



CORRESPONDENCE

Clinical Experience With Venetoclax-Based Combinations for Relapsed/Refractory or MRD-Positive *NPM1*-Mutated Acute Myeloid Leukemia

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To the Editor,

NPM1 mutations occur in ~30% of acute myeloid leukemia (AML) [1] and define a distinct entity according on current WHO and ICC classification [2, 3]. *NPM1*^{mut} AML shows heterogeneous clinical behavior: some patients respond well to chemotherapy with rapid measurable residual disease (MRD) clearance, while others experience primary refractoriness or early relapse. Co-mutations, particularly *FLT3*-ITD, contribute to this variability [4]. While first-line treatment of *NPM1*^{mut} AML is well established [1], optimal management of relapsed/refractory (R/R) disease remains uncertain. Furthermore, in case of primary refractory or early relapsing patients, chemoresistance represents an issue [5]. For patients treated with curative intent, salvage chemotherapy followed by allogeneic stem cell transplantation (ASCT) is standard approach, although such regimens are burdened by toxicities that delay or prevent ASCT in some cases. In elderly and HSCT ineligible patients, therapeutic options are limited. Recently, favorable toxicity profile and efficacy of venetoclax (VEN) in combination with hypomethylating agents (HMAs) or low-dose cytarabine (LDAC) in *NPM1*^{mut} AML was reported in first line [6, 7] and the R/R setting [8–10]. Moreover, considering the evolving therapeutic scenario, the role of MRD-directed intervention in *NPM1*^{mut} AML, based on early detection of impending relapse at the molecular level, is unclear [11].

We retrospectively analyzed 58 adult patients with R/R or MRD-positive *NPM1*^{mut} AML treated with VEN-based approaches at Careggi University Hospital, Florence (Italy) (*n* = 28) and Mayo Clinic, Rochester, Minnesota (US) (*n* = 30) between 2018 and 2025. Median age was 61.5 years (26–83); 31/58 (53.4%) patients were older than 60 years. Median follow-up duration was 26.2 months.

Most patients (*n* = 43, 74%) were treated for relapsed disease (1st relapse, *n* = 35; subsequent relapse, *n* = 8), that in most cases occurred after CR of short duration (< 6 months, *n* = 27; 6–12 months, *n* = 9; > 12 months, *n* = 5; unknown, *n* = 2). Three patients were primary refractory. Twelve patients received VEN-based treatment as an MRD-directed approach (MRD persistence after frontline chemotherapy, *n* = 8; MRD relapse, *n* = 4). Overall, 37 patients (64%) were treated with intention-to-transplant (ITT); ultimately, 29/37 patients (78%) were able to proceed to ASCT.

VEN-based treatments were mainly represented by VEN combined with HMA (*n* = 47/58; 81%; azacitidine, *n* = 30; decitabine, *n* = 17); other combinations including *FLT3* inhibitors (*FLT3*i), menin inhibitors, or intensive chemotherapy. Full details of patients' characteristics and treatment regimens can be found in Table S1.

Most frequently detected co-mutations were *FLT3*-ITDs ($n=24$; 41%; median allelic ratio 0.425 (0.002–1.500)), *DNMT3A* ($n=23$; 39%), *IDH1* and *IDH2* ($n=17$; 31%), *RAS* pathway ($n=14$; 24%), and *FLT3*-TKD ($n=6$; 10%) (Figure 1 and Table S2).

A total of 282 cycles were administered (with hospitalization, $n=46$; 16.3%); median cycle number per patient was 4 for non-ASCT patients and 3 for HSCT recipients. Reasons for treatment discontinuation were ASCT ($n=22$), treatment failure ($n=20$), excessive myelotoxicity ($n=5$), unknown reasons ($n=4$), medical decision ($n=3$), infection ($n=1$), death due to unrelated cause ($n=1$). At the time of data cut-off, two patients were still receiving treatment.

