Original Article

A retrospective study on effectiveness of combined recombinant human interferon-α-1b, interleukin-2, and thalidomide for the treatment of acute myeloid leukemia in various disease states

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Background: Interferon-α-1b, interleukin-2 combined with thalidomide (ITI) improved the outcome and prognosis of some acute myeloid leukemia (AML) patients, but the cases was insufficient. This study observed the efficacy and safety of this regimen in the treatment of numbers of AML patients in various disease states.

Methods: Starting in January 2014, patients with AML (n=188) were treated with ITI regimen, including 60 refractory/relapses patients in group A, 40 patients in group B remained minimal residual disease-positive (MRD) or changed from negative to positive again after consolidation therapy, and 88 patients in group C with initial complete remission of AML received the ITI treatment after routine consolidation therapy. Bone marrow, fusion gene and MRD were detected to judge the curative effect and the adverse reactions were observed. The remission rate, MRD status and long-term survival of three groups were analyzed. An AML mouse model was constructed to observe the anti-leukemia effect of the three drugs *in vivo*.

Results: Sixty patients with primary AML who were unable to receive chemotherapy, or with relapsed/refractory AML, showed a total response rate of 28.3% (17/60) after receiving the ITI regimen. Forty patients with morphologically complete remission and MRD-positive achieved a response rate of 77.5% (31/40); the MRD converted to negative in 19 patients and was mitigated in 12 patients. Among 88 patients with initial complete remission, 11 failed to maintain the negative MRD, and the relapse rate was 12.5%, which was significantly lower than that of the non-maintenance treatment group (54.3%). In the mouse model, interferon, interleukin-2, and thalidomide exerted an anti-leukemia effect, prolonged the survival time of the mice, and the anti-leukemia effect was further enhanced after administration of the combination ITI regimen.

Conclusions: For suitable patients, hematopoietic stem cell transplantation is still strong recommended. The ITI regimen may be an effective option for patients with AML who cannot tolerate conventional

chemotherapy, including those with relapsed/refractory disease, those with a complete remission status but are MRD-positive, or those who require maintenance treatment after consolidation therapy. However, a rigorous clinical randomized controlled trial and more in-depth mechanism exploration are still needed to verify this conclusion.

Keywords: Acute myeloid leukemia (AML); thalidomide; IFN-α1b; IL-2; minimal residual disease

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Introduction

Acute myeloid leukemia (AML) is a heterogeneous malignancy of the blood that is characterized by the uncontrolled clonal proliferation of abnormal myeloid progenitor cells (1). The overall prognosis of AML is poor, with a 5-year survival rate among young adults of 40–50%, which declines with age (2). Chemotherapy is the main treatment option for AML; however, for some patients with relapsed and refractory (R/R) AML, its therapeutic effect is not satisfactory (3). Although the recent introduction of new drugs and protocols has provided more options for patients with R/R-AML, the overall survival rate has not changed dramatically (4). In 2017, the United States Food and Drug Administration approved several targeted drugs for the treatment of AML. As an inhibitor targeting Bcl-2, venetoclax improves the treatment efficacy of AML patients who are not suitable for high-intensity chemotherapy, but its efficacy is affected by complex molecular clones (5-8). Minimal residual disease (MRD) is an independent risk factor affecting the prognosis of AML patients, hematopoietic stem cell transplantation (HSCT) may be the best treatment for these patients (9). But for patients who cannot receive transplantation, how to carry out follow-up intervention treatment has not reached a consensus. At the same time, for AML patients without HSCT, it is recommended to enter the observation period after 3 or 4 cycles of consolidation therapy with HD-Ara-C regimen, and maintenance therapy is generally not recommended, but some reports suggest that maintenance therapy can benefit patients, which is still controversial (10,11). Meanwhile, the high cost of the new drugs is prohibitive for many patients, especially those without medical insurance. In addition, patients with AML are at risk of complications after multiple cycles of chemotherapy, such as lung infection and cardiac dysfunction. These factors significantly limit the administration of further intensive treatments.

Multiple cytokines and immunomodulatory drugs have been applied to the treatment of AML, with beneficial therapeutic effects. Interferon (IFN) as medication was first used for treating AML in 1979 (12); however, its anti-leukemic effects, exerted through a variety of intracellular mechanisms, were limited (13). Nevertheless, for the subpopulation of patients who received allogeneic hematopoietic stem cell transplantation (allo-HSCT) and those with positive MRD, markedly higher disease-free and overall survival rates were associated with the administration of IFN-alpha. Furthermore, maintenance therapy with IFN-α may also prevent relapse in favorable-risk AML after consolidation chemotherapy (13-16). Similarly, some clinical trials have suggested that the therapeutic effect of interleukin (IL)-2 monotherapy is not ideal for the treatment of AML (17,18). However, a phase III clinical trial demonstrated that patients with initial complete remission (CR) had improved disease-free and overall survival rates when IL-2 was administered in combination with other drugs (19). Thalidomide is rarely used alone in acute myeloid leukemia but when combined with IFN can inhibit Kasumi-1 cell proliferation, and is effective and tolerable in AML patients when combined with azacytidine. The three above-mentioned drugs alone have a limited effect on AML, so we attempted to use them in combination (20,21). In the previous study, 20 patients with hematological remission but AML1-ETO fusion gene positive AML were treated with routine dose of ITI regimen, 10 patients were negative after 1 month, and 4 patients decreased significantly after 2.8 months. Thirty-seven patients in remission were treated with ITI regimen. The 2-year disease progression rates of patients with good prognosis and moderate prognosis were 25.0% and 21.1%, respectively, and the recurrence rates were 12.5% and 15.8%, respectively, which were lower than those of the previous historical control (22,23). This has achieved gratifying results in some patients. However, the

insufficient number of cases and the high heterogeneity of the disease affect the accurate evaluation of the effectiveness and safety of this treatment.

