Single-center retrospective clinical evaluation of venetoclax combined with HMAs and half-dose CAG in the treatment of unfit or refractory/relapsed AML

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Research Article

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Abstract

Purpose The prognosis of unfit or relapsed/refractory (R/R) AML patients remains poor. Venetoclax has been proved to have anti-leukemia stem cell activity. However, there is little published information on the efficacy and safety of VEN combined with both demethylated drugs and half-dose chemotherapy in the treatment of unfit or R/R AML patients.

Methods In this study, we retrospectively analyzed the clinical characteristics, treatment details, safety profile and clinical outcomes of unfit or R/R AML patients who were treated with VEN+ HMAs+ half-dose CAG.

Results A total of 21 AML patients were involved in the study, 11 patients (52.4%) were in the unfit group, while 10 patients (47.6%) were in R/R group. FLT3 (6/21, 28.6%) was the most common gene aberration. Patients in the R/R group were found more likely to carry KIT (5/ 10, 50%) when compared to the unfit group (0/11, 0%) (P = 0.012). The median duration of CR+ CRi was 11.5 months (95% CI 8.9 to 14.1). Twelve patients achieved CR (57.1%), two CRi (9.5%), and three PR (14.3%), for an ORR 80.9% (17/21) and cCR 66.7% (14/21). CR was observed in 100% (5/5) AML patients with *TP53*, 80% (4/5) AML patients with *IDH*, 75% (3/4) AML patients with *NPM1*, 50% (3/6) AML patients with *FLT3*, 40% (2/5) AML patients with *KIT*, 40% (2/5) AML patients with *DNMT3A*, and 20% (1/5) AML patients with *RUNX1*. The most common AEs during VEN+ HMAs+ half-dose CAG therapy were persistent cytopenia, infection and gastrointestinal symptoms (decreased appetite, nausea, vomiting and diarrhea).

Conclusion The results of this investigation show that VEN+ HMAs+ half-dose CAG demonstrated promising efficacy (even high-risk cytogenetics) and a tolerable safety profile in unfit or R/R AML patients.

1. Introduction

Acute myeloid leukemia (AML) is predominantly a disease of the elderly, with a median age of 68 years at diagnosis [1, 2]. Because of advanced age, high frequency of unfavorable genomic features and less favorable clinical characteristics, such as declining biological function and more complex medical comorbidities, elderly patients usually cannot be unable to tolerate a high dose of chemotherapy [3, 4]. Simultaneously, refractory/relapsed (R/R) AML is still associated with dismal prognosis [5]. In the population of R/R AML, the treatment options are limited, treatment benefits are inadequate and the cure rates remain as low as 10% [6, 7].

B-cell leukemia/lymphoma-2 (BCL2) family play a central role in regulating cell survival and apoptosis [8]. Moreover, AML stem cells express aberrantly high levels of the anti-apoptotic gene BCL2 and rely on the expression of BCL2 to maintain their survival [9]. Over-expressed of BCL2 has been shown to have a significant impact on inferior chemotherapeutic response and adverse overall survival in AML patients [10].

Venetoclax (VEN) is an oral, potent BCL-2 selective inhibitor, proven to have anti-leukemic activity and able to induce cancer cell death [11]. Recently, several small retrospective studies evaluated the efficacy and chemotherapy toxicity of VEN in combination with hypomethylating agents [HMAs, such as decitabine (DAC) or azacitidine (AZA)] or low-dose cytarabine (LDAC) [4]. For elderly/unfit or R/R AML patients, VEN combination therapy has been shown to improve the response rates and survival [12-14].

HMAs combined with CAG regimen [LDAC, aclarubicin and granulocyte colony-stimulating factor (G-CSF)] tends to improve the prognosis of older patients with high-risk AML [15]. In patients with R/R AML, it was observed that increased dosage of the conventional CAG regimen was more effective as salvage therapy [16].

