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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Abstract

Erythropoiesis-stimulating agents (ESA) achieve hematological improvement-erythroid (HI-E) in only 30% of ESA-naïve lower-risk myelodysplastic syndrome (LR-MDS) patients with anemia, highlighting the need for developing novel drugs or new treatment strategies to improve the outcome of these patients. We conducted this multicenter, single-arm trial to investigate the efficacy and safety of a triple regimen consisting of recombinant human erythropoietin (rhEPO), all-*trans* retinoic acid (ATRA) and testosterone undecanoate in patients with anemia due to lower-risk MDS based on Revised International Prognostic Scoring System. Eligible patients received rhEPO 10,000 IU/day, oral ATRA 25 mg/m^2/day and oral testosterone undecanoate 80 mg twice daily for 12 weeks. The primary endpoint was the proportion of patients achieving HI-E during 12 weeks of treatment. Of 52 eligible patients, 32 (61.5%, 95% confidence interval [CI]: 48.0-73.5%) achieved HI-E, meeting the primary endpoint. Fifteen patients (65.2% [15/23]) with baseline serum erythropoietin (EPO) ≤500 IU/L had HI-E *versus* 58.6% of those (17/29) with baseline serum EPO >500 IU/L. More patients with very low or low risk had HI-E than those with intermediate risk (73.3% *vs*. 45.5%; *P*=0.041) and fewer patients with mutated *ASXL1* had HI-E than those with wild-type *ASXL1* (33.3% *vs.* 70.0%; *P*=0.040). The regimen had an acceptable safety profile compatible with individual agents. In conclusion, the triple regimen of rhEPO combined with ATRA and testosterone undecanoate attained HI-E in approximately 61.5% of patients regardless of baseline serum EPO levels, supporting further development of this regimen for LR-MDS patients with anemia. This study was registered at http://www.chictr.org.cn (*Identifier: ChiCTR2000032845*).

Introduction

Myelodysplastic syndromes (MDS) are a group of clonal myeloid disorders that are characterized by ineffective hematopoiesis, persistent peripheral cytopenias, and a risk of progression to acute myeloid leukemia (AML). Symptomatic anemia is the most common cytopenia in lower-risk MDS (low- or intermediate-risk per the International Prognostic Scoring System [IPSS] or very low, low, or intermediate risk per the revised IPSS [IPSS-R])[1] and is associated with red blood cell (RBC) transfusion requirement, poor health-related quality of life (HRQoL) and multiple comorbidities such as cardiovascular disease.[2] Worsening anemia could render lower-risk MDS patients eventually dependent on RBC transfusions and negatively impacts on HRQoL and overall survival.[3,4]

Treatment goals for patients with lower-risk MDS include transfusion independence, improvement in cytopenia and hemoglobin levels, and amelioration of HRQoL.[5] Though the landscape of treatment for lower-risk MDS is rapidly evolving with the advent of transforming growth factor-β (TGF-β) inhibitor luspatercept, telomerase inhibitor imetelstat, lenalidomide, and hypomethylating agents,[6-8] they are not broadly applicable to lower-risk MDS patients with anemia. For instance, lenalidomide is approved for lower-risk MDS patients with chromosome 5q deletion (del[5q]) while luspatercept is approved for MDS with ring sideroblasts (MDS-RS). Currently, erythropoiesis-stimulating agents (ESA) including recombinant human erythropoietin (rhEPO) are the first-line treatment for non-del(5q) lower-risk MDS patients with anemia. They target early erythropoiesis by stimulating erythropoietin (EPO)-responsive erythroid precursor proliferation. However, ESA sustain a meaningful improvement in hemoglobin levels in only a small proportion of lower-risk MDS patients.[5,6,9,10] Higher serum EPO levels, especially when higher than 500 IU/L, are associated with lower response rates. Presently, ESA monotherapy is not recommended for patients with serum EPO levels above 500 IU/L.[5,6] Given limited treatment options, new treatment strategies are needed for anemia in lower-risk MDS patients.

An early interest in all-*trans* retinoic acid (ATRA) for the treatment of MDS emerged following the exemplary success of ATRA as a differentiation-induction therapy for acute promyelocytic leukemia, due to its role in regulating normal hematopoiesis by promoting hematopoietic cell differentiation and enhancing erythroid colony formation.[11-15] However, a flurry of trials revealed that ATRA only yielded modest activities in improving anemia in MDS.[16] It is of note that ATRA, when added to rhEPO, led to clinically significant erythroid responses in nearly half (48.0%, 13/27) of the patients with lower-risk MDS, which were sustained in over half of the responders (53.8%, 7/13) at 13 months of follow-up.[17] Testosterone undecanoate, a type of androgen, is known to be a potent stimulator of erythropoiesis by acting directly on bipotential hematopoietic precursor cells and potentially increasing the sensitivity of erythroid progenitor cells to EPO.[18-20] The combination therapy with ATRA and androgens has also been attempted for the treatment of anemia in patients with MDS.[21-23] Prompted by these findings and given the fact that the currently available treatment options fail to elicit a hematological improvement-erythroid (HI-E) response in a sizable proportion of lower-risk MDS patients with anemia, we hypothesized that a triple regimen consisting of rhEPO, ATRA and testosterone undecanoate could potentially improve the proportion of lower-risk MDS patients with anemia to achieve an HI-E response. This trial was conducted as a part of a prospective study to evaluate the efficacy and safety of rhEPO plus ATRA and testosterone undecanoate for lower-risk MDS patients with anemia.