Therefore, we applied this regimen to a greater number of patients with R/R-AML and others who were not suitable candidates for intensive chemotherapy. The regimen (registration number: ChiCTR-ONC-14004688; www.chictr.org.cn) was also given to MRD-positive patients with complete remission but without conditional transplantation, so that the rate of conversion to MRD negativity could be observed. Since previous studies have reported that IL-2 monotherapy had no significant long-term survival benefits in patients with CR (18,24), we also administered the ITI regimen to patients with initial CR who had completed consolidation therapy to observe their survival benefits. Herein, we present a retrospective analysis of the outcomes of 188 AML patients treated with the ITI regimen to investigate its therapeutic efficacy. And we observed the antileukemic effects of three drugs in mouse model. Meanwhile, we present the following article in accordance with the STROBE and ARRIVE reporting checklists (available at https://atm.amegroups.com/article/view/10.21037/atm-22-5520/rc).

Methods

Ethics considerations

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This retrospective study was approved by the Institutional Center Ethics Review Committee at the Affiliated Cancer Hospital of Zhengzhou University/Henan Cancer Hospital (No. 2014xjs15). All participating hospitals were informed and agreed the study. Written informed consent was obtained from all participants and they agreed to publish their individual data.

Study design

The study population included patients with AML who received the ITI regimen between January 2014 and December 2021 at the following 10 hospitals: Affiliated Cancer Hospital of Zhengzhou University/Henan Cancer Hospital; First People's Hospital of Pingdingshan City; No. 989 Hospital of Joint Logistic Support Force of the Chinese People's Liberation Army; First People's Hospital of Shangqiu City; First Affiliated Hospital and College of Clinical Medicine of Henan University of Science and Technology; Puyang People's Hospital; Luoyang Central Hospital; Huaihe Hospital of Henan University; Jiaozuo People's Hospital; and First Affiliated Hospital of Xinxiang Medical College.

In this study, the AML patients were classified into three groups (A, B, and C) according to their disease status and response to previous treatments, as specified below. Briefly, Group A comprised patients with R/R-AML or primary AML patients who were unable to receive chemotherapy. Group B included patients with both morphological CR and consistent positivity for MRD. Group C patients had achieved initial CR and negative MRD. At the same time, 81 AML patients with negative MRD who did not receive maintenance treatment were enrolled as the control group, and the recurrence rate and recurrence time were observed. The baseline clinical characteristics of the patients were collected, including sex, age, French-American-British (FAB) classification, WHO risk classification and so on. The groups were followed up, with a particular focus on their responses to the ITI regimen, rates of CR and partial remission, MRD status, quality of life, and long-term survival.

Potential subjects with any of the following characteristics were excluded from this analysis: serious allergy to thalidomide, interferon, or IL-2 that manifests clinically as anaphylactic shock and laryngeal edema; pregnant or nursing; malignancy in addition to AML or severe infection of the central nervous system; significant heart disease activity within the previous 6 months; ≥ two peripheral neuropathy events (25); poor liver function manifesting as alanine aminotransferase or aspartate aminotransferase ≥2.5-fold the upper limits of normal (ULN), or serum total bilirubin ≥1.5-fold the ULN; lung function decompensation; renal failure; or psychotic episodes.

Patients and subject groups

Group A included patients with R/R-AML or primary AML who were not suitable for chemotherapy. Relapsed AML was diagnosed in the presence of any of the following: leukemic cells in the peripheral blood; leukemic myeloid cell percentage >0.050; or the infiltration of extramedullary leukemia cells. Patients with normal myeloid cell proliferation were excluded by flow cytometric analysis. Refractory AML was diagnosed in patients with any of the following: primary AML without CR, even after two cycles

of standard regimen treatments; relapse within 12 months after CR and consolidation therapy; relapse after 12 months and no response to regular chemotherapy; ≥ two relapses; or persistent extramedullary leukemia. Patients in Group A met the following inclusion criteria: (I) not suitable for further intensive therapy due to severe cardiac dysfunction, pulmonary infection, or other severe diseases, or were not financially able to receive intensive therapy; (II) had not reached CR after chemotherapy and did not qualify for allo-HSCT; and (III) older than 18 years of age.

All patients in Group B (morphological CR/MRD-positive) received consolidation therapy. The proportion of leukemic cells in the bone marrow of these patients was <5%, but MRD remained positive. Also, these patients were not able to receive hematopoietic stem cell transplantation due to economic factors, poor physical condition, or the lack of an appropriate donor.

