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Combined Thalidomide and Recombinant Human Interferon-α-1b and Interleukin-2 for Acute Myeloid Leukemia of Various Disease Status: A Multi-Center Prospective Study

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Abstract

Objectives: This study investigated the therapeutic effects of combined recombinant human interferon-α-1b (IFN-α1b), thalidomide, and recombinant interleukin-2 (IL-2) in treating acute myeloid leukemia (AML) in patients of various disease status and vulnerabilities.

Methods: Patients with AML (*n* = 166) were treated with combined recombinant IFN-α1b, thalidomide, and recombinant IL-2 (ITI regimen). The rates of partial and complete remission, minimal residual disease (MRD) status, quality of life, and long-term survival were compared among 3 patient groups. Lymphocyte profiles and relevant cytokine levels determined from peripheral blood samples of patients (pre- and post-treatment) and healthy individuals were compared. (Registration number: ChiCTR-ONC-14004688; Registered 23 May 2014, www.chictr.org.cn)

Results: Sixty patients with primary AML who were unable to receive chemotherapy, or with relapsed/refractory AML, showed a total response rate of 30% after undergoing the ITI regime, and maintained a good quality of life. Eighteen patients with morphologically complete remission and consistently positive MRD achieved a response rate of 72.2%: the MRD converted to negative or was mitigated in 9 and 4 patients, respectively; 5 patients did not respond. For 88 patients with initial complete remission and negative MRD, 11 failed to maintain the negative MRD, and the relapse rate was 12.5%. The ITI regime was associated with substantial anti-leukemic changes in peripheral blood lymphocyte profiles and cytokine levels.

Conclusions: The ITI regimen may be an effective and affordable option for patients with AML who cannot tolerate conventional chemotherapy, including those with relapsed/refractory disease, or complete remission status but MRD-positive, or after initial complete remission.

Introduction

Acute myeloid leukemia (AML) is a heterogeneous malignancy of the blood that is characterized by uncontrolled clonal proliferation of abnormal myeloid progenitor cells(1). The overall prognosis of AML is poor. The 5-year survival rate of young adults with AML is 40–50%, and declines with age(2). Chemotherapy is the main treatment option for AML, but for some patients with relapsed and refractory (R/R) AML, the 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 rate of overall survival has not changed significantly(4). In 2017, several targeted drugs were approved by the United States Food and Drug Administration for the treatment of AML. However, the high cost of the new drugs is prohibitive for many patients, especially for those without medical insurance. In addition, patients with AML after multiple cycles of chemotherapy are at risk of complications such as lung infection and cardiac dysfunction. All of these factors profoundly 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 as a medication was first used for treating AML in 1979(5), but the anti-leukemic effects, exerted through a variety of intracellular mechanisms, were limited(6). Nevertheless, for the subpopulation given allogeneic hematopoietic stem cell transplantation (allo-HSCT) and with positive minimal

residual disease (MRD), significantly higher disease-free and overall survival rates were associated with administration of interferon alpha (IFN-α)(6, 7).

Similarly, some clinical trials suggest that the therapeutic effect of interleukin (IL)-2 monotherapy is not ideal for the treatment of AML(8, 9). However, a phase III clinical trial showed 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(10).

Thalidomide is used relatively rarely as an immunomodulatory drug in AML, and the results from the limited studies of its single use are not encouraging. However, when combined with lenalidomide and other agents, the results were more positive(11).

Currently, there are no reports concerning the combination of IFN-α, IL-2, and immunomodulatory drugs (thalidomide or lenalidomide) for the treatment of R/R-AML in a Chinese population. Yet, in China these drugs are affordable for most patients with AML. In addition, the adverse effects when any of these is used as monotherapy are mild. Therefore, the present clinical trial was conducted to evaluate the therapeutic efficacy of a combined ITI regimen (i.e., IFN-α1b, thalidomide, and IL-2) for the treatment of patients with R/R-AML and others who are not suitable candidates for intensive chemotherapy. The program (Registration number: ChiCTR-ONC-14004688; www.chictr.org.cn) was also applied to MRD-positive patients with complete remission but not conditional transplantation, so that the rate of conversion to MRD negativity could be observed. Since previous studies reported that IL-2 monotherapy had no significant long-term survival benefits in patients with CR(9, 12), we also applied the ITI regimen to patients with initial CR and completed consolidation therapy, to observe their survival benefits.

