Fludarabine, cytarabine, and idarubicin with or without venetoclax in patients with relapsed/refractory acute myeloid leukemia

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Abstract

Treatment options for relapsed and refractory acute myeloid leukemia patients (R/R AML) are limited. This retrospective cohort study compares safety and efficacy of fludarabine, cytarabine, and idarubicin (FLA-IDA) without or with venetoclax (FLAVIDA) in patients with R/R AML. Thirty-seven and 81 patients received one course FLA-IDA with or without a 7-day course of venetoclax, respectively. The overall response rate (ORR) was significantly higher in FLAVIDA compared to FLA-IDA-treated patients (78% *vs*. 47%; *P*=0.001), while measurable residual disease was negative at a similar proportion in responding patients (50% *vs*. 57%), respectively. Eighty-one percent and 79% of patients proceeded to allogeneic hematopoietic cell transplantation or donor lymphocyte infusion after FLAVIDA and FLA-IDA, respectively. Event-free and overall survival were similar in FLAVIDA- and FLA-IDA-treated patients. Refractory patients could be salvaged more successfully after FLA-IDA compared to FLAVIDA pretreatment. Neutrophil and platelet recovery times were similar in the venetoclax and the control group. In conclusion, short-term venetoclax in combination with FLA-IDA represents an effective treatment regimen in R/R AML identifying chemosensitive patients rapidly and inducing measurable residual disease-negative remission in a high proportion of R/R AML patients.

Introduction

Primary refractory disease is found in 10-20% of younger and 50% of older acute myeloid leukemia (AML) patients after two courses of intensive induction therapy, and 50-70% of patients who obtain complete remission (CR) will relapse.[1,2] The primary goal in these patients is to reliably determine eligibility for allogeneic hematopoietic cell transplantation (alloHCT) and donor availability.[3] Salvage treatment with intensive chemotherapy regimens like fludarabine, cytarabine, and idarubicin (FLA-IDA), high-dose cytosine arabinoside and mitoxantrone (HAM), or mitoxantrone, etoposide and intermediate-dose Ara-C (MEC) induce CR in 35-58%, and median overall survival (OS) of 6.5-11.9 months in patients undergoing alloHCT and of 1.5-11.9 months in patients not eligible for alloHCT.[4-9]

Lower-intensity regimens combining the B-cell lymphoma-2 (BCL-2) inhibitor venetoclax and hypomethylating agents (HMA) or low-dose cytarabine (LDAC) demonstrated high efficacy in AML.[10-12] Based on the results of the VIALE-A trial venetoclax combined with HMA is approved for newly diagnosed patients with AML who are 75 years old, or unfit for intensive chemotherapy providing a new robust standard of care option for this frail population.[10] Subgroup biomarker analyses identified an association between *NPM1*-, *IDH1*- or *IDH2*-mutated AML and favorable response, whereas outcomes were similar in patients irrespective of *FLT3*-internal tandem duplication (ITD) mutational status.[10,13,14] Further analyses suggested that outcomes of patients with poor-risk cytogenetics in the absence of mutated *TP53* were similar to those with intermediate risk when treated with azacitidine plus venetoclax.[15]

In order to improve response to induction or salvage chemotherapy, venetoclax has been recently combined with intensive chemotherapy. The addition of venetoclax to cytarabine and idarubicin (FLAVIDA) or FLAG-IDA induced remarkable response rates (composite CR [CRc] of 97% and 90%, respectively) and promising OS (median not reached [NR] and 11.2 months, respectively) in newly diagnosed AML patients.[16,17]

In a subsequent propensity score matched analysis the efficacy of venetoclax combined with cytarabine, idarubicin and either fludarabine or cladribine was compared to treatment with cytarabine, idarubicin and either fludarabine, cladribine or clofarabine without venetoclax. The analysis included 279 newly diagnosed AML patients, with 85 patients in the venetoclax and 194 in the control cohort.[18] Eighty-six percent of patients in the venetoclax cohort achieved measurable residual disease (MRD)-negative CRc compared to 61% in the control cohort (odds ratio [OR] =3.2 ; 95% confidence interval [CI]: 1.5-6.7; *P*=0.0028). While event-free survival (EFS) was significantly improved (median NR; 95% CI: NR-NR *vs*. 14.3 months; 95% CI: 10.7-33.5; hazard ratio [HR] =0.57; 95% CI: 0.34-0.95; *P*=0.03), OS was not significantly different between both cohorts (median NR; 95% CI: 24-NR *vs*. 32 months; 95% CI: 19-NR; HR=0.63; 95% CI: 0.35-1.1; *P*=0.13.

In R/R AML, 67% of patients treated with FLAG-IDA with venetoclax (FLAVIDA) achieved a CRc and 46% transitioned to alloHCT. Median OS in R/R AML patients was 13 months (95% CI: 7-NR). Due to high rates of sepsis in the first six patients treated with FLAG-IDA with a 21-day regimen, venetoclax was reduced to 200 mg days 1-14 in the subsequent patients.[17] In another retrospective analysis of 25 patients including mainly R/R AML patients, venetoclax combined with FLAG induced CRc in 72%. In this study, venetoclax was administered at a dose of 400 mg once daily for a median of 8 days. Three patients (12%) experienced early death within 30 days of therapy initiation and 1-year OS was 50%.[19] Zucenka *et al.*[20] retrospectively compared the outcomes of 20 patients receiving venetoclax plus low-dose cytarabine plus actinomycin D with 29 patients receiving FLAG-IDA as salvage therapy for R/R AML after alloHCT. The CR/CRp rate was significantly higher in the venetoclax (FLAVIDA) compared to the FLAG-IDA groups (70%, n=14/20 *vs*. 34% n=10/29, respectively; *P*= 0.02).

Yet, it remains unclear, whether the high-response rates of regimens combining purine analogues and cytarabine with venetoclax translate into better survival outcomes. We, therefore, compared patients treated with FLA-IDA with a short 7-day course of venetoclax as salvage regimen in R/R AML patients with a historical cohort of FLA-IDA treated patients from our institution.

Methods

Inclusion criteria

Patients aged 18 years or older with refractory or relapsed (R/R) AML, who had been treated with FLAVIDA between May 2018 and July 2021 and had been reported to the venetoclax registry (venreg.org) were included in the analysis. Patients with acute promyelocytic leukemia or significant cardiovascular, renal or hepatic comorbidities were excluded. All patients had given written informed consent to the off-label use of venetoclax, genetic analysis and use of clinical data according to the Declaration of Helsinki and institutional guidelines. The registry was approved by the local Ethics Review Committee (ethical vote No.7972_BO_K_2018).

Treatment administration

All patients had received venetoclax for salvage chemotherapy in combination with fludarabine, cytarabine, and idarubicin (FLA-IDA).[21] Venetoclax was administered without dose ramp-up at a dose of 100 mg instead of 400 mg once daily per orally (days 1-7) due to mandatory co-medication with a CYP3A4 inhibitor for fungal prophylaxis, primarily posaconazole. The control patients were selected from the in-house database of Hannover Medical School and were treated with FLA-IDA between 2000 and 2018 for relapsed or refractory AML. Eighty-one patients were identified that fulfilled the treatment criteria of the FLAVIDA patients and were compared to the FLAVIDA patients included in this analysis (detailed information about the dosing scheme is provided in the *Online Supplementary Appendix*).

Cytogenetic and molecular analysis

Molecular and cytogenetic analysis was performed centrally by G- and R-banding analysis and next-generation sequencing (NGS) using peripheral blood or bone marrow as reported previously.[22] Molecular analysis was performed before start of chemotherapy, at time of relapse, and at time of refractory disease. MRD was assessed on bone marrow specimens using either mutation-specific real-time quantitative polymerase chain reaction (qRT-PCR) for *NPM1*, capillary electrophoresis for *FLT3*-ITD, or NGS-based MRD detection for any other MRD marker as reported previously.[23-26] In patients without detectable mutations by NGS, multi-parameter flow cytometry using leukemia-associated immunophenotypes (LAIP) was performed before start of FLAVIDA chemotherapy and for detection of measurable residual disease after the first cycle. MRD assessment was performed at the end of cycle one. Details on MRD assessment are provided in the *Online Supplementary Appendix*.