Group C patients (initial CR/MRD-negative) included either those categorized into the favorable-risk group, or those with an intermediate or poor prognosis who could not receive hematopoietic stem cell transplantation, according to the World Health Organization (WHO) risk stratification criteria for AML (26). These patients achieved morphological remission after one or two cycles of induction chemotherapy and received at least four cycles of consolidation treatment. In addition, the MRD of these patients converted to negative during or after their consolidation therapy.

Administration of the ITI regimen

For patients in Groups A and B, recombinant human IFN-α1b (rhIFNα-1b; Shenzhen Ke Xing Bioengineering, China; 60 μg/time, every other day) was injected subcutaneously, followed by the administration of ibuprofen particles (Harbin Pharmaceutical Group Sanjing Pharmaceutical Nuojie, China) 30 min later. Recombinant human interleukin-2 (rhIL-2; Beijing Sihuan Biopharmaceutical, China) was injected subcutaneously once every other day at a dose of 1 million units/time. Thalidomide tablets (Changzhou Pharmaceutical Factory, China; 200 mg) were taken orally every night before sleep. Patients whose platelet count was $>50\times10^9$/L were recommended to take compound salvia tablets orally (Guangdong Baiyun Mountain Heji Huangpu Chinese Medicine, China; three tablets/time, three times daily) to prevent deep venous thrombosis. On day 28 of the treatment, bone marrow aspiration was performed for sampling and further evaluation of the therapeutic effect. For patients in Group C, the ITI regimen was initiated as a maintenance therapy 1 month after completing the final chemotherapy treatment, and the dose of each drug was the same as that in Groups A and B.

Follow-up and evaluation of the therapeutic effect

Patients were followed up by telephone and medical records until December 2021. Responses to treatment were recorded as CR, CR with incomplete blood count recovery (CRi), partial remission (PR), or no response (NR). CR and CRi were defined according to the standards of the International Working Group (27). PR was adjudged when the percentage of leukemic cells in the bone marrow was <20% but declined over 50% compared to the initial time point. NR was defined as no obvious decline in the percentage of leukemic cells in the bone marrow. Progression-free survival (PFS) was the interval from data collection until AML progression. Overall survival (OS) was the interval from the start of data collection until death due to any cause.

Safety assessment

Safety assessments were performed before and after each cycle of treatment. According to the criteria for evaluating adverse reactions established by WHO, adverse reactions were classified as grade 0–IV, and the main adverse reactions were fever, chills, rash, and muscle pain.

In vivo experiment

We selected 6–8-week-old female NCG mice with severe immunodeficiency. On the first day, a 100 μL cell suspension containing 2.5×10^6 cells MOLM-13 transfected with luciferase was injected into the tail vein of the mice to establish the leukemia mouse model. The changes in tumor formation and tumor load were observed using an *in vivo* imaging system (General Electric Company, USA). Except for the control group, 2×10^6 normal peripheral blood mononuclear cells were injected into the tail vein on the fifth day to aid in immune reconstruction, and a repeat injection was administered on the seventh day.

The animals were randomly divided into nine groups, with seven animals in each group, including a control group, a PBMC-only group (peripheral blood mononuclear cell), a PBMC + IFN-α group, a PBMC + IL-2 group, a PBMC + thalidomide group, a PBMC + IFN-α + IL-2

Table 1 General information of Group A patients

Characteristics	Value
Gender	
Male	32
Female	28
Medium age (y) [range]	54.5 [18-75]
FAB	
M0	2
M1	3
M2A	15
M2B	8
M4	13
M5	19
Enrollment reason	
Financial	22
Unfit	26
Unwilling	12
Therapeutic effect	
CR	4
CRi	6
PR	7
NR	43

FAB, the French-American-British classification system of hematologic diseases; CR, complete remission; CRi, complete remission with incomplete hematological recovery; PR, partial remission; NR, no remission.

group, a PBMC + IFN-α + thalidomide group, a PBMC + IL-2 + thalidomide group, and a PBMC + IFN-α + IL-2 + thalidomide group. The specific administration methods and doses were as follows: IFN-α was injected intraperitoneally for 10^5 IU/body once every other day, interleukin-2 was injected intraperitoneally for 2×10^3 IU/body once a day, and thalidomide was administered intragastrically for 10^5 IU/body. This was replaced with normal saline in the control and PBMC groups.

The body weights of the mice were monitored every other day, and the tumor load was observed by *in vivo* imaging every 3 days. On the seventh day after administration, three mice in each group were randomly selected, and the peripheral blood lymphocyte subsets were detected by flow cytometry. The proportion of $CD45^+$ cells in liver tissue was also detected by immunohistochemistry. A protocol of the animal experiment was prepared before the study, which was reviewed and agreed by the Ethics Review Committee of the Life Sciences Division of Zhengzhou University, in compliance with the Zhengzhou University institutional guidelines for the care and use of animals.