Methods

Study design and patients

This investigator-initiated, prospective, single-arm trial enrolled adult patients (18-80 years) with primary MDS according to the World Health Organization (WHO) 2016 criteria[24] at ten Chinese centers. The key inclusion criteria were: IPSS-R-defined very low-, low-, or intermediate-risk MDS;[25] bone marrow blasts <5%; baseline hemoglobin <100 g/L, regardless of EPO levels and RBC transfusion support; Eastern Cooperative Oncology Group (ECOG) performance status of 0-2; and adequate renal and hepatic function. The main exclusion criteria included del(5q) cytogenetic abnormality, prior stem cell transplant, and recent treatment with ESA, immunosuppressants, lenalidomide, or hypomethylating agents prior to enrolment. The full eligibility criteria are available in the *Online Supplementary Appendix*. The study protocol was 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 Good Clinical Practice guidelines. All patients provided written informed consent before any trial-related activities. This trial is registered at the Chinese Clinical Trial Registry (*Identifier: ChiCTR2000032845*).

Procedures

Patients received rhEPO 10,000 IU/day subcutaneously, oral ATRA 25 mg/m^2/day and oral testosterone undecanoate 80 mg twice daily for 12 weeks. Dose modification based on biweekly measurement of hemoglobin values was allowed according to a prespecified algorithm. After 12 weeks of treatment, patients with clinical response (HI-E response or transfusion independence) could continue triple treatment until disease progression or unacceptable toxicities. Patients who completed or discontinued treatment were followed up for 8 weeks.

Assessments and endpoints

Baseline assessments included bone marrow morphology, cytogenetics, flow cytometry, and next-generation sequencing of 20 MDS-related genes (*Online Supplementary Table S1*). Hemoglobin levels, transfusion requirements, and safety were assessed biweekly throughout the study period. Adverse events (AE) were monitored using the National Cancer Institute Common Terminology Criteria of Adverse Events version 4.0 (NCI CTCAE v4.0).

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.[26] Secondary endpoints included the median time to HI-E response and progression to higher-risk disease. Exploratory endpoints included achieving RBC transfusion independence for ≥8 weeks and progression to AML.

Statistical analysis

The sample size was calculated using the Simon two-stage optimal design,[27] aiming to differentiate an erythroid response rate of 60% from a minimal erythroid response rate of 40%, with 80% power, a one-sided α of 0.05, and a 10% dropout rate, resulting in approximately 52 evaluable patients. In this intention-to-treat study, efficacy analyses used the full analysis set (FAS), supported by the per-protocol set (PPS). Summary statistics were employed to describe patients' demographics and laboratory measurements, and a logistic regression model was utilized to identify risk factors for HI-E response. *Post hoc* subgroup efficacy analyses included age, hemoglobin (Hb), IPSS-R risk, and molecular profiles. Methodological details are provided in the *Online Supplementary Appendix*.

Results

Patient baseline and treatment characteristics

Between July 26, 2020 and August 4, 2023, of 55 patients assessed for eligibility, two did not meet the eligibility criteria, and one withdrew before the start of treatment. Finally, 52 patients who received study medications were included in the FAS. The PPS included 50 patients after exclusion of one patient who withdrew from the study due to lack of efficacy and one who discontinued the study treatment due to severe AE (Figure 1). The median age of the patients in the FAS was 65 years (range, 20-78) and 57.7% (30/52) of the patients were male. The median Hb was 62 g/L (range, 40-91), with 30 patients (57.7%) having Hb ≥60 g/L and 22 patients (42.3%) having Hb <60 g/L. Baseline serum EPO levels were distributed as follows: 55.8% (29/52) of patients had levels >500 IU/L, 15.4% (8/52) had levels between >200 and ≤500 IU/L, and 28.8% (15/52) had levels ≤200 IU/L. With regard to the IPSS-R categories, 57.7% (30/52) and 42.3% (22/52) of the patients were categorized in the very low- or low-risk group and the intermediate-risk group, respectively. Twenty-four patients (46.2%) had MDS-RS, five (9.6%) had MDS with single lineage dysplasia (MDS-SLD), and 23 (44.2%) had MDS with multilineage dysplasia (MDS-MLD). Twenty-five patients (48.1%) had no transfusion burden (NTB), defined as receiving no RBC transfusion in 8 weeks before treatment, and 27 (51.9%) had a transfusion burden (TB), defined as receiving one or more RBC units within 8 weeks before treatment. Most patients (47/52, 90.4%) had no prior ESA therapy, and no patient previously received lenalidomide, luspatercept, imetelstat or hypomethylating agents (HMA). Next-generation sequencing showed that *SF3B1* was mutated in 40.4% (21/52) of the patients. Other mutated genes included *ASXL1* (12/52, 23.1%), *TET2* (10/52, 19.2%), *U2AF1* (7/52, 13.5%), *DNMT3A* (7/52, 13.5%), and *SRSF2* (5/52, 9.6%) (Table 1).