Safety and efficacy assessment

The primary objectives included safety and tolerability of a 7-day venetoclax regimen with FLA-IDA and assessment of the overall response rate (ORR). Secondary objectives included assessment of the MRD-negative response rate and survival outcomes including OS (time from treatment start to death) and EFS (time from treatment start until refractory disease, relapse or death, whichever occurred first). The ORR was defined by European LeukemiaNet

2017 (ELN) criteria and included CR, CR with incomplete blood count recovery (CRi) (composite complete remission CRc=CR+CRi), and morphologic leukemia-free state (MLFS, defined as less than 5% blasts in an aspirate sample without hematological recovery).[27] Details on efficacy and safety assessment are provided in the *Online Supplementary Appendix*.

Statistical analysis

Safety and efficacy analyses were performed for all patients who received at least one cycle of FLAVIDA chemotherapy. Demographics were analyzed by descriptive statistics. The propensity score for each patient was calculated as a probability from a logistic regression model that included all covariates deemed likely to have affected treatment decisions and response including age, ELN 2017 risk, sex, AML type, prior alloHCT, and refractory *versus* relapsed disease. Estimated propensity scores were incorporated into survival models using Inverse Probability of Treatment Weighting (IPTW) (see the *Online Supplementary Appendix*). The statistical analyses were performed using statistical software environment R, version 3.5.1 using packages survival, survminer, PSweight and cmprsk (R Foundation for Statistical Computing, Vienna, Austria), and statistical software package SPSS 27.0 (IBM Corporation, Armonk, NY, USA).

Results

Patient characteristics

Thirty-seven and 81 sequentially treated patients received FLAVIDA and FLA-IDA, respectively, and had safety and efficacy outcomes reported. Baseline characteristics were similarly distributed between FLAVIDA and FLA-IDA patients (Table 1). A similar proportion of patients had refractory AML (49% and 42%) or relapsed AML (51% and 58%) in the FLAVIDA and FLA-IDA groups, respectively. Refractoriness was not uniformly defined due to the retrospective design of the study. However, the number of prior chemotherapy cycles was comparable in FLAVIDA and FLA-IDA treated patients (Table 1). In addition, bone marrow blasts in patients considered refractory after one cycle of chemotherapy were similar between FLAVIDA and FLA-IDA treated patients (Table 1). Thus, characteristics defining refractory disease were met by a similar number of patients in both cohorts. The number of patients with prior alloHCT in relapsed FLAVIDA-treated patients was six of 19 (32%), and was similar in FLA-IDA control patients with 21 of 47 (44%). The median duration of the most recent CR before FLA(V)IDA therapy was 13.3 months in relapsed FLAVIDA patients and 11.9 months in relapsed FLA-IDA control patients (Table 1). The number of prior lines of therapy was comparable between both groups (Table 1). Mutation analysis was available for 36 FLAVIDA and 63 FLA-IDA patients. Molecular aberrations were similarly distributed between FLAVIDA and FLA-IDA patients except a lower frequency of *SRSF2* mutations in the FLA-IDA control group (Table 1).

Treatment response

All patients received either one cycle of FLAVIDA or one cycle of FLA-IDA chemotherapy. The ORR was 78% (n=29) in the FLAVIDA group compared to 47% (n=38) in the FLA-IDA group (P=0.001). The CRc rate was 59% and 30% in FLAVIDA and FLA-IDA patients, respectively (P=0.003, Table 2; Figure 1).

MRD data after one treatment cycle was available for 26 and only 23 patients with overall response (OR) in FLAVIDA and FLA-IDA cohorts, respectively. MRD-negative response was achieved in a similar proportion of patients treated with the two regimens (FLAVIDA 50% and FLA-IDA 57%; P=0.65, Table 2; *Online Supplementary Figure S1*). Exploratory analysis of response in clinical and genetic subgroups showed a high consistency of the favorable response to FLAVIDA compared to FLA-IDA across most subgroups, notably in patients with ELN adverse risk, and *IDH1/2* mutations, while the benefit was less pronounced in patients with refractory disease, and *KRAS/NRAS* mutations (Figure 2).

In summary, significantly more patients treated with FLAVIDA compared to FLA-IDA achieved OR and CRc, while the MRD rate was similar among responding patients.

Allogeneic hematopoietic cell transplantation

After FLAVIDA chemotherapy, 30 (81%) patients transitioned to alloHCT (n=27) or received donor lymphocyte infusions (DLI, n=3). Among responding patients, 90% (n=26/29) proceeded to alloHCT or received DLI. In the FLA-IDA cohort, 64 (79%) patients proceeded to alloHCT (n=49) or received DLI (n=15) (*Online Supplementary Table S1*). Among relapsed and refractory patients, usage of alloHCT as well as treatment outcome was similar (*Online Supplementary Appendix*; *Online Supplementary Figures S2A, B* and *S3A, B*). Dose intensity for conditioning was comparable in the FLAVIDA and FLA-IDA groups (reduced-intensity conditioning [RIC] FLAVIDA *vs*. FLA-IDA 61% *vs*. 67%; myeloablative conditioning [MAC] FLAVIDA *vs*. FLA-IDA 39% *vs*. 33%) (Table 1). In summary, both regimens allowed bridging to alloHCT or DLI in a high proportion of patients.

Survival

OS rates at 1 and 2 years were 52% and 48% in the FLAVIDA cohort after a median follow-up of 22.4 months and 61% and 52% in the FLA-IDA cohort after a median follow-up of 62.9 months, respectively. Despite a higher ORR in FLAVIDA patients, OS was similar between FLAVIDA and FLA-IDA-treated patients after propensity score

Table 1. Patient demographics, baseline and treatment characteristics of FLAVIDA- and FLA-IDA-treated patients.

Baseline characteristics	FLAVIDA N=37	FLA-IDA N=81	*P*
Age in years, median (range)	54 (19-70)	52 (22-72)	0.95
Sex, N (%)			0.9
Male	21 (57)	45 (56)	
Female	16 (43)	36 (44)	
Type of AML, N (%)			0.53
De novo	26 (70)	62 (77)	
Secondary	10 (27)	15 (19)	
Therapy-related	1 (3)	4 (5)	
ELN 2017 risk group, N (%)			0.2
Favorable	6 (16)	20 (25)	
Intermediate	19 (51)	37 (46)	
Adverse	12 (33)	20 (25)	
Missing	0 (0)	4 (5)	
Treatment lines before FLA(V)IDA			0.91
Median (range)	1 (1-5)	1 (1-4)	
Salvage 1, N (%)	26 (70)	57 (70)	
Salvage 2, N (%)	9 (25)	16 (20)	
Salvage 3 or greater, N (%)	2 (5)	8 (10)	
Disease status, N (%)			0.5
Refractory AML	18 (49)	34 (42)	
Relapsed AML	19 (51)	47 (58)	
Refractory after cycles of chemotherapy	N=18	N=34	0.34
1 cycle, N (%)	14 (78)	28 (82)	
≥2 cycles, N (%)	4 (22)	6 (18)	
Bone marrow blasts in patients considered refractory after 1 cycle			0.94
Median, % (range)	55 (15-90)	44 (5-95)	
Median duration in months of most recent CR before FLA(V)IDA therapy	13.3	11.9	0.81
Molecular mutations, N (%)	N=36	N=63	
DNMT3A	13 (36)	21 (33)	0.78
NPM1	11 (31)	14 (22)	0.36
SRSF2	9 (25)	3 (5)	<0.01
FLT3-ITD	8 (22)	19 (30)	0.39
IDH1	2 (6)	5 (6)	0.66
IDH2	8 (22)	9 (14)	0.31
RUNX1	7 (19)	5 (8)	0.09
NF1	6 (17)	5 (8)	0.18
K/NRAS	4 (11)	8 (13)	0.82
TP53	2 (6)	4 (6)	0.87
Prior alloHCT before FLA(V)IDA, N (%)			
Overall (relapsed and refractory)	8 (22)	23 (28)	0.44
In relapsed patients	6/19 (32)	21/47 (44)	0.33
Conditioning regimen, N (%)	N=27	N=49	0.48
Reduced-intensity conditioning	16 (59)	33 (67)	
Myeloablative conditioning	11 (41)	16 (33)	
Use of G-CSF after FLA(V)IDA, N (%)			0.63
Yes	25 (68)	56 (69)	
No	12 (32)	20 (25)	
Missing	0 (0)	5 (6)	

FLA-IDA: fludarabine, cytarabine, and idarubicin; FLAVIDA: FLA-IDA with venetoclax; AML: acute myeloid leukemia; alloHCT: allogeneic hematopoietic cell transplantation; CR: complete remission; ELN: European LeukemiaNet; G-CSF: granulocyte colony stimulating factor; N: number; *P*: *P* value.