Statistical analysis

SPSS 22.0 software (International Business Machines Corporation, USA) was used for statistical analysis. The measurement data were expressed as the median (range), and the rank-sum test was used for comparison. For continuous data, the chi-square test was used for comparison between the groups. The log-rank test was applied to compare the overall and relapse-free survival using the Kaplan-Meier method, and a survival curve was drawn. P values were two-sided, and $P<0.05$ was considered a statistically significant difference.

Results

The ITI regimen improved the quality of life and prolonged the survival of Group A patients with R/R-AML

Group A initially comprised 68 patients, eight of whom died of severe complications before the completion of the first cycle of treatment (early mortality rate: 11.8%). Among the 60 remaining eligible patients, there were 32 men and 28 women, with a median age of 54.5 years (18–75 years). According to the FAB classification: M2 (23 cases), M5 (19 cases), M4 (13 cases) were the most, M0 (2 cases) and M1 (3 cases) were less. (*Table 1*)

Among these patients, 48 patients had refractory relapsed AML, and four patients achieved CR. All four of these patients relapsed after multiple cycles of chemotherapy, and had neutropenia, severe pulmonary infection, and elevated transaminase, which made it difficult to undergo intensive therapy. All patients achieved CR after two cycles of the ITI regimen. One patient survived for longer than 5 years and was MRD-negative for 8 months. Three patients achieved CRi after 2–6 cycles of treatment, and four patients achieved PR. The CR + CRi of 48 patients was 14.58% (7/48), PR was 8.33% (4/48), and the total effective rate was 22.92%. The median survival time of the 48 patients was 6.5 months (1.0–60.0 months), as shown in *Figure 1*.

After receiving ITI treatment, three patients achieved CRi and three patients achieved PR after 2–4 cycles of

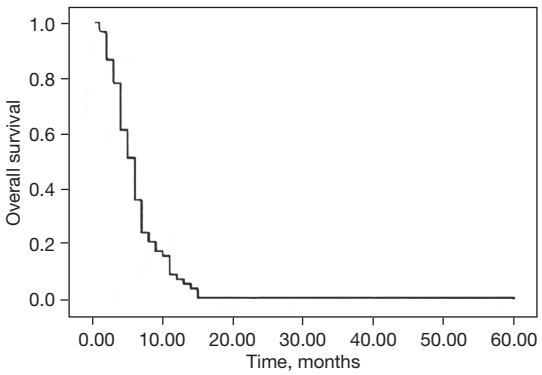

Figure 1 Survival of 48 patients with refractory and relapsed AML. AML, acute myeloid leukemia.

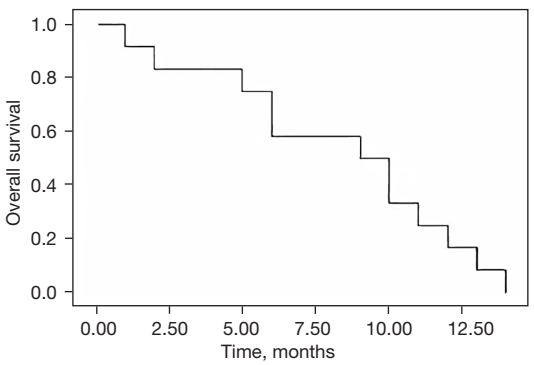

Figure 2 Survival of 12 patients with initially treated AML who were refractory to chemotherapy. AML, acute myeloid leukemia.

treatment. The CR + CRi was 25% (3/12), PR was 25% (3/12), and the total effective rate was 50%. The median survival time of 12 patients was 8.0 months (1.0–14 months), as shown in *Figure 2*. Of the 60 patients, 10 achieved CR + CRi, seven achieved PR, and the total effective rate was 28.3%, indicating that the ITI regimen could effectively treat some R/R-AML patients and prolong their survival time.

The ITI regimen benefited Group B MRD-positive AML patients with morphological CR

The 40 patients in Group B remained MRD-positive or changed from negative to positive again after routine consolidation therapy, there were 25 men and 15 women, with a median age of 43.5 years (14–70 years). According to the FAB classification: M2 (31 cases) was the most, while M5 (5 cases), M1 (2 cases) and M4 (2 cases) were less. Among these patients, 16 were at low risk of WHO, 18 were at moderate risk, and 6 were at high risk (26) (*Table 2*). The patients received the ITI regimen after consolidation therapy, and the protocol was the same as that for Group A. Diagnoses were made according to routine complete blood cell counts, bone marrow morphometry, flow immunophenotyping, cytogenetics, and molecular biology assays. Patients with fms-like tyrosine kinase 3-internal tandem duplication (FLT3-ITD) mutations were treated with oral sorafenib during early induction and consolidation treatment. Among the 40 patients with CR, 15 had continuously positive MRD, nine patients had MRD that converted from negative to positive, and 16 had unstable MRD during treatment. The response rate of the 40 patients in Group B was 77.5% (31/40), where MRD <0.01% was defined as the negative threshold. Specifically, 22 patients had MRD that converted to negative, nine patients had mitigated MRD, and nine patients did not respond.