Efficacy

In the FAS, 32 of 52 patients (61.5%, 95% CI: 48.0-73.5%) achieved an HI-E response lasting for ≥8 consecutive weeks during 12 weeks of treatment, thus rejecting the null hypothesis. The median follow-up time was 20 weeks (range, 4-20 weeks) after treatment initiation. The median time to achieve an HI-E response was 40 days (95% CI: 34.4-45.5 days). In the PPS, 64.0% of the patients (32/50, 95% CI: 50.1-75.9%) achieved an HI-E response lasting for ≥8 consecutive weeks during 12 weeks of treatment. Among responders achieving HI-E, the post-treatment median Hb was 95 g/L (range, 68-152) compared to 73 g/L (range, 46-91) in non-responders. Patients with baseline Hb ≥60 g/L

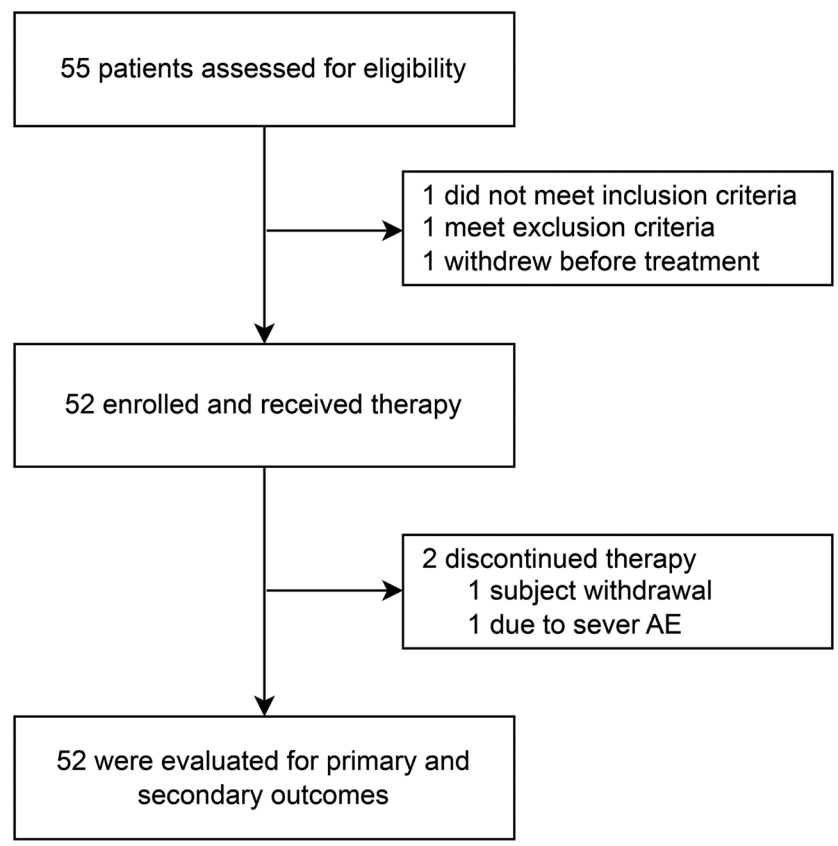

Figure 1. Patient disposition chart. AE: adverse event.

achieved a post-treatment median Hb of 92 g/L (range, 69-152), whereas those with baseline Hb <60 g/L had a lower post-treatment median Hb of 73 g/L (range, 46-106). Fifteen of 23 patients (65.2%) with baseline serum EPO ≤500 IU/L had an HI-E response as compared to 58.6% of those (17/29) with baseline serum EPO >500 IU/L (*P*=0.627). They had a shorter median time to an HI-E response as compared with those with baseline serum EPO >500 IU/L (27 days, 95% CI: 16.9-37.1 days *vs*. 44 days, 95% CI: 34.9-53.1 days; *P*=0.051). When evaluated according to the baseline transfusion burden, 63% (17/27) of the patients with transfusion burden achieved RBC transfusion independence (RBC-TI) lasting for ≥8 consecutive weeks. Furthermore, 55.6% (15/27) of the patients were transfusion-free for ≥8 weeks, and 48.1% (13/27) maintained RBC-TI lasting for ≥12 weeks. The maximum percentage change from baseline over 8 weeks in patients with RBC transfusion burden is shown in Figure 2. Among NTB patients at baseline, the median Hb level was 72 g/L (range, 60-91), which increased to a post-treatment median Hb of 92 g/L (range, 69-152). In *post hoc* subgroup analysis, when evaluated according to the IPSS-R risk category, the percentages of patients with an HI-E response were significantly higher in patients with very low or low risk than those with intermediate risk (73.3% [22/30], 95% CI: 55.6-85.8% *vs*. 45.5% [10/22], 95% CI: 25.1-67.3%; *P*=0.041). Additionally, the HI-E response rates were comparable between patients with and without ring sideroblasts (RS) (61.5% [16/26] *vs*. 61.5% [16/26]; *P*=1.000). No statistical difference was observed in the HI-E response rate when the patients were evaluated according to prior use of ESA (yes *vs*. no), age (<60 years *vs*. ≥60 years), serum Hb level (<60 g/L *vs*. ≥60 g/L), serum ferritin level (<500

Table 1. Patient demographic and baseline characteristics - full analysis set.