Overt R/R cohort: composite CR rate (CCR; CR + CR with incomplete counts recovery) was 65.2% (30/46), with 17/46 (36.9%) patients achieving CR and 13/46 (28.3%) CRi status. About 70% of complete responders achieved MRD negativity in bone marrow (BM) and/or peripheral blood (PB) by RT-qPCR (or flow cytometry, in case of noncanonical mutant *NPM1* transcripts). Best response occurred early during treatment (cycle 1, $n=20$; cycle 2, $n=4$; cycle 3, 4, and 6, $n=1$; unknown, $n=3$). No genetic biomarker (including aberrant karyotype, *FLT3*-ITD status, *IDH1/2* status, *RAS* pathway mutations) or prior exposure to HMA or *FLT3i* influenced CR

probability (Table S3). Patients with *FLT3* mutation (ITD or TKD) had a CCR of 63.6% (14/22; CR, $n=6$; CRi, $n=8$); restricting the analysis to *FLT3*-ITD cases, CCR was 55.5% (10/18; CR, $n=2/10$; CRi, $n=8/10$). Prior *FLT3i* exposure did not reduce CCR in *FLT3*-mutated patients. Eighteen of 25 (72%) patients treated with ITT were able to proceed to ASCT.

Median OS and EFS was 20.7 and 13 months, respectively (Figures 2 and 3). *IDH1* or *IDH2* mutation was associated with longer, though not statistically significant, EFS (45.7 vs. 9.7 months, $p=0.137$) and OS (24.5 vs. 9.5 months; $p=0.064$). Conversely, *FLT3*-ITD positivity was associated with shorter EFS (9.5 vs. 13.9 months) and OS (9.5 vs. 24.5 months). Patients undergoing had significantly longer EFS (26.8 vs. 5.7 months; $p=0.048$) and OS (median not reached vs. 9.5 months; $p=0.013$). A survival plateau for both EFS and OS was observed, with seven patients experiencing long-term survival at data cut (five off treatment). In multivariate analysis, both *IDH1/2* mutation and ASCT were independently associated with improved outcomes. *IDH1/2* mutation was associated with a > three-fold reduction in risk of death (OS: HR 0.324; 95% CI, 0.108–0.968; $p=0.043$) and a nonsignificant reduction in event risk (EFS: HR 0.400; 95% CI, 0.146–1.097; $p=0.075$). ASCT was significantly associated with improved OS (HR: 0.267; 95% CI: 0.103–0.690; $p=0.006$) and EFS (HR: 0.371; 95% CI: 0.103–0.885; $p=0.025$) (Table S4).