Table 2 General information of Group B patients

Characteristic	N
Gender	
Male	25
Female	15
Medium age (y)	
>60	6
≤60	34
FAB	
M0	0
M1	2
M2a	11
M2b	20
M4	2
M5	5
WHO risk classification	
Low risk	16
Medium risk	18
High risk	6

FAB, the French-American-British classification system of hematologic diseases.

In Group B, 33 patients received only conventional

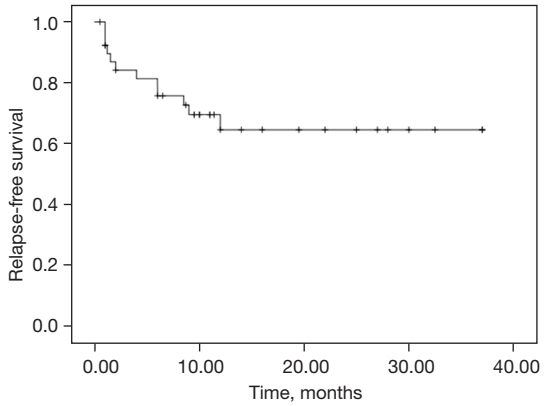

Figure 3 Relapse-free survival of 40 patients with morphologically complete remission and consistently positive MRD. MRD, minimal residual disease.

Table 3 General information of Group C patients

Characteristics	With maintenance treatment	Without maintenance treatment
Gender		
Male	52	42
Female	36	39
Medium age (y)		
>60	14	19
≤60	74	62
FAB		
M0	2	2
M1	7	5
M2a	32	33
M2b	11	17
M4	20	13
M5	16	11
WHO risk classification		
Low risk	26	32
Medium risk	55	39
High risk	7	10

FAB, the French-American-British classification system of hematologic diseases.

dosages. Seventeen patients had negative MRD after 1 or 2 months of treatment with the conventional ITI regimen dosage, while the median MRD of these 17 patients before treatment was 0.17% (0.03–0.58%), and no leukemia cells were identified in their bone marrow by morphological examination. The MRD levels of seven patients significantly decreased after 1 or 2 months, and their median MRD level was 0.14% (0.04–4.92%). Also, nine patients did not respond to treatment with the ITI regimen, and their median MRD before treatment was 1.05% (0.06–3.00%). Moreover, seven patients received the ITI regimen at increased dosages; that is, the administration of IFN-α1b and IL-2 was changed from once every other day to once daily, and the amount of thalidomide was not changed. The median MRD before treatment in these patients was 0.16% (0.02–1.18%). These patients also had elevated MRD levels after 2 months of application with the conventional doses, and therefore, the ITI regimen at the increased dosages was applied to them. The MRDs of five of these patients converted to negative at 3–7 months after switching to the higher doses, while the other two patients had significantly lower MRDs (>10-fold) at 1–3 months after application of the higher doses. Notably, one patient (No. 8) maintained a negative MRD for 17 consecutive months; however, the ITI dosing was discontinued for him, and his MRD turned positive again after 6 months. At present, this patient is receiving the ITI regimen at higher dosages. The median relapse-free survival of these 40 patients was 9.7 (0.5–37) months (*Figure 3*).

The ITI regimen improved the long-term survival of Group C patients with initial CR and MRD-negativity

Group C comprised 88 patients with initial CR of AML, there were 52 men and 36 women, with a median age of 44.5 years (18–66 years). According to the FAB classification: M2 (43 cases), M4 (20 cases) and M5 (16 cases) were the most, while M1 (7 cases) and M0 (2 cases) were less. Among these patients, 26 were at low risk of WHO, 55 were at moderate risk, and 7 were at high risk (*Table 3*). These patients received the ITI regimen treatment beginning 1 month after routine consolidation therapy, and the protocol of the ITI regimen was the same as that in Group A. Among the 88 patients with initial CR, 11 (12.5%) relapsed during the maintenance period (*Figure 4*); these 11 patients relapsed within 2 years, with a median recurrence period of 20 months, of which three, five, and four patients were considered at a favorable, intermediate, and high risk, respectively.

The recurrence rates of 81 AML patients without maintenance therapy during the follow-up period were

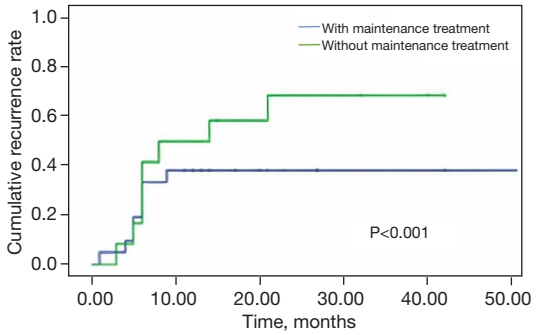

Figure 4 Relapse of MRD-negative AML patients with and without maintenance treatment following completion of consolidation therapy. MRD, minimal residual disease; AML, acute myeloid leukemia.

significantly higher than those of patients receiving ITI maintenance therapy (P<0.001). The median recurrence time was 6 months (3–16 months) in 44 patients, who were divided into low (n=11), medium (n=23), and high (n=10) risk groups.

