

Long-term safety and erythroid response with luspatercept treatment in patients with β -thalassemia

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Abstract

Background: β -thalassemia is a hereditary blood disorder resulting in ineffective erythropoiesis and anemia. Management of anemia with regular blood transfusions is associated with complications including iron overload. Here, we report long-term safety and efficacy results of the first clinical study of luspatercept in β -thalassemia, initiated in 2013, enrolling adults with both nontransfusion-dependent (NTD) and transfusion-dependent (TD) β -thalassemia.

Objectives: The objective was to report long-term safety data, for up to 5 years of treatment, for 64 patients with TD or NTD β -thalassemia, and long-term efficacy data for a subset of 63 patients with β -thalassemia who received high-dose luspatercept (0.6–1.25 mg/kg): 31 NTD and 32 TD patients.

Design: The study was a phase 2, noncontrolled, open-label trial comprising a dose-finding base phase and a 5-year extension phase.

Methods: Endpoints include safety; erythroid response over a continuous 12-week period [NTD: hemoglobin increase from baseline ≥ 1.0 or ≥ 1.5 g/dl; TD: red blood cell (RBC) transfusion burden reduction, $\geq 20\%$, $\geq 33\%$, or $\geq 50\%$]; and changes in biomarkers of ineffective erythropoiesis, iron metabolism parameters, Functional Assessment of Chronic Illness Therapy – Fatigue (FACIT-F) scores, and 6-min walking distance.

Results: Median duration of luspatercept exposure for NTD and TD patients was 910 days (range, 40–1850) and 433 days (range, 21–1790), respectively. Seventeen of 31 (54.8%) NTD patients achieved a mean hemoglobin increase of ≥ 1.5 g/dl and 19 of 32 (59.4%) TD patients achieved $\geq 50\%$ reduction in RBC transfusion burden, during any continuous 12-week period. Median cumulative duration of response was 1126 days (range, 127–1790) for NTD patients and 909 days (range, 87–1734) for TD patients. The most common treatment-related adverse events of any grade were bone pain, headache, and myalgia.

Conclusion: Long-term assessment of patients with β -thalassemia showed luspatercept was associated with sustained increases in hemoglobin levels in NTD patients and sustained transfusion burden reductions in TD patients.

Trial registration: (ClinicalTrials.gov Identifiers: NCT01749540 and NCT02268409).

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Plain Language Summary

Long-term safety and erythroid response with luspatercept treatment in patients with β -thalassemia

Background: β -thalassemia is a genetic blood disorder caused by mutations in the β -globin gene, which encodes one of the proteins that comprise hemoglobin, a key constituent

of red blood cells. Patients with β -thalassemia experience anemia, the main treatment for which is blood transfusions. Long-term repeated blood transfusions lower patients' quality of life, use hospital resources, and the resulting accumulation of excess iron can cause organ failure and decrease life expectancy. The severity of the anemia experienced by patients with β -thalassemia varies; patients with transfusion-dependent β -thalassemia require regular blood transfusions, compared with those with nontransfusion-dependent β -thalassemia who require infrequent transfusions, or even none at all, to manage their symptoms. Luspatercept (Reblozyl®) is an agent that stimulates the production of red blood cells and is used to treat anemia caused by β -thalassemia. However, the long-term effects of luspatercept treatment on patients with β -thalassemia are not known.

Objective: In this study, we report the long-term safety of luspatercept in 64 adult patients with either transfusion-dependent or nontransfusion-dependent β -thalassemia, and the long-term efficacy of high-dose luspatercept (0.6–1.25 mg/kg) in a subset of 63 patients.

Results: The average time period that patients were treated with luspatercept was 910 days for nontransfusion-dependent β -thalassemia and 433 days for transfusion-dependent β -thalassemia. We report that in patients with nontransfusion-dependent β -thalassemia, luspatercept treatment was associated with sustained increases, just over 3 years, in hemoglobin levels. Likewise, in transfusion-dependent β -thalassemia, luspatercept treatment was associated with a sustained reduction, 2.5 years, in the amount of blood transfusion required to manage their anemia. Long-term treatment with luspatercept was not associated with any new side effects compared with previous short-term treatment studies. The most common side effects were headache (27 patients), bone pain (20 patients), and muscle pain (14 patients) with more than 90% of these patients experiencing these side effects as mild severity.

Conclusion: The results of this study show that in patients with either transfusion-dependent or nontransfusion-dependent β -thalassemia, luspatercept provides lasting reduction in anemia with mostly mild and predictable side effects.

Keywords: anemia, luspatercept, thalassemia

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Introduction

β -thalassemia is a hereditary blood disorder caused by mutations in the β -globin gene and impaired synthesis of β -globin, eventually leading to ineffective erythropoiesis, chronic anemia, and a range of other clinical consequences that significantly impact disease-related morbidity and mortality.^{1–5} Patients with β -thalassemia have a reduced life expectancy because of complications of the disease and the effects of chronic red blood cell (RBC) transfusions, such as iron overload.^{6,7}

Patients can be classified as having a major, intermedia, or minor thalassemia phenotype based on clinical presentation and genotype. An alternate classification for the clinically relevant major and intermedia forms of β -thalassemia is based on transfusion requirements: patients with

transfusion-dependent (TD) β -thalassemia require regular RBC transfusions throughout their lives, whereas those with nontransfusion-dependent (NTD) β -thalassemia may need only occasional transfusions.⁸ Patients with TD β -thalassemia usually present with a thalassemia major phenotype, characterized by early presentation to clinical care with severe anemia that requires regular and lifelong transfusions.⁸ Improvements in the care of TD β -thalassemia have led to improved quality of life and long-term survival outcomes, but regular transfusions are still associated with significant risks.^{5,9,10} In contrast, patients with β -thalassemia intermedia are generally NTD, with regular transfusions being used to manage disease in a minority of adult patients.¹¹ Still, there is a trend to worsening of symptoms with a significant proportion of patients

becoming TD, and those who remain NTD suffer from reduced quality of life from complications due to chronic anemia, ineffective erythropoiesis, and iron overload.^{8,12–15} There remains a significant unmet need for treatment to reduce the need for transfusions and improve health-related quality of life (HRQoL); conventional treatments reduce regular blood transfusions, when needed, and iron chelation therapy (ICT) to minimize the toxic effects of iron accumulation.¹⁶