Methods

Ethics considerations

This prospective study was approved by the Institutional Center Ethics Review Committee at the Affiliated Cancer Hospital of Zhengzhou University/Henan Cancer Hospital. The study protocol was carefully explained to the participants who accepted voluntarily. 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 were administered with the ITI regimen between January 2014 and June 2017 from the following 10 hospitals: Affiliated Cancer Hospital of Zhengzhou University/Henan Cancer Hospital; People's Hospital of Pingdingshan City; People's Liberation Army 150 Hospital; First People's Hospital of Shangqiu City; First Affiliated Hospital of Henan University of Science and Technology; People's Hospital of Fuyang City; Center Hospital of Luoyang City; Huaihe Hospital of Henan University; People's Hospital Jiaozuo City; and First Affiliated Hospital of Xinxiang Medical College.

In this study, the patients with AML were classified into 3 groups (A, B, and C) according to their disease status and response to previous treatments as specified below. In brief, Group A comprised patients with R/R-AML, or primary AML who were unable to receive chemotherapy. Group B included patients with both morphological CR

and consistent positivity for MRD. In Group C, patients had achieved initial CR and negative MRD. The groups were followed with focuses on response to the ITI regimen, rates of CR and partial remission, MRD status, quality of life, and long-term survival. In addition, the peripheral blood lymphocyte profiles and relevant cytokine levels of the Group A patients, before and after receiving the ITI regime treatments, were compared to that of the control group, which included 10 healthy subjects selected from the physical examination center of Affiliated Cancer Hospital of Zhengzhou University.

Potential subjects with any of the following were excluded from this analysis: serious allergy to thalidomide, interferon, or IL-2 with clinical manifestation of 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; ≥2 peripheral neuropathy events(13); poor liver function manifested as alanine aminotransferase or aspartate aminotransferase ≥ 2.5-fold the upper limits of normal (ULN), or serum total bilirubin ≥ 1.5-fold ULN; lung function decompensation; renal failure; or psychotic episodes.

Patients and subject groups

The patients of Group A were with R/R-AML, or primary AML that were not suitable for chemotherapy. Relapsed AML was diagnosed in the presence of any of the following: leukemic cells in the periphery 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 for patients with any of the following: primary AML without CR, even after 2 cycles of standard regimen treatments; relapse within 12 months after CR and consolidation therapy; relapse after 12 months and no response to regular chemotherapy; ≥2 relapses; or persistent extramedullary leukemia. Patients in Group A met the following inclusion criteria: 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; had not reached CR after chemotherapy and were not qualified for allo-HSCT; and older than 18 years.

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

Patients of Group C (initial CR/MRD-negative) were either those categorized into the favorable-risk group, or those with intermediate- or poor-prognosis who could not receive hematopoietic stem cell transplantation, according to the criteria of risk stratification for AML of the World Health Organization (WHO)(14). These patients achieved morphological remission after 1 or 2 cycles of induction chemotherapy, and received at least 4 cycles of consolidation treatments. In addition, the MRD of these patients converted to negative during or after their consolidation therapy.

Administration of the ITI regimen

For patients in Group A and Group B, a subcutaneous injection of recombinant human IFN-α1b (rhIFNα-1b; Shenzhen Ke Xing Bioengineering; 60 μg/time, every other day) was given, which was followed by administration of ibuprofen particles (Harbin Pharmaceutical Group Sanjing Pharmaceutical Nuojie) 30 min later. Recombinant human interleukin-2 (rhIL-2; Beijing Sihuan Biopharmaceutical) was injected subcutaneously

once every other day at a dose of 1 million units/time. Thalidomide tablets (Changzhou Pharmaceutical Factory; 200 mg) were taken orally every night before sleep. If the patient's platelet count was 50 × 10^9/L, patients were recommended to take compound salvia tablets orally (Guangdong Baiyun Mountain Heji Huangpu Chinese Medicine; 3 tablets/time, 3 times/d) to prevent deep venous thrombosis. At 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 at 1 month after their completion of the last chemotherapy, and the dose of each drug was the same as that in Group A and Group B.

Evaluation of therapeutic effect

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 in accordance with the standards of the International Working Group(15). PR was adjudged when the percentage of leukemic cells in bone marrow was < 20%, but declined over 50% as compared with the initial timepoint. NR was defined as no obvious decline in the percentage of leukemic cells in 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 of any cause.

Collection and storage of peripheral blood samples

Peripheral blood specimens were collected before and 3 months after the start of ITI administration. Blood samples (4 mL/patient) were collected into K2-EDTA (ethylenediaminetetraacetic acid) anticoagulation vacuum tubes (BD Biosciences). Within 24 hours of collection, 2 mL of blood was processed and tested for peripheral blood lymphocyte subsets and granzyme B/perforin via flow cytometric analysis. Plasma from the remaining 2 mL of blood samples was separated under sterile conditions by centrifugation, and cryopreserved in − 80°C freezers for subsequent quantitation of cytokines.