VEN-HMAs/LDAC usually lead to profound and prolonged neutropenia, which increases the risk of getting infected in AML patients. In order to overcome the toxicity of VEN-HMAs/LDAC, we considered whether the treatment duration of VEN could be shortened and, in the meantime, combined with low-dose chemotherapy to maintain the response rate. There is little published information on the efficacy and side effects of VEN combined with HMAs and half-dose CAG.

Here we report the clinical characteristics, treatment details, clinical outcomes and safety profile of 21 consecutive patients with unfit or R/R AML who were treated with VEN+ HMAs+ half-dose CAG.

2. Methods

2.1. Patients

In this retrospective study, we analyzed consecutive AML patients who were treated at the First Affiliated Hospital of Harbin Medical University during the period of January 1, 2020 to January 1, 2022. Diagnostic procedures and genetic risk stratification were carried out according to European Leukemia Net (ELN) 2017 recommendations [17]. The unfit group included newly diagnosed AML patients who were considered to be ineligible for high-dose chemotherapy if they were ≥60 years old, the ECOG score≥3 or have complex medical comorbidities. This study included all the unfit or R/R AML patients who were treated with VEN+ HMAs+ half-dose CAG. This retrospective study was approved by the Medical Ethics Committee of the First Affiliated Hospital of Harbin Medical University and the informed consent was waived.

2.2. Treatment

VEN+ HMAs+ half-dose CAG regimens were administered as follows: all patients received VEN once a day, for 14 days. To prevent tumor lysis syndrome (TLS), the dose of VEN was given 100mg on day 1 and 200mg on day 2; on day 3, to elevate the concentration of VEN alongside routine antifungal prophylaxis, we combined CYP3A4 inhibitors fluconazole 400mg once a day [18]. After the application of VEN for five days, we measured the plasma concentration of VEN. HMAs therapy comprising DAC 20 mg/m^2/d intravenously for five days or AZA 75 mg/m^2/d subcutaneously for seven days. Half-dose CAG regimen consists of LDAC 10 mg/m^2/12h subcutaneously for fourteen days, aclarubicin 8 mg/m^2/d intravenously for four days, and G-CSF 200 μg/m^2/d subcutaneously for fourteen days. For patients with leukocytosis, whose white blood cell (WBC) count was greater than 10×10^9/L, hydroxyurea (1.0–3.0 g/day) was given orally, until the WBC count dropped below 10×10^9/L, and then the chemotherapy began. All patients did not receive other anti-leukemic agents (e.g. FLT3, IDH1, IDH2 inhibitors) at the same time. According to clinical manifestations and blood routine, appropriate anti-infective therapy and supportive treatment [red blood cell (RBC) and platelet (PLT) transfusions] was given.

2.3 Adverse events reporting

The severity of adverse events was estimated according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0[19]. Clinical and laboratory data about adverse events were collected from patients' files.

2.4 Treatment responses

Response to treatments was determined by bone marrow (BM) evaluation at the time of hematological recovery and adjudicated according to the recommendations of 2017 ELN [19]. Assessment of minimal residual disease (MRD) was conducted for all patients, including patients with incomplete hematological recovery. Complete response (CR) was defined as bone marrow blasts <5% with hematological recovery [absolute neutrophil count (ANC) $\geq1.0\times10^9$/L and PLT $\geq100\times10^9$/L] [3]. CR without minimal residual disease (CR_{MRD-}) was defined as CR with negativity for a genetic marker by RT-qPCR, or CR with negativity by MFC [3]. CR_i was defined as CR with incomplete hematological recovery, and partial response (PR) as bone marrow blast percentage to 5% to 25% or decrease of pretreatment bone marrow blast percentage by at least 50% [3]. Overall response rate (ORR) was the sum of the CR, CRi, and PR. Relapse was defined as bone marrow aspirate reappearance of leukemic blast (>5%) after a CR had been achieved [3].