Luspatercept (Reblozyl[®]) is a first-in-class erythroid maturation agent that binds to a subset of select transforming growth factor β ligands to inhibit aberrant Smad 2/3 signaling and enhance late-stage erythropoiesis.¹⁷ Luspatercept has recently been approved in the United States and Europe for the treatment of adult patients with TD β -thalassemia, based on the results of the phase 3 BELIEVE trial.¹⁸ In the BELIEVE trial, a significantly greater proportion of patients in the luspatercept group had a reduction in transfusion burden of $\geq 33\%$ from baseline during weeks 13–24, plus a reduction of ≥ 2 RBC units over this 12-week interval, compared with the placebo group [21.4% (48 of 224 patients) *versus* 4.5% (5 of 112); $p < 0.001$]. Adverse events more common with luspatercept than placebo were bone pain (19.7% *versus* 8.3%), arthralgia (19.3% *versus* 11.9%), dizziness (11.2% *versus* 4.6%), hypertension (8.1% *versus* 2.8%), and hyperuricemia (7.2% *versus* 0%).¹⁸ Before the BELIEVE trial, an open-label, randomized, dose-finding, phase 2 trial of luspatercept was performed in adults with TD and NTD β -thalassemia (NCT01749540).¹⁹ Luspatercept reduced transfusion burden in 81% [26 of 32; 95% confidence interval (CI): 63.6–92.88] of TD patients, increased hemoglobin in 58% (18 of 31; 95% CI: 39.1–75.5) of NTD patients, and improved HRQoL in seven out of nine (78%) NTD patients. In this trial, the most frequent adverse events were bone pain (33% grade 1–2; 5% grade 3), headache (25% grade 1–2; 2% grade 3), myalgia (20% grade 1–2; 0% grade 3), arthralgia (19% grade 1–2; 0% grade 3), musculoskeletal pain (16% grade 1–2; 0% grade 3), back pain (11% grade 1–2; 0% grade 3), and injection site pain (11% grade 1–2; 0% grade 3).¹⁹

The phase 2 trial included a long-term, nonrandomized extension phase (NCT02268409) in which patients could continue to receive luspatercept treatment for up to 5 years. Here, we report

the long-term safety and efficacy outcomes from both base and extension phases. These findings further support a randomized clinical trial to assess luspatercept efficacy and safety.

Methods

Patients

The study included adults with either TD or NTD β -thalassemia. Patients with NTD β -thalassemia were eligible if they had a mean hemoglobin concentration of < 10.0 g/dl and had received < 4 RBC units in the 8 weeks before the start of luspatercept treatment. Patients with TD β -thalassemia were eligible for inclusion if they had received an average of ≥ 4 RBC units every 8 weeks during the 6 months before starting treatment. The study was approved by the institutional review board or central ethics committee at each participating institution (Supplemental Tables S1 and S2) and was conducted according to the Declaration of Helsinki. All patients provided written informed consent. The results of this study are reported according to the Strengthening the Reporting of Observational studies in Epidemiology (STROBE) guidelines.²⁰ Adherence to the relevant STROBE guidelines is summarized in Supplemental Table S3.

Study design

The full methodology of the phase 2 base study (NCT01749540) and its extension phase (NCT02268409) have previously been reported.¹⁹ In brief, the study was a phase 2, open-label, nonrandomized study designed to evaluate the efficacy and safety of luspatercept in adults with either TD or NTD β -thalassemia (Supplemental Figure S1). There was no formal sample size calculation used in the base study. Enrolled patients received luspatercept, administered subcutaneously (s.c.) every 3 weeks, at a starting dose ranging from 0.2- to 1.25-mg/kg body weight in the base study for 12 weeks with a 8-week follow-up period. Patients who completed the base study were eligible to enroll in the 5-year extension study to evaluate the long-term efficacy and safety of luspatercept. Patients with no treatment interruption in the base study were eligible to continue into the extension study at a dose equal to their last dose level in the base study. Patients with treatment interruption were reassessed for transfusion status and eligibility for enrollment to the

extension study; the starting dose was 0.8 mg/kg s.c. every 3 weeks, which could be titrated to a maximum of 1.25 mg/kg. Here, we report efficacy data from 12 patients enrolled only in the base study and 51 patients who continued to the extension study, including 33 patients who were followed up longer term, all 63 of whom received high-dose luspatercept (0.6–1.25 mg/kg) at any time during either the base or the extension studies. Safety data are reported for the 64 patients enrolled in the base study if adverse events were experienced during the base or extension studies, or during long-term follow-up.

Endpoints

Primary endpoint. As previously reported,¹⁹ the primary endpoint in the base study was erythroid response, defined as a hemoglobin increase from baseline of ≥ 1.5 g/dl for ≥ 2 weeks (in the absence of RBC transfusions) for NTD patients and as a reduction in RBC transfusion burden over any continuous 12-week period of $\geq 20\%$ compared with pretreatment for TD patients. The primary endpoint in the extension study was safety and tolerability of luspatercept in patients with β -thalassemia who were previously enrolled in the base study.

Secondary/exploratory endpoints. Key secondary endpoints for the extension phase included erythroid response over a continuous 12-week period: NTD patients were evaluated for a hemoglobin increase from baseline of ≥ 1.0 or ≥ 1.5 g/dl; TD patients were assessed for a $\geq 33\%$ or $\geq 50\%$ reduction in RBC transfusion burden over any continuous 12-week period *versus* pretreatment. Additional key secondary endpoints included the following: time to erythroid response during any 12-week interval, calculated using the first day of the first 12-week response period; duration of erythroid response; mean hemoglobin increase (in NTD patients); change in RBC transfusion burden over 8- and 12-week intervals [in TD patients who do not achieve RBC transfusion independence (RBC-TI)]; and the proportion of patients who achieved RBC-TI (i.e. required no RBC transfusions for ≥ 8 consecutive weeks) in TD patients. Duration of exposure was calculated as the number days between the first and last dose dates plus 21 days.