Table 2. Treatment response after one cycle of FLAVIDA and FLA-IDA treatment.

	FLAVIDA N=37	FLA-IDA N=81	OR (95%CI)	*P*
ORR (CRc+MLFS), N (%)	29 (78)	38 (47)	0.001	4.1 (1.67-10.1)
CRc (CR+CRi), N (%)	22 (59)	34 (42)	0.003	2.0 (1.0-4.5)
CR	20 (54)	24 (30)		
CRi	2 (5)	10 (12)		
MLFS (%)	7 (19)	4 (5)	0.02	4.5 (1.23-16.5)
MRD- ORR	13/26 (50)	13/23 (57)	0.65	0.77 (0.25-2.4)
No Response (PR+SD+RD), N (%)	7 (19)	42 (52)	0.001	0.24 (0.1-0.6)
Early death (within 30 days), N (%)	1 (3)	1(1)	0.6	2.2 (0.14-36.5)

FLA-IDA: fludarabine, cytarabine, and idarubicin; FLAVIDA: FLA-IDA with venetoclax; CI: confidence interval; CR: complete remission; CRc: composite complete remission; CRi: complete remission with incomplete hematological recovery; MLFS: morphologic leukemia-free state; MRD: measurable residual disease; N: number; OR: odds ratio; ORR: overall response rate; *P*: *P* value; PR: partial remission; RD: refractory disease; SD: stable disease.

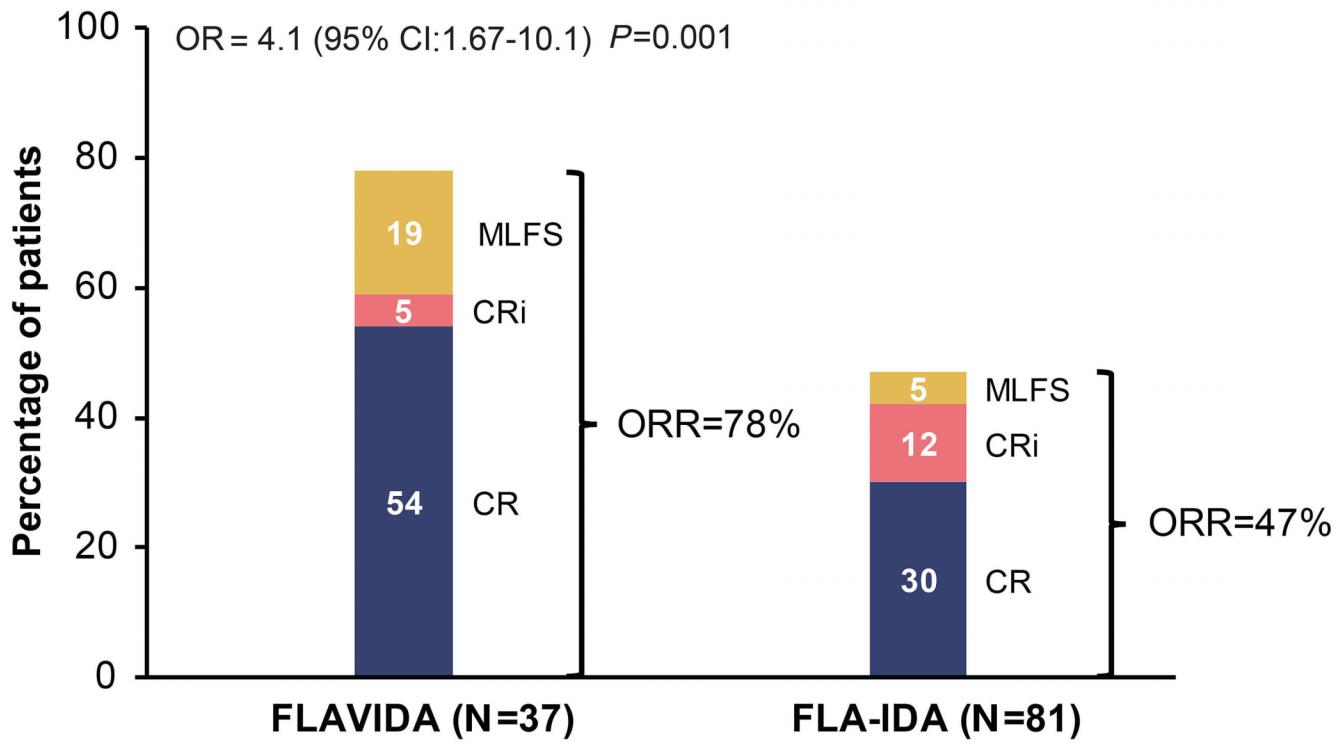


Figure 1. Frequency of complete remission, complete remission with incomplete hematological recovery, morphologic leukemia-free state, and overall response rate in FLAVIDA- (N=37) and FLA-IDA- (N=81) treated patients. FLA-IDA: fludarabine, cytarabine, and idarubicin; FLAVIDA: FLA-IDA with venetoclax; CR: complete remission; CRi: complete remission with incomplete hematological recovery; MLFS: morphologic leukemia-free state; ORR: overall response rate; CI: confidence interval.

weighting (HR=1.25; *P*=0.4) (Figure 3A; *Online Supplementary Table S2*). Median EFS was comparable between FLAVIDA and FLA-IDA patients (11.3 months *vs*. 6.9 months; HR=0.7; *P*=0.1) (Figure 3B; *Online Supplementary Table S2*). Survival outcomes and response rates were also similar when excluding patients treated with FLA-IDA before 2007, when antifungals were not available (*Online Supplementary Appendix*; *Online Supplementary Figure S4A, B*).

In the 26 responding FLAVIDA patients with available MRD data, survival was significantly longer in MRD-negative patients compared to MRD-positive patients (HR=0.1; 95% CI: 0.01-0.59; *P*=0.01) (Figure 4A). MRD was not prognostic for OS in responding FLA-IDA patients, while it should be recognized that MRD was available only for 28% of FLA-IDA-treated patients due to missing samples (HR=0.41; 95% CI: 0.1-1.7; *P*=0.23) (Figure 4B). However, OS rates at 1 year and 2 years were similar in MRD-negative FLAVIDA and FLA-IDA patients (OS at 1 year and 2 years FLAVIDA 89% and 89% *vs*. FLA-IDA 85% and 77%; *P*=0.4) (*Online Supplementary Figure S5*).

While RFS appeared superior by trend for MRD-negative compared to MRD-positive CR/CRi patients in the FLAVIDA group, RFS was comparable between MRD-negative and MRD-positive CR/CRi patients in the FLA-IDA group (FLAVIDA: HR for relapse or death, MRD^- *vs*. MRD^+ 0.2 ; 95% CI: 0.04-1.0; *P*=0.11; FLA-IDA: HR for relapse or death, MRD^- *vs*. MRD^+ 0.76; 95% CI: 0.27-2.1; *P*=0.59) (Figures 4C, D).