2.5 Statistical analysis

Patient characteristics were summarized using descriptive statistics (medians or frequencies with ranges). For binary variable, Chi-squared or Fisher's exact test were used to analyze the differences between the two subgroups. Time-to-event endpoints

were measured using Kaplan-Meier method and the log-rank test was used to compare the statistically differences between subgroups. Data were statistically analyzed with SPSS version 22.0 (IBM SPSS Statistics, IBM Corporation, Armonk, NY).

3. Results

3.1. Patients

From January 2020 to January 2022, a total of 21 AML patients were treated at our institution with VEN+ HMAs+ half-dose CAG regimens. Eleven patients (52.4%) were enrolled in the unfit group and ten patients (47.6%) in R/R group (relapsed, n = 8; refractory, n = 2) (Table 1). Table 1 summarized the patients' details at the time of VEN+ HMAs+ half-dose CAG initiation. The median age was 55 years (range, 21-72 years), and 9/21 (42.9%) patients were≥ 60 years. Age of unfit group was significantly higher than that of R/R group (*P*=**0.016**). The differences of other clinical features between the two groups did not reach statistical significance (Table 1).

Table 1. Clinical characteristics of 21 AML patients.

Characteristic	All(n=21)	unfit（n=11）	R/R（n=10）	P vaule
Age, years	55(21-72)	62(43-72)	47(21-68)	**0.016**
≥ 60	9(43%)	7(64%)	2(20%)	
＜60	12(57%)	4(36%)	8(80%)	
Gender				
Male	10(48%)	5(45%)	5(50%)	0.590
Female	11(52%)	6(55%)	5(50%)	
ECOG performance status				
0-1	11(52%)	4(36%)	7(70%)	0.198
2-3	10(48%)	7(64%)	3(30%)	
WBC count, ×109/L				
Median (range)	34.8(1.01-151.21)	43.3(1.01-151.21)	25.3(1.22-101.57)	0.973
PLT count, ×109/L				
Median (range)	74.9(3-390)	117.4(8-390)	27.8(3-69)	0.072
HGB level (g/L)				
Median (range)	80.3(54-112)	84.8(60-112)	75.2(54-98)	0.605
Bone marrow blast count				
<30%	9(43%)	5(45%)	4(40%)	0.630
≥30 to <50%	4(19%)	1(9%)	3(30%)	
≥50%	8(38%)	5(45%)	3(30%)	
2017 ELN risk stratification by genetics				
Favorable	3(14%)	2(18%)	1(10%)	0.876
Intermediate	2(10%)	1(9%)	1(10%)	
Adverse	16(76%)	8(73%)	8(80%)	
Overall response	16(76%)	9(82%)	7(70%)	0.258
CR	12(57.1%)	8(72.7%)	4(40%)	
CRi	2(9.5%)	1(9.1%)	1(10%)	
PR	3(14.3%)	0(0%)	3(30%)	

One patient had previously undergone allogeneic HSCT, but early recurrence occurred after transplantation. Nine patients received prior therapy with HMAs. For R/R group, the median number of prior therapies was 2 (range, 1-5). Nine R/R patients (81.8%) received VEN+ HMAs+ half-dose CAG therapy as the first salvage treatment.

3.2. Biological Characteristics

Molecular aberrations were identified using next-generation sequencing. Cytogenetic and molecular aberration results are available in all AML patients, and they are summarized in Fig. 1. Nearly half of all patients (10/21) had complex karyotype (≥3 abnormalities). As for molecular aberrations, ranging from 1 to 8 gene mutations per patient were detected. The average

mutational burden was 4 (Figure 1). In our study, *FLT3* (6/21, 28.6%) was the most common gene aberration (Figure 1). The second frequently mutated gene was *KIT*、*RUNX1*、*DNMT3A*、*TP53* and *IDH* (5/21, 23.8%) (Figure 1).

