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– ORIGINAL PAPER –

Triple Therapy with Azacitidine, Venetoclax and Gilteritinib as a Therapeutic Option for Adult Patients with Refractory/Relapsed FLT3 Mutated Acute Myeloid Leukemia – A Single Center Experience

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Abstract

Introduction: *FLT3 mutations occur in approximately 30% of acute myeloid leukemia (AML) cases and are associated with high relapse rates and poor prognosis. While second-generation inhibitors like gilteritinib have improved outcomes in the relapsed/refractory (R/R) setting, therapeutic resistance remains a significant challenge. This study evaluates the real-world experience of a single center using an off-label triplet regimen (Azacitidine, Venetoclax and Gilteritinib) as a bridge to allogeneic hematopoietic stem cell transplantation (allo-HSCT).*

Materials and Methods: *We conducted a retrospective, observational study between March 2022 and September 2025. Out of 78 identified FLT3-positive AML patients, 8 patients with R/R disease received the triplet therapy following partial response or disease progression on gilteritinib monotherapy.*

Results*: Within the subgroup who experienced disease progression under gilteritinib (n = 4 of 8), all patients achieved complete remission (CR) after receiving triple therapy and successfully underwent allogeneic hematopoietic stem cell transplantation (allo-HSCT). Regarding the safety profile, 63% of patients developed grade 3–4 hematologic toxicities (febrile neutropenia, anemia, thrombocytopenia), while hepatic cytolysis remained mild (grade 1–2), although it was observed in all patients (n = 8). At a median follow-up of 9 months, 75% of patients who underwent allo-HSCT post-triplet therapy remained alive.*

Conclusions*: Our findings suggest that the triplet combination of azacitidine, venetoclax and gilteritinib is a highly effective salvage strategy. Although careful monitoring is required due to moderate toxicities, particularly hematologic adverse events, it is a viable bridge to transplant for R/R FLT3-mutated AML patients who fail standard monotherapy.*

Keywords: *relapsed/refractory acute myeloid leukemia, FLT3 mutation, gilteritinib, venetoclax, azacitidine, triple therapy, allogeneic hematopoietic stem cell transplantation;*

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Introduction

In acute myeloid leukemia (AML), FLT3 mutations are among the most common genetic associated aberrations, being present in approximately 30% of newly diagnosed cases. Regarding age distribution, younger adults (aged 18–39 years) tend to have the highest prevalence of FLT3 mutations of all age groups evaluated in recent reviews, with paediatric and older populations having lower prevalence [1], [2].

From a prognostic perspective, FLT3 internal tandem duplications (ITDs), which occur in about 20–25% of adult AML patients, are most strongly associated with an aggressive disease course, increased relapse risk and inferior survival outcomes. In contrast, tyrosine kinase domain (TKD) point mutations are present in 5–10% of cases; however, their prognostic impact has not yet been clearly established. Both ITD and TKD mutations promote ligand-independent activation of FLT3 and persistent downstream signaling through the STAT5 and PI3K/AKT pathways. Furthermore, molecular features such as high variant allele frequency (VAF ≥ 0.5) and increased ITD length have been shown to correlate with poorer clinical outcomes. These parameters are therefore incorporated into the latest European Leukemia Net (ELN) risk stratification framework for AML (2022) and the risk classification for patients receiving less-intensive therapies (ELN 2024 Less-Intensive), where FLT3 mutations are classified as intermediate risk, regardless of the FLT3-ITD allelic ratio or NPM1 co-mutation status [1], [2], [3], [4].

Furthermore, it seems that FLT3 mutations, particularly FLT3-ITD, are frequently acquired as late events in leukemogenesis and often exist as subclonal lesions rather than founding mutations. Next-generation sequencing (NGS) has demonstrated that the mutation can be present in only a fraction of leukemic cells at diagnosis and minor FLT3-ITD-positive subclones may expand and become dominant at relapse, indicating dynamic clonal evolution, and, as a result, the need to retest patients at relapse [5], [6].