In order to better understand why a higher ORR and longer OS of MRD^- patients did not result in a survival benefit in

Characteristic	FLAVIDA (N=37) Patients N (%)	FLAVIDA (N=37) Events N (%)	FLA-IDA (N=81) Patients N (%)	FLA-IDA (N=81) Events N (%)	Odds ratio [95% CI]
Age in yrs					
Age≤60	25 (68)	21 (84)	57 (70)	28 (49)	5.44 [1.66-17.85]
Age>60	12 (32)	8 (67)	24 (30)	10 (42)	2.80 [0.66-11.92]
Sex					
Female	16 (43)	13 (81)	36 (44)	15 (42)	6.07 [1.47-25.09]
Male	21 (57)	16 (76)	45 (56)	23 (51)	3.06 [0.96-9.78]
AML type					
De novo	26 (70)	23 (88)	62 (77)	32 (52)	7.19 [1.95-26.43]
sAML/tAML	11 (30)	6 (55)	19 (24)	6 (32)	2.60 [0.56-12.02]
ELN					
Favorable	6 (16)	5 (83)	20 (25)	11 (55)	4.09 [0.40-41.66]
Intermediate	19 (51)	14 (74)	37 (46)	19 (51)	2.65 [0.79-8.87]
Adverse	12 (33)	10 (83)	20 (63)	7 (35)	9.29 [1.57-54.77]
Complex karyotype					
No	31 (84)	25 (81)	68 (84)	35 (52)	3.93 [1.43-10.79]
Yes	6 (16)	4 (67)	10 (12)	3 (30)	4.67 [0.53-40.89]
AML status					
Refractory	18 (49)	13 (72)	34 (42)	17 (50)	2.60 [0.76-8.91]
Relapsed	19 (51)	16 (84)	47 (58)	21 (45)	6.60 [1.69-25.74]
NPM1					
Wild-type	25 (69)	20 (77)	49 (78)	29 (43)	4.37 [1.56-12.26]
Mutated	11 (31)	9 (82)	14 (22)	9 (64)	2.50 [0.38-16.42]
FLT3-ITD					
No	28 (78)	22 (76)	44 (70)	28 (41)	3.82 [1.42-10.24]
Yes	8 (22)	7 (88)	19 (30)	10 (53)	6.30 [0.64-61.63]
IDH1/2					
Wild-type	26 (72)	20 (74)	49 (78)	33 (49)	2.94 [1.10-7.88]
Mutated	10 (28)	9 (90)	14 (22)	5 (36)	16.20 [1.56-167.74]
DNMT3A					
Wild-type	24 (64)	18 (75)	42 (67)	26 (43)	3.92 [1.37-11.27]
Mutated	13 (36)	11 (85)	21 (33)	12 (57)	4.13 [0.73-23.43]
TET2					
Wild-type	29 (81)	25 (83)	49 (78)	33 (49)	5.15 [1.76-15.06]
Mutated	7 (19)	4 (57)	14 (22)	5 (36)	2.40 [0.38-15.32]
KRAS/NRAS					
Wild-type	32 (89)	26 (79)	55 (87)	33 (45)	4.50 [1.74-11.68]
Mutated	4 (11)	3 (75)	8 (13)	5 (63)	1.80 [0.12-26.20]

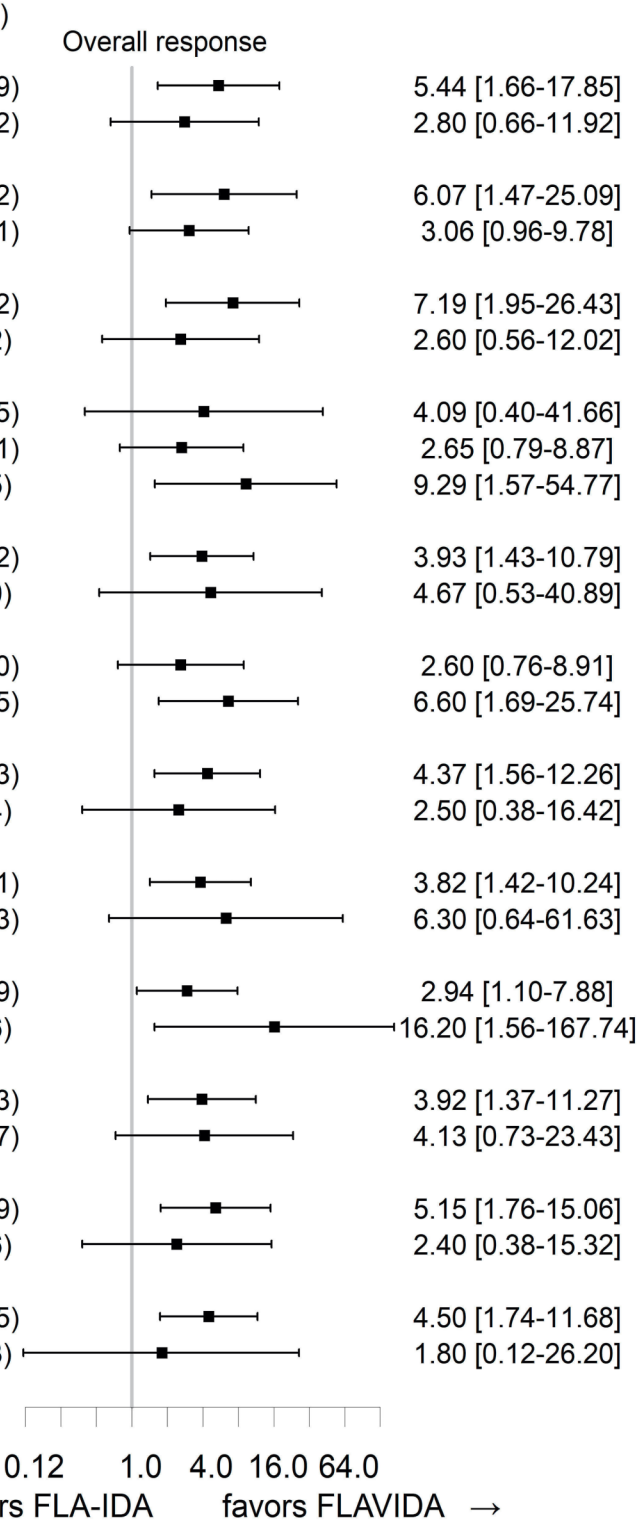


Figure 2. Impact of patient and disease characteristics on achieving overall response in FLAVIDA and FLA-IDA patients. *TP53* status not shown due to small number of mutated patients (FLAVIDA *TP53*^{mut} N=2; FLA-IDA *TP53*^{mut} N=4). FLA-IDA: fludarabine, cytarabine, and idarubicin; FLAVIDA: FlA-IDA with venetoclax; CI: confidence interval; sAML: secondary acute myeloid leukemia; tAML: therapy-related AML; ELN: European LeukemiaNet.

FLAVIDA treated patients, we evaluated survival differences across clinical and genetic subgroups between FLAVIDA and FLA-IDA regimens. EFS in IDH1/2 mutated patients favored FLAVIDA over FLA-IDA, while OS was similar in these patients (*Online Supplementary Figures S6* and *S7*). The only subgroup with different EFS and OS between FLAVIDA and FLA-IDA was the patient group not responding to these regimens, in which FLA-IDA treatment was associated with improved EFS and OS compared to FLAVIDA treatment (*Online Supplementary Figures S6* and *S7*).

Median OS from the time of non-response was significantly longer in non-responding FLA-IDA compared to non-responding FLAVIDA patients (HR=0.3; 95% CI: 0.14-0.71; *P*<0.001), suggesting that FLA-IDA treated patients could be salvaged more successfully (Figure 4). In addition, non-responding FLA-IDA patients numerically more often received alloHCT/DLI (FLA-IDA cohort 81%, n=35/43 *vs*. FLAVIDA cohort 50%, n=4/8; *P*=0.05), and had less pre-treatment lines (FLA-IDA median lines 1; range, 1-4 *vs*. FLAVIDA median lines 2; range 1-5; *P*=0.02) (*Online Supplementary Tables S3* and *S4*).

Safety

Most commonly observed treatment-related toxicities of

any grade were hematological adverse events (AE) being reported in all patients. Non-hematological AE of all grades included bacteremia, sepsis, and fungal pneumonia occurring in 27%, 11%, and 11%, respectively. One patient (3%) died within 30 days after start of FLAVIDA treatment from multiorgan failure following pneumonia. The most common grade 3 and 4 all-causality event was neutropenic fever (97%), while thrombocytopenia, anemia, and neutropenia occurred in all patients at nadir (*Online Supplementary Table S5*).

