Subcutaneous azacitidine maintenance in transplant-ineligible patients with acute myeloid leukemia: a single-center retrospective study

Acute myeloid leukemia (AML) is an aggressive hematological malignancy more common in the elderly. While selected older patients can receive intensive chemotherapy resulting in a 50-60% complete remission (CR) rate, they are generally not eligible for hematopoietic stem cell transplantation (HSCT) and median overall survival (OS) is less than 1 year due to frequent relapse. Therefore, renewed interest in maintenance strategies recently emerged to improve survival among this population. Several maintenance approaches including treatment with histamine dihydrochloride-IL2, gemtuzumab ozogamycin, cytarabine or norethandronolone showed limited impact.[1–3] Hypomethylating agents (azacitidine [AZA] and decitabine) are DNA methyltransferase inhibitors with a favorable efficacy/safety profile in the treatment of myelodysplastic syndromes and AML.[4] Recently, AZA maintenance demonstrated survival gain in AML, either using a conventional subcutaneous administration route or a newly available oral formulation (CC-486) in clinical trials.[5–7] In this study, we report our single-center experience on the use of subcutaneous AZA maintenance in a real-world cohort of 39 patients with AML in first CR after intensive induction chemotherapy, with a focus on the impact of leukemic cell genotype on survival.

We retrospectively investigated a cohort of AML patients treated with intensive chemotherapy frontline, who received subcutaneous AZA maintenance after CR achievement from 2012 to 2022.

Characteristics of the 39 patients treated from 2012 to 2022 are reported in Table 1. Median age was 72 years (range, 23-78) and 44% had secondary AML. Conventional cytogenetic analysis was available at diagnosis for 36 patients, who were classified according to the national comprehensive cancer network (NCCN) cytogenetic risk categories[8] as favorable, intermediate or adverse in 5%, 72% and 15%, respectively. Moreover, most patients were classified as intermediate (41%) or adverse (44%) risk according to the 2017 European Leukemia Network (ELN) guidelines.[9] The genomic landscape of leukemic samples at diagnosis is provided (*Online Supplementary Figure S1*). All patients were in CR after one (87%) or two (13%) cycles of intensive induction chemotherapy with idarubicin (64%) or daunorubicin (26%) combined with cytarabine, and 33% received at least one consolidation cycle before AZA maintenance initiation (Table 1). During AZA maintenance, 14 patients received other treatments including venetoclax (n=5), tyrosine kinase inhibitor (n=4) and other (n=5), and two patients had HSCT after one and two AZA cycles. Patients received a median number of six (range, 1-38) AZA maintenance cycles. Initial AZA dosing was 75 mg/m^2/day during 7 days in all patients, and dose-reduction occurred in 36% of patients during follow-up (*Online Supplementary Table S1*). The most common adverse events during AZA maintenance were thrombocytopenia and/or neutropenia, leading to AZA dose reduction and discontinuation in 23% and 8% of patients, respectively (*Online Supplementary Table S1*). After a median follow-up of 20.2 (range, 4-102) months, only 10% patients remained on-therapy and the main causes of AZA discontinuation were relapse (36%) and persistence of CR (18%) (Figure 1; *Online Supplementary Table S1*).

From AZA maintenance onset, median RFS and OS were 18 and 30 months, respectively, for the whole cohort (Figure 2A, B). We performed subgroup analyses and observed using a univariate Cox model that presence of a *TP53* alteration (mutation and/or deletion), adverse ELN 2017 or cytogenetic NCCN risk categories, and administration of less than six cycles of AZA maintenance were significantly associated with decreased RFS and OS probabilities (Figure 2C, D). Particularly, ELN 2017 risk classifier, which aggregates several of these variables was highly predictive of survival (Figure 2E, F). Finally, comparison of variant allele frequency at diagnosis and post-AZA relapse suggested patterns of clonal evolution with reappearance of the initial clone in most cases and occasional subclone loss or gain (*Online Supplementary Figure S2*).

In our single-center retrospective real-world study, we found that subcutaneous AZA maintenance therapy was feasible in selected elderly patients with AML in CR after intensive induction chemotherapy. Moreover, the 2-year survival probabilities were similar to those observed in prospective clinical trials including the HOVON97 (subcutaneous AZA, 2-year RFS 44%) and QUAZAR-AML-001 (CC-486, median OS 24 months) trials.[5,7] In contrast to a recent large real-world study on AZA treatment in MDS/AML showing inferior survival in comparison with results from clinical trials,[10] our retrospective study confirmed the results of two large prospective phase III trials of AZA maintenance in AML, suggesting that the benefits of this strategy is also found in the routine practice. This could also have reflected an important selection of patients eligible for this strategy both in clinical trials and in routine practice due to the use of intensive chemotherapy induc-

Table 1. Patient characteristics.

