



## ORIGINAL PAPER

# A venetoclax and azacitidine bridge-to-transplant strategy for *NPM1*-mutated acute myeloid leukaemia in molecular failure

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## Summary

*NPM1*-mutated acute myeloid leukaemia (*NPM1*<sup>mut</sup> AML) represents a mostly favourable/intermediate risk disease that benefits from allogeneic haematopoietic stem cell transplantation (HSCT) in case of measurable residual disease (MRD) relapse or persistence after induction chemotherapy. Although the negative prognostic role of pre-HSCT MRD is established, no recommendations are available for the management of peri-transplant molecular failure (MF). Based on the efficacy data of venetoclax (VEN)-based treatment in *NPM1*<sup>mut</sup> AML older patients, we retrospectively analysed the off-label combination of VEN plus azacitidine (AZA) as bridge-to-transplant strategy in 11 *NPM1*<sup>mut</sup> MRD-positive fit AML patients. Patients were in MRD-positive complete remission (CR<sub>MRDpos</sub>) at the time of treatment: nine in molecular relapse and two in molecular persistence. After a median number of two cycles (range 1–4) of VEN–AZA, 9/11 (81.8%) achieved CR<sub>MRD</sub>-negative (CR<sub>MRDneg</sub>). All 11 patients proceeded to HSCT. With a median follow-up from treatment start of 26 months, and a median post-HSCT follow-up of 19 months, 10/11 patients are alive (1 died from non-relapse mortality), and 9/10 patients are in MRD<sub>neg</sub> status. This patient series highlights the efficacy and safety of VEN–AZA to prevent overt relapse, achieve deep responses and preserve patient fitness before HSCT, in patients with *NPM1*<sup>mut</sup> AML in MF.

## KEY WORDS

AML, HSCT, MRD, venetoclax

## INTRODUCTION

In the clinical management of acute myeloid leukaemia (AML), measurable/minimal residual disease (MRD) has gained increasing importance for the assessment of therapeutic responses, as dynamic on-treatment parameters and for disease risk definition.<sup>1–3</sup> Among the advantages of MRD monitoring, the possibility to identify an impending relapse and enable early treatment intervention is clearly stated by

the European LeukemiaNet (ELN) 2022 recommendations, defining the novel treatment setting of molecular failure (MF).<sup>2,3</sup> The feasibility of systematic molecular monitoring to identify relapsing patients to allocate to pre-emptive therapy has been demonstrated in the setting of acute promyelocytic leukaemia and has become routine practice also in other molecular categories.<sup>4,5</sup> Although important studies have addressed the point of pre-emptive intervention in delaying or preventing haematological relapse in MRD-positive

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(MRD<sub>pos</sub>) AML patients, clinicians have little indications on how to act on MRD data and clinical trials to understand the role of early intervention in MF are ongoing.<sup>2,6,7</sup>

The presence of detectable MRD positivity before haematopoietic stem cell transplantation (HSCT) is associated with an increased risk of relapse and death in AML patients.<sup>8–11</sup> However, delaying transplantation to achieve MRD negativity is not recommended.<sup>2</sup> Recently, data presented at ASH2022 from the phase III as soon as possible (ASAP) randomized trial in relapsed/refractory (R/R) AML showed no benefit from bridging chemotherapy as compared to sequential conditioning and immediate HSCT, underlining the importance of proceeding ASAP to transplantation.<sup>12</sup> Whether this applies to MRD<sub>pos</sub> relapsing AML needs to be evaluated. However, transplant organization may be time-consuming, thus strategies aimed at preventing disease progression in the peri-transplant setting are required.

*NPM1*-mutated (*NPM1*<sup>mut</sup>) AML is a defined WHO category, which represents the largest molecular subgroup of AML.<sup>13–17</sup> In *NPM1*<sup>mut</sup> AML, MRD is evaluated with quantification of *NPM1*<sup>mut</sup> transcript as it is a stable and highly sensitive leukaemia-specific molecular marker analysed by quantitative real-time polymerase chain reaction (qRT-PCR).<sup>18</sup> Persistent *NPM1*<sup>mut</sup> MRD after intensive induction chemotherapy or recurrence during post-treatment monitoring identifies an unfavourable subgroup of patients at risk of impending relapse.<sup>10,19–21</sup> These patients benefit from HSCT to eradicate leukaemia.<sup>13,22,23</sup> Moreover, the presence of detectable *NPM1*<sup>mut</sup> MRD before transplantation is an independent unfavourable predictor of post-HSCT outcome.<sup>9,11,20</sup> In the absence of *NPM1*-targeted agents currently available in clinical daily practice, therapeutic interventions for *NPM1*<sup>mut</sup> relapsing AML patients are variable and data are sparse, ranging from direct HSCT to salvage chemotherapy or hypomethylating agents (HMA)-based combinations, while trials with targeted agents (i.e. Menin inhibitors) are available only in the R/R AML setting.<sup>7,11,24–27</sup>