The effects of luspatercept treatment on levels of biomarkers of ineffective erythropoiesis [serum

erythropoietin (EPO), soluble transferrin receptor 1 (sTfR1), lactate dehydrogenase (LDH), reticulocytes, and bilirubin], iron metabolism parameters (serum ferritin, calculated transferrin saturation), and liver iron concentration [LIC; assessed by magnetic resonance imaging (MRI)] were also assessed.

Exploratory endpoints include the impact of luspatercept on HRQoL, as measured using the Functional Assessment of Chronic Illness Therapy – Fatigue (FACIT-F), a validated patient-reported outcome measure consisting of a 13-item questionnaire regarding anemia-related symptoms such as fatigue and weakness.²¹ FACIT-F scores range from 0 to 52, with higher scores indicating better quality of life. Changes from baseline in 6-min walking distance (6MWD) were also assessed.

The safety and tolerability of luspatercept were evaluated throughout the study, including adverse events, clinical laboratory tests, and vital signs.

Statistical methods

Erythroid response, the primary efficacy endpoint, was summarized as the percentage of responders \pm the exact 95% CI. For secondary and exploratory efficacy endpoints, any continuous measurement data, including LIC, hemoglobin, and duration of erythroid response, were summarized using the mean change from baseline and corresponding standard deviation (SD) or 95% CI, as required. For dichotomized response measurement data, including $\geq 20\%$, $\geq 33\%$, and $\geq 50\%$ RBC transfusion reduction from baseline, results were summarized by percentage of response and 95% CI. For HRQoL measures, a minimal clinically meaningful improvement was defined as a difference >3 compared with baseline scores. The correlation between improvement in FACIT-F and hemoglobin change was calculated using the Pearson correlation coefficient and the significance of this correlation was determined. All *p* values result from a two-sided test; nominal values of *p* < 0.05 were considered statistically significant without adjustment for multiplicity. SAS statistical software was used (version 9.4; SAS Institute, Cary, NC). When calculating the primary endpoint, a patient without adequate data for evaluation was considered a nonresponder. For other patient data, including LIC and quality of life, no value imputation was

Table 1. Patient demographics and baseline characteristics of patients who received high-dose luspatercept.

Parameter ^a	Overall (N = 63)	TD (n = 32)	NTD (n = 31)
Age (years), median (range)	38.0 (20–62)	38.5 (21–55)	38.0 (20–62)
Female, n (%)	30 (47.6)	19 (59.4)	11 (35.5)
Time from diagnosis (year), median (range)	28.6 (1.1–51.3)	32.1 (3.1–51.3)	27.5 (1.1–47.8)
Splenectomy, n (%)	42 (66.7)	21 (65.6)	21 (67.7)
NTD, n (%) ^b	31 (49.2)	0	31 (100)
Hb (g/dl ^c), median (range)	NA	NA	8.5 (6.5–9.8)
TD, n (%) ^b	32 (50.8)	32 (100)	0
RBC transfusion burden (units/12 week ^d), median (range)	NA	8.0 (4–18)	NA
ICT, n (%)	46 (73.0)	30 (93.8)	16 (51.6)
LIC ^e (mg/g dw), mean (SD)	5.0 (4.14)	4.7 (4.65)	5.3 (3.62)
EPO ^f (IU/L), mean (SD)	113.36 (113.37)	104.76 (118.35)	122.24 (109.22)
Serum ferritin ^g (µg/L), mean (SD)	807.71 (614.55)	985.45 (737.66)	624.24 (386.98)

dw, dry weight; EPO, erythropoietin; Hb, hemoglobin; ICT, iron chelation therapy; LIC, liver iron concentration; NA, not available; NTD, nontransfusion dependent; RBC, red blood cell; SD, standard deviation; TD, transfusion dependent.

^aFor patients who rolled over to the extension study without interruption, the baseline for the base study was used as the baseline for the extension study for all measurements. For patients who entered the extension study with interruption, the last value on or before the first dose of the extension study was considered the baseline unless otherwise specified. The base study baseline was used for interrupted patients who do not have an extension study baseline.

^bDirect rollover patients are defined as TD if the total RBC transfusion is ≥ 11 units during 26 weeks on or before the base study cycle 1 day 1. For interrupted patients, if a patient was TD in the base study, then this patient was also considered TD for the extension study. If a patient was NTD in the base study, this patient could be considered TD in the extension if the baseline RBC transfusion was ≥ 4 units in 8 weeks on or before cycle 1 day 1 of the base study.

^cAverage Hb values within 28 days before the first dose, excluding values < 14 days after RBC transfusion.

^dTotal amount of RBC units transfused over the 12 weeks before cycle 1, day 1.

^eLIC levels in healthy adults without β -thalassemia range from 0.2 to 2 mg/g dw.²²

^fEPO levels in healthy adults without β -thalassemia range from 5.8 to 10.6 IU/L.²³

^gSerum ferritin levels in healthy adults without β -thalassemia range from 24 to 300 µg/L.²⁴

performed. Some patients experienced multiple erythroid responses. The cumulative duration of response was the sum of all response durations and was summarized descriptively.

Results

Patient characteristics

As of 18 June 2020, data were available for 64 patients who enrolled only in the base study, continued to the extension study, or were followed up after the extension study. Sixty-three of 64 (98.4%) patients received high-dose luspatercept

(0.6–1.25 mg/kg) during either the base study or the extension study. Overall, 31 of 63 (49.2%) patients who received high-dose luspatercept were NTD and 32 of 63 (50.8%) were TD. The clinical characteristics of patients who received high-dose luspatercept included in this long-term follow-up analysis of the phase 2 study are summarized in Table 1.

