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Scientific Letter

Treating Relapsed/Refractory Acute Myeloid Leukemia with Chidamide, Fludarabine, Cytarabine and Granulocyte-Colony Stimulating Factor with Subsequent Bridging to Myeloablative Allogeneic Hematopoietic Stem Cell Transplantation

Keywords: Chidamide; Fludarabine; Cytarabine; Granulocyte colony-stimulating factor; Relapsed/refractory acute myeloid leukemia; Allogeneic hematopoietic stem cell transplantation; Survival.

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To the editor.

Despite the significant progress made in treating acute myeloid leukemia (AML) in the last decade, 10%–40% of the patients with standard induction chemotherapy still did not achieve complete remission (CR),[1] and 50%-70% of the patients in the first CR were at risk for relapse.[2] Although few of these patients can be cured with conventional salvage therapy,[2,3] they need to be evaluated regarding eligibility for allogeneic hematopoietic stem cell transplantation (HSCT), the most potent therapeutic strategy for patients who achieve CR after relapse.[2] Before transplantation, salvage chemotherapy regimens need to be employed to reduce the leukemia burden.

In relapsed/refractory AML, fludarabine, cytarabine, combination with granulocyte colony-stimulating factor (G-CSF) (FLAG) were used as a reinduction therapy and resulted in only 38.2% CR.[4] Wrzesień-Kuś A et al. utilized the combination of cytarabine, cladribine, and G-CSF as the induction therapy in patients with refractory or early relapsed AML, obtaining a 50% CR rate with 17% early death.[5] Subsequent studies evaluated FLAG plus idarubicin (FLAG-Ida) or FLAG-Ida plus gemtuzumab ozogamicin (FLAGO-Ida) in adult patients with refractory/relapsed AML, showing that the CR/CR with an incomplete blood count recovery (CRi) rate was 51%, with 9% of induction deaths.[6] These data demonstrated the therapy limitation of FLAG in relapsed/refractory AML and the prospects of FLAG combination with other specific drugs.

Chidamide is a new histone deacetylase (HDAC) inhibitor of the benzamide class, and it has been approved by China Food and Drug Administration (CFDA) in treating peripheral T-cell lymphoma in China. Additionally, evidence demonstrated that chidamide combined with cytarabine synergistically enhanced apoptosis in AML cell lines.[7] Therefore, we speculated that the addition of chidamide in the FLAG combination might improve its efficacy in relapsed/refractory AML. Herein, we evaluate the efficacy and toxicity of chidamide-FLAG (Chi-FLAG) reinduction treatment in patients with relapsed/refractory AML and the potential for subsequent HSCT.

Material and Methods.

Patients. AML was defined by the criteria of the World Health Organization.[8] Furthermore, genetic risk grouping and remission criteria were defined according to the European Leukemia Network (ELN).[9] Additionally, the selected patients were required to have an adequate hepatic and renal function and no uncontrolled infections with ECOG scores of 0–2 and could not receive investigational agents within 30 days of enrollment or myelosuppressive therapy within 14 days. Based on these, a total of 14 consecutive patients, median age 28 years (range from 14 to 52 years, four female and ten male), with refractory/relapsed AML were enrolled in our current prospective study (ChiECRCT-20180058), which had passed Ethical Committee in our hospital.

Treatment plan. Chidamide 30 mg was given orally on days 1, 4, 8, and 11. Fludarabine 30 mg/m^2 and cytarabine 2 g/m^2 were given for 5 days, from day 4 to 8. G-CSF was given at a 5–10 (g/kg body weight) dose, which started 24 h prior to fludarabine until neutrophil recovery (Chi-FLAG regimen). Patients who were found to have CR/CRi, PR, or even NR, as tolerated and

disposed of, received HSCT. Adverse events were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events Version 4.0.

All patients were treated according to standardized institutional treatment and supportive care algorithms. Patients were maintained on infectious disease prophylaxis, including a broad-spectrum fluoroquinolone, anti-fungal agent, and acyclovir. Transfusions were given according to institutional guidelines.

Statistical analysis. Continuous variables were summarized using descriptive statistics such as median and interquartile range (IQR). Impact on response rate was assessed using the Mann–Whitney U test. Categorical variables were presented as percentages and compared using Fisher's exact test. Finally, survival curves were compared using the Log-rank test. Statistical analysis was conducted using Stata# data analysis and statistical software (version 14.0; Stata-Corp LP, College Station, TX).

Results.

Patient characteristics. The demographic characteristics of the 14 patients are shown in **Table 1**. The median age was 28 years (range, 14–55). Six patients had a primary refractory disease at the time of enrollment, seven were experiencing a first relapse, and one was experiencing a second relapse. The relapse always occurred before 12 months. Among the eight patients in the ELN favorable-risk group, six patients had t(8;21)(q22;q22) translocation; one had a normal karyotype with NPM1 mutation and one with CEBPA double mutations. The intermediate group consisted of one patient with t(8;21)(q22;q22) translocation with C-kit mutation and three patients with normal karyotypes, including one with NPM1 FLT3-ITD mutations. Finally, the poor-risk group contained two patients with abnormal karyotypes with FLT3-ITD mutations (**Table 1**).