Clinically, FLT3-mutated AML is frequently characterized by pronounced hyperleukocytosis and a high burden of circulating and bone marrow blasts [7]. This presentation, together with the frequent association with myelomonocytic differentiation (FAB M5), confers a substantial risk of leukostasis and subsequent multi-organ dysfunction. The elevated tumor burden further predisposes these patients to tumor lysis syndrome, cytokine release syndrome and disseminated intravascular coagulation. Extramedullary involvement is also a recognized feature, with central nervous system (CNS) infiltration occurring at a notable frequency. In light of this evidence, a lumbar puncture for evaluation of leptomeningeal disease should be considered in patients presenting with extramedullary disease, leucocytosis at diagnosis (white blood cell count ≥50 × 109), FLT3–ITD mutated acute myeloid leukaemia or acute myeloid leukaemia with monocytic differentiation (ie, French-American-British classification M4 or M5). If a lumbar puncture is performed, prophylactic intrathecal cytarabine or methotrexate is recommended [2], [8].

Over the past two decades, FLT3 inhibitors have evolved from exploratory use to becoming an integral component of targeted therapy in FLT3-mutated AML. Midostaurin, a first-generation type I multikinase inhibitor active against both FLT3-ITD and TKD mutations, demonstrated a survival benefit in combination with standard chemotherapy in newly diagnosed patients in the RATIFY trial and has become the standard of care for fit patients in combination with the “7+3” regimen [9].

In the relapsed/refractory setting, gilteritinib — a more selective type I inhibitor targeting both ITD and TKD mutations—improved overall survival compared with salvage chemotherapy (9 months vs 5 months), achieved higher complete remission rates (34% vs 15%) in the ADMIRAL trial and remains the standard of care, although resistance and QTc prolongation still pose important challenges [2], [7].

Regarding long-term administration, in the ADMIRAL study, gilteritinib was continued in responding patients, and 38 patients remained on treatment at the time of publication, compared with none in the chemotherapy arm, demonstrating that prolonged FLT3 inhibition was tolerable and could be sustained in remission [9].

Overall, the proposed treatment algorithms for relapsed/refractory AML recommend at the moment consideration of FLT3-targeted therapy with gilteritinib in patients harboring FLT3 mutations, regardless of fitness status, as a preferred salvage strategy and potential bridge to allogeneic HSCT [10].

Building on the synergy between the inhibition of FLT3 and BCL-2, triplet regimens combining azacitidine, venetoclax and gilteritinib have been investigated. A phase 1b/2 study reported high remission rates in both newly diagnosed and relapsed/refractory FLT3-mutated AML, although treatment was associated with significant myelosuppression and infectious complications [11]. Similarly, a physician-choice–based study confirmed the activity of gilteritinib-based triplets, while emphasizing

the ongoing need to balance efficacy with toxicity, particularly in relation to venetoclax duration. Overall, the combination of a FLT3 inhibitor, venetoclax and a hypomethylating agent appears to be an effective option in relapsed/refractory AML and a valuable bridge to allogeneic HSCT [12].

As far as the maintenance therapy post-transplant is concerned, the MORPHO trial [13], randomized 356 patients with FLT3-ITD AML who underwent allo-HSCT to gilteritinib or placebo. Even though the relapse-free survival, the primary endpoint, was not met, there was a trend favoring gilteritinib. Subgroup analyses revealed that patients who were MRD-positive prior to or after transplant derived significant benefit from gilteritinib maintenance, whereas MRD-negative patients did not [14]. Subsequent analysis demonstrated that conditioning intensity modulated the impact of gilteritinib, with the greatest benefit observed in patients receiving reduced-intensity conditioning [15].

Given the lack of published national data, there is a clear need to document and evaluate the real-world experience of centers administering this triplet therapy off-label, particularly in Romania, in order to inform and optimize future therapeutic strategies in FLT3-mutated AML.

Material and methods

This is a retrospective, observational study conducted between March 2022, the date of regulatory approval of gilteritinib in Romania, and September 2025. The study population included adult patients (≥18 years) diagnosed with acute myeloid leukemia (AML) according to the World Health Organization (WHO) classification, with positive FLT3 mutations. Eligible patients had relapsed or refractory disease after at least one prior line of AML therapy and had previously received treatment with a FLT3 inhibitor according to national guidelines (gilteritinib monotherapy). The intervention consisted of off-label triple therapy with azacitidine, venetoclax and gilteritinib, administered as a bridge to allogeneic HSCT. Data collection and analysis were performed retrospectively using institutional medical records. Microsoft Office software (Word and Excel) was used for data processing, statistical analysis and manuscript preparation. Relapse-free survival (RFS) and overall survival (OS) were estimated using the Kaplan–Meier method. All patients provided written informed consent for treatment and data use in accordance with institutional and ethical standards.