Safety assessment

Of the 68 AML patients who were refractory/relapsed or had poor physical condition and could not tolerate chemotherapy, 8 patients died of severe complications before completing the first cycle. The main non-hematological adverse reactions of other patients were fever, constipation, rash, lethargy, joint pain, peripheral neurotoxicity, edema and so on, which resolved with symptomatic management.

The main hematologic adverse reactions were myelosuppression, which occurred in about 1/3 of patients with grade III or higher myelosuppression. It was mainly concentrated in refractory/relapsed AML patients and those with poor physical status, while myelosuppression was mild in MRD-positive patients and maintenance treatment group. The incidence of infection was low, with no significant pulmonary, gastrointestinal, or soft tissue skin infections.

In vivo experiment

The flow chart of *in vivo* experiment is shown in *Figure 5A*. We found that the survival times of mice in the treatment group were significantly longer than those in the control group. On the 20th day, the ITI group had the greatest number of surviving mice. At the end of the experiment, it was suggested that the ITI group could prolong the survival time of mice compared with the two drug groups, although there was no statistical difference in the survival times of the mice (*Figure 5B*). After treatment, the fluorescence intensity of the PBMC and treatment groups were significantly lower than that of the control group (P<0.001), while that of the combination group (including two drugs and three drugs) was markedly lower than that of the control group (P<0.001) (*Figure 5C,5D*).

On the seventh day after treatment, the livers of mice in each group were taken and CD45$^+$ positive leukemic cells were detected by immunohistochemistry. The results showed that the infiltrating leukemic cells in the mice livers were hCD45 positive, suggesting that the tumor cells were of human origin. The number of CD45$^+$ positive leukemic cells in the PBMC + IFN-α + IL-2 + thalidomide group was the lowest (*Figure 5E*). After tumor formation, the body weights of mice in all groups showed a downward trend; the weight loss of mice in the control group was the most obvious, and there were statistical differences between the experimental (including the PBMC and treatment groups) and control groups (*Figure 5F*). The proportion of CD3$^+$CD4$^+$ and CD3$^+$CD8$^+$ T lymphocytes in the peripheral blood of mice in the experimental group (including the PBMC and treatment groups) increased, and the expression levels of dendritic cells, perforin, and granzyme-B increased significantly, especially in the PBMC + IFN-α + IL-2 + thalidomide group. There were also notable differences between the treatment and PBMC groups (*Figure 5G-5J*).

Discussion

In general, ~40–45% of younger and 10–20% of older adults with AML can be cured with the current standard chemotherapy (2). Most patients with AML experience relapse or primary drug resistance and are then considered as having R/R-AML. At present, R/R-AML is the most significant challenge for hematologists worldwide. For decades, there has been no revolutionary improvement in the efficacy of AML treatments, and experts have been exploring strategies to overcome R/R-AML. Currently, there are several new drugs and novel chemotherapy combinations. However, patients with poor physical conditions and economic limitations can at best only be given symptomatic treatment and supportive care. Therefore, we tried to improve the quality of life of these R/R-AML patients via the application of a new combination