The median time to neutrophil recovery (>500/nL) in responding FLAVIDA-treated patients was 33 days (95% CI: 30-36) and 28 days (95% CI: 23-33) in the FLA-IDA cohort (*P*=0.94) from day 1 of chemotherapy (*Online Supplementary Figure S8A*). Recovery times for ANC >1,000/nL for responding FLAVIDA patients were 35 days (95% CI: 34-36), and were similar to those in the FLA-IDA cohort (34 days; 95% CI: 30-38; *P*=1.0) (*Online Supplementary Figure S8B*). Median time for platelet recovery (>50/nL) was 35 days (95% CI: 32-38) in the FLAVIDA cohort *versus* 34 days (95% CI: 27-41) in the FLA-IDA cohort (*P*=0.85) (*Online Supplementary Figure S8C*). Recovery time for platelet counts >100/nL for responding FLAVIDA patients was 36 days (95% CI: 33-39) compared to 34 days (95% CI: 31-37) in the FLA-IDA cohort (*P*=0.86) (*Online Supplementary Figure S8D*). Thus, absolute neutrophil

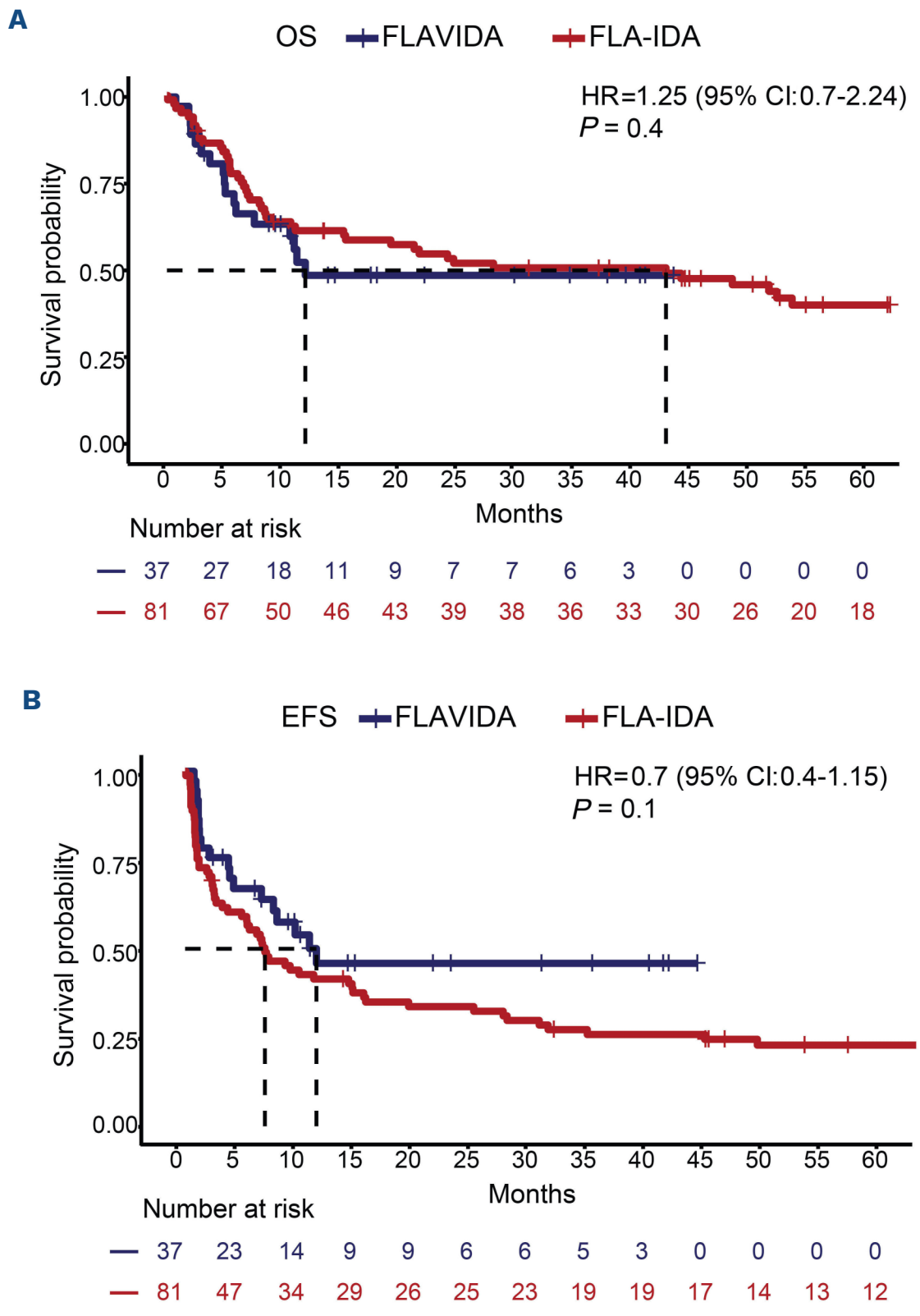
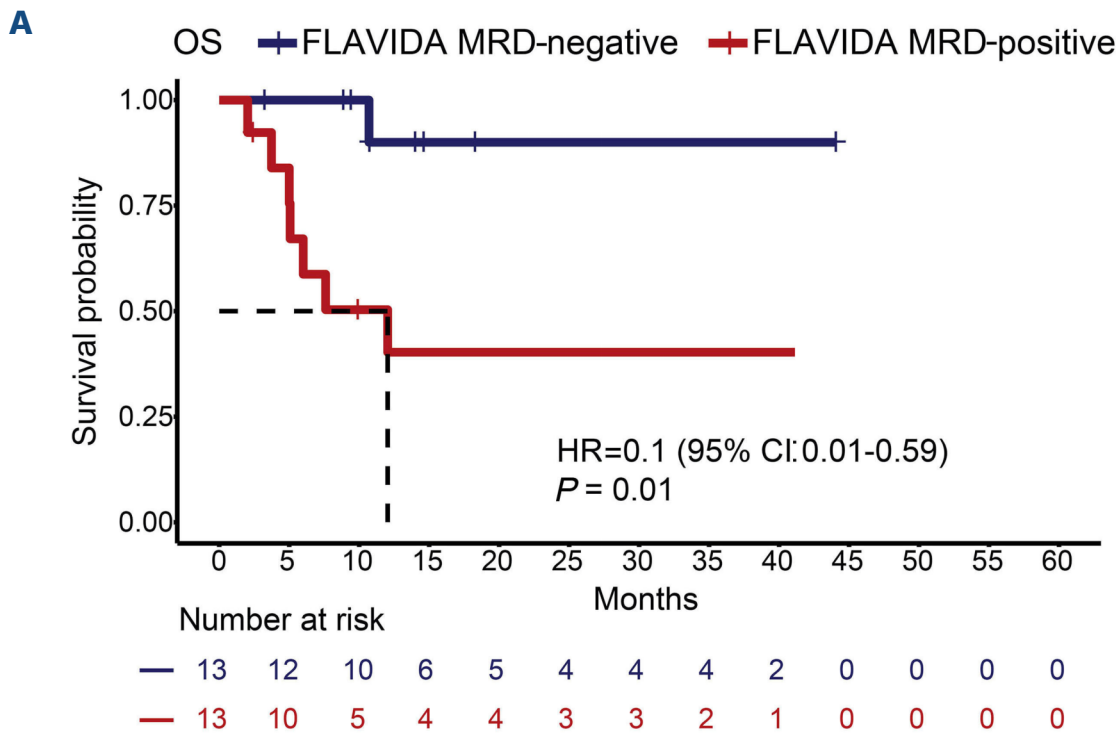