As reported in previous studies, we obtained the same results: cytogenetics and molecular characteristics were balanced between the two AML groups. The incidence of high-risk cytogenetics was the same in the unfit group (8/11) and R/R group (8/10) (P = 0.876, Table 1). In addition, patients in the R/R group were found more likely to carry *KIT* (5/ 10, 50%) when compared to the unfit group (0/11, 0%) (P = 0.012, Figure 1). The distribution of other mutations didn't reach statistically significant difference.

3.3. Response

Median follow-up for 21 AML patients was 13.6 months (range, 0.1-22 months), and the median duration of CR+ CRi was 11.5 months [95% confidence interval (CI) 8.9 to 14.1]. Twelve patients achieved CR (57.1%), two CRi (9.5%), and three PR (14.3%). We observed an ORR of 80.9% (17/21), with 66.7% (14/21) of the patients achieving cCR (Figure 1 and Table 1). Ten patients (6 in the unfit group and 4 in R/R group) achieved MRD negative status evaluated by flow cytometry. In the unfit group, 9/11 (81.8%) patients achieved response: eight CR and one CRi; while 8/10 (80%) R/R patients achieved response: four CR, one CRi and three PR. The details of responders were provided in Table 2.

In our study, CR was observed in 100% (5/5) AML patients with *TP53*, 80% (4/5) AML patients with *IDH*, 75% (3/4) AML patients with *NPM1*, 50% (3/6) AML patients with *FLT3*, 40% (2/5) AML patients with *KIT*, 40% (2/5) AML patients with *DNMT3A*, and 20% (1/5) AML patients with *RUNX1* (Figure 1).

Table 2. Characteristics of responding patients (CR, CRi, PR)

Patient	Group	Cytogenetics	Molecular type (VAF)	Best response	VEN cycles
43y/F	Unfit	Normal	*IDH2、NPM1 、FLT3*	CR	2
51y/F	Unfit	inv(16)	*CBFb-MYH11*	$CR_{、MRD-、}$	4
55y/F	Unfit	Normal	*PBRM1、CSMD1、BRAF、ATRX、DNMT3A、ASXL1、SF3B1、BCOR*	$CR_{、MRD-、}$	1
64y/F	Unfit	Complex	*RUNX1、IDH2、NRAS、FLT3、MPL*	$CR_{、MRD-、}$	3
70y/M	Unfit	-Y	*CEBPA、IDH1、NPM1、ARID1A、PTPN11*	$CR_{、MRD-、}$	1
58y/F	Unfit	Normal	*GATA2、CALR、SF3B1、ASXL1*	$CR_{、MRD-、}$	2
65/F	Unfit	Complex	*ASXL1、NRAS、KMT2A、GATA2、CALR、TP53*	CRi	
63y/M	Unfit	Normal	*IDH2、TP53*	$CR_{、MRD-、}$	2
71y/M	Unfit	Complex	*BRAF、TP53、PBRM1*	CR	1
43y/F	Relapsed	Complex	*RUNX1、KMT2A、KIT*	PR	2
63y/F	Relapsed	Complex	*TET2、TP53、DNMT3A、FLT3*	Cri	2
47y/M	Relapsed	Complex	*KIT、KRAS*	PR	1
48y/F	Relapsed	Complex	*TET2、BCOR、CEBPA、KIT、WT1、GATA2*	$CR_{、MRD-、}$	1
68/M	Relapsed	Normal	*SF3B1、DNMT3A、RUNX1、BCORL1、FLT3*	PR	1
27y/F	Refractory	Normal	*WT1、CEBPA、BCOR*	$CR^{、MRD-、}$	4
21y/M	Refractory	Complex	*MLL-AF9、TP53、KIT*	$CR^{、MRD-、}$	2
56y/M	Relapsed	Normal	*NPM1、TET2*	$CR^{、MRD-、}$	1

On average, two cycles (range,1–4) of VEN+ HMAs+ half-dose CAG therapy were given, and the median response time (from the beginning of treatment to bone marrow blasts <5%) was 1.2 months (range, 0.6–2.2 months), or 1.4 cycle (range,1–2). Using Kaplan–Meier survival analysis, we compared the survival rate of AML patients in the unfit or R/R groups. There was no statistical difference between the two groups (P=0.853) (Figure 2).

