Recombinant human erythropoietin plus all-*trans* retinoic acid and testosterone undecanoate for the treatment of anemia in patients with lower-risk myelodysplastic syndromes: a multicenter, single-arm, prospective trial

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Methods

Study design and patients

This investigator-initiated, prospective, single arm trial conducted at 10 sites across China enrolled adult patients between 18 and 80 years of age who had primary myelodysplastic syndrome (MDS) according to the World Health Organization (WHO) 2016 criteria[1] and were required to have baseline hemoglobin < 100 g/L regardless of the levels of endogenous serum erythropoietin and red blood cell (RBC) transfusion support. Patients were considered to have no transfusion burden if they had received no RBC transfusion in the 8 weeks before treatment and have transfusion burden if they required 1 or more RBC units in the 8 weeks before treatment.

Inclusion criteria:

Each potential subject must satisfy all of the following criteria to be entered in the study.

(1) Primary MDS classified as very low, low, or intermediate-risk by the Revised International Prognostic Scoring System (IPSS-R)[2] with <5% bone marrow blasts;

(2) Aged from 18 to 80 years, male or non-pregnant, non-lactating female;

(3) Eastern Cooperative Oncology Group (ECOG) performance status 0, 1, 2;

(4) Adequate renal and hepatic function was defined as serum creatinine ≤ 1.5 times of the upper limit of normal (× ULN), blood urea nitrogen (BUN) ≤ 1.5 × ULN, alanine aminotransferase (ALT) ≤ 2 × ULN, aspartate aminotransferase (AST) ≤ 2 × ULN, and total bilirubin ≤ 1.5 × ULN;

(5) Hemoglobin < 100 g/L, platelet ≥ 30×10^9/L and neutrophil absolute count ≥ 0.5 ×10^9/L;

(6) No erythropoiesis-stimulating agent (ESA), all-trans retinoic acid (ATRA), or

androgen treatment within 30 days prior to the first day;

(7) Ability to follow the study visit schedule and comply with all protocol requirements;

(8) Capacity to take oral medication and willingness to provide informed consent;

(9) Fertile individuals and their spouses must agree to use effective contraception during the trial and for at least 3 months post-last study drug administration.

Exclusion criteria:

Any potential subject who meets any of the following criteria will be excluded from participating in the study.

(1) Presence of del(5q) cytogenetic abnormality;

(2) Prior stem cell transplant;

(3) Treatment with granulocyte colony-stimulating factor (G-CSF), thrombopoietin, or thrombopoietin receptor agonists within 8 weeks prior to the first day;

(4) Treatment with anti-thymocyte globulin, azacytidine, decitabine, cyclosporine, thalidomide, lenalidomide within 12 weeks before the first day;

(5) Receipt of any trial drug within 28 days before the first day;

(6) Any active malignant tumor except for localized non-metastatic squamous cell or basal cell skin cancer or carcinoma in situ;

(7) Active infection requiring systemic antibiotic treatment;

(8) Life expectancy < 6 months;

(9) History of epilepsy;

(10) Thromboembolic events within six months prior to the first day;

(11) Currently receiving anticoagulant therapy;

(12) Known human immunodeficiency virus (HIV), hepatitis B, or hepatitis C infection;

(13) Severe heart disease, including New York Heart Association (NYHA) class III or IV congestive heart failure, uncontrolled hypertension or hypotension, or severe valve or endocardial disease;

(14) Clinically significant or uncontrolled persistent inflammatory/autoimmune diseases;

(15) Currently alcohol or drug abuse.

Study approval and registration

The study protocol and subsequent amendments were approved by the Clinical Research Ethics Committee of the First Affiliated Hospital, Zhejiang University School of Medicine (Reference number: 197). The study was conducted in accordance with the principles of the Declaration of Helsinki and the Good Clinical Practice guidelines. All patients provided written informed consent before any trial-related activities. This trial is registered with Chinese Clinical Trial Registry (ChiCTR2000032845).

Procedures

Patients received recombinant human erythropoietin (rhEPO, 3SBio, Shenyang, China) 10000 IU/day subcutaneously, oral ATRA (Liangfu, Shandong, China) 25 mg/m^2/day and oral testosterone undecanoate (Catalent, Beinheim S.A, France) 80 mg twice daily for 12 weeks. Dose modification based on central or local biweekly measurement of hemoglobin values was allowed according to a prespecified algorithm. Treatment with rhEPO 10000 IU was adjusted to three times weekly if hemoglobin levels increased to ≥100 g/L and interrupted if hemoglobin levels increased to ≥ 120g/L. All patients who completed or discontinued treatment were followed up for 8 weeks, resulting in a total study duration of 20 weeks (12 weeks of treatment plus 8 weeks of follow-up). At the end of the 12-week treatment, patients who had attained clinical response

(hematological improvement-erythroid [HI-E] response or transfusion independence) were allowed to continue treatment with rhEPO, ATRA and testosterone undecanoate. These patients received continuous treatment with the prior regimen, with dosages adjusted based on hemoglobin levels, until disease progression or unacceptable toxicities occurred, rather than repeating the initial 12-week cycle. Beyond the 20-week timeframe, treatment continuation was at the discretion of patients and their treating physicians but was not protocol-mandated nor systematically tracked.