Overall, the median (range) patient age in the extension study population was 38.0 (20–62) years and 42 (66.7%) of the patients had previously undergone splenectomy. For NTD patients, the baseline median (range) hemoglobin level was

8.5 (6.5–9.8) g/dl and the mean (SD) baseline serum ferritin level was 624.24 (386.98) μ g/L. For TD patients, the median (range) baseline transfusion burden was 8.0 (4–18) units every 12 weeks, and the mean (SD) serum ferritin level was 985.45 (737.66) μ g/L.

Drug exposure

The median (range) duration of exposure to luspatercept was 510 (21–1850) days overall, 910 (40–1850) days for NTD patients and 433 (21–1790) days for TD patients. The average dose of luspatercept was approximately 1.1 mg/kg by month 12 and remained close to this level throughout the study (Supplemental Figure S2).

Rates of discontinuation

Overall, 3 of 64 (4.7%) patients discontinued during the base study for nonmedical reasons. Ten of 61 (16.4%) patients declined to participate in the extension, the main reason for which was difficulty in reconciling a demanding study protocol with work or study commitments; no patients declined participation in the extension study for medical reasons. During the extension study, 18 of 51 (35.3%) patients discontinued, of which 12 (23.5%) were because of patient decisions or protocol noncompliance, 4 (7.8%) were because of a medical reason or adverse events, and 2 (3.1%) patients each experienced grade 2 or 3 adverse events. One (3.2%) NTD patient, with a history of arrhythmias, died of asystole during the study (Supplemental Figure S1).

Long-term efficacy

Hemoglobin response in patients with NTD β -thalassemia. Twenty-two of 31 (71.0%) NTD patients achieved a mean increase in hemoglobin of ≥ 1.0 g/dl over any 12-week window compared with baseline, including 17 of 31 (54.8%) who achieved a mean increase of ≥ 1.5 g/dl (Table 2). The median (range) maximum increase in hemoglobin was 1.8 (–0.7–3.5) g/dl during any 12-week period and 1.3 (–1.2–3.3) g/dl during weeks 37–48 specifically. The mean increase in hemoglobin in patients with NTD β -thalassemia exceeded 1.0 g/dl by week 6 and was sustained throughout the extension study (Figure 1). The median (range) time was 8 (7–92) days for the first hemoglobin increase of ≥ 1.0 g/dl and 9 (7–791) days

for the first hemoglobin increase of ≥ 1.5 g/dl. The median (range) cumulative duration was 1266.5 (120–1879) days for a hemoglobin increase of ≥ 1.0 g/dl and 1126 (127–1790) days for a hemoglobin increase of ≥ 1.5 g/dl. In total, 16 of 27 NTD patients (59.3%) had a mean increase in hemoglobin of ≥ 1.0 g/dl in weeks 37–48 and 8 of these patients (29.6%) had an increase of ≥ 1.5 g/dl during weeks 37–48 (Table 2).

Transfusion burden reduction in TD patients.

Twenty-five of the 32 (78.1%) TD patients achieved a reduction in transfusion burden of $\geq 20\%$ over any 12-week interval compared with pretreatment levels, including 22 (68.8%) and 19 (59.4%) patients who achieved a reduction of $\geq 33\%$ and $\geq 50\%$, respectively (Table 2). The median (range) time was 1 (1–227) day for the first transfusion burden reduction of $\geq 33\%$ and 1 (1–378) day for the first transfusion burden reduction of $\geq 50\%$ (Table 2). The median (range) cumulative duration was 1145.5 (91–1750) days for first transfusion burden reduction of $\geq 33\%$ and 909 (87–1734) days for first transfusion burden reduction of $\geq 50\%$ (Table 2). Nine of 32 (28.1%) patients achieved RBC-TI. Twelve of 29 TD patients (41.4%) achieved a $\geq 33\%$ reduction in transfusion burden from baseline over weeks 37–48 including eight patients (27.6%) who achieved a $\geq 50\%$ reduction. The mean maximum change in transfusion burden was –37.6% (SD, 36.89%) (Table 2).

Exploratory endpoints

Improvement in markers of ineffective erythropoiesis. The mean change from baseline in serum EPO, reticulocyte, and sTfR1 levels in TD and NTD patients by erythroid response is shown in Figure 2, with responders achieving $\geq 33\%$ reduction in RBC transfusions for TD patients, or a hemoglobin increase of ≥ 1.0 g/dl for NTD patients, during any continuous 12-week period. In NTD patients, an early increase in serum EPO, sTfR1, and reticulocyte level was observed from weeks 12–24; these changes were more evident in nonresponders [Figure 2(a), (c), (e)]. Long-term, NTD responders showed a trend toward decreasing EPO levels [Figure 2(a)]. Similar changes were observed in TD patients, with only sTfR1 levels differing between responders and nonresponders [Figure 2(d)]. An initial rise in mean serum EPO occurred in TD responders

Table 2. Erythroid response in patients with β -thalassemia during long-term treatment with high-dose luspatercept.