Results

Seventy-eight patients diagnosed with FLT3-positive AML were identified over a period of 3 years and 6 months, of whom 10% (8 patients) received triple therapy. Among these, 37% (n = 3 of 8) were male and 63% (n = 5 of 8) were female. Patient age ranged from 19 to 62 years, with a median age of 44 years. The main characteristics of the analyzed patients are shown in Table 1.

Table 1. *Baseline characteristics of the study cohort*

Characteristic	***Relapsed/ Refractory AML (N = 8)***
Age, years	
Median (range)	*44 (19–62)*
Sex	
Female	*5 (62.5%)*
Male	*3 (37.5%)*
AML FAB subtype	
M0	*1 (12.5%)*
M1	*1 (12.5%)*
M2	*1 (12.5%)*
M3	*1 (12.5%)*
M4	*1 (12.5%)*
M5	*1 (12.5%)*
Not available	*2 (25%)*
FLT3 mutation status	
FLT3 mutated	*8 (100%)*
Cytogenetic abnormalities (FISH)	
del(17p)	*3 (37.5%)*
Trisomy 8	*2 (25%)*
Not available	*3 (37.5%)*

Response to gilteritinib monotherapy	
Partial response	*3 (37.5%)*
Progressive disease	*4 (50%)*
No response	*1 (12.5%)*
Allogeneic hematopoietic stem cell transplantation (HSCT)	
Performed	*4 (50%)*
Not performed	*4 (50%)*
Conditioning regimen for HSCT	
Reduced-intensity conditioning (RIC)	*3 (37.5%)*
Myeloablative conditioning (MAC)	*1 (12.5%)*

Regarding FAB classification, no subtype of AML showed a higher prevalence.

As first-line treatment, the majority of patients (38%, n = 3 of 8) received 7+3 induction therapy combined with the FLT3 inhibitor midostaurin. Other first-line therapeutic options used in these patients included 7+3 induction without FLT3 inhibitor, 2+5 induction with midostaurin, hypomethylating agents (HMA) plus venetoclax, depending on patient comorbidities and availability of FLT3 inhibitors.

The median duration of first remission was 6.5 months (range, 0-17 months). Relapse-free survival (RFS) was estimated using the Kaplan–Meier method in Figure 1.

At relapse, patients received FLT3 inhibitor therapy with gilteritinib as monotherapy (120 mg per day), according to guideline recommendations. Half of the patients achieved partial response (n = 4 of 8), while the other half experienced disease progression under gilteritinib treatment (n = 4 of 8). After disease assessment, off-label triple therapy (azacitidine 75 mg/mp for 7 days + venetoclax for 7, 14 or 21 days + gilteritinib 80 mg or 120 mg per day for 28 days) was initiated for all patients (n = 8). This therapeutic strategy was used as bridge therapy for patients with disease progression, who were scheduled for HSCT (n = 4 of 8). All patients in this subgroup received at least two treatment cycles and achieved CR before undergoing transplantation in our center.

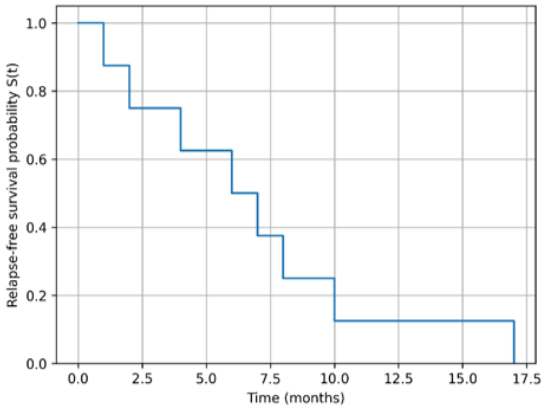

Figure 1. *Kaplan–Meier curve for relapse-free survival for first remission*

Regarding triple therapy tolerability, according to CTCAE definitions (v5.0), all patients (n = 8) experienced grade 1–2 hepatic cytolysis, 63% (n = 5 of 8) developed grade 3-4 hematologic toxicity (including anemia, thrombocytopenia, febrile neutropenia), and 13% (n = 1 of 8) presented grade 2 pruritic maculopapular rash.