Best supportive care, including RBC transfusion and antimicrobial therapy, was permitted to ensure patient safety. According to the RBC transfusion guideline in China[3], the transfusion threshold for chronic anemia is hemoglobin level below 60 g/L. However, transfusion decisions should be individualized. For patients with clinically significant symptoms such as dyspnea, syncope, or impaired functional capacity, RBC transfusion can be considered even if their hemoglobin levels are ≥ 60 g/L.

Assessments

All patients were assessed for bone marrow morphology, cytogenetics, and flow cytometry at baseline. Furthermore, bone marrow mononuclear cells were obtained at baseline for mutational analyses by next-generation sequencing for a panel of 20 genes frequently mutated in MDS (**Supplementary table 1**). Additional baseline evaluations included comprehensive biochemistry panels, electrocardiograms (ECGs), echocardiography, and vascular ultrasound scans. Hemoglobin levels, blood transfusion requirements and safety were assessed biweekly until 8 weeks after the end of treatment. Other tests including pulmonary CT scans and cranial MRI scans were conducted at the discretion of the physician based on clinical need. Adverse Events (AEs) were monitored using the National Cancer Institute Common

Terminology Criteria of Adverse Events (NCI CTCAE), version 4.0 and described in MedDRA version 23.0 preferred terms and CTCAE grade. The occurrences, frequencies, and severities of AEs were summarized.

Endpoints

The primary efficacy endpoint was the proportion of patients who had achieved an HI-E response, defined as a hemoglobin increase ≥ 15 g/L from baseline lasting for ≥ 8 consecutive weeks in the absence of RBC transfusions during 12 weeks of treatment according to the modified 2006 International Working Group (IWG) response criteria[4]. Secondary endpoints included the median time to HI-E response and the percentage of progression to higher-risk disease. Exploratory endpoints included the percentage of patients achieving RBC transfusion independence for 8 consecutive weeks or longer, and the percentage of patients who progressed to AML.

Statistical analysis

A Simon two-stage optimal design was used to determine whether the triple regimen had sufficient activity to warrant further development[5]. An HI-E response rate ≤ 40% for the triple regimen was assumed unacceptable (null hypothesis) whereas an HI-E rate of ≥ 60% warranted further study (alternative hypothesis). Assuming a power (1-β) of 0.80 and one-sided $\alpha = 0.05$, target accrual was a minimum of 16 patients in Simon stage 1. If 7 patients achieved an HI-E response, 30 additional patients were recruited in Simon stage 2. Assuming a dropout rate of 10%, a population of 52 patients was required.

The study followed the intention-to-treat principle. The Full Analysis Set (FAS) included all patients who received at least one dose of the study medications and had a baseline assessment and at least one post-baseline assessment. The Per Protocol Set (PPS) included all patients who met the study eligibility criteria, had completed all

scheduled visits and laboratory studies, showed good compliance, and had no major study protocol violations. Good compliance was defined as ≥ 90% medication adherence, 100% completion of critical study visits, and ≤ 1 minor protocol deviation. These criteria were established to ensure the robustness of the dataset and to minimize potential biases that could arise from non-adherence, in accordance with Good Clinical Practice (GCP) guidelines. Efficacy analysis was mainly based on the FAS and supported by the PPS. Last observation carried forward (LOCF) was used for missing efficacy data.

Data were summarized by median (range or interquartile range [IQR]) for continuous variables and frequency (percentage) for categorical values. Risk factors for an HI-E response were performed using a logistic regression model. Time-to-event analyses were performed using the nonparametric tests. Post hoc subgroup efficacy analyses by prior use of ESAs (yes *vs*. no), age (< 60 years *vs.* ≥ 60 years), serum hemoglobin level (< 60 g/L *vs*. ≥ 60 g/L), serum ferritin level (< 500 ng/mL *vs.* ≥ 500 ng/mL), baseline serum erythropoietin levels (> 500 IU/L *vs.* ≤ 500 IU/L), bone marrow fibrosis (grade 0 *vs.* grade 1-3), bone marrow blast percentage (≤ 2% vs. > 2 - < 5%), MDS subtypes (MDS-RS *vs.* MDS with single lineage dysplasia/ multilineage dysplasia [SLD/MLD]), IPSS-R risk category (very low or low risk *vs.* intermediate risk), IPSS R karyotype (very good/good *vs.* intermediate), baseline transfusion burden (no *vs.* yes) and gene mutational status (yes *vs.* no). The safety set included all patients who had received at least one dose of the study medications and had at least one safety evaluation. AEs were analyzed using descriptive statistics. Statistical analyses were performed using SAS version 9.1.3 (The SAS Institute, Cary, NC, USA) and GraphPad Prism (version 8.0). A two-sided α level of 0.05 was considered significant.

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Supplementary Table 1. List of genes in the targeted next-generation sequencing panel

ASXL1	***RUNX1***	***U2AF1***	***TP53***	***TET2***
DNMT3A	*STAG2*	*SETBP1*	*BCOR*	*SRSF2*
IDH2	*EZH2*	*NRAS*	*ZRSR2*	*IDH1*
ETV6	*SF3B1*	*FLT3*	*CBL*	*JAK2*