Parameter ^a	Response rate		
	Any 12 weeks	Weeks 13–24 ^b	Weeks 37–48 ^b
NTD patients (<i>n</i> = 31)			
Mean Hb increase of ≥ 1.0 g/dL, <i>n/N</i> (%) (95% CI)	22/31 (71.0) (52.0–85.8)	16/30 (53.3) (34.3–71.7)	16/27 (59.3) (38.8–77.6)
Time to first response (days), median (range) ^c	8 (7–92)	NA	NA
Duration of response (days), median (range) ^d	1267 (120–1879)	NA	NA
Number of responses, median (range) ^e	1 (1–6)	NA	NA
Cumulative duration of response (days), median (range)	1266.5 (120–1879)	NA	NA
Mean Hb increase of ≥ 1.5 g/dL, <i>n/N</i> (%) (95% CI) ^c	17/31 (54.8) (36.0–72.7)	12/30 (40.0) (22.7–59.4)	8/27 (29.6) (13.8–50.2)
Time to first response (days), median (range) ^c	9 (7–791)	NA	NA
Duration of response (days), median (range) ^d	939 (127–1790)	NA	NA
Number of responses, median (range) ^e	1 (1–9)	NA	NA
Cumulative duration of response (days), median (range)	1126 (127–1790)	NA	NA
Maximum increase in Hb (g/dL), median (range)	<i>n</i> = 29 1.8 (–0.7 to 3.5)	<i>n</i> = 27 1.3 (–1.0 to 3.2)	<i>n</i> = 22 1.3 (–1.2 to 3.3)
TD patients (<i>n</i> = 32)			
RBC transfusion reduction $\geq 20\%$, <i>n/N</i> (%) (95% CI)	25/32 (78.1) (60.0–90.7)	14/29 (48.3) (29.5–67.5)	12/29 (41.4) (23.5–61.1)
RBC transfusion reduction $\geq 33\%$, <i>n/N</i> (%) (95% CI)	22/32 (68.8) (50.0–83.9)	12/29 (41.4) (23.5–61.1)	12/29 (41.4) (23.5–61.1)
Time to first response (days), median (range) ^c	1 (1–227)	NA	NA
Duration of response (days), median (range) ^d	206 (91–1732)	NA	NA
Number of responses, median (range) ^e	2.5 (1–42)	NA	NA
Cumulative duration of response (days), median (range)	1145.5 (91–1750)	NA	NA
RBC transfusion reduction $\geq 50\%$, <i>n/N</i> (%) (95% CI)	19/32 (59.4) (40.6–76.3)	8/29 (27.6) (12.7–47.2)	8/29 (27.6) (12.7–47.2)
Time to first response (days), median (range) ^c	1 (1–378)	NA	NA

(Continued)

Table 2. (Continued)

Parameter ^a	Response rate		
	Any 12 weeks	Weeks 13–24 ^b	Weeks 37–48 ^b
Duration of response (days), median (range) ^d	147 [86–1732]	NA	NA
Number of responses, median (range) ^e	5 [1–42]	NA	NA
Cumulative duration of response (days), median (range)	909 [87–1734]	NA	NA
Maximum transfusion burden change (%), mean (SD)	NA	<i>n</i> = 23 –31.5 (39.50)	<i>n</i> = 19 –37.6 (36.89)

CI, confidence interval; Hb, hemoglobin; NA, not available; NTD, nontransfusion dependent; RBC, red blood cell; SD, standard deviation; TD, transfusion dependent.

^aAll changes are compared with baseline. 95% CI is the exact 95% CI based on the binomial distribution.

^bOnly patients with the records in the entire fixed window period are included.

^cTime to response is defined as the first date of any 12-week interval of erythroid response – first dose date + 1.

^dDuration of response is defined as the time from the starting date of the first rolling 12-week window achieving response to the last date of the consecutive rolling 12-week window achieving response. When there are multiple disjoint intervals with response, the longest interval is used.

^eThe number of separate responses of 12 weeks or more punctuated by a decrease in Hb of <1 or <1.5 g/dl for NTD patients or a transfusion burden reduction of <33% or <50% for TD patients.

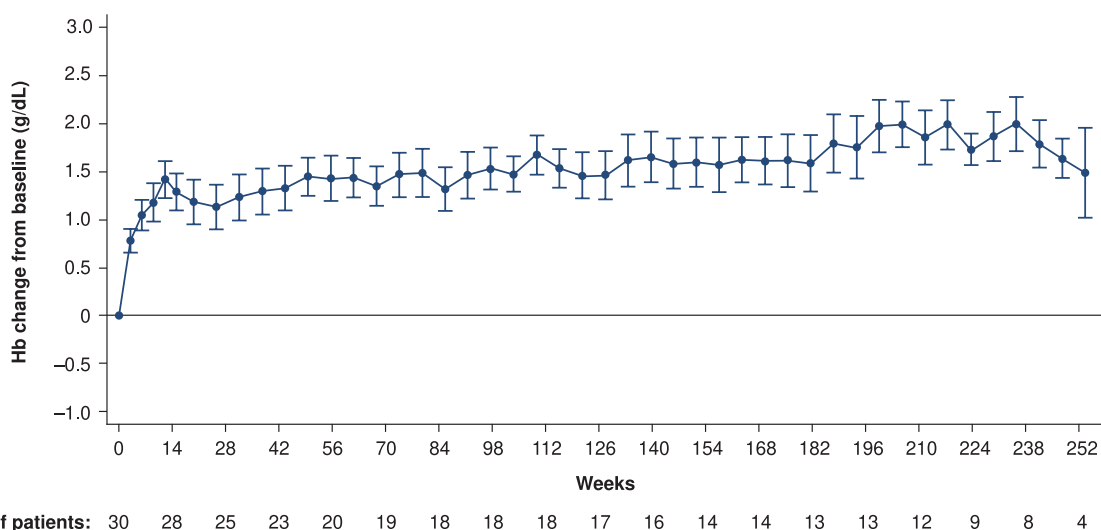


Figure 1. Mean (\pm 95% CI) change in the Hb level relative to baseline during long-term treatment with luspatercept in NTD patients with β -thalassemia (*n* = 27).