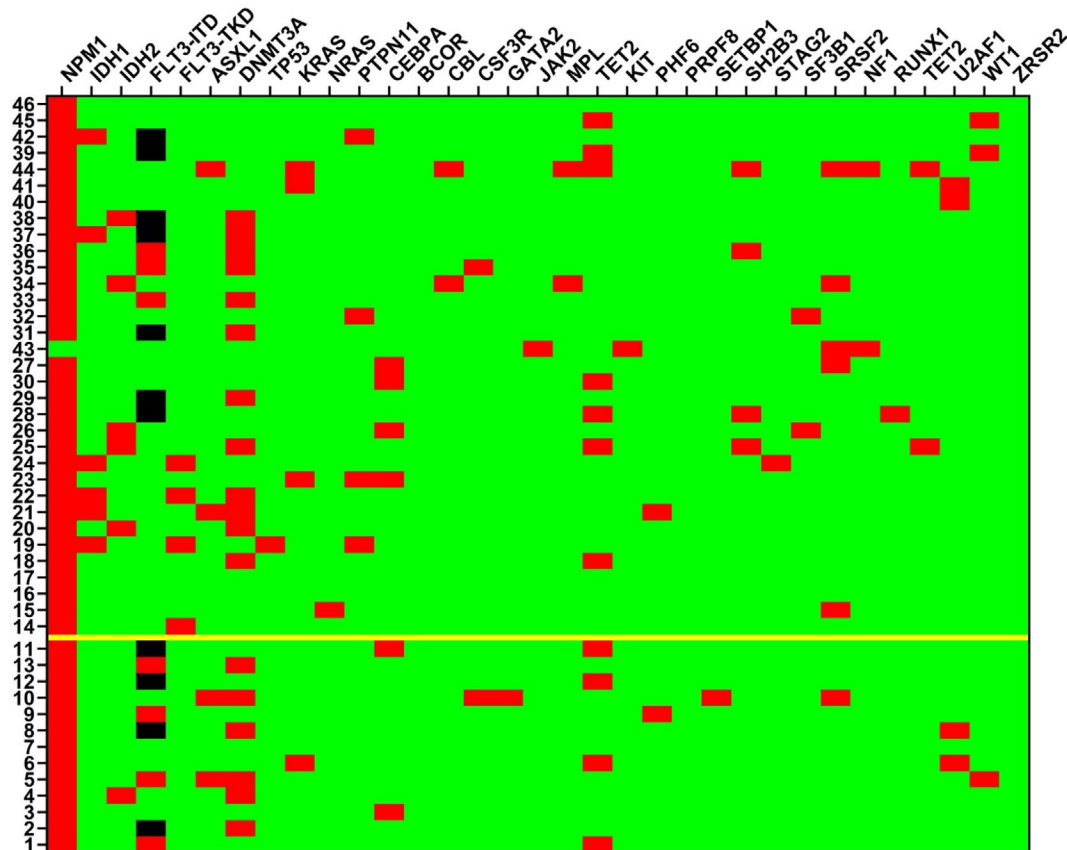


FIGURE 1 | Heatmap depicting the molecular characteristics of the 46 patients treated for overt relapsed/refractory disease. Complete responders are represented above the golden line, while refractory patients are listed below. Red box = mutated; green box = wild type; black box = *FLT3*-ITD positive with allelic ratio > 0.8.

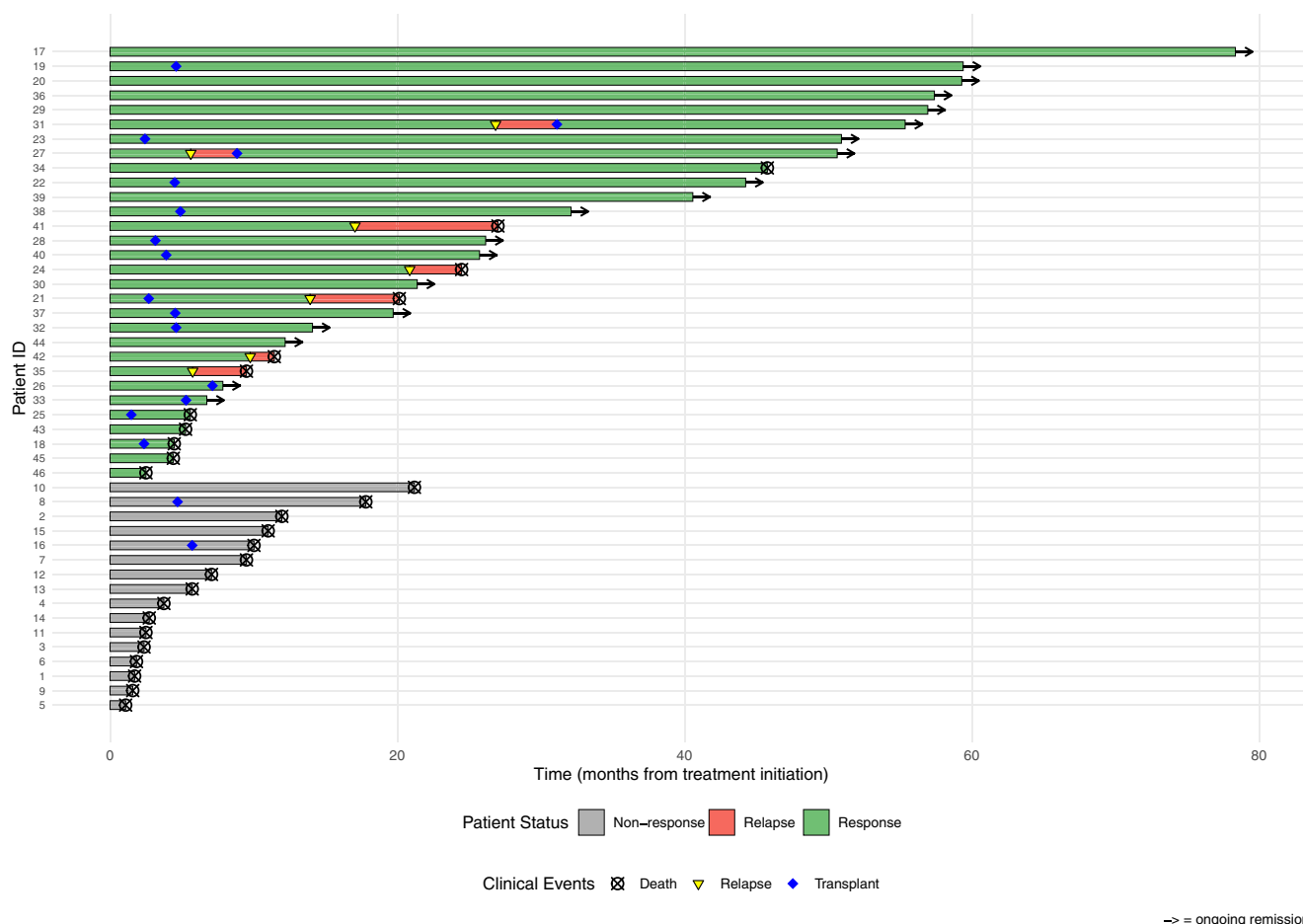


FIGURE 2 | Swimmer plot representing the clinical course and treatment-related outcomes of 46 patients treated for over relapsed/refractory disease.