Venetoclax (VEN) is a potent and selective oral inhibitor of BCL-2 with remarkable synergic activity in association with azacitidine (AZA), that has revolutionized the management of treatment-naïve elderly/unfit AML patients.<sup>28</sup> *NPM1*<sup>mut</sup> patients seem to particularly benefit from this approach in both frontline, with a composite complete remission (CR) rate of 91.5% and a substantial proportion of complete MRD remissions (75%), and R/R setting.<sup>29–31</sup> VEN-AZA has a favourable toxicity profile in terms of haematological and infective adverse events (AEs).<sup>27,29</sup>

The ideal bridge-to-transplant strategy should quickly and effectively prevent overt relapse, while preserving the patient's fitness for HSCT and VEN-AZA could potentially meet both requirements.<sup>27</sup> Moreover, in a population of previously chemotherapy-exposed patients, a different pharmacological approach may overcome the chemo-resistance of the residual leukaemic clone. Therefore VEN-AZA could represent the ideal therapeutic combination for peri-transplant MRD management in *NPM1*<sup>mut</sup> AML.

This study aimed to explore the feasibility of VEN-AZA as a bridge-to-transplant disease-control intervention in *NPM1*<sup>mut</sup> AML patients with MRD<sub>pos</sub>.

## MATERIALS AND METHODS

Patients with newly diagnosed *NPM1*<sup>mut</sup> AML who had received at least two cycles of intensive chemotherapy including anthracycline and cytarabine, were included in this analysis. Patients had to have experienced either molecular persistence/progression during treatment, defined as any detectable transcript level after completion of chemotherapy, or molecular reoccurrence during post-treatment follow-up, as defined by ELN guidelines.<sup>1,2</sup> All patients were eligible for SCT and had an available donor at study entry as VEN-AZA treatment was conceived as a bridge-to-transplant strategy. This study was approved by the local ethics committee and procedures were conducted in compliance with the Declaration of Helsinki and Good Clinical Practices. Informed consent was waived for this retrospective study.

Patients were treated with venetoclax off-label at the dosage of 400 mg/die PO for 14–28 days in combination with azacitidine (75 mg/m<sup>2</sup> SC for 5–7 days) between December 2019 and July 2022. VEN daily dosage was reduced to 100 mg if concurrent prophylaxis with strong CYP3A4 inhibitor posaconazole was used.<sup>32</sup> No tumour lysis syndrome prophylaxis was given considering the low disease burden. Three-day VEN ramp-up was performed in 8/11 (72.7%) patients.

All participating centres routinely monitored MRD during chemotherapy and post-treatment follow-up, to intercept MF. Disease was assessed on bone marrow (BM) with *NPM1*<sup>mut</sup>-specific qRT-PCR assay as previously described.<sup>18</sup> *NPM1*<sup>mut</sup> transcript levels are reported as the normalized values of *NPM1*<sup>mut</sup> copy number/*ABL* copy number multiplied by 100 to report a percentage. The quantitative detection limit of the assays was 0.01%. Complete response with measurable residual disease negativity (CR<sub>MRDneg</sub>) was defined as a ratio *NPM1*<sup>mut</sup>/*ABL* × 100 transcript <0.01%.<sup>18,33</sup> *FLT3* mutational status was assessed on mononuclear cells from BM blood with PCR amplification of exons 14 and 15 of *FLT3* and analysed using capillary electrophoresis, with a sensitivity of 10<sup>-2</sup>.

Baseline characteristics have been presented by means of descriptive statistics: median and its interquartile range have been reported for continuously distributed variables, while frequencies and relative percentages have been adopted for categorical distributions. Survival analysis applying the Kaplan–Meier method has been adopted for time-to-event endpoints: overall and relapse-free survivals. The Cox semi-parametric regression analysis has been adopted to calculate hazard ratios with their 95% confidence intervals (CIs). All variables objected to statistical inference have been reported with their 95% CI. MRD relapse-free survival (RFS<sub>MRD</sub>) has been defined as time from achievement of CR<sub>MRDneg</sub> to molecular MRD relapse, as defined by ELN2022 guidelines.