3.5. Adverse Events (AEs)

The most common AEs during VEN+ HMAs+ half-dose CAG therapy were persistent cytopenia and infection. Febrile neutropenia, transfusion-dependent anemia and thrombocytopenia occurred in 19 (90%) AML patients (Table 3). Prolonged pancytopenia (requiring treatment delay >14 days) was experienced by ten (48%) AML patients. Hematological count recovery was observed only in patients who responded to chemotherapy (n = 9). Among these patients, the median time to neutrophil recovery (ANC >1.0×10^9/L) was 24 days (range, 15-36 days), and the median time to PLT recovery (PLT >100×10^9 /L) was 32 days (range, 22-58 days). Nineteen AML patients (90%) occurred Grade 3 or higher neutropenia.

Grade 3 or higher infection was observed in twelve patients (57%) during the initial VEN cycle, including seven patients with pneumonia, two patients with perianal infection, two patients with urinary tract infection, two patients with sepsis and one patient with skin infection. During VEN+ HMAs+ half-dose CAG therapy, a total of eleven AML patients (52%) had probable invasive fungal infection. Three (14%) AML patients were treated with micafungin and eight (38%) received voriconazole for prophylaxis. No patients experiencing breakthrough fungal infection (BFI) and no patient had tumor lysis syndrome (TLS). Hemorrhagic complications occurred in three patients (14%), including gastrointestinal hemorrhage and hematuria. The most common non-haematological AEs was gastrointestinal symptoms (decreased appetite, nausea, vomiting and diarrhea) (11/21,

52%). Early mortality (death within 30 days of therapy initiation) occurred in two (10%) patients—one experienced a septic shock and acute left heart failure followed by multiple organ dysfunction syndrome and DIC; the other patient developed severe pneumonia complicated with ARDS. Three additional patients in the R/R group did not achieve remission. Overall, five (24%) deaths were recorded. The most notable adverse events are outlined in Table 3.

Table 3. The most notable adverse events (grade 3 or higher)

Adverse events	All(n=21)	Unfit（n=11）	R/R（n=10）
Length of stay in hospital, days			
Median (range)	29（4-48）	26（4-36）	32（26-48）
Duration of neutropenia			
Median (range)	17（2-27）	15（2-25）	18（12-27）
All adverse events			
Hematologic adverse events	19(90%)	9(82%)	10(100%)
Thrombocytopenia	19(90%)	9(82%)	10(100%)
Anemia	19(90%)	9(82%)	10(100%)
Febrile Neutropenia	19(90%)	9(82%)	10(100%)
Nonhematologic adverse events			
Gastrointestinal (Decreased appetite /Nausea/ Vomiting/ Diarrhea/ Constipation)	11(52%)	5(45%)	6(60%)
Gastrointestinal Hemorrhage	3(14%)	1(9%)	2(20%)
Hypokalemia	9(43%)	4(36%)	5(50%)
Hypoproteinemia	7(33%)	5(45%)	2(20%)
Elevated transaminases	3(14%)	2(18%)	1(10%)
Infections	12(57%)	8(73%)	4(40%)
Pneumonia	7(33%)	5(45%)	2(20%)
Perianal infection	2(10%)	1(9%)	1(10%)
Skin and soft tissue infections	1(5%)	1(9%)	0(0%)
Urinary tract infection	2(10%)	1(9%)	1(10%)
Sepsis	2(10%)	2(18%)	0(0%)