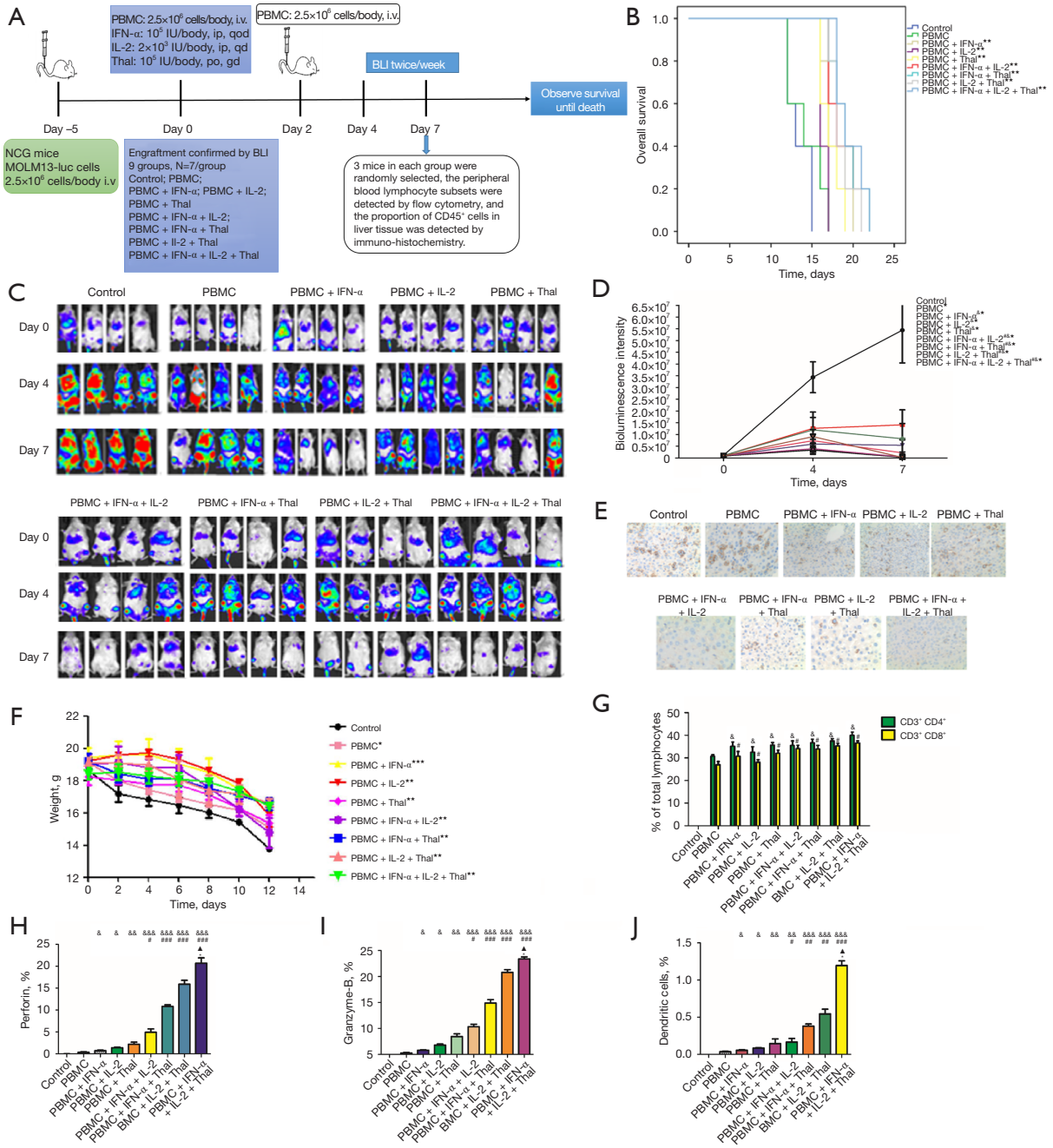

Figure 5 Effect of interferon-α-1b, interleukin-2, and thalidomide on AML xenograft mice. (A) Mouse grouping (n=7 mice per group) and experimental procedures for the administration of IFN-α (10^5 IU, intraperitoneally, once every other day), interleukin-2 (2×10^3 IU, intraperitoneally, once daily), thalidomide (10^5 IU, intragastrically) in AML xenograft mice. (B) Survival curve of AML xenograft mice. (C) The tumorigenesis of AML xenograft mice was monitored regularly. (D) Fluorescence intensity was analyzed in AML xenograft mice. (E) The hCD45$^+$ immunohistochemical staining results of the livers in AML xenograft mice on day 7 (magnification, ×200). (F) Changes in the body weights of mice were monitored after injection of the cells. (G) Changes in the lymphocyte proportion on the 7th day after administration. (H) Expression level of perforin in each group on the 7th day after administration. (I) Expression level of granzyme-B in each group on the 7th day after administration. (J) Proportion of dendritic cells in each group on the 7th day after administration. *, treatment group *vs.* control group, *P<0.05, **P<0.01, ***P<0.001; *, "PBMC + IFN-α + IL-2 + Thal" group *vs.* "PBMC + IFN-α + Thal" group or "PBMC + IL-2 + Thal" group, P<0.05; $^{\blacktriangle}$, "PBMC + IFN-α + IL-2 + Thal" group *vs.* "PBMC + IFN-α + IL-2" group, P<0.05; combination group *vs.* single medication group, $^{\#}$P<0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001; treatment group *vs.* PBMC group, $^{\&}$P<0.05, $^{\&\&}$P<0.01, $^{\&\&\&}$P<0.001. AML, acute myeloid leukemia; PBMC, peripheral blood mononuclear cell; IFN-α, interferon-α; IL-2, interleukin-2; Thal, thalidomide.

of affordable old drugs. The present study determined that this novel ITI regime can provide an affordable treatment option for patients with R/R-AML, and efficiently drives MRD negativity. In addition, the ITI regimen was associated with improved quality of life and longer survival in AML patients with CR. In addition, these three drugs are relatively cheap in China and the related medical expenses are accessible to most patients.

Our results suggested that the CR/CRi rate was only 11.7% when the ITI regimen combining rhIFNα-1b, rhIL-2, and thalidomide was used for R/R-AML salvage therapy. However, this approach does not provide an advantage over some intensive chemotherapy regimens, such as CLAG (cladribine, cytarabine, and granulocyte colony-stimulating factor), in terms of the remission rate (28,29). However, chemotherapy proved ineffective for some patients in the present trial, and others were unable to receive chemotherapy due to physical or economic factors. The National Comprehensive Cancer Network (NCCN) guidelines only recommend best supportive care for R/R-AML patients. However, patients who are unable to avoid blood transfusion and require long-term hospitalization will inevitably experience a degradation in their quality of life. In the present study, these patients received the ITI regimen, and 12 (20%) were rescued from the need for blood transfusions and received subcutaneous injections and oral medications only as outpatients. The survival time and quality of life for these patients were significantly improved.

Numerous new drugs have been used in the treatment of R/R-AML with specific targets, and the curative effect has been remarkable in some cases. However, multi-drug combinations are urgently needed for R/R-AML with no specific targets. Current combinations of new drugs inevitably cause severe bone marrow suppression (29,30). In the present study, some of the included patients who received the ITI regime showed a decrease in their routine blood counts during the malignant cell elimination stage and required the transfusion of blood components. However, patients who achieved CR did not need blood component transfusions, or needed only a small amount of blood transfusion due to the acceptable cell number decline caused by interferon.