Characteristics	N=52
Median age, years (range)	65 (20-78)
≥60, N (%)	35 (67.3)
<60, N (%)	17 (32.7)
Sex, N (%)	
Male	30 (57.7)
Female	22 (42.3)
Time since MDS diagnosis, N (%)	
>6 months	4 (7.7)
≤6 months	48 (92.3)
Prior use of ESA, N (%)	
Yes	5 (9.6)
No	47 (90.4)
ECOG performance status, N (%)	
0	25 (48.1)
1	21 (40.4)
2	6 (11.5)
White blood cell count, $\times10^9$/L, median (range)	3.0 (1.8-9.9)
Neutrophil count, $\times10^9$/L, median (range)	1.51 (0.8-5.57)
Platelet count, $\times10^9$/L, median (range)	133 (39-399)
Hemoglobin level, g/L, median (range)	62 (40-91)
≥60, N (%)	30 (57.7)
<60, N (%)	22 (42.3)
LDH, IU/L, N (%)	
≤250	40 (76.9)
>250	12 (23.1)
Serum ferritin concentration, ng/mL, N (%)	
≥500	35 (67.3)
<500	17 (32.7)
Baseline serum EPO level, IU/L, N (%)	
>500	29 (55.8)
>200-≤500	8 (15.4)
≤200	15 (28.8)
Bone marrow fibrosis, N (%)	
Grade 0	39 (75.0)
Grade 1-3	13 (25.0)

Characteristics	N=52
Bone marrow blast, N (%)	
≤2%	40 (76.9)
>2-<5%	12 (23.1)
WHO (2016) classification of MDS, N (%)	
MDS-SLD	5 (9.6)
MDS-RS	24 (46.2)
MDS-MLD	23 (44.2)
Ring sideroblasts (RS), N (%)	
RS-positive*	26 (50.0)
RS-negative**	26 (50.0)
IPSS-R karyotype, N (%)	
Very good/good	40 (76.9)
Intermediate	12 (23.1)
IPSS-R risk category, N (%)	
Very low/low	30 (57.7)
Intermediate	22 (42.3)
RBC transfusion burden, N (%)	
No transfusion burden†	25 (48.1)
Transfusion burden‡	27 (51.9)
Selected gene mutations, N (%)	
SF3B1	21 (40.4)
ASXL1	12 (23.1)
TET2	10 (19.2)
U2AF1	7 (13.5)
DNMT3A	7 (13.5)
SRSF2	5 (9.6)

*RS-positive is defined as the presence of ring sideroblasts (RS) in patients, including those diagnosed with myelodysplastic syndrome with ring sideroblasts (MDS-RS) and patients exhibiting RS without fulfilling the diagnostic criteria for MDS-RS. **RS-negative is defined as the absence of ring sideroblasts in patients. †No transfusion burden is defined as no red blood cell (RBC) transfusion within 8 weeks before study treatment. ‡Transfusion burden is defined as requiring 1 or more RBC units within 8 weeks before study treatment. ESA: erythropoesis-stimulating agent; agente stimolante l'eritropoiesi; ECOG: Eastern Cooperative Oncology Group; LDH: lactate dehydrogenase; MDS-MLD: myelodysplastic syndrome with multilineage dysplasia; MDS-SLD: MDS with single lineage dysplasia; WHO: World Health Organization; IPSS-R: Revised International Prognostic Scoring System-Revised; EPO: erythropoietin.

ng/mL *vs*. ≥500 ng/mL), bone marrow fibrosis grade (0 *vs*. 1-3), bone marrow blast percentage (≤2% *vs*. >2-<5%), MDS subtypes (RS *vs*. SLD/MLD), IPSS-R karyotype (very good/good *vs*. intermediate), and baseline transfusion burden (no *vs*. yes) (Figure 3).

The patients were also evaluated according to gene mutational status. Patients with mutated *ASXL1* had a significantly lower HI-E response rate than those with wild-type *ASXL1* (33.3% [4/12], 95% CI: 11.3-64.6% *vs*. 70.0% [28/40], 95% CI: 54.6-81.9%; *P*=0.040). The percentages of patients with an HI-E response were higher in patients harboring *SF3B1* mutation than those with wild-type *SF3B1*, but without statistical difference (71.4% [15/21], 95% CI: 50.0-86.2% *vs*. 54.8% [17/31], 95% CI: 37.8-70.8%; *P*=0.228). No statistical difference was observed in the percentages of patients with an HI-E response with wild-type *TET2, U2AF1*, *DNMT3A*, and *SRSF2* as compared to those with mutated *TET2, U2AF1, DNMT3A*, and *SRSF2*.