Flow cytometric analysis of lymphocytes in blood samples

Blood samples (~ 100 μL) were mixed with the appropriate amount of fluorescence-conjugated monoclonal antibodies. All monoclonal antibodies were used in saturating concentrations. For surface staining, cells were stained with the indicated antibodies in staining buffer (phosphate buffered saline supplemented with 2% fetal bovine serum) for 20 minutes at 4°C. The lysis of red blood cells was implemented with the addition of ACK Lysing Buffer (ThermoFisher Scientific). The remaining white blood cells were fixed in 2% paraformaldehyde and stored for subsequent flow cytometry.

For intracellular staining, cells were stained with the indicated antibodies against surface markers, fixed with Cytofix/Cytoperm buffer for 1–2 hours at 4°C, and incubated with Cytoperm Plus buffer (BD Biosciences, both) for 15 minutes at room temperature. After re-fixing for 15 minutes at room temperature, cells were incubated with antibodies (anti-Perforin and anti-Granzyme B) or isotype controls for 20 minutes.

All FACS (flow cytometry and fluorescence-activated cell sorting) analyses were performed on a BD Calibur or a Canto II flow cytometer, and data were analyzed with FlowJo software. The following anti-human antibodies were used in this study: phycoerythrin (PE)-conjugated CD8, from Beckman Coulter (Catalog No. A07757); and peridinin-chlorophyll-protein (PerCP)-conjugated CD45, allophycocyanin (APC)-conjugated CD3, fluorescein isothiocyanate (FITC)-conjugated CD4, APC-conjugated CD56, PE-conjugated perforin, and FITC-conjugated

granzyme B (from BD Biosciences, catalog No. 652803, 652815, 340133, 341025, 51-65995x, and 560211, respectively).

Quantitation of VEGF, IFN-γ, TNF-α, and IL-6 in serum

The following cytokines were measured in blood sera using a Cytometric Beads Assay kit (CBA Flex Sets, BD Biosciences): VEGF (vascular endothelial growth factor); IFN-γ (interferon-γ); TNF-α (tumor necrosis factor); and IL-6 (interleukin-6). The processing of specimens was conducted in strict accordance with the instructions of the manufacturer. Data were analyzed using FCAP Array software (BD Biosciences).

Statistical analysis

All the data are presented as mean ± standard deviation. Unless otherwise indicated, 2-tailed Student's *t*-tests were used to compare group pairs. Statistical significance was considered $P < 0.05$.

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, of whom 8 died of severe complications before the completion of the first cycle of treatment, an early mortality rate of 15.4%. Of the 60 eligible patients in the present analysis there were 32 men and 28 women, with a median age of 54.5 years (18–75 y; Table 1).

Of the 60 patients, 4 achieved CR. Of these, 2 did not receive any treatments in the early stage, but refused chemotherapy for financial reasons and received the ITI regimen. CR was achieved after they were treated with 1 and 2 courses, respectively. The same regime was applied for more than 1 year, and they were still in morphological remission.

The remaining 2 patients who had achieved CR relapsed after multiple cycles of treatments. Because of neutropenia and severe pulmonary infection and elevated aminotransferase, they were not suitable for intensive therapy. The ITI regimen was applied and they reached CR after 2 courses of treatments. One of them survived for more than 4 years; the MRD converted and remained negative for 8 months. The other patient experienced a recurrence of AML at 7 months after CR. Six patients achieved CRi within 2 to 6 months after application of the ITI regimen.

Thirty-six patients had previously received induction therapy or salvage treatment, but did not achieve morphological remission. The ITI regimen was accepted by these patients due to poor physical condition or financial problems. Of them, 2 and 3 patients reached CR or CRi, respectively, resulting in a total remission rate (CR + CRi) of 13.8%. When 3 patients with PR are considered, the overall response rate (CR + CRi + PR) was 22%.

Moreover, 24 patients had not received previous intensive chemotherapy but only support therapy. Of theses, 2 and 3 experienced CR or CRi, respectively, with a total effective rate of 20.8%. If 4 patients with PR are included, then the total response rate was 37.5%. The median survival time of patients with PR was 3.5 (1–5) months.

Although the overall response rate to the ITI program was only 30%, a number of patients had a better quality of life and prolonged survival. Among them, 3, 4, and 5 patients with CR, CRi, and PR, respectively, were not transfused with any red blood cells or platelet components. No serious complication, such as severe pulmonary infection, was found in patients with initial pulmonary infection before treatments. After the recovery of the hemogram, these patients were able to avoid hospitalization, and took medications outside the hospital. Collectively, these results suggested that the ITI regimen improved the quality of life and prolonged the survival of Group A patients with R/R-AML.