## RESULTS

We retrospectively identified 11 patients (age 43–68 years) with *NPM1*<sup>mut</sup> AML who received off-label treatment with VEN–AZA combination for MRD<sub>pos</sub> status at 4 Italian Haematology Institutions as bridge-to-transplant strategy. Eight patients were classified as low risk and three as intermediate risk, for concomitant high *FLT3*-ITD allelic ratio, according to ELN2017; conversely, six were classified as low and five as intermediate (concomitant *FLT3*-ITD) according to ELN2022. One patient in the intermediate group presented concomitant *ZRSR2* mutation. Concerning *FLT3* mutational status, 5 of 11 (45.5%) patients harboured *FLT3*-ITD mutation and 1 patient (9.1%) *FLT3*-TKD; 9 patients had available *IDH1/2* mutational status: 2 patients presented *IDH1* R132 and 1 patients *IDH2* R140Q mutation. *IDH1/2* mutations and *FLT3*-ITD were mutually exclusive in this patient set. All patients received anthracycline-based first-line intensive chemotherapy and the six patients with concomitant *FLT3* mutation received in association with the *FLT3*-inhibitor (*FLT3i*) Midostaurin in induction and consolidation (Table 1).

Nine patients were treated for molecular relapse and two for molecular persistence, both after having received three cycles of intensive chemotherapy. For patients treated for molecular recurrence, the median time from the end of chemotherapy to molecular relapse was 11.50 months (interquartile range [IQR] 5.67–17.40). A median number of days intercurrent from molecular relapse/persistence to treatment start was 28 (range 1–81).

The median *NPM1*<sup>mut</sup>-MRD level prior to VEN–AZA intervention was 3.66 (range 0.032–368). No patients had detectable *FLT3* mutation. Patients' characteristics and previous chemotherapy treatment are detailed in Table 1.

## Treatment

Patients received a median of three cycles (range 1–4) of AZA–VEN before SCT. All patients were treated as outpatients, and none required transfusions. No patients required AZA dose reduction, while median VEN exposure varied among cycles (Table 2). Reduction of VEN exposure was in 50% of cases due to cytopenia  $\geq$ G3 and in 50% of cases due to imminent HSCT. All patients were bridged to HSCT, and no patients required subsequent therapy before transplantation.

## Safety

VEN–AZA treatment was demonstrated to be well tolerated. The most frequent AEs  $\geq$ G3 were cytopenias in 5 of 11 (45.4%) patients, which were managed with dose reduction and delay among cycles (maximum 14 days, until neutrophil recovery). During C1 neutropenia  $\geq$ G3 occurred in 4/11 (36.6%) patients and thrombocytopenia  $\geq$ G3 in 1 patient (9.0%); during C2, C3 and C4, neutropenia  $\geq$ G3 occurred in

33.3%, 16.6% and 25% of patients, respectively. Other AEs  $\geq$ G3 were three febrile neutropenia, managed outpatient and 1 deep vein thrombosis related to the presence of a peripherally inserted central catheter (DVT PICC-related).

## Efficacy

No patients experienced a morphological relapse while on VEN–AZA treatment. Nine of 11 (81.8%) patients become MRD<sub>neg</sub> while on treatment. MRD<sub>neg</sub> status was achieved in 4 (44.4%) patients after cycle 1 (C1), for 3 (33.3%) patients after C2, for 1 (11.1%) patient after C3 and 1 (11.1%) after C4; thus 77.7% of patients achieved MRD negativity within the first two cycles (Figure 1). Patients' characteristics in relation to responses are detailed in Table 3. The first patient (case 8) who did not clear MRD had *FLT3*-ITD AML and experienced initial molecular reduction after C1 (*NPM1*<sup>mut</sup> 0.220–0.0526), but subsequent molecular progression after C3 (*NPM1*<sup>mut</sup> 3.0067). *FLT3*-ITD always tested negative. The second patient (case 9) who did not achieve MRD negativity obtained a consistent log-reduction of 3.99, from *NPM1*<sup>mut</sup> 368 to 0.038 after one single cycle and was then assigned directly to HSCT. At the time of HSCT 8/11 (72.7%), patients were MRD<sub>neg</sub>; of the three MRD<sub>pos</sub> patients, two have been already described, while the third one had MRD detectable at low level (LL-MRD: *NPM1*<sup>mut</sup> 0.0107). All patients tested negative for *FLT3* mutational status at pre-transplant evaluation.

The median time from VEN–AZA start of treatment to HSCT was 120 days (IQR 79.59, 148.00). No patients delayed HSCT due to AEs encountered during treatment and patient fitness was preserved. All patients tested MRD<sub>neg</sub> after transplantation. The timing of HSCT was mostly based on the time needed for donor confirmation and preparation and not on response to VEN–AZA treatment.