MRD relapse/persistence cohort: All patients achieved complete MRD clearance by RT-qPCR, including 7 with *FLT3* mutations (*FLT3*-TKD, $n = 1$; *FLT3*-ITD, $n = 6$). Best response occurred at cycle 1 in 5 patients, cycle 2 in 3, cycle 3 in 3, and cycle 4 in 1. All patients were successfully bridged to ASCT, except for a 57-year-old woman diagnosed with end-stage cholangiocarcinoma (Table S3). Median OS and EFS were not reached (Figure 3), and most OS events were due to transplant-related mortality.

The safety profile was consistent with previous reports, with no treatment-related deaths. Nonhematologic adverse events are reported in Table S5. Notably, in the MRD relapse/persistence cohort, VEN-based regimens (VEN-AZA, $n = 9$; VEN-AZA + ziftomenib, $n = 2$; VEN + gilteritinib, $n = 1$) were particularly well tolerated, with cytopenias (mainly neutropenia) as the most frequent treatment-related AE and only one case of G3 febrile neutropenia.

To our knowledge, this represents one of the largest series evaluating outcomes of R/R and MRD positive *NPM1*^{mut} AML patients treated with VEN-based combinations. The observed CCR rate is comparable to that reported for conventional salvage regimens in R/R AML patients [12], including *NPM1*^{mut}

cases, despite most patients in our cohort would be ineligible for intensive chemotherapy due to age or comorbidities and having short prior CR duration, a setting typically associated with poor outcomes.

Although the study was not powered to assess the impact of co-mutations on CR achievement and survival, improved outcomes were observed in patients with *IDH1* or *IDH2* mutations, consistent with previous reports [7, 9, 10]. In *FLT3*^{mut} patients, while *FLT3* inhibitor-based regimens remain preferred, VEN-based approaches may represent a potential option. Notably, patients treated for MRD relapse or persistence achieved high MRD clearance and favorable survival. The tolerability and activity of VEN-based regimens support their potential role in MRD-directed therapy, currently being investigated in a phase II GIMEMA (Gruppo Italiano Malattie Ematologiche dell'Adulto) trial (NCT04867928).

The favorable toxicity profile of VEN-based regimens may offer an advantage over conventional salvage chemotherapy, particularly as bridge-to-transplant in R/R *NPM1*^{mut} AML. Interestingly, the durable CRs were observed in some patients even without ASCT, warranting further investigation.