The persistence of positive MRD is an independent prognostic factor for long-term survival in AML patients (31). The earlier the MRD of these patients turns negative, the better chance they have of long-term survival. Patients with persistent positive MRD during the course of treatment or MRD that turns from negative to positive are more likely to experience morphological recurrence, and allo-HSCT may be the best option to improve the survival of these patients. The ITI regimen described herein may be the best choice for patients who are not able to undergo allo-HSCT due to financial limitations or poor physical condition. Notably, the ITI regimen efficiently drives MRD negativity, which benefits the patient's fitness for allo-HSCT and betters the chance of long-term survival. In this study, after receiving the ITI regime, 19 of the 40 MRD-positive patients became MRD-negative, while the MRD decreased significantly in 12 patients, and the response rate of the 40 patients in Group B was 77.5% (31/40). Twenty patients from previous studies with hematologic remission but a positive AML-ETO fusion gene were treated with the ITI regimen in our center; 90% (18/20) of the patients showed varying levels of decline in the fusion gene level, and 10 of them became completely negative (22). This suggests that the ITI regimen can be an alternative economical option that can substantially improve the long-term survival rate of patients who are unable to undergo allo-HSCT.

Currently, the maintenance treatment of AML patients after allo-HSCT (32,33) is afforded greater research attention compared with patients after the first CR. In the present study, rhIFNα-1b, rhIL-2, and thalidomide were combined for the maintenance therapy of patients with initial CR. Compared with other reports (34-38), the cumulative recurrence rate was relatively low (3 years, 17.7%), and the ITI regimen can be implemented in a much simpler manner.

A previous experiment has shown that IFN exerts its antitumor effect mainly by directly acting on immune cells, and indirectly through immunomodulatory effects (13). IFN can potently stimulate immune responses in dendritic cells, T cells, and natural killer cell (NK) , which are key to promoting anti-tumor immune responses that enhance the killing of leukemic cells (13). The immune responsiveness of patients may be severely damaged by immune dysfunction in these cells, while the ability of type I IFN to restore their defective immune functions further underscores its biological basis for treating leukemia. In addition, IFN can promote IL-2-mediated T-cell proliferation and survival, thereby enhancing the cytotoxic effect of T cells on hematological malignancies. IL-2 is considered the most important regulatory factor in the immune network, as it has a major role in the growth and proliferation of many immune cells, including NK and T cells (39). In addition to inhibiting tumor angiogenesis, thalidomide has a strong immunomodulatory effect. On the one hand, thalidomide,

as a costimulator, increases the stimulating effect of T cells on T cell receptor-mediated antigen stimulation and promotes the proliferation of T cells to produce more IL-2, IL-10, and IFN (40). On the other hand, it reduces the production of inflammatory cytokines, such as IL-5, IL-6, IL-8, and IL-12. *In vivo* experiments also proved that the combination of the three drugs was effective in AML and prolonged survival. At the same time, the proportion of CD3+CD4+ and CD3+CD8+ T cells in the peripheral blood of experimental group mice (including the PBMC and treatment groups) were increased, and the expression levels of dendritic cells, perforin, and granzyme B were significantly increased, especially in the PBMC + IFN-α + IL-2 + thalidomide group. These factors further support the rationale for combining these three drugs in the treatment of AML.

It is important to note that the major limitation of this study is the relatively small sample size, in which referral bias is possible. Meanwhile, since there is no control group receiving standard treatment, this study still needs a rigorous clinical randomized controlled trial and more in-depth mechanism exploration to verify this conclusion. In addition, because the prices of the three drugs are low, the financial burden of patients is small, and the adverse reactions of the drugs are mild, there is no need for more expenditure to deal with complications. However, there is a lack of more detailed data to support it, so whether it is a cost-effective or affordable regimen is open to question.

Conclusions

In summary, for suitable patients, we still recommend allo-HSCT to improve the clinical effect. But for patients who can't get a transplant, ITI regimen can provide a new treatment option for patients with R/R-AML and those with AML who cannot tolerate conventional chemotherapy. In patients with CR status but MRD positivity, the ITI regimen can cause MRD to turn negative and improve the short-term survival of patients. As a maintenance treatment option, it can also benefit the survival of AML patients after initial CR.

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Footnote

Reporting Checklist: The authors have completed the STROBE and ARRIVE reporting checklists. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-5520/rc

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-5520/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This retrospective study was approved by the Institutional Center Ethics Review Committee at the Affiliated Cancer Hospital of Zhengzhou University/Henan Cancer Hospital (No. 2014xjs15). All participating hospitals were informed and agreed the study. Written informed consent was obtained from all participants and they agreed to publish their individual data. A protocol of the animal experiment was prepared before the study, which was reviewed and agreed by the Ethics Review Committee of the Life Sciences Division of Zhengzhou University, in compliance with the Zhengzhou University institutional guidelines for the care and use of animals.

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