4. Discussion

Both unfit and R/R AML are still associated with a dismal prognosis[3, 20, 21]. Previous studies have reported that for unfit or R/R AML patients the remission rate of common chemotherapy regimens remains low, with a CR rate of 32% for HD-Ara-C [22], 46.5% for CAG regiment [23, 24], and 48% for FLAG [25]. Recently, BCL-2 inhibitor VEN has been approved for AML patients and is widely used in combination with demethylated drugs (azacitidine or decitabine) [26]. Meanwhile CAG regimen is widely used to treat patients with MDS and AML in China. However, whether VEN or CAG regimen alone apply to unfit or R/R AML patients, the remission rate is still unsatisfactory [27, 28]. VEN combined with cytotoxic chemotherapy can induce synergistic leukemic killing effects, so as to improve the clinical efficacy [29]. The molecular mechanism is increased DNA damage and down-regulation of Mcl-1, in turn increasing the concentration of intracellular apoptosis activator Bim and apoptosis [30, 31]. Here, by using VEN+

HMAs+ half-dose CAG as the backbone of treatment, we report a CR rate of 66.7%, which has a distinct advantage over conventional treatment procedures. To our knowledge, this is the first study to evaluate the therapeutic efficacy and side effects of VEN+ HMAs+ half-dose CAG treated unfit or R/R AML patients.

In our study, the clinical characteristics of the two groups were analyzed, and only the difference of age reached statistical significance (*P*=**0.016**, Table 1). As for genetic characteristics, only *KIT* mutation (*P*= 0.012) reaches statistical difference between the two AML groups; patients in R/R group were much more likely to carry *KIT* mutation. The ELN risk stratification between the two groups was the same (*P*= 0.876), and patients in the unfit or R/R group were more likely to be in the high-risk state of ELN risk stratification (16/21, 76%).

The biomarker analyses of our research demonstrate that VEN+ HMAs+ half-dose CAG was effective in most unfit or R/R AML patients, including those harboring high-risk molecular features. Previous studies have showed that mutations such as *TP53* [32] and *FLT3-ITD* [33, 34] are associated with poor prognosis in AML patients. The CR rate of traditional chemotherapy in AML patients with *TP53* mutant was as low as 28%. Kim K et al. reported that the overall response rate of VEN combined with decitabine in the treatment of AML patients with *TP53* was 66% [4, 12, 35-37]. Patients with TP53 AML have lower response rates and shorter survival with VEN. The addition of VEN to standard treatment regimens did not improve the prognosis of TP53 AML patients[38, 39]. Surprisingly patients with T*P53* performed particularly well in all outcome measures, which achieved a CR/CRi rate of 100%. A study of 125 elderly AML patients treated with D-CAG showed that D-CAG tends to improve the prognosis of *TP53* mutated patients[40]. This result suggests that the VEN+ HMAs+ half-dose CAG regimen may be an effective rescue regimen for *TP53*^{mut} AML patients, but the sample size needs to be further expanded to verify this finding. Some studies have shown that patients with *IDH1/2* mutation have a favorable prognosis [11, 41]. However, in other studies, the prognosis was poor or depends on the type of *IDH* [42]. In this study, we reported a CR rate of 80% in AML patients with *IDH1/2*, which may indicate that VEN is highly sensitive in AML patients with *IDH* ^{mut}. The mechanism may be that the accumulation of tumor metabolite 2-HG inhibits cytochrome C oxidase and effectively primes AML blasts to Bcl2 inhibition[43]. In addition, AML patients with *NPM1*^{mut} (CR rate of 75%) also seem to have a relatively good prognosis under the treatment of VEN+ HMAs+ half-dose CAG.

The ORR of VEN+ HMAs+ half-dose CAG was 80.9% (CR+ Cri, 66.7%, PR, 14.3%) and the median duration of CR+ CRi was 11.5 months (95% CI 8.9 to 14.1). The high CR rate of AML patients with high-risk mutations may represent a proof that VEN in combination with chemotherapy is capable of overcoming some mechanisms of conventional chemotherapy resistance.