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

The 18 patients of Group B remained MRD-positive or changed from negative to positive again after routine consolidation therapy. Patients underwent the ITI regimen after consolidation therapy, and the protocol was the same as for Group A (Tables 2a and 2b). Diagnoses were performed by routine complete blood cell counts, bone marrow morphometry, flow immunophenotyping, cytogenetics, and molecular biology assays. Ten of the 18 patients were men. According to the criteria of the WHO risk stratification(14), 7 were favorable with a low risk, 8 were at intermediate risk, and 3 were poor and at high risk. Patients with FLT3 (fms-like tyrosine kinase 3)-ITD (internal tandem duplication) mutations were treated with oral sorafenib during early induction and consolidation treatment.

Of 17 patients with CRi, 4 and 5 patients had continuously positive MRD, or MRD converted from negative to positive, respectively, and 7 patients had unstable MRD during treatment, and 1 patient reach first remission with MRD-positivity after 3 cycles of induction. One patient (No.7) showed a secondary remission.

The response rate of the 18 patients in Group B was 72.2%, where MRD < 0.01% was defined as the negative threshold. Specifically, 9 and 4 patients had MRD that converted to negative or had mitigated MRD, respectively, and 5 patients did not respond.

In Group B, 15 patients received only conventional dosages. Seven patients had a negative MRD after 1 or 2 months of treatment with the conventional dosage of the ITI regimen, while the median MRD of these 7 patients before treatment was 0.13% (0.02–0.58%), and no leukemia cell was identified in their bone marrow by morphology. The MRD levels of 3 patients significantly decreased after one or two months, and their median MRD level was 2.09% (1.20–4.92%). Of these 3 patients, one showed no leukemia cells, and the remaining had morphological leukemia cells in percentages of 4.5% and 5%, respectively, in bone marrow.

Five patients did not respond to treatment with the ITI regimen. Their median MRD before treatment was 2.50% (0.06-3.00%). Among these 5 patients, 2 had no leukemia cells in bone marrow, and 3 had increased percentages of morphological leukemia cells in bone marrow (2.4, 3.5, and 5%, respectively).

Three patients received the ITI regimen with increased dosages, that is, administration of IFN-α1b and IL-2 was changed from once every other day to once per day, and the amount of thalidomide was not changed. These 3 patients had elevated MRD levels after 2 months of application with conventional doses. Therefore, the ITI regimen with increased dosages was applied to them. The MRD of 2 of these patients converted to negative at 3 and 7 months, respectively, after switching to the higher doses. The other patient had significantly lower MRD

(by > 10-fold) at 1 month 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. Currently, this patient is receiving the ITI regimen with the higher dosages.

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 (Table 3). They received the treatments of the ITI regimen 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 (Fig. 1). All of these 11 patients relapsed within 2 years, with a median recurrence period of 20 months, of which 3, 5, and 4 patients, respectively, were considered at favorable, intermediate, and high risk.

All patients who met the criteria of Group C were given maintenance treatment without prospective controlled observation.

The ITI regimen drove changes in lymphocyte profiles of peripheral blood from patients with AML

Since T and natural killer (NK) lymphocytes have crucial functions in eliminating leukemic cells, the ITI regimen was investigated for its potential influence on the peripheral blood lymphocyte profiles of 10 patients in Group A who achieved CR or CRi. The percentages of $CD3^+CD4^+$ T cells, $CD3^+CD8^+$ T cells, and $CD56^+$ NK cells among total leukocytes ($CD45^+$ cells) were measured by flow cytometry.

It was found that the percentage of $CD4^+$ T cells and ratio of $CD4^+$ to $CD8^+$ in the patients before treatment were significantly lower than that of the healthy control group (Table 4). Two months after therapy with the ITI regime, these parameters were significantly higher compared with the pretreatment values. However, the percentage of $CD3^+CD8^+$ T cells after the ITI regimen were not significantly higher, although the average number seemed to be slightly elevated (28.33 for pretreatment compared with 29.49 posttreatment).

Similar to $CD3^+CD4^+$ T cells, $CD56^+$ NK cells were also lower in percentage in the patients before ITI therapy compared with the healthy control group (Table 4). In the patients, the pre-ITI regimen ratio of NK cells (7.40 ± 6.63) was higher after treatment (10.0 ± 8.51), and almost equivalent to that of the control group (9.13 ± 5.58). Similar trends were observed with regard to the ratio of perforin-positive and granzyme B-positive NK cells. The frequency of both $perforin^+$ and granzyme B^+ NK cells of the patients was significantly lower than that of the patients, while the posttreatment levels of the patients were similar to that of the controls.