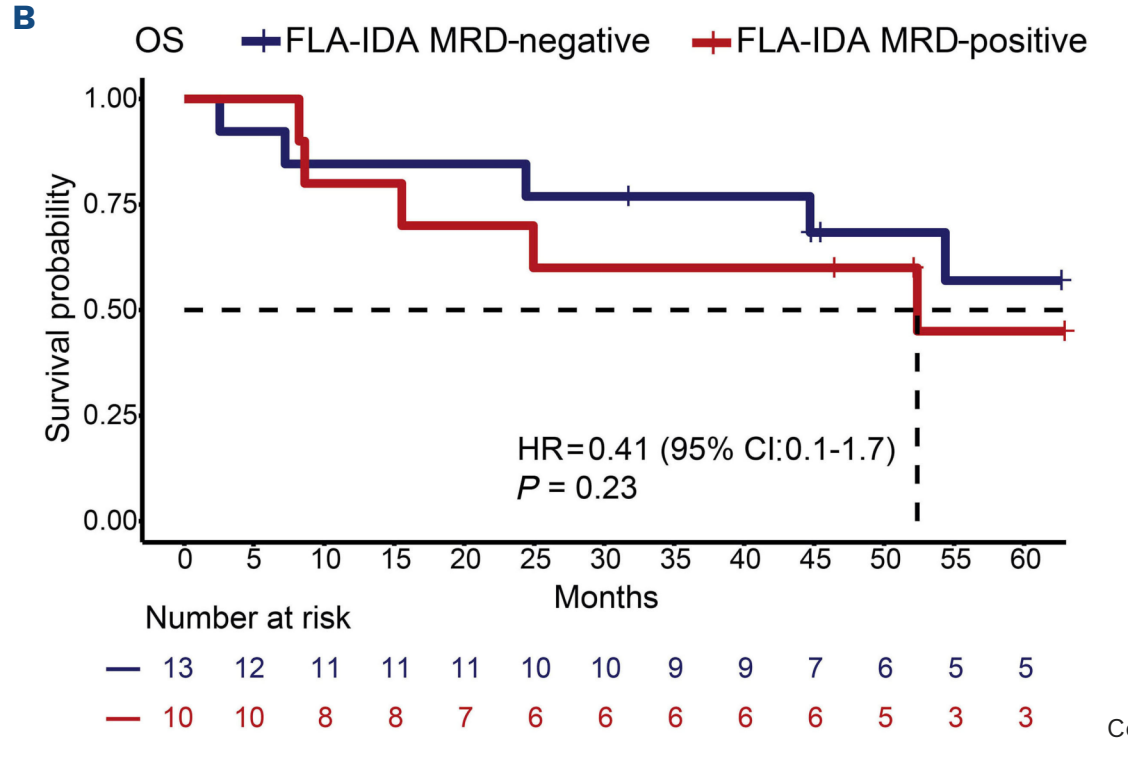
Figure 3. Survival outcomes in FLAVIDA- and FLA-IDA-treated patients. (A) Inverse probability of preatment weighted Kaplan Meier curves of overall survival (OS) in FLAVIDA- and FLA-IDA-treated patients. (B) Inverse probability of treatment weighted Kaplan Meier curves of event-free survival (EFS) in FLAVIDA- and FLA-IDA-treated patients. FLA-IDA: fludarabine, cytarabine, and idarubicin; FLAVIDA: FlA-IDA with venetoclax; HR: hazard ratio; CI: confidence interval.

count (ANC) and platelet recovery times were comparable between FLAVIDA and FLA-IDA regimens (*Online Supplementary Table S6*). Recovery times were also similar between both regimens when including patients with MLFS (FLAVIDA *vs*. FLA-IDA: ANC recovery >500/nL 35 days *vs*. 32 days, *P*=0.43; ANC recovery >1,000/nL 36 days *vs*. 38 days, *P*=0.86; PLT recovery >50/nL 36 days *vs*. 34 days, *P*=0.39; PLT recovery >100/nL 38 days *vs*. 34 days, *P*=0.22) suggesting similar hematologic toxicity of the two regimens.

Discussion

In this study, short-term venetoclax in combination with FLA-IDA salvage chemotherapy was well tolerated and demonstrated higher response rates compared to FLA-IDA, while OS was similar for the two regimens. High response rates were observed in patients with *de novo*, secondary and therapy-related AML, and across ELN and mutational subgroups. However, due to the small sample size subgroup analyses should be interpreted with caution. The response rate in the FLA-IDA-treated cohort was consistent with the previously reported response rates for this regimen.[28,29] Responding patients with available MRD data attained MRD negativity in a similar proportion in the FLAVIDA and FLA-IDA groups, respectively. DiNardo and colleagues[17] reported flow cytometry MRD negativity in 69% of responding patients who were treated with FLAG-IDA and venetoclax. Lachowiecz and colleagues[18] reported higher rates of MRD-negative remission in patients receiving intensive chemotherapy with *versus* without venetoclax during first line treatment (86% *vs*. 61%). In their study





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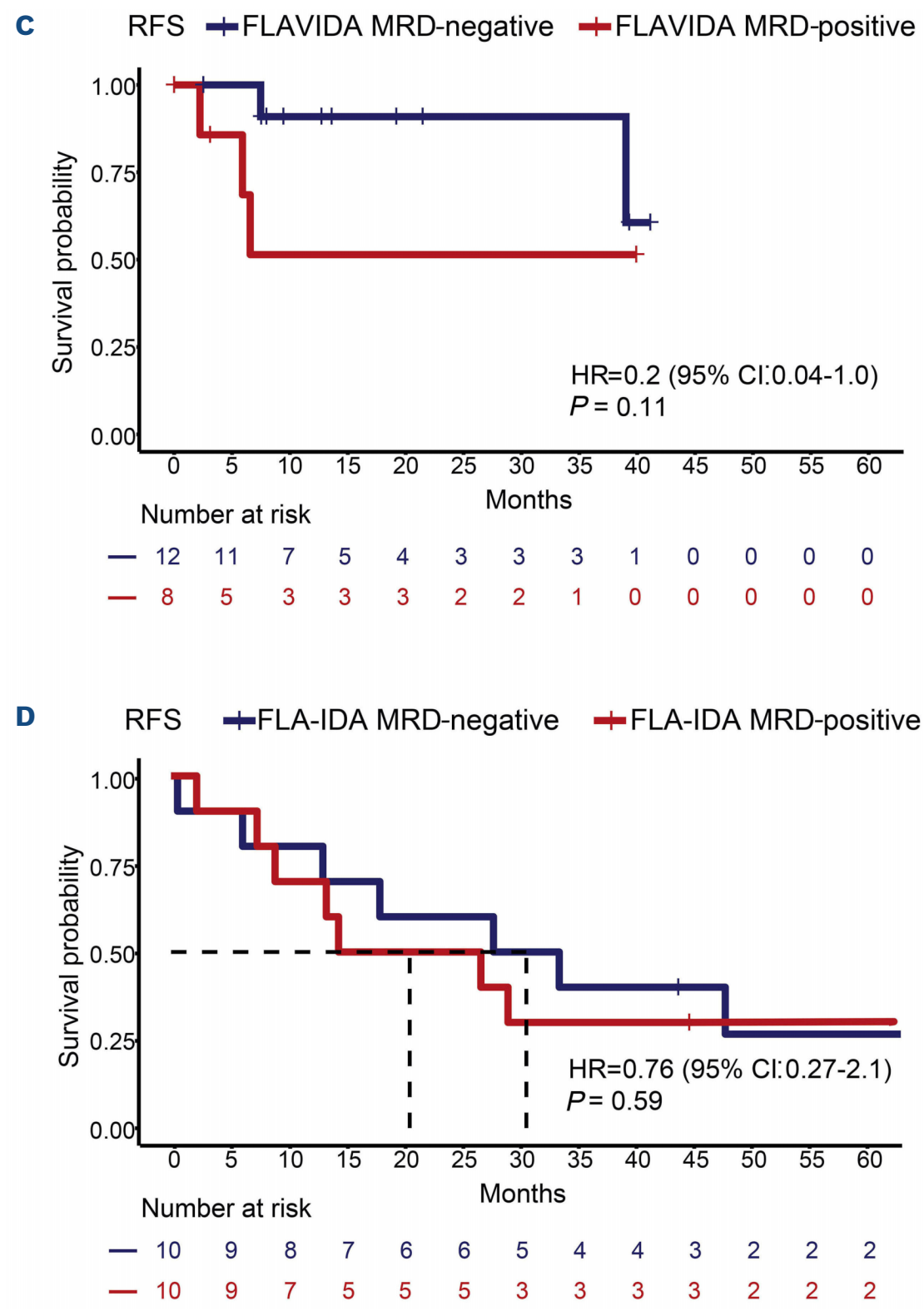

Figure 4. Survival outcomes in FLAVIDA- and FLA-IDA-treated patients depending on measurable residual disease status. (A) Overall survival (OS) in FLAVIDA-treated patients with measurable residual disease (MRD)-negative or MRD-positive overall response (including CR, CRi, and MLFS). Landmark analysis was performed from time of overall response. (B) OS in FLA-IDA-treated patients with MRD-negative or MRD-positive overall response (including CR, CRi, and MLFS). Landmark analysis was performed from time of overall response. (C) Relapse-free survival in FLAVIDA patients with MRD-negative or MRD-positive CR/CRi. Landmark analysis was performed from time of CR/CRi. (D) Relapse-free survival in FLA-IDA patients with MRD-negative or MRD-positive CR/CRi. Landmark analysis was performed from time of CR/Cri. FLA-IDA: fludarabine, cytarabine, and idarubicin; FLAVIDA: FlA-IDA with venetoclax; CR: complete remission; CRc: composite complete remission; CRi: complete remission with incomplete hematological recovery; MLFS: morphologic leukemia free state

a higher rate of MRD negativity was only found in ELN adverse-risk patients.