Characteristics	N=39
Median age at diagnosis in years (min-max)	72 (23-78)
Sex, N (%)	
Male	21 (54)
Female	18 (46)
Hematological parameters at diagnosis, median (range)	
Bone marrow blasts %	55 (20-100)
WBC x10^9/L	4.3 (0.8-290)
Blood blasts %	15 (0-97)
Hemoglobin g/L	90 (36-130)
Platelets x10^9/L	100 (8-311)
Type of AML, N (%)	
De novo	22 (56)
Secondary	17 (44)
WHO 2016 classification, N (%)	
AML with recurrent genetic abnormalities	2 (5)
AML with myelodysplasia-related changes	18 (46)
Therapy-related myeloid neoplasms	5 (13)
AML NOS	14 (36)
ELN 2017 risk stratification, N (%)	
Favorable	5 (13)
Intermediate	16 (41)
Adverse	17 (44)
Unknown	1 (2)
NCCN cytogenetic risk, N (%)	
Favorable	2 (5)
Intermediate	28 (72)
Adverse	6 (15)
Unknown	3 (8)
Induction protocol, N (%)	
Daunorubucin + cytarabine	10 (26)
Idarubicin + cytarabine	25 (64)
Other#	4 (10)
Intensive treatment cycles, N (%)	
1 induction	34 (87)
≥2 inductions	5 (13)
≥1 consolidation	13 (33)
Remission type, N (%)	
CR	24 (62)
CRi	9 (23)
Unknown	6 (15)
Post-induction MRD, N (%)	
Positive	8 (20)
Negative	12 (31)
Unknown	19 (49)
Combined with AZA, N (%)	
Venetoclax	5 (13)
Anti-FLT3	4 (10)
Other	5 (13)
No treatment	25 (64)

#miniCIA (Idarubicin 12/mg/m^2 day 1+ subcutaneous cytarabine 40 mg/m^2/d day 1-5) or CPX-351. CRi: complete response with incomplete hematologic recovery defined according to the European LeukemiaNet (ELN) as CR criteria except for residual neutropenia (<1.0×10^9/L) or thrombocytopenia (<100×10^9/L); AML: acute myeloid leukemia; WHO: World Health Organization; NOS: not otherwise specified; NCCN: National Comprehensive Cancer Network; MRD: minimal residual disease; AZA: azacitidine.

tion. Representing a potential bias to this study, 36% patients received concomitant treatment during AZA maintenance including 13% with venetoclax. However, these time-limited associations had no impact on survival in this small cohort (Figure 2C, D).

HOVON97 and QUAZAR-AML-001 trials focused on the comparison of AZA maintenance with observation or placebo, respectively, and did not specifically investigate variables predicting AZA efficacy. Here, we showed that adverse ELN 2017 risk group predicted shorter survival upon AZA maintenance therapy even in a small-sized cohort of patients with AML. While ELN 2017 risk categories are strongly predictive of treatment response in patients with AML undergoing intensive chemotherapy,[11] the validation of our finding in larger cohorts could represent an important predictive tool in the context of AZA mainten-