The median follow-up time from the start of VEN–AZA therapy is 26 months (IQR 20–30). No patients experienced a morphological relapse. Concerning survival analysis, 10 of 11 (90.9%) patients are alive (Figure 2). One patient, MRD<sub>neg</sub> at the time of HSCT, died from non-relapse mortality (NRM) due to pulmonary aspergillosis. Median follow-up from HSCT is 19.2 months (IQR 11.9–24.3). Median RFS<sub>mol</sub> is not reached, with two patients experiencing post-HSCT molecular relapse (Figure 3). Of these, case 8, notably the *FLT3*-ITD-positive at diagnosis patient in molecular progression at the pre-transplant evaluation, resulted in MRD<sub>neg</sub> at 3 months, and MRD<sub>pos</sub> (*NPM1*<sup>mut</sup> 0.04, *FLT3* unmutated) at 6 months from transplantation. This patient received post-HSCT intervention with immunosuppressive therapy withdrawal and pre-emptive treatment with sorafenib, currently ongoing, with the achievement of MRD negativity.<sup>34</sup> Patient 8 status at the last follow-up is of MRD negativity. The other patient (case 3), also *FLT3*-ITD-positive at diagnosis, experienced molecular relapse at 6 months from HSCT, with subsequent confirmed *FLT3*-ITD positivity and started gilteritinib; the patient is currently MRD<sub>pos</sub>.<sup>35</sup> Three patients

TABLE 1 Patient characteristics.

Patient characteristics	Level	Overall
<i>n</i>		11
Sex (%)	F	5 (45.5)
	M	6 (54.5)
Age at AML diagnosis, years (median [IQR])		56.4 [49.9, 61.4]
Hb at AML diagnosis, g/dL (median [IQR])		7.5 [6.5, 9.0]
WBC at AML diagnosis, $\times 10^9/L$ , (median [IQR])		51.8 [30.2, 103.8]
Plt at AML diagnosis, $\times 10^9/L$ (median [IQR])		48.5 [37.5, 62.0]
BM blast cell, % (median [IQR])		75 [65, 88]
Karyotype (%) <sup>a</sup>	Normal	10 (100)
<i>NPM1</i> type mutant transcript (%) <sup>a</sup>	A	9 (90.0)
	B	1 (10.0)
<i>NPM1</i> <sup>mut</sup> /ABL $\times 100$ transcript at diagnosis (median [IQR])		565.00 [335.37, 641.00]
<i>FLT3</i> mutational status at diagnosis (%)	ITD	5 (45.5)
	TKD	1 (9.1)
	Wild Type	5 (45.5)
<i>IDH1/2</i> mutational status (%) <sup>b</sup>	<i>IDH1</i> R140Q	2 (22.2)
	<i>IDH2</i> R132	1 (11.1)
	Wild Type	6 (66.7)
ELN2017 (%)	Intermediate	3 (27.3)
	Low	8 (72.7)
ELN2022 (%)	Intermediate	5 (45.5)
	Low	6 (54.5)
Induction chemotherapy (%)	3 + 7	2 (18.2)
	3 + 7 + Mido	6 (54.5)
	Fluda-based	2 (18.2)
	3 + 7 + GO	1 (9.1)
No. of chemotherapy cycles (median [IQR])		4 [3, 4]
No. of chemotherapy cycles, <i>n</i> (%)	2	1 (9.1)
	3	3 (27.3)
	4	5 (45.5)
	5	2 (18.2)
<i>NPM1</i> <sup>mut</sup> best response post-chemotherapy (%)	MRD <sub>neg</sub>	9 (81.8)
	MRD <sub>pos</sub>	2 (18.2)
Time from last chemotherapy to MF, months (median [IQR])		11.50 [5.67, 17.40]
Age at VEN-AZA treatment, years (median [IQR])		58.38 [50.84, 62.85]
MRD status at VEN-AZA treatment, <i>n</i> (%)	Persistence	2 (18.2)
	Relapse	9 (81.8)
<i>NPM1</i> <sup>mut</sup> /ABL $\times 100$ transcript at MF (median [IQR])		3.66 [0.04, 10.90]
BM blast cell at MF, % (median [IQR])		0.40 [0.00, 1.90]
<i>FLT3</i> mutational status at MF, <i>n</i> (%)	Unmutated	11 (100)

Abbreviations: AML, acute myeloid leukaemia; AZA, azacitidine; BM, bone marrow; ELN, European LeukemiaNet; FLT3, FMS-like tyrosine kinase 3; Fluda, fludarabine; GO, gemtuzumab ozogamicin; Hb, haemoglobin; IDH, isocitrate dehydrogenase; ITD, internal tandem duplication; IQR, interquartile range; MF, molecular failure; Mido, midostaurin; MRD, measurable residual disease; NPM1, nucleophosmin; Plt, platelets; TKD, tyrosine kinase domain; VEN, venetoclax; WBC, white blood cells.