The high response rate enabled a high transplant/DLI rate of 81%, which was similar to the transplant rate in FLA-IDA-treated patients (79%; *P*=0.88).

Despite a significantly higher response rate in the FLAVIDA cohort, EFS and OS were similar in FLA-IDA-treated patients. Only *IDH1/2*-mutated patients showed an improved EFS with FLAVIDA, consistent with previous studies showing high sensitivity of these patients towards venetoclax, while other clinical and genetic subgroups showed similar EFS and OS with FLAVIDA or FLA-IDA.[13,30] The previously reported median OS of R/R AML patients treated with FLAG-IDA and venetoclax of 13 months and 12 months were similar to the median OS of 12 months found in our study.[17,19] In first line treated AML patients, the addition of venetoclax to intensive chemotherapy improved EFS but not OS.[18] Improved OS was only observed in the subgroup of ELN adverse risk patients.[18]

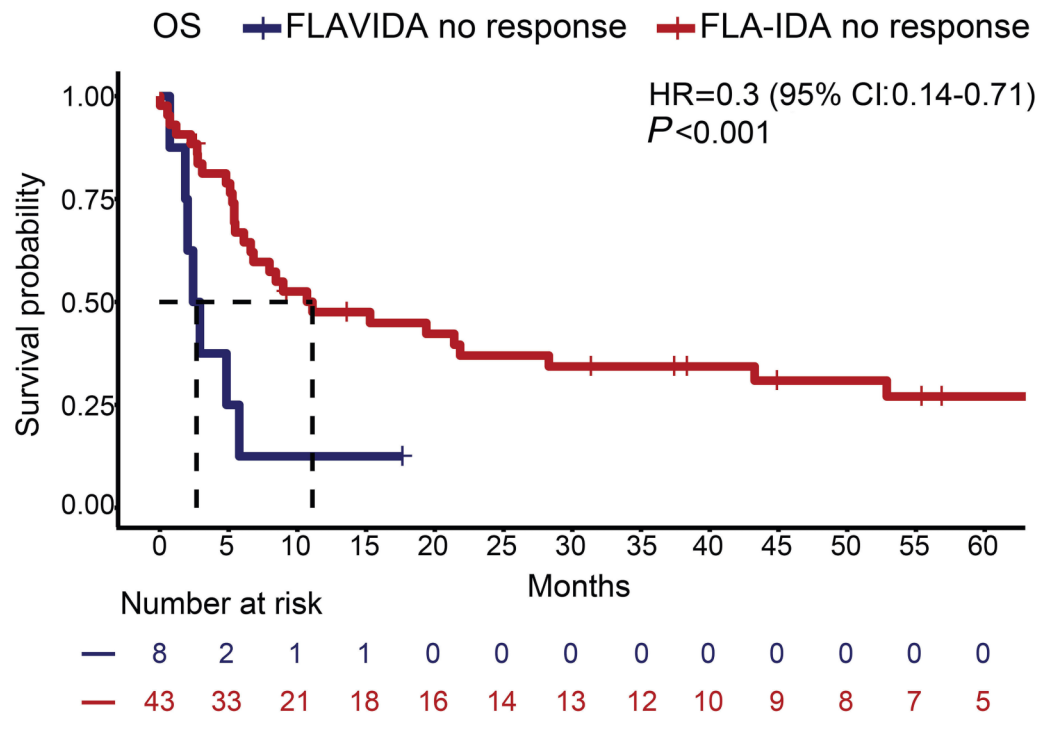

Figure 5. Overall survival in non-responding FLAVIDA- or FLA-IDA-treated patients. Landmark analysis was performed from time of first response assessment. OS: overall survival; HR: hazard ratio; CI: confidence interval. FLA-IDA: fludarabine, cytarabine, and idarubicin; FLAVIDA: FlA-IDA with venetoclax.

In the subgroup of patients with response to either regimen, RFS and OS was similar between FLAVIDA and FLA-IDA-treated cohorts. MRD-negative patients in the FLAVIDA group had a favorable 2-year-OS of 89%, while the 2-year OS of MRD-positive patients was 40%. Based on the higher response rate and comparable MRD negativity rate in FLAVIDA compared to FLA-IDA-treated patients, more patients with MRD$^-$ response can be expected from FLAVIDA treatment (corresponding to 39% *vs*. 27%), if our results are extrapolated to all treated patients.

We further evaluated how non-responding patients to either regimen survived after being refractory. We show that non-responding patients in the FLA-IDA cohort could be salvaged more effectively than non-responding patients in the FLAVIDA cohort. This is partially explained by a higher use of salvage alloHCT and fewer lines of prior treatments in FLA-IDA treated patients and is consistent with the dismal outcome in unfit AML patients who failed treatment with a hypomethylating agent and venetoclax.[31] These data suggest that FLAVIDA efficiently selects the most chemotherapy refractory patients, while some non-responding patients in the FLA-IDA group may still be rescued with additional alloHCT.

Our study raises important questions. First, it will be important to improve the outcome of MRD-positive patients in the FLAVIDA group for example with donor-lymphocyte infusions or maintenance treatment after alloHCT. Second, patients not responding to FLAVIDA have a dismal outcome and these patients should be treated in clinical trials. Third, it is of interest, whether MRD-negative patients in the FLAVIDA group benefit from alloHCT or can also achieve long-term remission with additional cycles of FLAVIDA since MRD negativity in the relapse setting has not yet proven its clinical value.

Since toxicity is a major concern when adding novel drugs to existing regimens, safety and toxicity are important outcomes of this study. Common grade 3 and 4 AE occurring in the FLAVIDA cohort were hematological toxicity and febrile neutropenia, which were managed with transfusions and anti-infective therapy. The early death rate was low with both treatment regimens. Comparison of ANC and platelet count recovery were overall comparable between FLAVIDA and FLA-IDA regimens suggesting that a 7-day course of venetoclax does not add significant hematologic toxicity to the FLA-IDA regimen. As the response and survival rates were similar to other published studies using FLAG-IDA and longer duration venetoclax (days 1-14 during induction and days 1-7 during consolidation when combined with FLAG-IDA; days 1-14 when combined with cytarabine+idarubicin 5+2), treatment with 7 days venetoclax simultaneously with chemotherapy appears similarly effective as venetoclax for longer periods of time.[17-19] Based on emerging data we now use venetoclax 50 mg combined with posaconazole or voriconazole.

Limitations of our study include the moderate sample size in the FLAVIDA group, retrospective analysis of FLAVIDA-treated patients, the non-randomized comparison to the FLA-IDA cohort and the long period in which FLA-IDA patients were treated. Using historical controls bears the risk of historical bias. However, since rates of alloHCT and OS were similar between the two cohorts, the historical control appears a valid comparator.

Our data demonstrate that FLAVIDA is an effective intensive salvage treatment option for R/R AML, particularly as a bridge to alloHCT, inducing high ORR including molecular remissions and allowing high rates of alloHSCT in this difficult to treat AML population. From a patient perspective it may seem preferable to identify chemotherapy respon-

sivess rapidly with a regimen like FLAVIDA instead of sequencing multiple salvage regimens until best response is achieved. In light of comparable toxicity this currently argues for the continued use of FLAVIDA in relapsed and refractory AML patients who are eligible for allogeneic transplantation.

Disclosures

RS declares honoraria from Abbvie and Jazz Pharmaceuticals. MH declares honoraria from Abbvie, Eurocept, Jazz Pharmaceuticals, Janssen, Novartis and Takeda; paid consultancy for Abbvie, Agios, BMS, Daiichi Sankyo, Glycostem, Jazz Pharmaceuticals, Kura Oncology, Novartis, Pfizer, PinotBio, Roche and Tolremo; research funding to his institution from Abbvie, Agios, Astellas, Bayer Pharma AG, BergenBio, Daiichi Sankyo, Glycostem, Jazz Pharmaceuticals, Loxo Oncology, Novartis, Pfizer, PinotBio and Roche. The other authors have no conflict of interest to disclose.