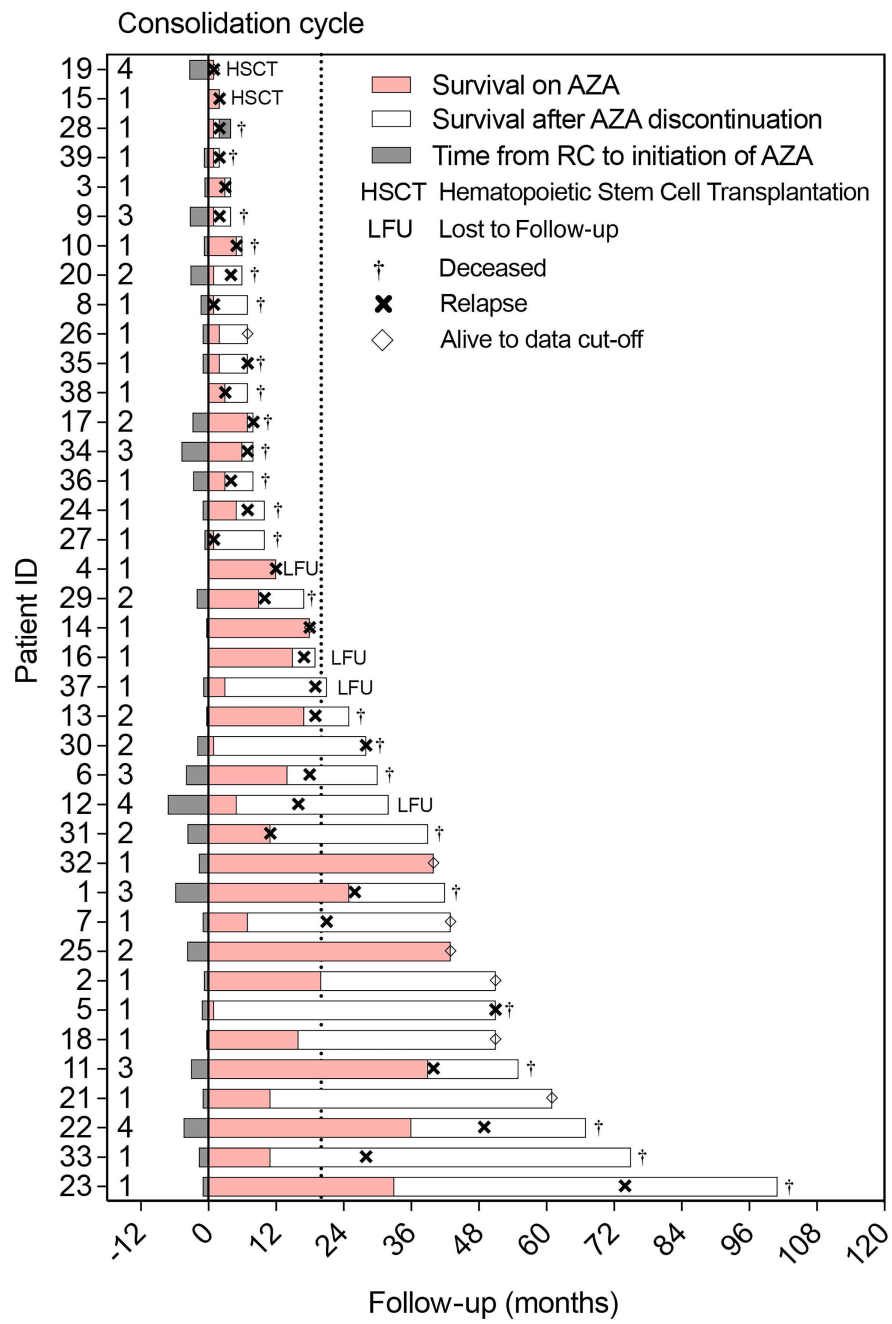
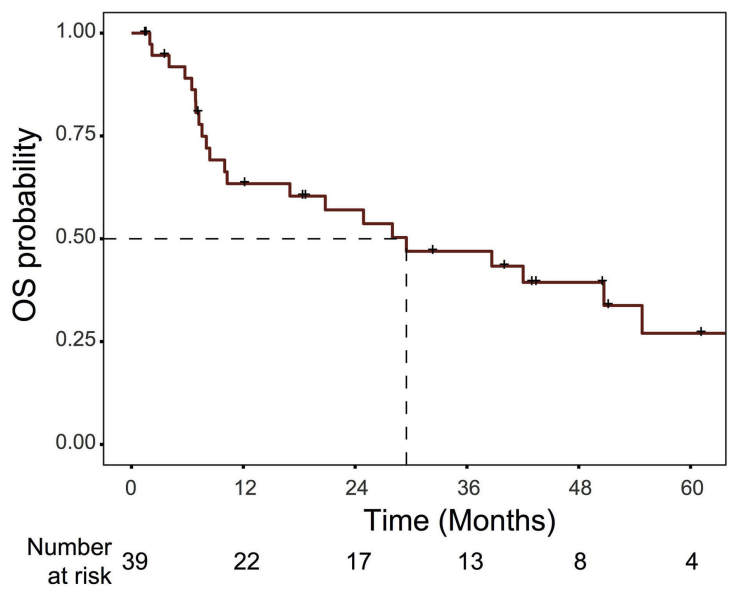


Figure 1. Swimmer plot representing time from CR (complete remission) to initiation of azacitidine (AZA), duration of AZA maintenance (beginning on day 1 of cycle one and ending at day 1 of the last cycle), survival after AZA discontinuation and time of confirmed relapse. Some patients who were lost to follow-up may have relapsed, and some may have relapsed after the end of follow-up (relapse date unknown).