Safety

All 52 patients received at least one dose of the study medications and were included in the safety set. Fifty patients completed 12 weeks of treatment; two patients withdrew after receiving 4 and 8 weeks of treatment, respectively.

Primary toxicity data were systematically collected throughout the entire 20-week study period, including the initial 12-week treatment phase and the subsequent 8-week safety follow-up. Treatment-related AE (TRAE) of any grade occurred in all 52 patients (100%). Fatigue was the most frequently reported TRAE (38.4%), followed by dry skin (36.5%), headache (15.4%), edema (13.4%) and hypertriglyceridemia (11.5%). Grade 3 TRAE occurred in four patients (7.7%). Grade 3 infections (pneumonia or skin and soft tissue infections) occurred in three patients (5.8%) and deep vein thrombosis was reported in one patient (1.9%) (Table 2). Three patients (5.8%) had dose reduction, and one (1.9%) discontinued treatment due to grade 3 deep vein thrombosis. No new or worsening toxicities attributable to the triple regimen were observed during the 20-week study period. No progression to higher-risk MDS or AML, and no death was reported throughout the study period.

Discussion

Current treatment options for lower-risk MDS patients with anemia remain limited. In this trial, the triple regimen of rhEPO plus ATRA and testosterone undecanoate achieved an HI-E response lasting for ≥8 consecutive weeks during 12 weeks of treatment in 61.5% of the patients with non-del (5q) lower-risk MDS with anemia, meeting the primary study endpoint. Besides, the triple regimen was well-tolerated, and the toxicity profiles were consistent with those of individual components in the regimen. No unexpected toxicities emerged during the trial. The findings indicated that the study regimen could offer an effective and safe therapeutic option for lower-risk MDS patients with anemia, supporting further exploration of the regimen in advanced trials.

Currently, ESA monotherapy as the first-line treatment for non-del(5q) lower-risk MDS patients with anemia remains unsatisfactory. In a randomized trial, epoetin-α led to an HI-E response in 31.8% of the patients with lower-risk MDS patients with no or moderate transfusion burden.[28] In a separate trial, 14.7% of lower-risk MDS patients with no or

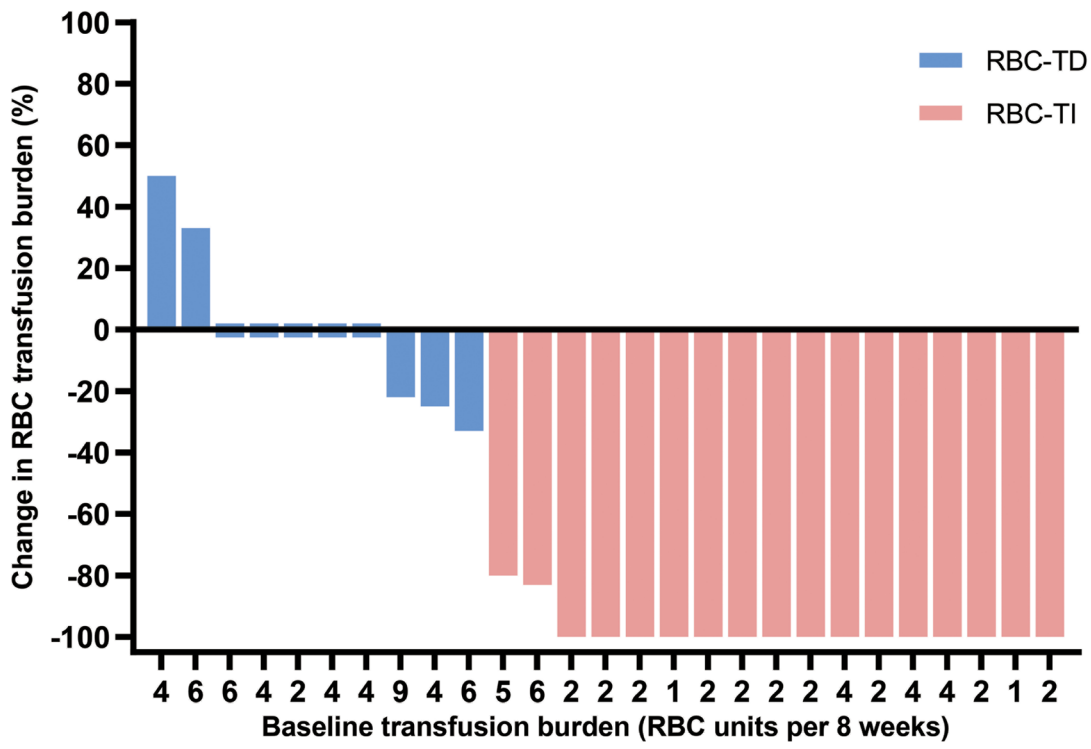

Figure 2. Maximum percentage change in red blood cell transfusion burden in previously transfused patients (N=27). Of these 27 patients, 13 (48.1%) had a low transfusion burden (LTB), defined as receiving <4 red blood cell (RBC) units in 8 weeks, and 14 (51.9%) had a high transfusion burden (HTB), defined as receiving ≥4 RBC units in 8 weeks. TD: transfusion dependence; TI: transfusion independence.