<sup>a</sup>Data not available for one patient.

<sup>b</sup>Data available for nine patients.

experienced transient LL-MRD positivity during post-transplantation monitoring, not confirmed at a second determination, and thus did not meet the criteria for molecular

relapse, nor received post-HSCT treatment intervention. Ten of 11 (90.9%) patients are in CR<sub>MRDneg</sub> at the time of the last follow-up visit, as detailed in Figure 1.



## DISCUSSION

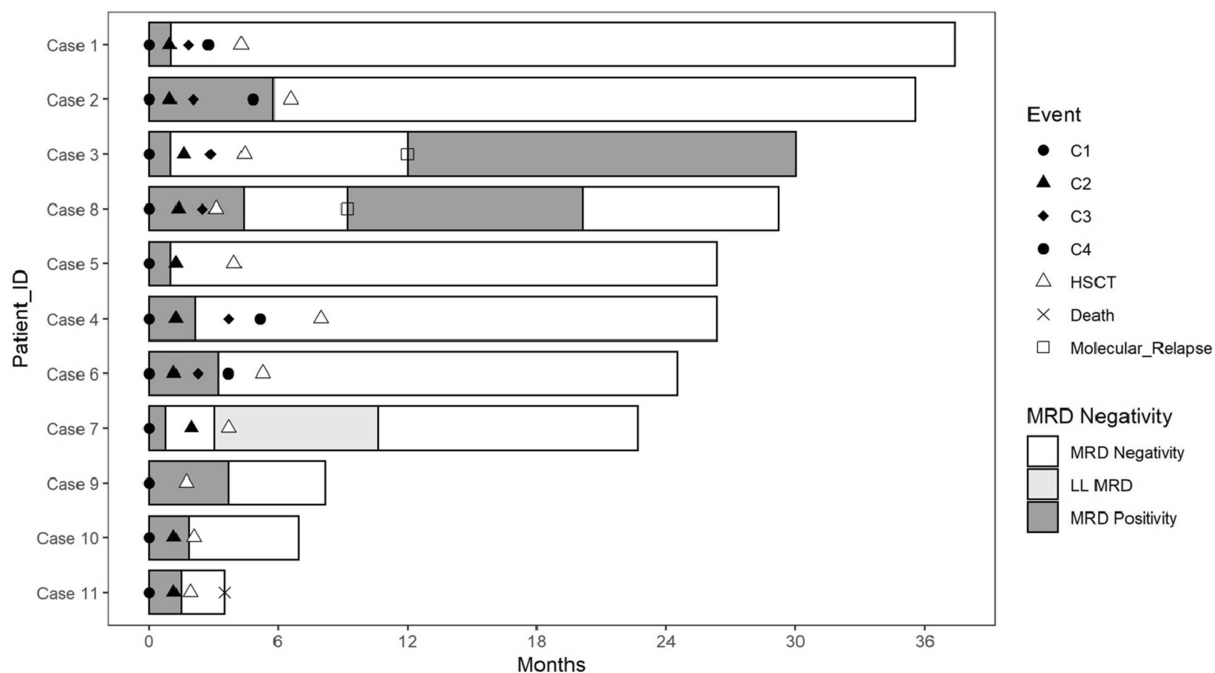
*NPM1*<sup>mut</sup> AML is a prognostically favourable entity in the absence of *FLT3*-ITD mutations or adverse cytogenetics, for which transplant in CR1 is not recommended.<sup>23</sup> However, in case of relapse and successful salvage therapy, this patient group benefits from transplantation in CR2 as elegantly demonstrated in a Mantel-Byar analysis performed by Burnett et al.<sup>22</sup> Likewise, intermediate-risk patients, such as *NPM1*<sup>mut</sup> with concomitant *FLT3*-ITD have a stronger indication to HSCT if not performed in CR1 and represent a higher risk patient group for which the achievement of MRD negativity before transplantation significantly impacts on outcome.<sup>9,11</sup> In the UK NCRI AML17 study, among the 107 *NPM1*<sup>mut</sup> AML patients allocated for

**TABLE 2** Venetoclax exposure during cycles.

Variables		No. of patients per cycle
Total VEN-AZA cycles <i>n</i> (median [IQR])	3 [2, 4]	
VEN exposure C1, days (median [IQR])	28.0 [22.5, 28.0]	11
VEN exposure C2, days (median [IQR])	21.0 [14.0, 28.0]	10
VEN exposure C3, days (median [IQR])	24.5 [17.3, 28.0]	6
VEN exposure C4, days (median [IQR])	25.5 [22.0, 28.0]	4

Abbreviations: AZA, azacitidine; C, cycle; IQR, interquartile range; VEN, venetoclax.