Contributions

RS and MH designed the study. RS, MH, and GB controlled the database. PK, LD, CA, MW and YA contributed to the collection of clinical and biological data. GB, RG, AS, AK, CK, MS, GG, BS, ZL, LD, PK, CA MW, YA, AB, MS, AG, and FT contributed to the analysis of clinical and biological data. RS, RG and MH performed the statistical analysis. RS and MH interpreted the data and wrote the manuscript. All authors read and agreed to the final version of the manuscript.

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Data-sharing statement

Individual patient data will not be made available in order to maintain health information privacy. De-identified MRD and mutation information will be shared upon reasonable request to the corresponding author.

References

1. Döhner H, Wei AH, Appelbaum FR, et al. Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. Blood. 2022;140(12):1345-1377.
2. Walter RB, Othus M, Burnett AK, et al. Resistance prediction in AML: analysis of 4601 patients from MRC/NCRI, HOVON/SAKK, SWOG and MD Anderson Cancer Center. Leukemia. 2015;29(2):312-320.
3. Heuser M, Ofran Y, Boissel N, et al. Acute myeloid leukaemia in adult patients: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up [published correction appears in Ann Oncol. 2021;32(6):821]. Ann Oncol. 2020;31(6):697-712.
4. Thol F, Heuser M. Treatment for relapsed/refractory cute myeloid leukemia. Hemasphere. 2021;5(6):e572.
5. Nath R, Reddy V, Kapur A, et al. Survival of relapsed/refractory acute myeloid leukemia (R/R AML) patients receiving stem cell transplantation (SCT). Biol Blood Marrow Transplant. 2019;25(3):S125.
6. Westhus J, Noppeney R, Dührsen U, Hanoun M. FLAG salvage therapy combined with idarubicin in relapsed/refractory acute myeloid leukemia. Leuk Lymphoma. 2019;60(4):1014-1022.
7. Marconi G, Talami A, Abbenante MC, et al. MEC (mitoxantrone, etoposide, and cytarabine) induces complete remission and is an effective bridge to transplant in acute myeloid leukemia. Eur J Haematol. 2020;105(1):47-55.
8. Canaani J, Nagar M, Heering G, et al. Reassessing the role of high dose cytarabine and mitoxantrone in relapsed/refractory acute myeloid leukemia. Oncotarget. 2020;11(23):2233-2245.
9. Megías-Vericat JE, Martínez-Cuadrón D, Sanz MÁ, Montesinos P. Salvage regimens using conventional chemotherapy agents for relapsed/refractory adult AML patients: a systematic literature review. Ann Hematol. 2018;97(7):1115-1153.
10. DiNardo CD, Jonas BA, Pullarkat V, et al. Azacitidine and venetoclax in previously untreated acute myeloid leukemia. N Engl J Med. 2020;383(7):617-629.
11. DiNardo CD, Maiti A, Rausch CR, et al. 10-day decitabine with venetoclax for newly diagnosed intensive chemotherapy ineligible, and relapsed or refractory acute myeloid leukaemia: a single-centre, phase 2 trial. Lancet Haematol. 2020;7(10):e724-36.
12. Wei AH, Montesinos P, Ivanov V, et al. Venetoclax plus LDAC for newly diagnosed AML ineligible for intensive chemotherapy: a phase 3 randomized placebo-controlled trial. Blood. 2020;135(24):2137-2145.
13. Lachowiez CA, Loghavi S, Kadia TM, et al. Outcomes of older patients with NPM1-mutated AML: current treatments and the promise of venetoclax-based regimens. Blood Adv. 2020;4(7):1311-1320.
14. Konopleva M, Thirman MJ, Pratz KW, et al. Impact of FLT3 mutation on outcomes after venetoclax and azacitidine for patients with treatment-naïve acute myeloid leukemia. Clin Cancer Res. 2022;28(13):2744-2752.
15. Pollyea DA, Pratz KW, Wei AH, et al. Outcomes in patients with poor-risk cytogenetics with or without TP53 mutations treated with venetoclax and azacitidine. Clin Cancer Res. 2022;28(24):5272-5279.
16. Chua CC, Roberts AW, Reynolds J, et al. Chemotherapy and venetoclax in elderly acute myeloid leukemia Trial (CAVEAT): a phase Ib dose-escalation study of venetoclax combined with modified intensive chemotherapy. J Clin Oncol. 2020;38(30):3506-3517.
17. DiNardo CD, Lachowiez CA, Takahashi K, et al. Venetoclax combined with FLAG-IDA induction and consolidation in newly diagnosed and relapsed or refractory acute myeloid leukemia. J Clin Oncol. 2021;39(25):2768-2778.
18. Lachowiez CA, Reville PK, Kantarjian H, et al. Venetoclax combined with induction chemotherapy in patients with newly diagnosed acute myeloid leukaemia: a post-hoc, propensity

score-matched, cohort study. Lancet Haematol. 2022;9(5):e350-e360.
19. Wolach O, Frisch A, Shargian L, et al. Venetoclax in combination with FLAG-IDA-based protocol for patients with acute myeloid leukemia: a real-world analysis. Ann Hematol. 2022;101(8):1719-1726.
20. Zucenka A, Vaitekenaite V, Maneikis K, et al. Venetoclax-based salvage therapy followed by Venetoclax and DLI maintenance vs. FLAG-Ida for relapsed or refractory acute myeloid leukemia after allogeneic stem cell transplantation. Bone Marrow Transplant. 2021;56(11):2804-2812.
21. Shahswar R, Beutel G, Klement P, et al. FLA-IDA salvage chemotherapy combined with a seven-day course of venetoclax (FLAVIDA) in patients with relapsed/refractory acute leukaemia. Br J Haematol. 2020;188(3):e11-e15.
22. Heuser M, Gabdoulline R, Löffeld P, et al. Individual outcome prediction for myelodysplastic syndrome (MDS) and secondary acute myeloid leukemia from MDS after allogeneic hematopoietic cell transplantation. Ann Hematol. 2017;96(8):1361-1372.
23. Gabert J, Beillard E, van der Velden VHJ, et al. Standardization and quality control studies of 'real-time' quantitative reverse transcriptase polymerase chain reaction of fusion gene transcripts for residual disease detection in leukemia - a Europe Against Cancer Program. Leukemia. 2003;17(12):2318-2357.
24. Thol F, Gabdoulline R, Liebich A, et al. Measurable residual disease monitoring by NGS before allogeneic hematopoietic cell transplantation in AML. Blood. 2018;132(16):1703-1713.
25. Krönke J, Schlenk RF, Jensen K-O, et al. Monitoring of minimal residual disease in NPM1-mutated acute myeloid leukemia: a study from the German-Austrian acute myeloid leukemia study group. J Clin Oncol. 2011;29(19):2709-2716.
26. Schuurhuis GJ, Heuser M, Freeman S, et al. Minimal/measurable residual disease in AML: a consensus document from the European LeukemiaNet MRD Working Party. Blood. 2018;131(12):1275-1291.
27. Döhner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood. 2017;129(4):424-447.
28. Bergua JM, Montesinos P, Martinez-Cuadrón D, et al. A prognostic model for survival after salvage treatment with FLAG-Ida +/- gemtuzumab-ozogamicine in adult patients with refractory/relapsed acute myeloid leukaemia. Br J Haematol. 2016;174(5):700-710.
29. Roboz GJ, Rosenblat T, Arellano M, et al. International randomized phase III study of elacytarabine versus investigator choice in patients with relapsed/refractory acute myeloid leukemia. J Clin Oncol. 2014;32(18):1919-1926.
30. DiNardo CD, Pratz K, Pullarkat V, et al. Venetoclax combined with decitabine or azacitidine in treatment-naive, elderly patients with acute myeloid leukemia. Blood. 2019;133(1):7-17.
31. Maiti A, Rausch CR, Cortes JE, et al. Outcomes of relapsed or refractory acute myeloid leukemia after frontline hypomethylating agent and venetoclax regimens. Haematologica. 2021;106(3):894-898.