moderate transfusion burden who were treated with darbepoetin α had an HI-E response.[10] These findings highlight the inadequacy of ESA monotherapy for lower-risk MDS patients with anemia. In a study on dual agent therapy for lower-risk MDS patients with anemia, ATRA plus rhEPO led to clinically significant erythroid responses, defined as increases of hemoglobin levels of ≥1 g/dL or reduction of transfusion needs, in nearly half (48.0% [13/27]) of the

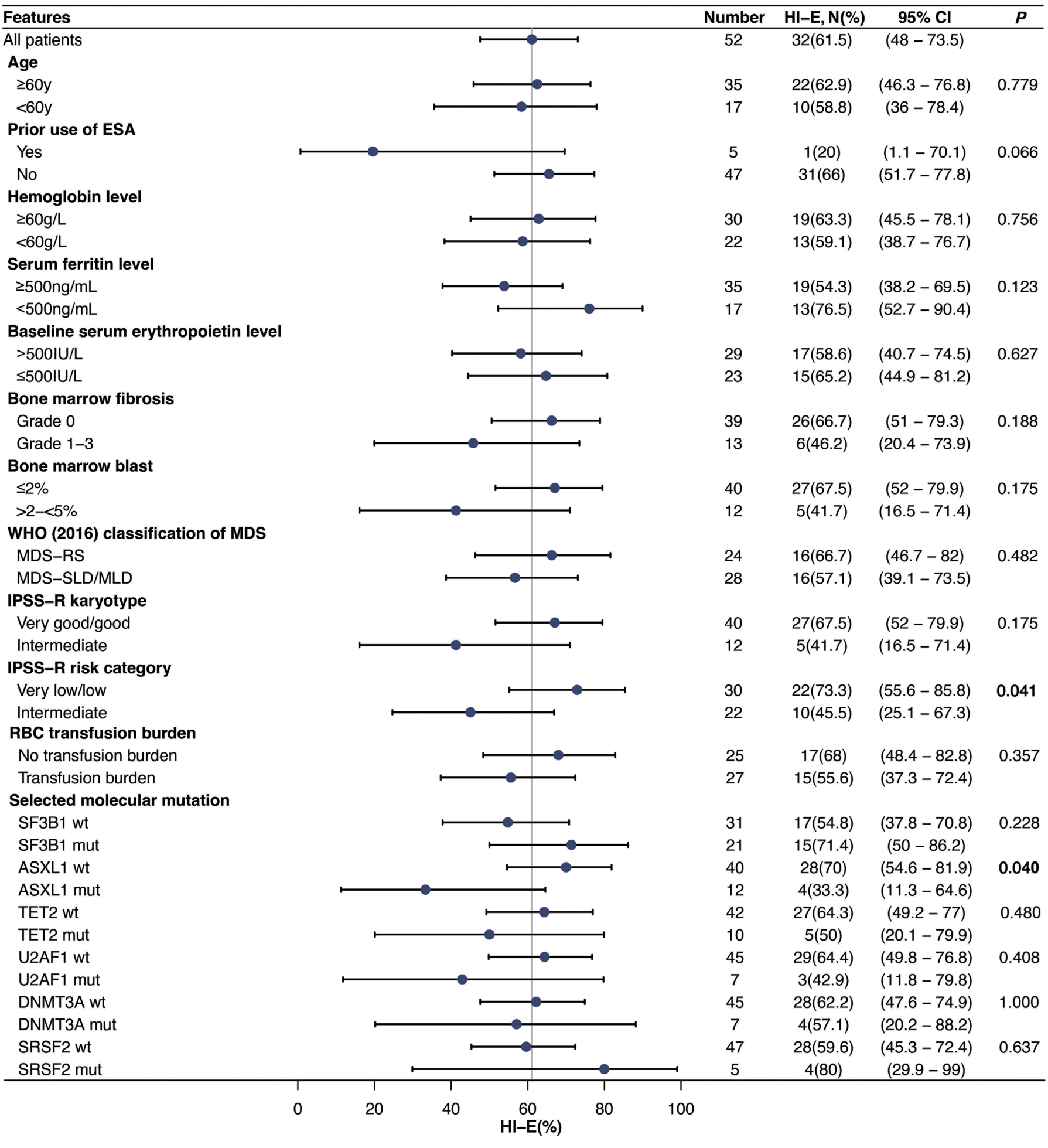

Figure 3. Forest plots of hematological improvement-erythroid responses in subgroups of patients. HI-E: hematological improvement-erythroid; ESA: erythropoiesis-stimulating agents; WHO: World Health Organization; MDS-RS: myelodysplastic syndrome with ringed sideroblasts; MDS-SLD/MLD: MDS with single lineage dysplasia/multilineage dysplasia; IPSS-R: Revised International Prognostic Scoring System; RBC: red blood cell; mut: mutation; wt: wild-type.