CI, confidence interval; Hb, hemoglobin; NTD, nontransfusion dependent.

from weeks 0–48, followed by a return to baseline levels by weeks 84–96 [Figure 2(b)]. Reticulocyte levels in NTD erythroid responders, after an initial rise from baseline, showed a decreasing trend, whereas in nonresponders, the rising trend lasted throughout the base study and extension phase [Figure 2(c)]. Similarly, mean sTfR1 levels plateaued in NTD responders, whereas in

nonresponders, a rising trend persisted long term. TD patients showed an increase in mean sTfR1 levels with no significant differences between responders and nonresponders [Figure 2(f)]. Erythroid response-dependent changes in other markers of ineffective erythropoiesis (bilirubin and LDH) were also observed in both NTD and TD patients. A trend toward an increase was seen

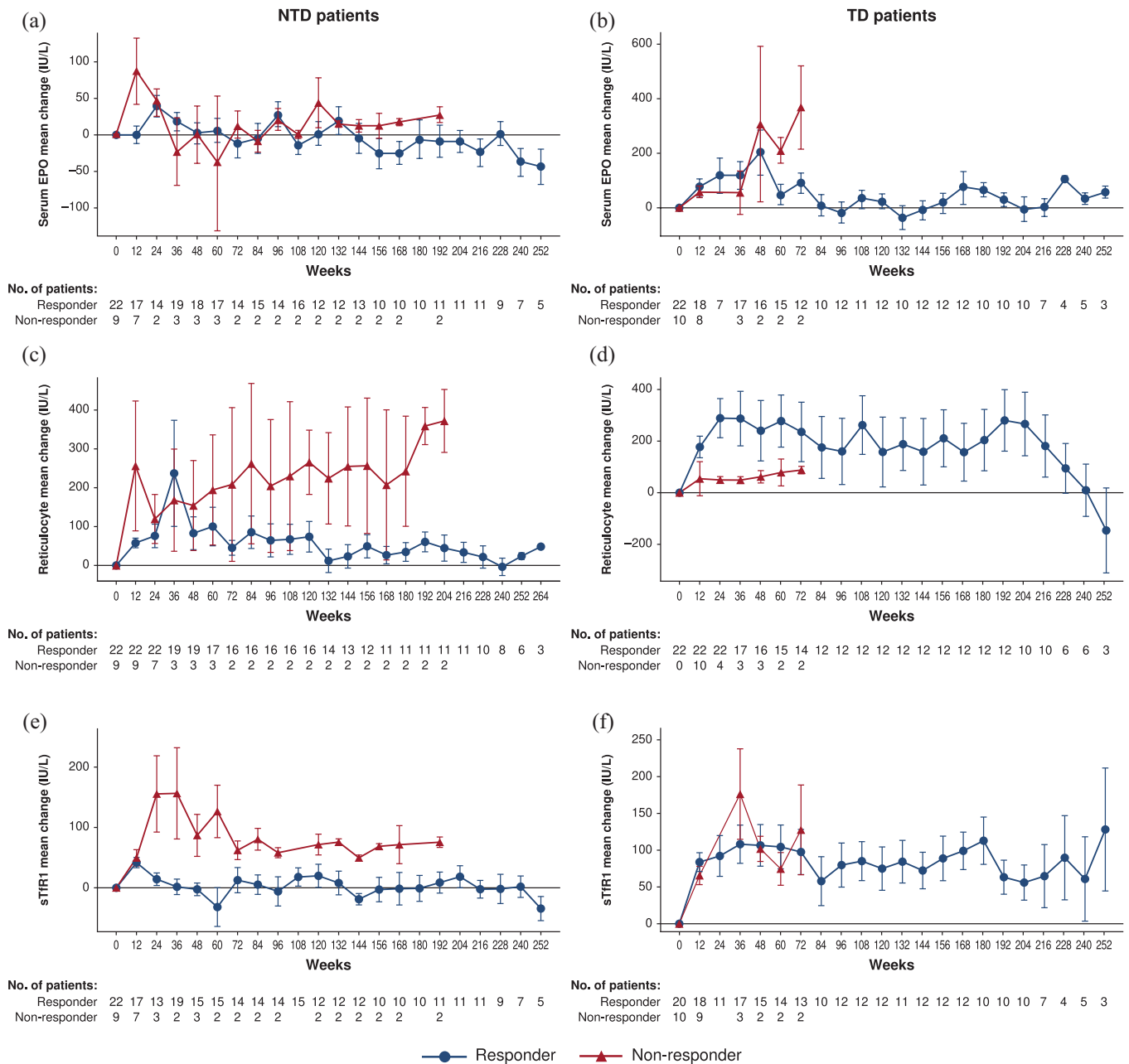


Figure 2. Mean change from baseline in serum EPO levels (a and b), reticulocyte levels (c and d), and sTfR1 levels (e and f) in NTD (a, c, and e) and TD (b, d, and f) erythroid responders and nonresponders. NTD responders defined as mean hemoglobin increase of ≥ 1.0 g/dl during any continuous 12-week period; TD responders defined as $\geq 33\%$ reduction in RBC transfusions during any continuous 12-week period.

EPO, erythropoietin; NTD, nontransfusion dependent; RBC, red blood cell; sTfR1, soluble transferrin receptor 1; TD, transfusion dependent.

with bilirubin (Supplemental Figure S3A, B), whereas a trend toward a decrease was seen with LDH (Supplemental Figure S3 C, D).

Iron metabolism. Mean transferrin saturation levels were stable in TD patients and a transient rise was observed in NTD patients, with only slight

variations between responders and nonresponders [Figure 3(a) and (b)], whereas mean serum ferritin showed a decreasing trend in all patients [Figure 3(c) and (d)], as did LIC (Supplemental Figure S4A, B). LIC decreased in 7 of 11 TD patients with LIC ≥ 3 mg/g dry weight (dw), versus 4 of 12 (33.3%) TD patients with LIC