The ITI regimen changed the levels of cytokines, including VEGF, IFN-γ, TNF-α and IL-6, in the sera of patients with AML

Cytokines that have been reported associated with the pathogenesis of AML were investigated to determine the potential influence of the ITI regimen. There were, specifically, leukemia-promoting (VEGF and IL-6) and leukemia-inhibiting (TNF-α and IFN-γ) factors in plasma (Table 4). In the patients, the posttreatment plasma

concentrations of both VEGF and IL-6 were much lower than before treatment, while the posttreatment TNF-α and IFN-γ percentages were, respectively, ~ 2- and 3-fold higher.

Discussion

In general, ~ 40 to 45% of younger and 10 to 20% of older adults with AML can be cured with 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 biggest challenge for hematologists worldwide. For decades, there has been no revolutionary improvement in the efficacy of treatments for AML, and experts have been looking for ways to conquer R/R-AML. Currently, there are many new drugs and new chemotherapy combinations. However, patients with poor physical condition and economic burden can only be given, at best, symptomatic treatment and supportive care. Therefore, we tried to improve the quality of life of these patients with R/R-AML by application of a new combination of affordable old drugs. The present study determined that this new ITI regime can provide a new, 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 of patients with AML with CR.

Our results suggest that the CR/CRi rate was only 13.4%, when the ITI regimen with a combination of rhIFNα-1b, rhIL-2 and thalidomide was used for the salvage therapy of R/R-AML. This does not hold an advantage over some intensive chemotherapy regimens such as CLAG (cladribine, cytarabine, granulocyte colony-stimulating factor), in terms of remission rate(16, 17). However, chemotherapy proved ineffective for the patients in the present trial, or patients were unable to receive chemotherapy due to physical or economic reasons. The guidelines of the National Comprehensive Cancer Network (NCCN) recommend only best supportive care for patients with R/R-AML. However, if patients are unable to avoid blood transfusion and require long-term hospitalization, this always degrades their quality of life. In the present study, these patients received the ITI regimen and 12 (20%) were freed from blood transfusions and received subcutaneous injection and oral medication only as outpatients. The survival time and quality of life for these patients was significantly improved.

Many 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. Yet, multi-drug combinations are urgently needed for R/R-AML with no specific targets. Current combinations of new drugs always cause severe bone marrow suppression(17, 18).

In the present study, some of the patients showed a decrease in blood routine counts during the stage of malignant cell elimination when undergoing the ITI regime, and these patients required transfusion of blood components. However, the patients who achieved CR did not need transfusion of blood components, or they needed only a small amount of blood transfusion, due to the acceptable limit of cell number decline caused by interferon.

The persistence of positive MRD is an independent prognostic factor for long-term survival of patients with AML(19). 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 treatments, 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 those who are not able to undergo allo-HSCT due to financial burden or poor physical condition. Also notable, the ITI regimen efficiently drives MRD negativity, which benefits the patient's fitness for allo-HSCT and betters the chance of long-term survival. After receiving the ITI regime, 9 of the 18 MRD-positive patients (66.7%) became MRD-negative, while in 4 patients the MRD decreased significantly. This suggests that the ITI program can be an alternative economic option that can substantially improve the long-term survival rate of patients who are unable to undergo allo-HSCT.

Currently in AML, the maintenance treatment of patients after allo-HSCT(20, 21) is given more research attention compared with patients after the first CR. In the present study, rhIFNα-1b, rhIL-2, and thalidomide were combined toward maintenance therapy for patients with initial CR. The cumulative recurrence rate was relatively low (3 years, 17.7%) compared with other reports(22–26), and the ITI regimen can be implemented in a much simpler manner.

In vitro cytological experiments have shown that interferon exerts its antitumor effect mainly by directly acting on immune cells, and indirectly through immunomodulatory effects(6). Interferon can potently stimulate immune responses in dendritic cells, T cells, and NK cells, which are key to promoting anti-tumor immune responses that enhance the killing of leukemic cells(6). The immune responsiveness of patients may be severely damaged by immune dysfunction in these cells, while the ability of type I interferon in restoring their defective immune functions further underscores its biological basis for treating leukemia. In addition, interferon can promote IL-2-mediated T cell proliferation and survival, thus 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(27). 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 interferon(28). On the other hand, thalidomide reduces the production of inflammatory cytokines, such as IL-5, IL-6, IL-8, and IL-12.

The results of the present study suggest that the ITI regimen, comprising thalidomide, rhIFNα-1b, and rhIL-2, raised the ratio of $CD4^+$ to $CD8^+$ T cells and the percentage of NK cells in peripheral blood. Moreover, the ITI regimen was associated with increased levels of perforin and granzyme-B in NK cells, increased plasma concentrations of IFN-γ and TNF-α, and decreased plasma concentrations of VEGF and inflammatory IL-6. This implies the enhancement of antitumor ability in these patients. These factors further support the rationale for applying the combination of these 3 drugs in the treatment of AML.