A



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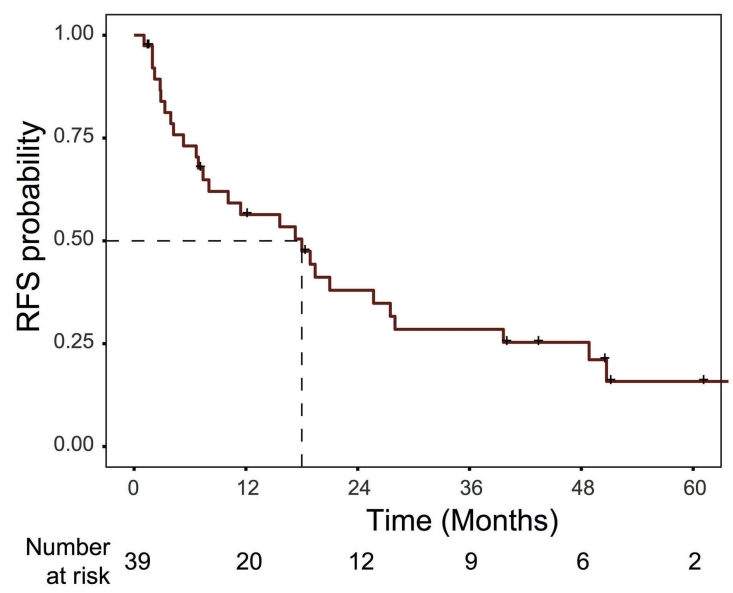


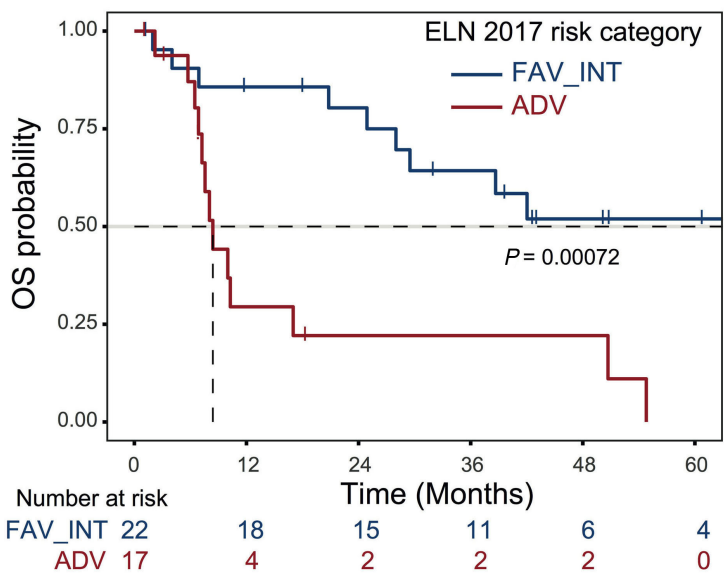
C

Variable	HR	95% CI	*P*
DNA methylation[1]	0.73	0.32-1.7	0.4
Chromatin-cohesin[2]	1.1	0.44-2.5	0.9
Splicing[3]	0.78	0.32-1.19	0.5
RTK-RAS signaling[4]	0.57	0.25-1.3	0.1
Transcription[5]	0.8	0.31-2.1	0.6
TP53[6]	5.2	1.5-18	**0.009**
NPM1[7]	0.43	0.13-1.5	0.1
ELN2017 adverse	4.2	1.7-10	**0.001**
NCCN adverse	5.5	1.7-17	**0.003**
Secondary AML	2.2	0.95-4.9	0.06
Other treatment	0.83	0.34-2	0.6
> 6 AZA	0.2	0.08-0.5	**0.0005**
MRD+	0,84	0.25-2.8	0.7

D

Variable	HR	95% CI	*P*
DNA methylation[1]	0.64	0.3-1.4	0.25
Chromatin-cohesin[2]	1.2	0.54-2.6	0.69
Splicing[3]	0.53	0.22-1.3	0.15
RTK-RAS signaling[4]	0.53	0.25-1.1	0.11
Transcription[5]	0.65	0.27-1.5	0.32
TP53[6]	5.2	1.7-16	**0.0041**
NPM1[7]	0.31	0.093-1	0.057
ELN2017 adverse	3.5	1.6-7.6	**0.0014**
NCCN adverse	4.2	1.4-13	**0.011**
Secondary AML	1.5	0.7-3.1	0.31
Other treatment	1	0.45-2.3	0.96
> 6 AZA	0.21	0.092-0.48	**0.00023**
MRD+	0.99	0.33-3	0.99