HSCT beyond CR1, 48 received salvage therapy for morphological or molecular relapse, and 27/48 (56%) achieved MRD negativity. The outcome for MRD<sub>neg</sub> patients at the time of HSCT was favourable, with an overall survival (OS) at 2 years of 80%. Of these 30/48 were transplanted for molecular relapse, and 27/30 received salvage therapy in MRD setting, achieving 16/27 (59%) MRD negativity. OS differed importantly based on MRD levels with MRD<sub>neg</sub>, MRD<sub>pos</sub>-low, and MRD<sub>pos</sub>-high experiencing 2-year OS of 83%, 63% and 13%, respectively. These data support the indication to procrastinate HSCT after CR1 in case of disease recurrence, to offer sequential monitoring to allow early intervention and to act therapeutically before transplantation with the aim to lower/eradicate MRD. In fact, different groups have suggested a benefit for pre-emptive intervention, exploring various MRD-directed strategies, ranging from direct HSCT, and salvage chemotherapy to less intensive strategies such as HMA with or without venetoclax.<sup>7,20,25,26</sup> Pre-emptive therapy resulted in prolonged RFS and improved overall outcomes, when compared to patients receiving salvage therapy for overt haematological relapse.<sup>25,26</sup> The benefit of HMA in MF has been demonstrated in two recent trials.<sup>7,36</sup> *NPM1*<sup>mut</sup> AML patients in molecular relapse, who received pre-emptive 5-azacitidine in the RELAZA2 trial showed a lower incidence of delayed haematological relapse as compared to historical controls.<sup>7</sup> In the phase 3 QUAZAR AML-001 trial, oral azacitidine significantly improved OS in *NPM1*<sup>mut</sup> AML, in both MRD-negative and MRD-positive patients. Median OS for *NPM1*<sup>mut</sup> MRD<sub>pos</sub> patients treated with oral azacitidine was of 46.1 months versus 10.0 months with placebo.<sup>36</sup> The benefit of oral azacitidine was evident also in *FLT3* AML, regardless of MRD status, in terms of both OS and RFS.



**FIGURE 1** Swimmer plot of patients treated with venetoclax and azacitidine. C, cycle; HSCT, haematopoietic stem cell transplantation; LL-MRD, low level MRD; MRD, measurable residual disease.

TABLE 3 Summary of patients' characteristics, and response to VEN-AZA and HSCT.

Case	Age/Sex	Cytogenetics/molecular features <sup>a</sup>	ELN2017/ELN2022	Best MRD response to chemo	MRD level pre-VEN	No cycles AZA-VEN	Best MRD response (%)	MRD status at HSCT	HSCT conditioning	HSCT donor	RFS (months)	MolRFS (months)	Post HSCT intervention
1	44/M	NK	Low/low	MRD neg	0.024	4	MRD neg	MRD neg	MAC	Sibling 10/10	37.9	36.8	-
2	58/F	NK/FLT3-TKD, IDH1 R142, DNMT3A	Low/low	0.047	0.047	4	MRD neg	MRD neg	MAC	Haplo	36.0	30.1	-
3	64/M	FLT3-ITD	Low/int	MRD neg	0.04	3	MRD neg	MRD neg	MAC	MUD 9/10	30.4	11.1	Gilteritinib
4	64/M	NK/FLT3-ITD	Low/int	MRD neg	3.659	4	MRD neg	MRD neg	MAC	Haplo	26.7	24.5	-
5	58/M	NK	Low/low	MRD neg	19.811	2	MRD neg	MRD neg	MAC	Haplo	26.7	25.7	-
6	56/M	NK/IDH2 R140Q	Low/low	MRD neg	6.682	4	MRD neg	MRD neg	MAC	MUD 7/8	24.8	21.6	-
7	69/F	NK/IDH1 R132, DNMT3A, CBL	Low/low	MRD neg	0.032	2	MRD neg	0.0107	RIC	MUD 9/10	23.0	34.3	-
8	48/F	NK/FLT3-ITD	Int/int	0.220	0.220	3	0.0526	3.0067	MAC	Sibling 10/10	20.4	4.9	Sorafenib
9	53/F	NK/PTPN11, TET2	Low/low	MRD neg	368	1	0.038	0.038	MAC	MUD 10/10	8.3	4.5	-
10	62/F	NK/FLT3-ITD, NRAS	Low/int	MRD neg	11.519	2	MRD neg	MRD neg	RIC	MUD 10/10	7.1	5.2	-
11	49/F	NK/FLT3-ITD, ZRSR2, DNMT3A	Low/int	MRD neg	10.280	2	MRD neg	MRD neg	MAC	MUD 9/10	3.5 <sup>b</sup>	2.0 <sup>b</sup>	-

Abbreviations: AZA, azacitidine; ELN, European Leukaemia Net; haplo, haploidentical donor; HSCT, haematopoietic stem cell transplantation; MRD, measurable residual disease; MUD, matched unrelated donor; NK, normal karyotype; NRM, non-relapse mortality; RFS, relapse free survival; VEN, venetoclax.