The current investigation has several strengths and limitations. To the best of our knowledge, this is the first report of the combined application of thalidomide, rhIFNα-1b, and rhIL-2 in Chinese patients with AML. The major limitation of the current study is the relatively small sample size, in which referral bias is possible.

In summary, the ITI regimen can provide a new, affordable 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 patients' short-term survival. As a maintenance treatment option, it can also benefit the survival of patients with AML after initial CR.

Declarations

Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

Competing interests

The authors have declared that no competing interests exist.

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Authors' contributions

Conception and design: Xudong Wei, Ruihua Mi, Lin Chen; Development of methodology: Xiaojiao Wang, Lin Chen; Acquisition of data: Ruihua Mi, Lin Chen, Qingsong Yin, Zhanfang Wang , Xiaomiao Ma , Yulin Xu , Shuxia Chen , Genjie Wang , Haiping Yang , Zhichun Li , Huirui Wang , Hongmiao Zhao , Shuli Guo , Qinglin Song , Weiyong Li , Jingdong Li; Analysis and interpretation of data: Ruihua Mi, Lin Chen , Xiaojiao Wang; Writing, review, and/or revision of the manuscript: Ruihua Mi, Lin Chen; Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): Xudong Wei, Lin Chen; Study supervision: Xudong Wei, Yongping Song.

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Tables

Table 1. General information of Group A patients.

Gender, male/female (*n*/*n*)		32/28
Medium Age (year)		54.5 (22-71)
FAB (*n*/%)	M0	2/3.3
	M1	3/5.0
	M2A	15/25.0
	M2B	8/13.3
	M4	13/21.7
	M5	19/31.7
Enrollment reason	Financial	22/36.7
	Unfit	26/43.3
	Unwilling	12/20.0
Therapeutic effect	CR	4/6.7
	CRi	6/10
	PR	8/15
	No remission	41/68.3

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

Table 2a. General information of Group B patients before treatment.

	Gender	Age, y	FAB	Chromosome	Gene mutation	WBC *	WHO risk
1	F	53	M2b	46, XX, t(8;21)[7]/46, XX[3]	Negative	1.30	Favorable
2	M	33	M2b	46, XX, t(8;21)[6]/46, XX[10]	c-KIT	25.32	Intermediate
3	M	45	M4Eo	46, XY, inv(16)(p131q22)[17]/46.XY[3]	IZKF1, ERG, MLL-PTD	18.30	Favorable
4	M	28	M2b	46, XY, t(8;21)(q22;q22)[10]	Negative	1.54	Favorable
5	M	54	M2a	46, XY[10]	Negative	3.05	Intermediate
6	F	45	M2a	46, XX[20]	Negative	18.20	Intermediate
7	M	59	M2a	46, XX[20]	FLT3-ITD, IDH1, NPM1	66	Intermediate
8	M	28	M2a	46, XY[18]	Negative	1.75	Intermediate
9	M	37	M2b	46, XY[20]	IKZF1	9.67	Favorable
10	F	56	M1	46, XX[20]	FLT3-ITD	215	Poor
11	F	27	M5	46, XX[20]	K-RAS, RUNX1	16.47	Poor
12	F	58	M2b	46, XX, t(8;21)[8]/46, XX[5]	Negative	3.08	Favorable
13	M	31	M4Eo	46, XY, inv(16)(p13q22)[10]	Negative	46.8	Favorable
14	F	64	M2a	46, XX[20]	DNMT3A	1.90	Intermediate
15	F	52	M2a	46, XX[20]	TET2	1.79	Intermediate
16	M	25	M5	46, XY[20]	ASXL1	6.80	Poor
17	F	19	M5	46, XX[20]	Negative	9.88	Intermediate
18	M	58	M2a	no mitotic phase	NPM1(with fibrosis)	9.79	Favorable

* Initial white blood cell count, $\times 10^9$/L

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

Table 2b. Therapeutic effects in Group B patients after treatment.