patients with lower-risk MDS, which were sustained in over half of the responders (53.8% [7/13]) at 13 months of follow-up,[17] suggesting that combination therapy might lead to improved treatment response among patients with lower-risk MDS patients with anemia. In our study, 61.5% (32/52) of the patients achieved an HI-E response lasting for ≥8 consecutive weeks during 12 weeks of treatment, and the response rates were 68% (17/25) in patients with no transfusion burden at baseline and 55.6% (15/27) in those with transfusion burden. While these response rates numerically exceed historical ESA monotherapy data,[10,28] our regimen yielded a lower post-treatment median hemoglobin level (95 g/L) compared to previous studies (106 g/L). Cross trial comparisons are inherently limited by differences in trial design and patient populations. For instance, our study utilized higher ESA dosing (70,000 IU/week *vs*. ≤40,000 IU/week in ESA monotherapy trials) and shorter response evaluation period (12 weeks *vs*. 24-48 weeks in ESA trials). Key population differences included a lower baseline median Hb level in our study (62 g/L *vs*. 90 g/L in ESA trials), the absence of EPO level restrictions (55.8% >500 IU/L *vs*. 0% in ESA trials), distinct genetic profiles (0% del[5q] MDS *vs*. 3.8-8.9% in ESA trials), prior ESA exposure in 9.6% of patients (*vs*. 0% in ESA trials), and a more varied IPSS-R risk distribution (42.3% intermediate-risk *vs*. 8.9-54.6% in ESA trials). Additionally, differing definitions of transfusion dependence across studies further limit direct efficacy comparisons. Rigorous clinical trials are required to determine whether the triple regimen is more effective than ESA monotherapy or ESA-based dual agent therapy for lower risk MDS patients with anemia.

The effectiveness of treatment for non-del(5q) MDS patients with anemia is impacted by serum EPO levels, with a strong inverse correlation with Hb levels[29-31] and a lower response rate for those with >500 IU/L.[6] Given that only 10-20% of patients with serum EPO levels above 500 IU/L would likely respond to ESA,[32,33] the National Comprehensive Cancer Network guidelines do not recommend ESA monotherapy for this patient subpopulation.[34] Nevertheless, it is interesting to note that in our study, patients were included regardless of baseline serum EPO levels, and there was no statistical difference in the HI-E response rates and the median time to achieve an HI-E response between patients with serum EPO levels >500 IU/L and those ≤500 IU/L. The two randomized trials of epoetin-α and darbepoetin α excluded patients with baseline serum EPO ≥500 IU/L[10,28] while our study included patients regardless of baseline serum EPO levels. In a prospective study, ATRA plus rhEPO led to an HI-E response in 19% of the patients with baseline serum EPO >500 IU/L.[35] In our study, 58.6% (17/29) of the patients with baseline serum EPO >500 IU/L showed an HI-E response to the triple regimen, suggesting that the study regimen could also benefit this subgroup of lower-risk MDS patients. Our analysis further revealed that HI-E rates were not significantly dependent on Nordic Score prognostic factors.[36] However, the response rates observed in our cohort differed from those reported by Fenaux *et al*. (e.g., High Nordic Score: 68.8% *vs*. 44.7%), which may be due to variations in study design and patient populations. We speculated that the triple regimen may improve or restore the response to ESA in lower-risk MDS patients with anemia with baseline serum EPO >500 IU/L, possibly due to synergy among the individual agents or enhancement of erythroid colony formation by ATRA and increment of sensitivity to EPO by testosterone undecanoate.[12,37] The mechanisms underlying these clinical observations require further in-depth studies. Our *post hoc* subgroup analysis also showed that the triple regimen conferred benefits across diverse subgroups of patients with regards to prior use of ESA, age, baseline serum hemoglobin level, baseline serum ferritin level, baseline serum EPO levels, bone marrow fibrosis, bone marrow blast percentage, MDS subtypes, IPSS-R karyotype, and baseline transfusion burden. One notable exception is the IPSS-R risk category. Patients with intermediate risk had a significantly lower HI-E response rate than those with very low or low risk (45.5% *vs*. 73.3%; *P*=0.041). The finding is consistent with a previous study showing higher IPSS-R risk may be associated with a lower response rate to ESA,[38] and such patients are recommended to receive alternative treatments. Patients with lower-risk MDS-RS have limited treatment options, except luspatercept,[39,40] if they do not respond to ESA. In the current trial, two thirds (66.7% [16/24]) of the MDS-RS patients responded to the triple regimen, suggesting that the triple regimen could be an effective treatment option for lower-risk MDS-RS patients with anemia.

Table 2. Treatment-related adverse events in the safety set.