<sup>a</sup>NGS analysis is available for cases 1, 2, 7, 8, 9, 10 and 11; karyotype analysis is not available for case 8.

<sup>b</sup>Patient died of NRM (pulmonary aspergillosis).

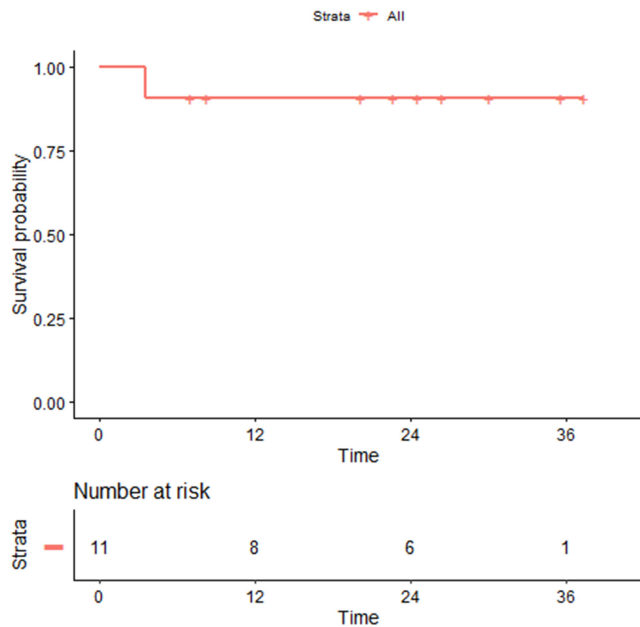


FIGURE 2 Overall survival.

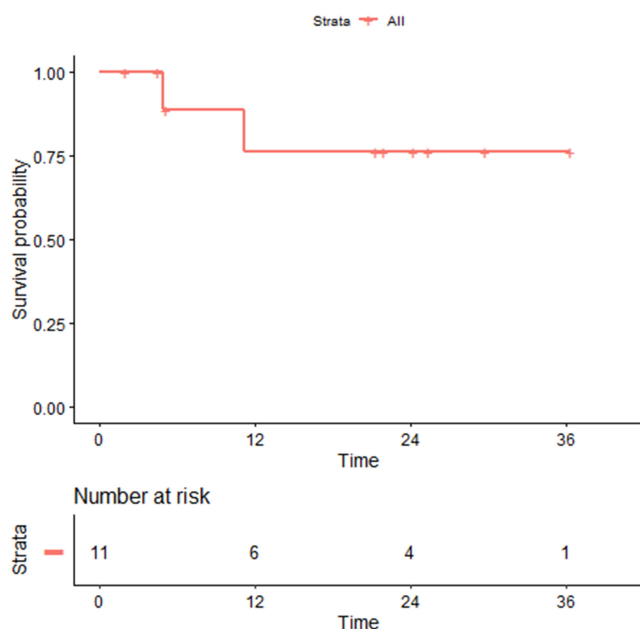


FIGURE 3 Molecular relapse free survival.

Considering the exquisite sensitivity of *NPM1*<sup>mut</sup> AML to venetoclax, the rationale to explore the molecule in association with HMA in the MF setting is strong. Tiong et al. reported promising results in 12 patients treated with VEN plus HMA for molecular persistence and molecular relapse/progression, with 11/12 (92%) patients achieving MRD<sub>neg</sub>.<sup>27</sup> Of these, four proceeded to HSCT: one transplanted in MRD<sub>pos</sub> relapsed with newly emergent clone *FLT3*-ITD, one died from NRM and two were alive at the last follow-up (10 and 9 months from HSCT).

To our knowledge, this case series of 11 patients treated with VEN-AZA and subsequent HSCT consolidation,

represents the first detailed report focused on a pre-emptive bridge-to-transplant venetoclax-based strategy.