	Pre-treatment	MRD status	MRD Pre	Bone marrow morphology	Dosage *	MRD Post	Follow-up
1	CAG, MA, DA, AA, MA, CAG, AA, HA, MA, DA, HE, CAG	Sustained positive	0.02%	Significantly active, 2.0% of promyelocyte, no abnormal Bm	Regular/2 mo	Negative	Continue same regimen
2	IA, HD-Ara-C, DCAG, DCHA, HD-Ara-C, MA	Repeated positivity during CT	0.09%	Active, no abnormal Bm	Regular/1 mo	Negative	Continue same regimen
3	HAA, HD-Ara-C, HD-Ara-C, DCAG, DCAG, HD-Ara-C	Repeated positivity during CT	0.10%	Active, no abnormal naive myelocyte	Regular/1 mo	Negative	Continue same regimen
4	IA, HD-Ara-C, HD-Ara-C, DCAG, DCHAG,	Sustained positive	0.13%	Active, no abnormal Bm	Regular/1 mo	Negative	Continue same regimen
5	DCAG, ID-Ara-C, ID-Ara-C, DCAG, DCHG, AA	Turned positive at 6 mo after last CT	0.49%	Active, 0.2% promyelocyte	Regular/1 mo	Negative	Continue same regimen
6	DA, HD-Ara-C, HD-Ara-C, DCAG, HD-Ara-C, HA	Repeated positivity during CT	0.56%	Active, 0.5% promyelocyte	Regular/1 mo	Negative	Continue same regimen
7	IA, HA, HD-Ara-C, DCAG	Hematologic remission after recurrence, MRD positive	0.58%	Active, 0.5% promyelocyte	Regular/1 mo	Negative	Continue same regimen
8	DA, DA, HD-Ara-C, HA, HA, HD-Ara-C, HA, HD-Ara-C, HD-Ara-C	Turned positive at 3 mo after last CT	0.06%	Active, 0.2% promyelocyte	Regular/2 mo	0.15%	Increased dosing for 7 mo, MRD turned negative
9	IA, HD-Ara-C, CAG, CHAG,	Repeated positivity during CT	0.01%	Active, no abnormal Bm	Regular/2 mo	0.06%	Increased dosing for 1 mo, MRD=0.0061%

	MA, CHAG, DCHAG, DCHAG, HD-Ara-C						
10	IA, HA, DCAG, DCAG,	Sustained positive	0.06%	Less active, no promyelocyte	Regular/2 mo	Morphological relapse	Other regimen
11	IA, DCAG, DCHAG, DCHAG, HD-AraC, HD-AraC	Sustained positive	0.17%	Active, 2.4% primordial monocytes	Regular/1 mo	Morphological relapse	Give up therapy
12	IA, ID-Ara-C, ID-Ara-C, ID-Ara-C, IA, CHG	Repeated positivity during CT	1.09%	Active, no abnormal Bm	Regular/1 mo	1.42%	Increased dosing for 3 mo, MRD turned negative
13	DHA, DHA, HD-Ara-C, EA, MA, DA	Repeated positivity during CT	1.20%	Active, no abnormal naive myelocyte	Regular/2 mo	0.13%	Continue same regimen
14	DCAG, ID-Ara-C, ID-Ara-C, ID-Ara-C, CHG, DA	Turned positive at 1 mo after last CT	2.09%	Active, 5% promyelocyte	Regular/1 mo	1.18%	Continue same regimen
15	DCAG, MAC, DCCHAG	Remission after 3 cycles, MRD did not turn negative	4.92%	Less active, 4.5% promyelocyte	Regular/2 mo	1%	Continue same regimen
16	IA, HD-Ara-C, HD-Ara-C, HD-Ara-C, AA, HA	Turned positive at 3 mo after last CT	2.50%	Active, 3.5% promyelocyte	Regular/1 mo	Morphological relapse	Gave up therapy
17	IA, HD-Ara-C, HD-Ara-C, HD-Ara-C,	Turned positive at 1 mo after last CT	2.68%	Significantly active, no Primordial monocytes	Regular/1 mo	Morphological relapse	Salvage CT was ineffective
18	IA, DCAG, DCHAG, HD-Ara-C, HD-Ara-C, HD-Ara-C, HA, DCAG	Repeated positivity during CT	3%	Active, 5% promyelocyte	Regular/1 mo	Morphological relapse	Gave up therapy

* Dosage reported as dose/duration; Bm, basophilic myelocyte; CT, chemotherapy; Pre, pretreatment; Post, posttreatment ; CAG, low dose cytarabine (Ara-C), aclarubicin (Acla) and Granulocyte Colony Stimulating Factor (G-CSF); MA, mitoxantrone (Mito) and Ara-C; DA, daunorubicin (DNR) and Ara-C; AA, Acla and Ara-C; HA,

homoharringtonine (HHT) and Ara-C; HE, HHT and etoposide (VP-16); IA, idarubicin (IDA) and Ara-C; HD-Ara-C, high dose Ara-C; DCAG, decitabine (DEC) and CAG; DCHAG, HHT+DCAG; HAA, HHT and Acla and Ara-C; DCHG, lows dose Ara-C, HHT, G-CSF and DEC; EA, VP-16 and Ara-C; MAC, Mito and Ara-C and cyclophosphamide (CTX); DCCHAG, chidamide (CHI) and DCHAG; mo, month;

Table 3. General information of Group C patients.