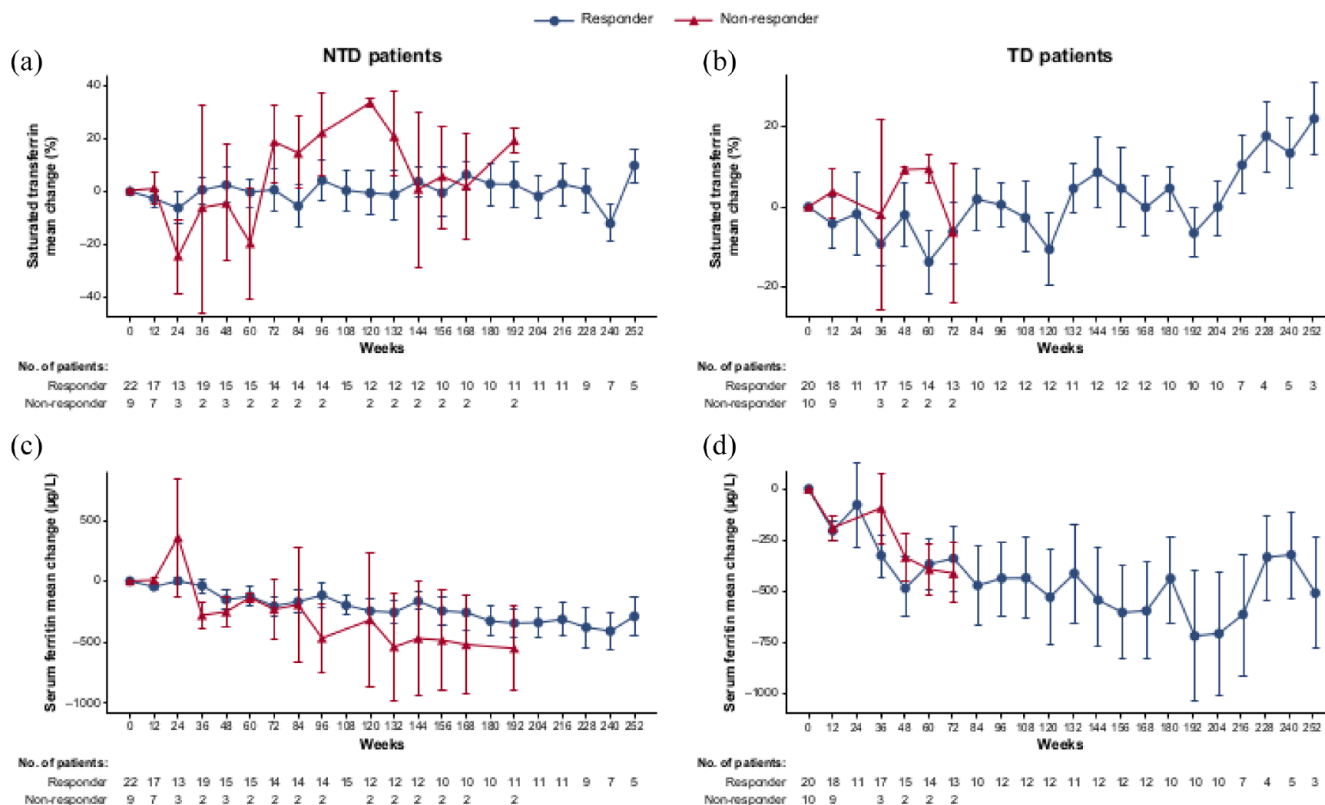


Figure 3. Mean change from baseline in levels of saturated transferrin (a and b) and serum ferritin (c and d) in patients with NTD β -thalassemia (a and c) and TD β -thalassemia (b and d). NTD responders defined as mean hemoglobin increase of ≥ 1.0 g/dl during any continuous 12-week period; TD responders defined as $\geq 33\%$ reduction in RBC transfusions during any continuous 12-week period. NTD, nontransfusion dependent; RBC, red blood cell; TD, transfusion dependent.

< 3 mg/g dw (Supplemental Figure S4C, D). In 11 TD patients and 12 NTD patients, LIC levels were ≥ 3 mg/g dw for the duration of the study; after luspatercept treatment, LIC decreased in 7 of 11 (63.6%) TD patients and 6 of 12 (50.0%) NTD patients (Supplemental Figure S4C, D).

HRQoL

Changes in HRQoL during the extension phase were measured using the FACIT-F patient-reported outcome tool. In NTD patients, a clinically meaningful (minimal clinically important difference > 3) mean increase in the FACIT-F score of 5 from baseline was observed at 96 weeks [Figure 4(a)]. In all patients with a baseline FACIT-F score of < 44 , a clinically meaningful improvement in FACIT-F was achieved by week 12 and maintained during the course of the study [Figure 4(b)]. The improvement in FACIT-F scores correlated with the change in the hemoglobin level at week 24

($r = 0.7081$; $p = 0.0015$) [Figure 4(c)]. An improvement from baseline in mean 6MWD of 50 m in NTD patients was observed at weeks 16–24 and was maintained throughout the study (Supplemental Figure S5A, B).

Safety

Luspatercept had an acceptable long-term safety profile, comparable to that previously reported.¹⁹ Fifty-six of 64 (87.5%) patients, including 1 patient receiving low-dose luspatercept (< 0.6 mg/kg), reported a treatment-emergent adverse event (TEAE) of any grade, while 8 (12.5%) patients reported a grade 3 or higher TEAE (Table 3). The most common related TEAEs of any grade were bone pain [27 of 64 (42.2%)] and headache [20 of 64 (31.3%)], which occurred at grade 3 or 4 severity in 3 (4.7%) patients and 1 (1.6%) patient, respectively (Table 3), and grade 1 or 2 myalgia [14 of 64 (21.9%)]. Four (6.3%)