E



F

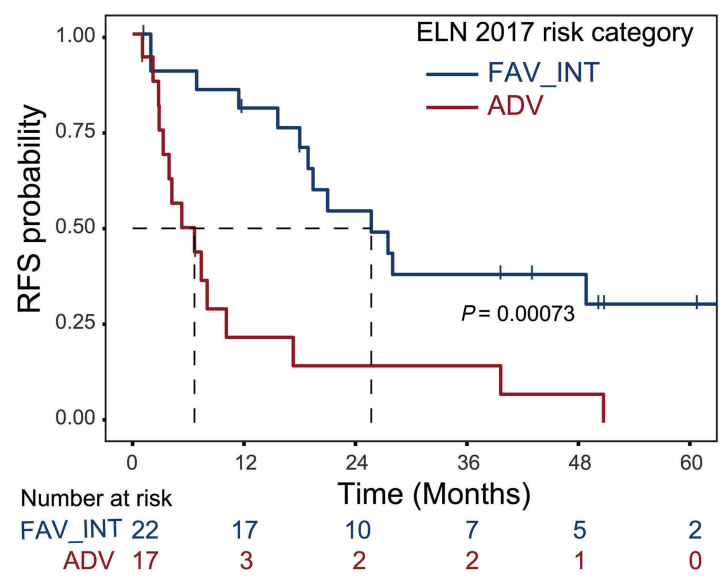


Figure 2. Survival analysis of patients with acute myeloid leukemia receiving azacitidine maintenance. (A, B) Survival analyses: from the onset of azacitidine (AZA) maintenance until the next event, defined as death for overall survival (OS) (A), and as death or disease progression for relapse-free survival (RFS) (B), and estimated using the Kaplan-Meier method. Patients were censored at the time of allogenic stem cell transplantation. (C, D) Univariate subgroup analysis of OS (C), and RFS (D). HR: hazard ratio; CI: confidence interval, 1: mutation in *IDH1, IDH2, TET2* or *DNMT3A*; 2: mutation in *ASXL1, EZH2, BCORL1, BCOR* or *STAG2*; 3: mutation in *SRSF2, SETBP1, SF3B1, ZRZR2* or *U2AF1*; 4: mutation in *FLT3, KRAS, NRAS, PTPN11, CBL, JAK2, CSF3R, MPL* or *RIT1*; 5: mutation in *CEBPA, RUNX1, GATA2, ETV6* or *WT1*; 6: mutation/deletion in *TP53*; 7: *NPM1* mutation; >6 AZA: patients who received more than 6 AZA maintenance cycles; MRD$^+$: positivity of minimal residual disease. (E, F) Survival stratified on ELN 2017 risk category. Kaplan Meyer representations for OS (E) and RFS (F).

ance as well. We also identified that patients receiving more than six cycles of AZA maintenance had improved survival, suggesting either that early treatment failure led to AZA discontinuation, or that the anti-leukemic activity of AZA required sufficient exposure to reach significant clinical benefit in patients. In addition, we observed that mutational status correlated with survival in our cohort, especially the presence of *TP53* alterations. We also observed that *NPM1* mutations were associated with a trend to improved RFS, in agreement with the results of the QUAZAR-AML-001 trial.[12] Together these results suggest that cytogenetic and molecular biomarkers, such as aggregated in the ELN 2017 risk stratification represent important predictive tools in the context of AZA maintenance therapy in AML. Recently, the combination of AZA with venetoclax (VEN), a BCL-2 inhibitor demonstrated a high activity in elderly patients with AML.[13] This strategy opens new perspectives currently evaluated in clinical trials, including the comparison of single-agent oral AZA maintenance *versus* oral AZA+VEN maintenance in older patients and the comparison of subcutaneous AZA+VEN in patients under 65 years old *versus* observation after CR (*clinicaltrials gov. Identifiers*, respectively: *NCT04102020, NCT05404906*), and will represent new opportunities to investigate predictive biomarkers as initiated in our current study.

To summarize, we showed that subcutaneous AZA maintenance is an effective option in patients with AML achieving CR in a real-world setting. We also observed that the ELN 2017 classifier efficiently predicted survival during AZA maintenance. While the duration of maintenance remains controversial, our data suggest that at least six cycles of AZA should be given to patients with AML achieving post-induction CR. The recent availability of oral AZA will probably result in a switch to a large use of this more convenient formulation, and future studies should investigate the impact of cytogenetic and molecular markers in large cohorts of patients with AML treated with oral AZA.

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Disclosures

No conflicts of interest to disclose.

Contributions

NJ collected clinical data. NJ and MT collected biological data. NJ, JT and JD performed statistical analyses and contributed to the analysis of the results. NJ, CF, IB, JT and JD designed the figures. NJ, JT and JD drafted the manuscript. All authors have reviewed and approved the final manuscript.

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Data-sharing statement

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

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