Gender, *n*	Male	52
	Female	36
Medium age, y		44.5 (18-66)
	< 60	74
FAB, *n*	M0	2
	M1	7
	M2a	32
	M2b	11
	M4	20
	M5	16
WHO risk classification, *n*	Favorable	23
	Intermediate	55
	Poor	7

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

Table 4. The ITI regime was associated with substantial anti-leukemic changes in peripheral blood lymphocyte profiles and cytokine levels.

		Pre-treatment	Post-treatment	Control
T cell subsets, %	$CD3^+CD4^+$	21.93 ± 8.77 [a]	29.81 ± 9.13 [b]	33.39 ± 6.94
	$CD3^+CD8^+$	28.33 ± 7.56	29.49 ± 9.57	26.54 ± 6.33
T cell subsets, ratio	$CD4^+/CD8^+$	0.77 ± 0.41 [a]	1.01 ± 0.37 [a,b]	1.26 ± 0.33
NK cells, %	NK/leukocytes	7.40 ± 6.63 [a]	10.0 ± 8.51 [b]	9.13 ± 5.58
	$Perforin^+$/NK	11.61 ± 7.39 [a]	14.21 ± 6.70 [b]	13.16 ± 7.01
	Granzyme B^+/NK	13.54 ± 7.59 [a]	17.99 ± 11.38 [b]	17.11 ± 8.53
Cytokines, pg/mL	VEGF	61.53 ± 30.71 [a]	40.96 ± 38.56 [a,b]	5.17 ± 3.09
	IL-6	8.58 ± 3.59 [a]	6.32 ± 3.75 [a]	3.81 ± 2.90
	TNF-α	8.60 ± 5.97 [a]	16.23 ± 11.39 [a]	3.22 ± 1.91
	IFN-γ	10.53 ± 8.93	27.52 ± 16.55 [a,b]	13.01 ± 7.23

[a] Compared with the control group, $P < 0.05$; [b] compared with the pre-treatment group, $P < 0.05$;

Pre- and post-treatment groups, n = 10 each; control group, n = 10.

Figures

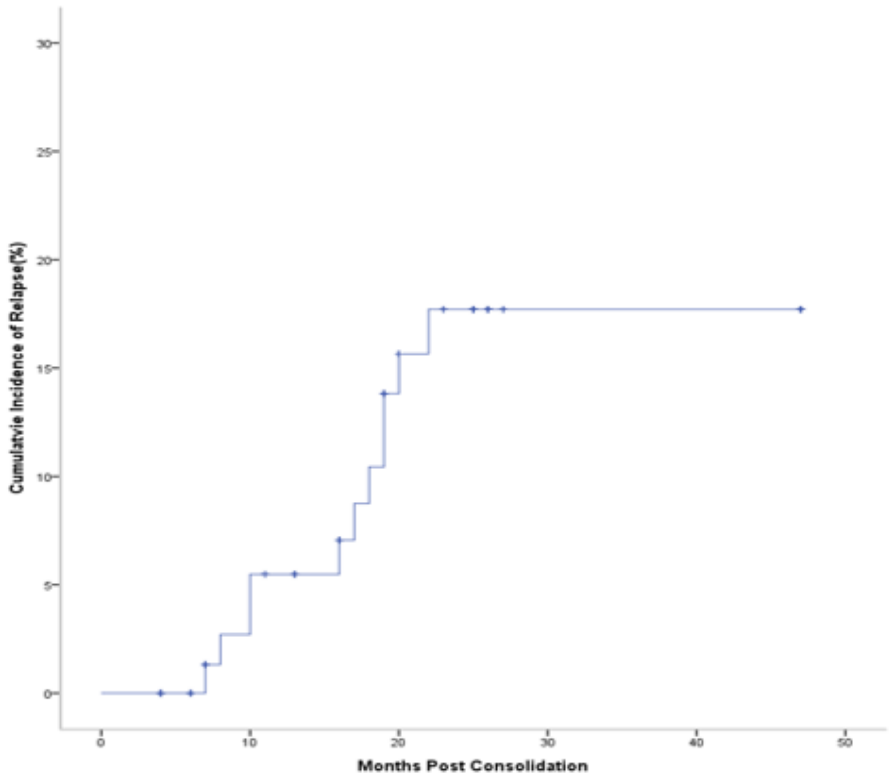

Figure 1

Cumulative incidence of relapse of patients in Group C patients with AML with initial CR. The x-axis indicates the time after consolidation therapy for patients with AML with initial CR (n = 88). The y-axis denotes the cumulative incidence of relapse.