Despite receiving a combination of chemotherapeutic drugs, VEN+ HMAs+ half-dose CAG showed tolerable and mostly reversible side effects. Common grade 3/4 AEs were mostly hematologic and similar to those reported with VEN in combination with azacitidine or decitabine [4, 44, 45]. Cytopenia mainly improved at response onset, although it is usually incomplete. In subsequent VEN cycles, recurrent grade 3/4 neutropenia was treated by suspending VEN, shortening the application time of VEN, prolonging the treatment interval and using growth factor. Only one patient discontinued chemotherapy because of excessive myelosuppression. All patients received blood transfusion support. The type and interval of blood transfusion were evaluated according to the blood cell count. Non-hematological toxicity was acceptable, and no significant end-organ damage events were noted. The low frequency of fungal infections can be attributed to the routine combination of CYP3A inhibitor azole antifungals to reducing VEN doses and treatment cost. Therefore, the toxicity associated with VEN+ HMAs+ half-dose CAG regimen was acceptable.

In summary, this study showed the VEN combined with HMAs and half-dose CAG regimen has promising efficacy and tolerable safety profile in unfit or R/R AML patients. Even in patients with high-risk molecular features and adverse prognostic factors, response was still observed. This chemotherapy regimen increases the opportunity for high-risk patients to proceed to allo-HSCT. Notably, VEN+ HMAs+ half-dose CAG was well tolerated, showing an acceptable safety profile. Despite the retrospective nature of this study that represents its major limitation, our results warrant further evaluation of VEN+ HMAs+ half-dose CAG in a prospective setting with larger AML population.

Abbreviations

AML Acute myeloid leukemia

BFI Breakthrough fungal infection

TLS Tumor lysis syndrome

VEN Venetoclax

AEs Adverse Events

R/R Relapsed/refractory

HSCT Hematopoietic stem cell transplant

BCL2 B-cell leukemia/lymphoma-2

HMAs Hypomethylating agents

LDAC Low-dose cytarabine

HD High-dose

DAC Decitabine

AZA Azacitidine

ELN European Leukemia Net

WBC White blood cell

PLT Platelet

CTCAE Common Terminology Criteria for Adverse Events

MRD Minimal residual disease

CR Complete response

ANC Absolute neutrophil cell

ORR Overall response rate

BM Bone marrow

Declarations

Author contributions CXT conceived, designed, analyzed the data, and wrote the manuscript. FSJ analyzed data and is the corresponding author. ZYQ and LQ participated in data collection. All authors reviewed the manuscript. All authors read and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethical approval This study protocol was discussed and approved by the Medical Ethics Committee of the First Affiliated Hospital of Harbin Medical University who waived the need for patient informed consent for this retrospective analysis.