Response. Response rates of the 14 patients are also shown in **Table 1**. Eight patients (8/14, 57.1%) achieved CR and no patients had CRi or PR as their best response. ORR was also 57.1%, Responses occurred in 71.4% (five of seven) of patients with first relapse, 0.0% with second relapse, and 50.0% (three of six) with refractory AML (P = 0.417). Patients with relapsed AML had a similar CR compared to those with refractory AML (62.5% vs. 50%; P = 1.000). Favorable, intermediate and poor risk patients had CR of 75.0%, 50.0% and 0.0%, respectively (P = 0.152).

Survival. The Kaplan–Meier survival curve results showed that the 1-year overall survival (OS) rate of patients uncensored for transplant was remarkably higher than that of patients censored for transplant (77.8% vs. 20%, p = 0.001, **Figure 1**). The 1-year OS rate of all patients was 55.6%. (95% CI 26.4%–77.2%); In multivariable analysis examining outcomes, neither age, sex, ELN grouping, nor disease status impacted survival significantly. Ultimately, four patients (28.6%) died of progressive disease.

Allogeneic transplant. Allogeneic transplant is the favored long-term strategy for disease control and prolonged survival,[9] seen in this population. Nine patients (9/14, 64.3%) in this cohort received an allogeneic transplant. The median age was 22 years

Table 1. Patient Characteristics.

ID No	Age (years)	Sex	Gene mutations	ELN grouping	Disease Status	Response	Subsequent HSCT	Relapse	Outcome	Survival (days)
1	52	F	FLT3-ITD	Poor	1st relapse	NR	Yes	No	Died of transplantation complications	249
2	16	M	AML/ETO	Favorable	1st relapse	CR	Yes	No	alive	519
3	21	M	AML/ETO	Favorable	1st relapse	CR	Yes	No	died of transplantation complications	247
4	22	F	AML/ETO	Favorable	1st relapse	CR	Yes	No	alive	429
5	38	M	FLT3-ITD	Poor	Refractory	NR	No	/	died of AML	81
6	14	F	AML/ETO	Favorable	1st relapse	CR	Yes	No	alive	378
7	43	F	NPM1, FLT3-ITD	Intermediate	Refractory	CR	Yes	Yes	alive	368
8	33	M	AML/ETO	Favorable	1st relapse	NR	Yes	No	alive	357
9	35	M	AML/ETO C-KIT	Intermediate	Refractory	NR	No	/	died of AML	96
10	23	M	none	Intermediate	Refractory	NR	Yes	No	alive	313
11	55	M	CEBPA double mutation	Favorable	1st relapse	CR	No	Yes	died of AML	181
12	18	M	none	Intermediate	Refractory	CR	Yes	No	alive	288
13	19	M	AML/ETO	Favorable	2nd relapse	NR	No	/	died of AML	48
14	47	M	NPM1	Favorable	refractory	CR	No	No	alive	188

ELN, European Leukemia Network; F, Female; M, Male; CR, Complete Remission; NR, Non-Remission; HSCT, Hematopoietic Stem Cell Transplantation

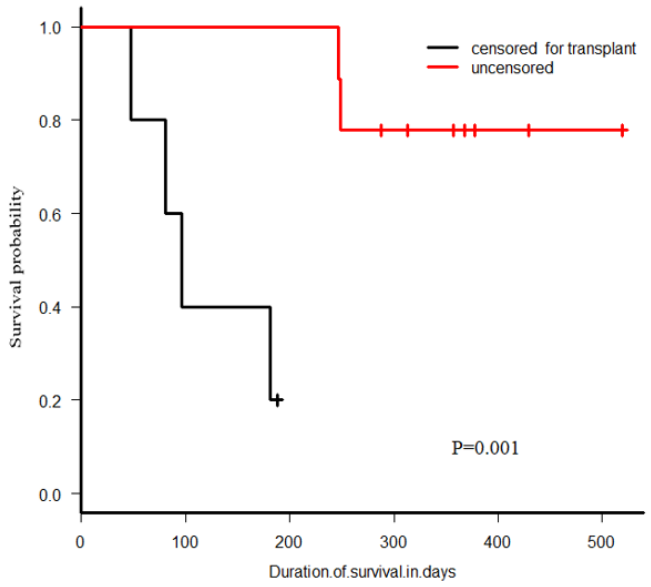

Figure 1. The results of Kaplan–Meier survival curve of the 1-year overall survival (OS) rate of patients uncensored for transplant (transplanted) and censored for transplant (not transplanted).

(range, 14–52 years) (**Table 1**). Among these nine patients, four had ELN intermediate/poor-risk disease, and five had ELN favorable-risk disease. Six patients were in CR, and three were in NR prior to transplant. One patient had a matched related donor, another had a haploidentical donor, and seven were transplanted with umbilical cord blood stem cells. The 1-year OS after allogeneic transplant was 77.8% (95% CI 36.5%–93.9%), and 1-year disease-free survival was 66.7% (95% CI 28.2%–87.8%). Two patients were disease-free at about one year, of the three patients in NR at the time of transplant.

