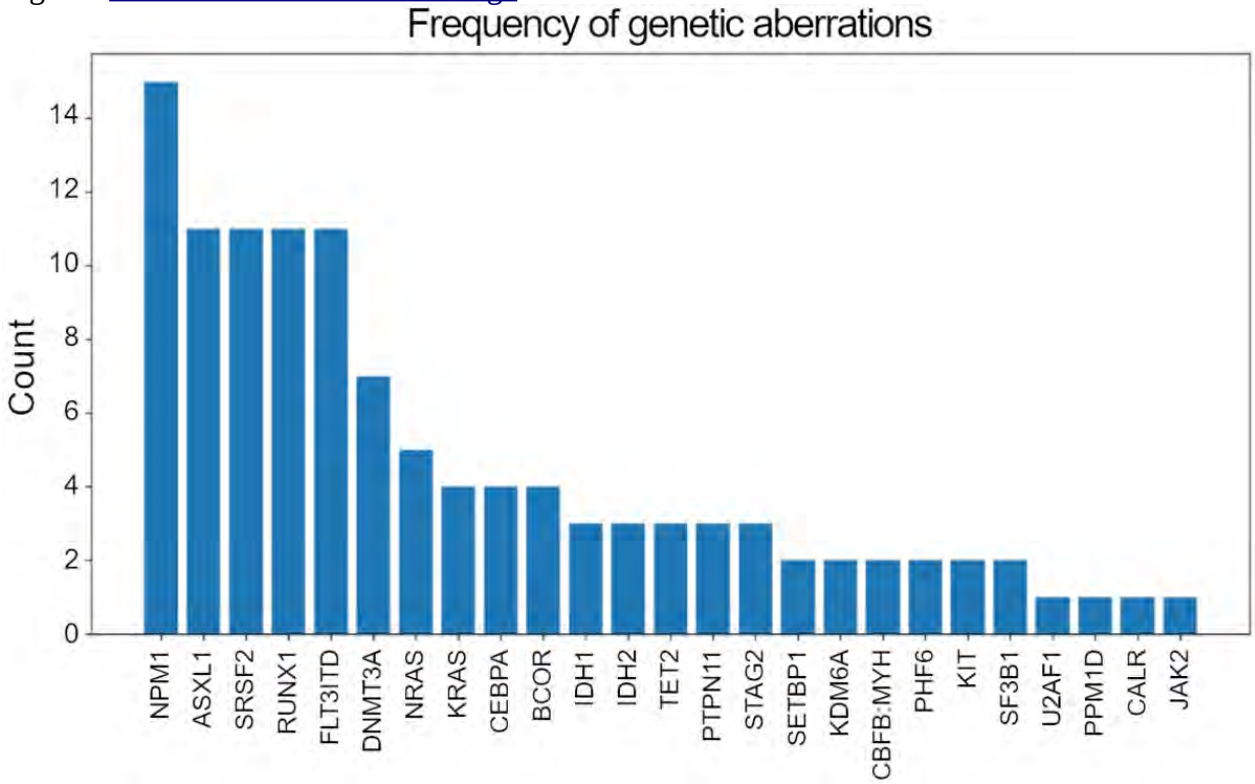

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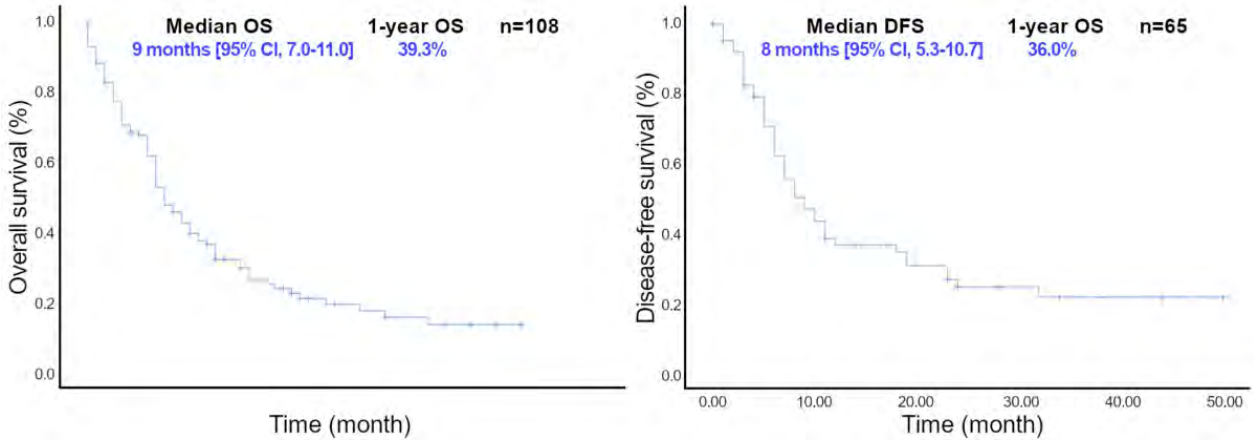

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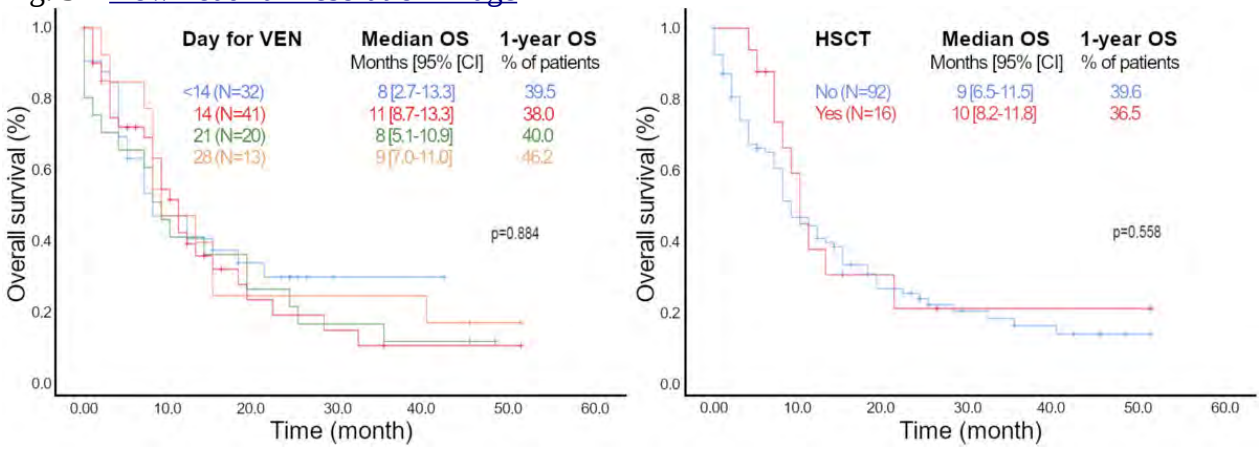

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