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Figures

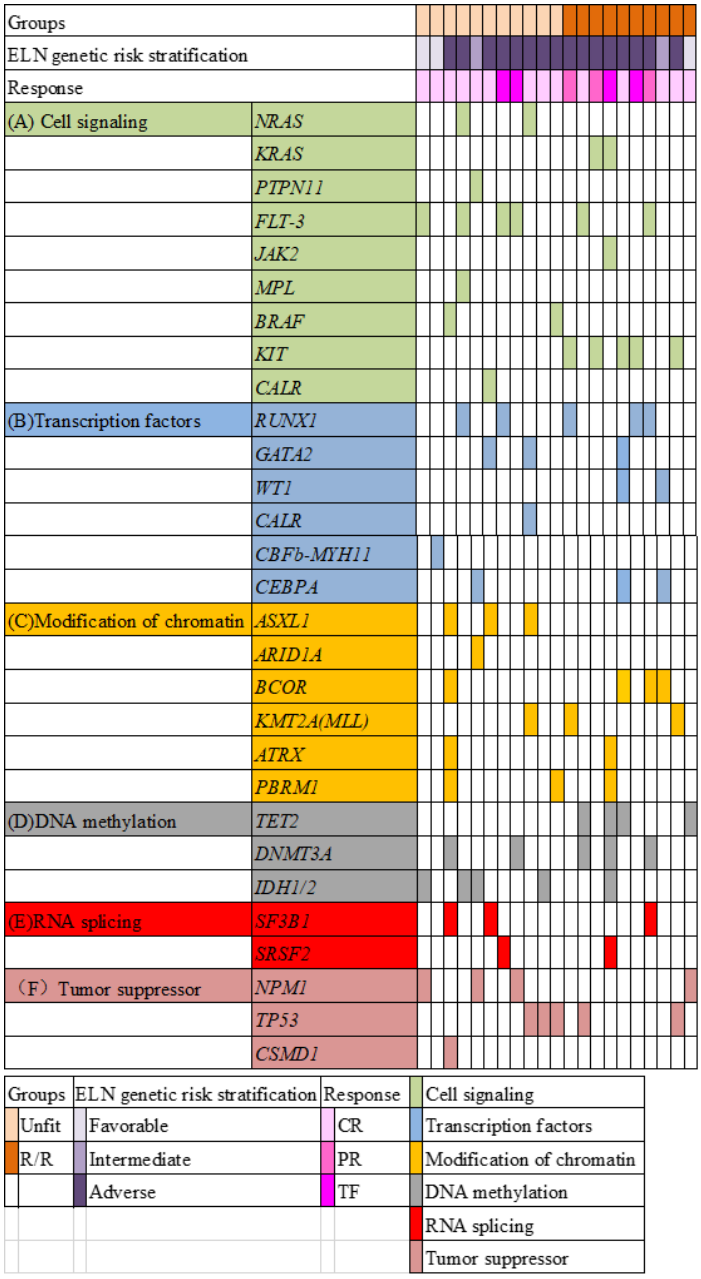

Figure 1

Distribution of mutated genes in unfit or R/R AML patients. Each column represents a patient. The first line was the grouping of each AML patient, the second line was the ELN genetic risk stratification of each AML patient, the third line was the treatment response of each patient. In the fourth to the thirty-second line each row represents a gene and each coloured box indicates one mutated gene.

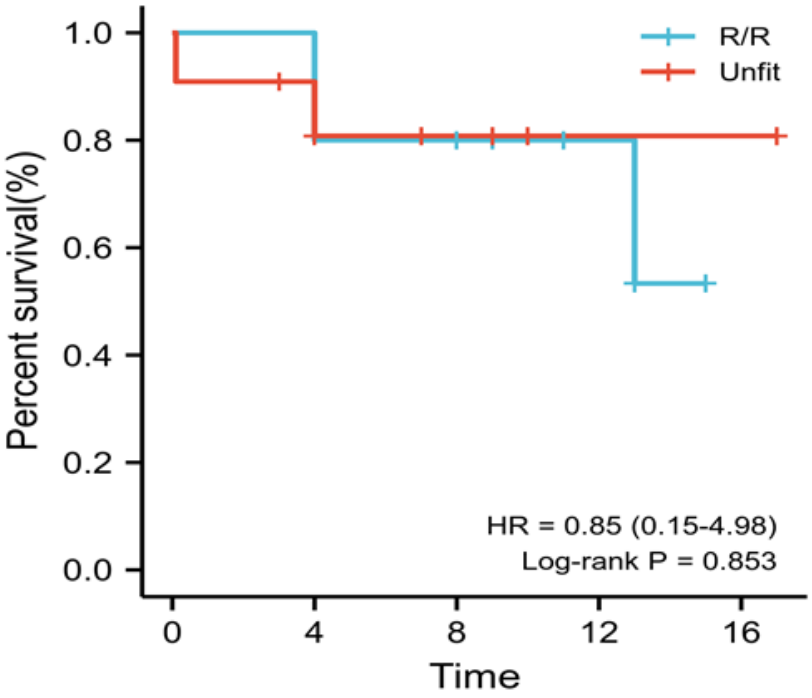

Figure 2

Kaplan–Meier survival curves for the survival rate in the unfit or R/R AML patients. (*P*= 0.853)