Toxicity. Four of 14 patients (28.6%) had a grade 3–4 nonhematologic toxicity within 30 days (based on NCI CTACE v4.0).[13] The most common toxicity was fatigue and nausea. Other toxicities included sepsis secondary to pneumonia, neutropenic fever without source, and thrombocytopenia. The readmission rate was 100%. Causes of hospitalization were febrile neutropenia (50.0%), proven infection (42.9%), cytopenia (7.1%). No patients died within 30 days of treatment due to toxicity. No significant differences in toxicity were observed in patients who achieved CR compared with those who did not (NR).

Discussion. In this prospective analysis, we report the results of a phase I study to assess the safety and activity of chidamide combined with the FLAG regimen in all subsets of patients with relapsed/refractory AML. Interestingly, the overall CR rate (57.1%) was comparable to prior analysis of relapsed/refractory patients with FLAG-Ida (51%) but showed lower treatment-related mortality (0% vs. 9%).[6] In addition, the overall CR rate in the present study was improved compared with previous studies of intensive reinduction regimens with high dose cytarabine with (44%) or without (32%) mitoxantrone[11] or FLAG (33.3%).[12] Collectively, the present work demonstrated that the combination of chidamide and FLAG shows a promising application prospect in relapsed/refractory AML.

Numerous HDAC inhibitors are in clinical trials, and the reported response rates are unsatisfactory for relapsed or refractory AML. Vey et al. reported a dose-escalation study of oral abexinostat to treat patients with relapsed/refractory AML. It is frustrating that the best response was stable disease in one patient.[13] Kirschbaum et al. demonstrated that no CR or PR had been seen in a phase 2 study of relapsed/refractory AML patients administrated with belinostat.[14] Gojo et al. found that only seven out of 21 attained a CR/CRi in relapsed/refractory AML patients when treated with the combination of vorinostat, cytarabine, and etoposide.[15] Moreover, Walter et al. suggested that among 43 older patients with relapsed/refractory AML treated with vorinostat combined with gemtuzumab ozogamicin and azacitidine, 10 achieved CR, 8 achieved CRi, and the overall response rate was only 41.9%.[16]

Chidamide is a new HDAC inhibitor of the benzamide class that specifically inhibits HDAC 1, 2, 3[7,17] and has been approved by the CFDA in treating peripheral T-cell lymphoma in China. A previous study demonstrated that Chidamide significantly increased the expression of suppressors of cytokine signaling 3, reduced the expression of Janus activated kinases 2 and signal transducer and activator of transcription 3 (STAT3), and inhibited STAT3 downstream genes, including c-Myc, Bcl-xL, and Mcl-1, which are involved in cell cycle progression and anti-apoptosis, thereby inducing G0/G1 phase arrest and apoptosis in AML cells.[18] Notably, chidamide synergistically enhances apoptosis combined with cytarabine,[19] decitabine,[20] or MLL-menin interaction targets[21] in leukemia cell lines.

At present, there are only two reports on the application of chidamide in relapsed/refractory AML patients. Lun et al. reported that one patient with MLL-AF9 attained complete molecular remission after treatment with chidamide combined with CAG regimen chemotherapy.[22] The other report was that chidamide and decitabine combined with the CHAG priming regimen for eight patients, five achieved CR, one achieved PR, one's disease progressed, and one died from complications of chemotherapy.[23] Our regimen, the first to be used in relapsed/refractory AML, was generally well tolerated with relatively high CR.

The Chi-FLAG regimen may be particularly useful for patients with intermediate- or high-risk disease characteristics, especially as a bridge to HSCT. In our series, the OS of patients with an intermediate/poor ELN risk was at least as high as those with favorable risk (50.0% vs. 60.0%, p = 0.750). No significant differences

in OS were observed in NR patients after reinduction chemotherapy (33.3%) compared with those who achieved CR (72.9%, P = 0.108). However, 64.3% of all patients underwent transplantation, and the OS was significantly higher in patients uncensored for subsequent transplant (77.8%) than in censored patients (20.0%). Additionally, the OS rate of all patients in our study (55.6%) was slightly higher than that of other previous series of relapsed/refractory patients (22.0%, uncensored for the subsequent transplant).[24] These differences may be related to the higher response rates to the Chi-FLAG regimen in patients with intermediate/poor-risk disease and their ability to undergo subsequent transplantation.

Our series adds to the growing literature supporting the use of the Chi-FLAG regimen in patients with relapsed/refractory AML and as a bridge to potentially curative allogeneic transplant. However, the limitation of this paper is the insufficient sample size. Further delineation of molecular and cytogenetic subsets associated with higher response rates to chidamide will be of value as future prospective trials of chidamide in combination with new molecularly targeted agents are designed.

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Compliance with Ethical Standards. The protocol was approved by our Ethics Committee and registered at Chictr.org (ChiCTR1800015871). Informed consent, and assent when appropriate, was obtained from all patients.

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