AE	Grade 1-2, N (%)	Grade 3, N (%)
Fatigue	20 (38.5)	0
Dry skin	19 (36.5)	0
Headache	8 (15.4)	0
Edema peripheral	7 (13.4)	0
Hypertriglyceridemia	6 (11.5)	0
Mucositis	5 (9.6)	0
Nausea	5 (9.6)	0
Liver enzymes increased	4 (7.7)	0
Infection	0	3 (5.8)
Rash	1 (1.9)	0
Myalgias	1 (1.9)	0
Hypertension	1 (1.9)	0
Creatinine increased	1 (1.9)	0
Deep vein thrombosis	0	1 (1.9)

No grade 4 or 5 treatment-related adverse events (AE).

SF3B1 was the most frequently mutated gene in the study cohort, occurring in 40.4% of the patients. Despite a higher HI-E response rate in patients with mutated *SF3B1*, no statistical difference was observed from that of patients with wild-type *SF3B1* (71.4% *vs*. 54.8%). This trend is similar to the differential efficacy of luspatercept, approved for lower-risk MDS with *SF3B1* mutation,[41] which demonstrated superior erythroid response in *SF3B1*-mutated *versus* wild-type patients (70% *vs*. 42%).[40] In contrast, epoetin α monotherapy in the COMMANDS trial obtained similar erythroid response rates in patients with and without *SF3B1* mutation (31% *vs*. 32%).[40] Cross-trial comparisons must be interpreted with caution. The COMMANDS trial exclusively enrolled transfusion-dependent patients requiring two to six RBC units every 8 weeks pre-treatment and utilized stringent endpoints defined as RBC transfusion independence for at least 12 weeks with a concurrent mean hemoglobin increase of at least 1.5 g/dL (weeks 1-24).[40] In contrast, our study included both transfusion-independent and dependent patients, and applied IWG 2006 criteria (≥8-week HI-E within 12 weeks). The non-randomized design of our study limits definitive attribution of efficacy differences to the triple regimen, rather than to inherent population biases. Although the higher HI-E response rate with the triple regimen for *SF3B1*-mutated patients suggests that it could be a promising treatment option, similar to luspatercept, this finding remains exploratory. Randomized controlled trials directly comparing EPO-based combinations with luspatercept in molecularly stratified cohorts are essential to clarify their therapeutic roles.

ASXL1 is mutated in approximately 20% of patients with MDS and a predictor of an adverse prognostic outcome.[42] In the current trial, *ASXL1* was mutated in 23.1% of the patients and was associated with a significantly lower HI-E response rate as compared with wild-type *ASXL1* (33.3% *vs*. 70.0%; *P*=0.040). In the W-JHS MDS01 trial, *ASXL1* mutation was associated with a worse response rate to ESA monotherapy in patients with baseline serum EPO levels ≥100 IU/L (mutated *ASXL1* 0% *vs*. wild-type *ASXL1* 60.0%).[43] *ASXL1* loss was shown to hinder erythroid development and differentiation, indicating that ineffective erythropoiesis of MDS may occur as a result of *ASXL1* mutation.[44] However, the precise mechanism with regards to poor response to ESA could not be identified for *ASXL1*-mutated subjects and needs to be explored in future studies. Therefore, when lower-risk MDS patients have a predictor of poor response such as *ASXL1* mutation, especially when concurrent with high baseline serum EPO levels, it would be advisable to initiate treatment with other therapeutic options.

The study has several limitations. The trial design was a single-arm study with no comparator. The study findings await confirmation in randomized controlled trials with a larger patient population. Most patients had a relatively short diagnostic duration (≤6 months) and received no prior treatment, which may limit the conclusions of the study. Sustaining an HI-E response remains an important goal of treatment for lower-risk MDS with anemia and impacts on the HRQoL of the patients. Given the short duration of the study, the durability of the treatment response observed in the patients and the long-term safety of this combination therapy remained unknown. While the IWG 2006 criteria were rigorously applied, there has been limited clinically meaningful improvement in some patients, highlighting the need for future trials to adopt more comprehensive response criteria to better evaluate therapeutic impact and clinical benefit.

In conclusion, the triple regimen of rhEPO, ATRA and testosterone undecanoate led to an HI-E response in 61.5% of the patients with lower-risk MDS with anemia regardless of baseline serum EPO levels and demonstrated broad activity across diverse patient subgroups except those in the IPSS-R intermediate-risk category or with mutated *ASXL1*. The triple regimen could offer a meaningful treatment option for lower-risk MDS patients with anemia. These findings should be taken into consideration for the design of randomized, controlled trials aimed at evaluating the efficacy and safety of the triple regimen for lower-risk MDS.

Disclosures

No conflicts of interest to disclose.

Contributions

HT and CM designed the study. CM, GX, CZ, and YX analyzed the data. CM and GX wrote the manuscript. CM, GX, WW, YL, and LY performed the research. CZ, YX, ML, YS, RY, ST, WJ, JG, ZZ, XZ, LM, CY, WY, and WX collected the data. JJ and HT guided the project design and provided administrative support. All authors reviewed and approved the manuscript.

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Data-sharing statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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