Treatment-related toxicities are summarized in Table 2. ECG was performed periodically in all patients and no QT interval prolongation was reported. Dose adjustments were made for venetoclax in patients receiving simultaneous azole treatment. Overall, triple therapy was well tolerated and the adverse reactions were manageable.

Table 2. *Toxicities of triple therapy*

Outcomes	Details	N (%)
Hepatocellular toxicity	Grade 1-2	100%
Hematological toxicity	Grade 3-4	63%
Skin rash	Grade 2	13%

As far as the transplantation procedure is concerned, 75% (n = 3 of 4) received reduced-intensity conditioning (RIC) and underwent allogeneic HSCT from a matched unrelated donor, while 25% (n = 1 of 4) received myeloablative conditioning (MAC) and underwent haploidentical transplantation from a related donor. All patients got maintenance therapy with gilteritinib after HSCT.

At the time of this retrospective analysis, the outcomes of patients who underwent HSCT (as per Table 3) were as follows: one patient who was MRD-positive by PCR prior to bone marrow transplantation (BMT) experienced disease relapse at 4 months post-transplant, with persistent MRD positivity. Another MRD-positive patient prior to BMT remains under follow-up at 6 months post-transplant. One patient who was MRD-negative before BMT died at 11 months post-transplant due to a cause unrelated to the hematological disease (probably an acute viral infection), with MRD remaining negative at hospital admission. Another MRD-negative patient before BMT experienced disease relapse at 26 months post-transplant, while MRD status remained negative.

Table 3. *Outcomes of patients who underwent HSCT*

Patient no.	***MRD PCR before HSCT***	***Time until event***	***Event***	***MRD PCR at event***
1	+	*4 months*	*Relapse*	+
2	+	*6 months*	*Still under follow-up*	*NA*
3	-	*11 months*	*Death*	-
4	-	*26 months*	*Relapse*	-

Among patients who did not undergo transplantation, clinical outcomes were as follows: two patients experienced disease relapse. One relapsed after the first cycle of triple therapy, developed neurological involvement with a fatal outcome. The second patient relapsed after four cycles of triple therapy, developed bronchopneumonia and subsequently died. The other two patients are currently alive and continuing treatment, having completed three cycles of triple therapy to date with good tolerability and are scheduled for disease reassessment in the following month.

Survival analysis was performed using the Kaplan–Meier method (Figure 2) to evaluate overall survival from the time of diagnosis to the date of data collection in this retrospective study. During this period, patients received multiple lines of treatment, not limited to the treatment line specifically investigated in the study (triple therapy). The survival curve demonstrated a gradual stepwise decline over time, reflecting the occurrence of death events. The estimated survival probability showed a decrease after approximately 12 months from diagnosis, with additional reductions observed around 19–21 months. Censored observations corresponded to patients who were alive at the last follow-up or who had not experienced the event by the time of analysis. The majority of events occurred within the first 25 months of follow-up.

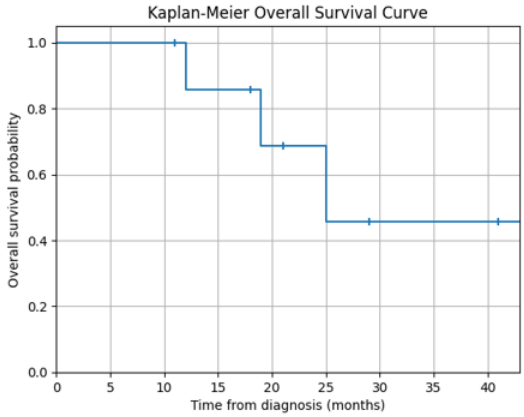

Figure 2. *Overall survival from diagnosis to the time of data collection*

Discussions

Our response rates were slightly lower (50%) compared to previous studies on relapsed/refractory cohorts, for example, Short et al. who reported a response rate of almost 70% (27% with CR/CRi; 41% with morphologic leukemia–free state) [11].