The ideal bridging therapy in the perspective of HSCT requires efficacy and safety, in order to prevent overt relapse, reduce MRD and preserve patient fitness for transplantation. In our experience, 9 of 11 (82%) patients achieved MRD<sub>neg</sub> while on treatment and 8/11 (73%) were MRD<sub>neg</sub> at the pre-transplant evaluation, providing promising efficacy data in eradicating *NPM1*<sup>mut</sup> MRD-positivity in patients in MF after intensive chemotherapy. Moreover, this data compares favourably with the 27 patients receiving salvage chemotherapy for MRD<sub>pos</sub> in the NCRI AML17 trial, where MRD<sub>neg</sub> was achieved in 59% of patients.<sup>11</sup> No patients relapsed morphologically while on treatment, allowing all patients to proceed to HSCT without requiring further therapy. Responses were rapid as 77.7% of patients achieved MRD<sub>neg</sub> within cycle 2. Among the three MRD<sub>pos</sub> patients at pre-transplantation evaluation, one had MRD-LL and two patients, had respectively *NPM1*<sup>mut</sup> transcripts of 3.007 and 0.038. This latter patient achieved a 3.99 log reduction after one single cycle of VEN-AZA and then proceed directly to HSCT. Whether a second cycle could have achieved MRD<sub>neg</sub> cannot be known, however, the decrease of *NPM1*<sup>mut</sup> log reduction highlights the great sensitivity to the regimen also in this patient. Moreover, in no case in this patient series, HSCT was delayed for toxicities related to the VEN-AZA regimen and eligibility for transplantation was preserved.

All patients achieved MRD<sub>neg</sub> after transplant underlining the dual role of conditioning regimen and immunological disease control in this patient setting. Concerning post-transplant intervention, only two patients (18.2%) experienced molecular relapse and require further therapy after transplantation. Both patients presented concomitant *FLT3*-ITD mutation at diagnosis, confirming the higher risk of relapse for *FLT3*-ITD positive disease and the role of *FLT3* mutations in mediating resistance to VEN.<sup>30,34</sup> Although both patients tested unmutated for *FLT3* mutational by capillary electrophoresis at the time of VEN-AZA MRD intervention, we cannot exclude that *FLT3*-ITD may have tested positive with more sensitive techniques. Whether these patients may benefit from *FLT3*-directed therapy in the setting of MRD-positivity before HSCT needs to be explored. Numerous studies are reporting the value of a *FLT3*-inhibitors for relapse prevention in the post-HSCT maintenance setting and data from randomized trials will respond to this issue.<sup>34,37</sup> With a median follow-up from treatment start of 26 months and a median post-HSCT follow-up of 19 months, 10/11 patients are alive and 9/10 patients are in MRD<sub>neg</sub> status.

The principal limitations of this study include the small cohort of patients analysed, the retrospective nature, the inevitable patient selection bias performed by the treating physician and the timing of HSCT, which depended mostly on the availability of the procedure. However, MRD negativity was rapidly achieved in most patients at the expense of little toxicity.

In conclusion, our experience provides promising efficacy and safety data of a venetoclax-based intervention, followed by transplantation, for *NPM1*<sup>mut</sup> patients in MF, that needs to be explored in prospective clinical trials. On the basis of biological and clinical insights that support such a strategy, the GIMEMA AML2521 trial (ClinicalTrials.gov Identifier: NCT04867928) is currently enrolling patients with *NPM1*<sup>mut</sup> AML in MF after intensive chemotherapy, fit for HSCT, to receive venetoclax and azacitidine as bridge-to-transplant strategy.

## AUTHOR CONTRIBUTIONS

C. Sartor, M. Cavo, C. Papayannidis and A. Curti designed the study, performed the research, analysed the data and wrote the paper. L. Brunetti, E. Audisio, A. Cignetti, V. Cardinali, S. Sciolacci, M. P. Martelli, L. Zannoni, G. Cristiano, J. Nanni, R. Ciruolo, contributed patients and analysed data. E. Ottaviani, L. Bandini, D. Forte and A. Patuelli contributed essential analytical tools. All authors approved the final version of the manuscript.

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## CONFLICT OF INTEREST STATEMENT

CS, LB, EA, AC, LZ, JN, GC, RC, FZ, DF, LB, EO, AP, SS and VC declare no conflict of interest. MPM declares honoraria/consultancy at scientific advisory board for AbbVie, Amgen, Celgene, Janssen, Novartis, Pfizer and Jazz Pharmaceuticals. CP received honoraria from Amgen, Astellas, Pfizer and Novartis; participated in advisory boards with Pfizer, Janssen, AbbVie and Novartis. AC received honoraria from Novartis, Pfizer, AbbVie and acted as speaker in Advisory Board for Novartis and AbbVie. MC acted as consultant for and received honoraria from AbbVie, Glaxo Smith Kline, Bristol-Myers Squibb, Adaptive Biotechnologies, Takeda, Janssen, Celgene, Amgen and is in the speakers' bureau of AbbVie and Glaxo Smith Kline.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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