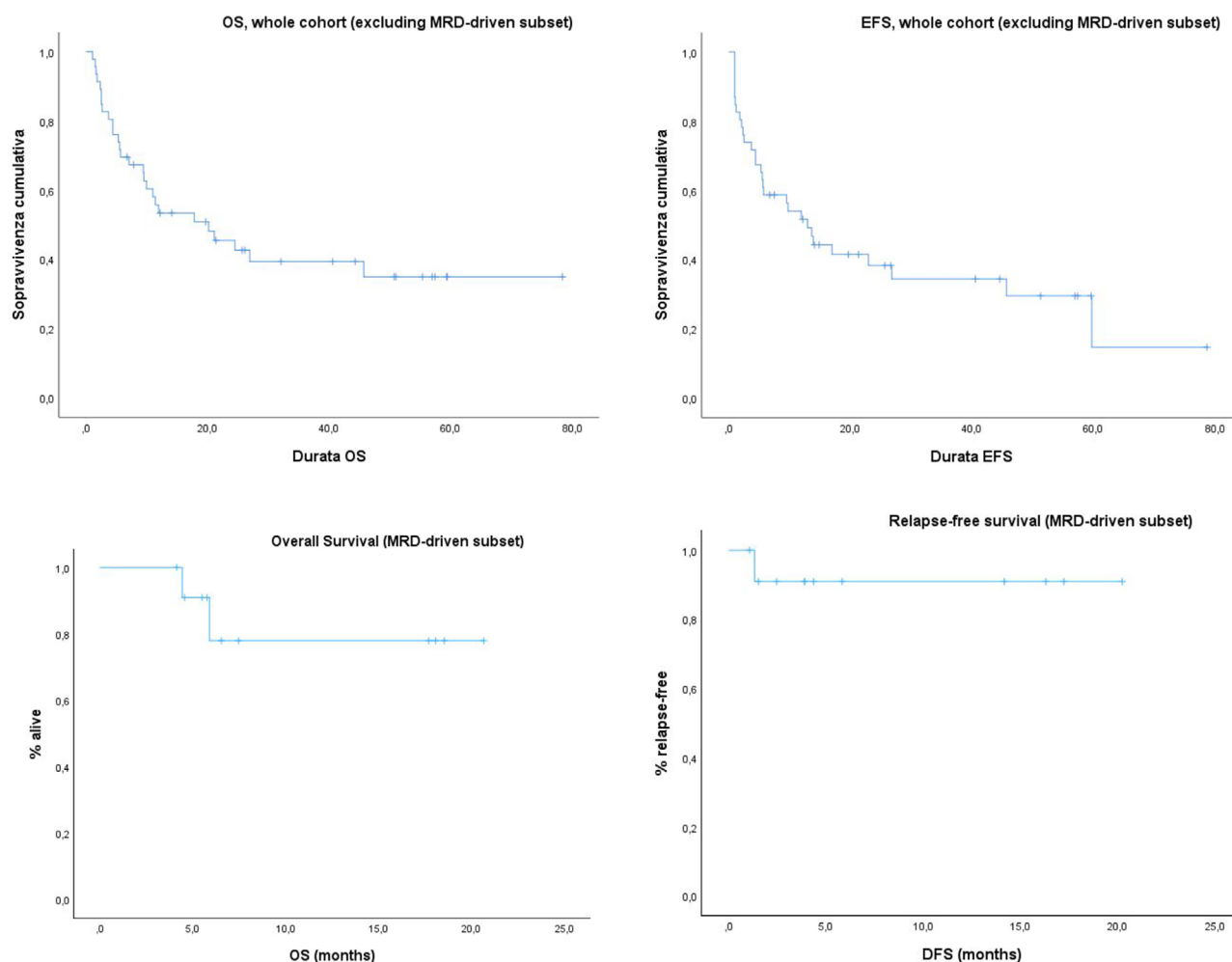


FIGURE 3 | Kaplan-Meier plots representing the survival outcomes for patients treated for overt relapsed/refractory disease (top) and MRD-positivity (bottom).

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Ethics Statement

This study was reviewed and approved by the Institutional Review Board (Comitato Etico Area Vasta Centro—CEAVC).

Consent

Written informed consent was obtained by patients according to the Declaration of Helsinki.

Conflicts of Interest

M.P.: Speaker bureau AbbVie Pharmaceuticals, Astellas, Astrazeneca, SERB, Amgen, Incyte. N.G.: Advisory Board for DISC Medicine and Agios. F.M.: Speaker bureau Blueprint, Novartis, Abbvie, Servier, GSK, Astellas. A.M.V.: honoraria from AbbVie, Novartis, Blueprint, GSK.

Data Availability Statement

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

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** Clinical and demographic characteristics of the study population. CR, complete remission; FLT3i, FLT3 inhibitor; HSCT, hematopoietic stem cell transplantation; ITD, internal tandem duplication; ITT, intention-to-transplant; MRD, minimal residual disease; TKD, tyrosine kinase domain; VEN, venetoclax. **Table S2:** Cytogenetic and molecular characteristics of the whole patient population. Full details regarding co-mutations can be found in Figure 1. **Table S3:** Response rates and kinetics with VEN-based regimens and HSCT bridging data in relapsed/refractory and MRD-positive patients sub-cohorts. CCR, composite complete remission; CR, complete remission; CRi, complete remission with incomplete counts recovery; FLT3i, FLT3 inhibitor; HMA, hypomethylating agent; HSCT, hematopoietic stem cell transplantation; ITT, intention-to-transplant; MRD, minimal residual disease. **Table S4:** Survival data according to relevant clinical and molecular covariates. EFS, event-free survival. HSCT, hematopoietic stem cell transplantation. HR, hazard ratio. OS, overall survival. **Table S5:** Nonhematological adverse events (whole cohort). Grading according to common terminology criteria for adverse events (CTCAE) version 5.0.