The most common adverse events of grade 3 with triple therapy were hematological (febrile neutropenia, anemia, thrombocytopenia), consistent with the known safety profile of venetoclax and the results of the ADMIRAL study for gilteritinib [7], [16]. In the latter, the most common serious adverse events that were considered to be related to gilteritinib therapy were febrile neutropenia, increase in the alanine aminotransferase level and increase in the aspartate aminotransferase level, while in our study, the reported hepatic cytolysis was mild (grade 1-2) for the triplet combination [7]. Moreover, several patients experienced prolonged cytopenias during response, suggesting that certain strategies should be considered, according to previous studies: delaying the initiation of subsequent cycles until achieving ANC > 500/µL and platelets > 50,000/µL, allowing longer cycles (4-6 weeks) for count recovery, shorter duration of venetoclax treatment and lower gilteritinib dose in those with persistent cytopenias after achieving CR [17].

With a median follow-up of 9 months, 75% of patients who received allo-HSCT post-triple therapy were alive, suggesting that triple therapy could be an effective bridge to transplant in young/fit patients with relapsed FLT3 mutated AML.

Limitations

The findings of this study are limited by the small sample size. Moreover, the study was conducted in the era of widespread midostaurin use in first-line therapy, which may potentially contribute to resistance to FLT3-targeted agents and consequently influence the activity of gilteritinib, as mentioned in previous studies [7], [16].

It is also notable that our population was enriched with patients who had received previous gilteritinib (the entire cohort) and/or hypomethylating agent plus venetoclax. The triplet regimen may be a reasonable option for patients with no previous exposure to any of these agents – given that median OS of 10.3 months is mentioned in the literature for this group of patients [11] - although it remains unclear whether the use of azacitidine provides additional efficacy, beyond what could be achieved with a gilteritinib and venetoclax doublet regimen.

Conclusions

In conclusion, our results support existing literature data regarding the role of triple therapy as an effective bridge to allogeneic hematopoietic stem cell transplantation in relapsed/refractory patients with FLT3-mutated AML who fail to respond to gilteritinib monotherapy. Although this regimen is designed for outpatient administration, careful monitoring is required due to moderate toxicities, particularly hematological adverse events and the associated infectious risk.

Our centre's experience represents the first reported dataset in Romania and may contribute to the development of future clinical trials evaluating therapeutic strategies for patients with relapsed/refractory FLT3-mutated AML, an area of significant unmet medical need.

Abbreviations

AML – Acute myeloid leukemia
ANC – Absolute neutrophil count
BCL-2 – B-cell lymphoma 2
BMT – Bone marrow transplantation
CNS – Central nervous system
CR – Complete remission
CRi – Complete remission with incomplete hematologic recovery
CTCAE – Common Terminology Criteria for Adverse Events
ELN – European Leukemia Net
FAB – French-American-British classification
FISH – Fluorescence in situ hybridization
FLT3 – FMS-like tyrosine kinase 3
• **ITD** – Internal tandem duplications
• **TKD** – Tyrosine kinase domain
HMA – Hypomethylating agents
HSCT – Hematopoietic stem cell transplantation
MAC – Myeloablative conditioning
MRD – Minimal residual disease
NGS – Next-generation sequencing
NPM1 – Nucleophosmin 1
PCR – Polymerase chain reaction
RIC – Reduced-intensity conditioning
RFS – Relapse-free survival
OS – Overall survival
WHO – World Health Organization

Ethics Statement and Conflict of Interest Disclosures
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Ethics Consideration: The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as

revised in 2008(5), as well as the national laws. Written informed consent was provided by the participants in this study.

Conflict of interest: No known conflict of interest correlated with this publication.

Availability of data and materials: The data used and/or analyzed throughout this study are available from the corresponding authors upon reasonable request.

Competing interests: The authors declared that they have no competing interests.

During the preparation of this manuscript ChatGPT 5 was used for the purposes of language editing.

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