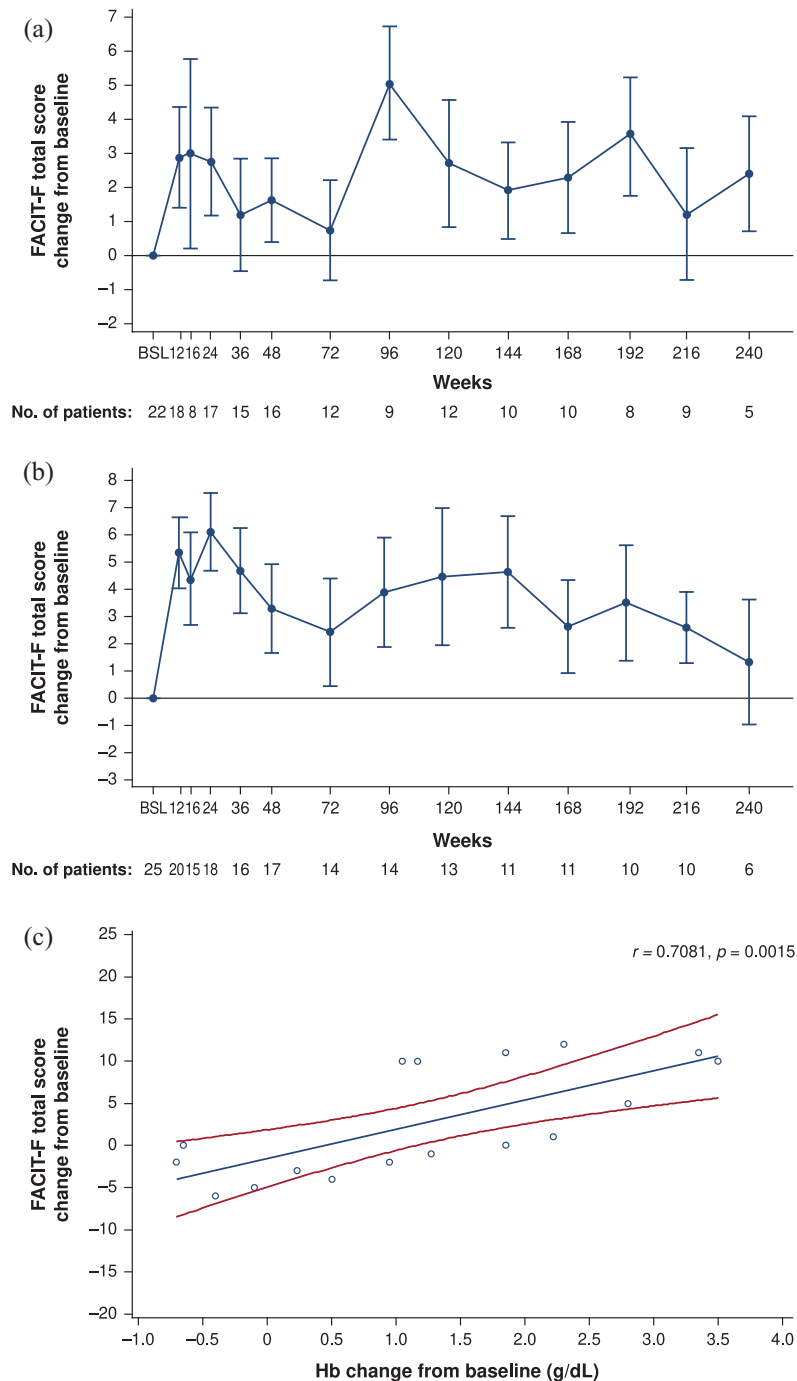


Figure 4. Mean change in FACIT-F scores over time in all NTD patients (a) and all patients with baseline FACIT-F score <44 (b), and FACIT-F score change from baseline *versus* Hb change from baseline in all patients (c). FACIT-F, Functional Assessment of Chronic Illness Therapy – Fatigue; Hb, hemoglobin; NTD, nontransfusion dependent.

patients also had extramedullary hematopoiesis, all of which were grade 1 or 2; however, follow-up information is not available for these patients; a baseline assessment was not available as it was

not included in the study protocol. Extramedullary erythropoiesis, if investigated, is a common finding in both adult TD and NTD patients with thalassemia.^{25,26} In addition, one (1.6%) patient

Table 3. AEs considered related to treatment that occurred during the study.

AE preferred term	Related TEAE, n (%)	
	Any grade (N=64)	Grade 3 or 4 (N=64)
Any TEAE	56 (87.5)	8 (12.5)
Bone pain	27 (42.2)	3 (4.7)
Headache	20 (31.3)	1 (1.6)
Myalgia	14 (21.9)	0
Arthralgia	12 (18.8)	0
Musculoskeletal pain	11 (17.2)	0
Asthenia	9 (14.1)	2 (3.1)
Injection site pain	9 (14.1)	0
Back pain	6 (9.4)	0
Extramedullary hematopoiesis	4 (6.3)	0

AE, adverse event; TEAE, treatment-emergent AE.

experienced a grade 3 or 4 serious TEAE related to luspatercept (biliary colic) and the death of one (1.6%) patient was reported because of a grade 5 serious TEAE (cardiac arrest).

Discussion

In this long-term follow-up of patients enrolled in the phase 2 trial of luspatercept for the treatment of β -thalassemia, luspatercept treatment was associated with sustained increases in hemoglobin levels in NTD patients and sustained reductions in transfusion burden in TD patients.

More than half of the NTD and TD patients receiving high-dose luspatercept achieved the primary endpoint of erythroid response, defined as a hemoglobin increase from baseline of ≥ 1.5 g/dl for ≥ 2 weeks (in the absence of RBC transfusions) for NTD patients and as a reduction in RBC transfusion burden over any continuous 12-week period of $\geq 20\%$ compared with pretreatment for TD patients. Among NTD patients, mean hemoglobin exceeded 1.0 g/dl by week 6 and was sustained throughout the extension study; 28.1% of TD patients achieved RBC-TI during any 12-week period. Notably, the erythroid response to luspatercept was sustained long term; the cumulative duration of response was 2.5 years in TD patients and

just over 3 years in NTD patients. During weeks 37–48, the proportion of NTD patients who had a hemoglobin increase of ≥ 1.5 g/dl, and the proportion of TD patients who had a $\geq 50\%$ reduction in RBC-TI, appears to be lower than that during weeks 13–24. While a statistical analysis was not performed, the small patient numbers and overlapping 95% CI of response between the time periods suggest this difference is not statistically significant. This result could be due to coincidence or a placebo effect, which is possible in a nonrandomized, noncontrolled study, although the latter is unlikely.

In the BELIEVE study, 158 of 224 (70.5%) patients achieved a reduction in transfusion burden of $\geq 33\%$ from baseline during any 12-week interval, and 48 of 224 (21.4%) patients and 44 of 224 (19.6%) patients achieved the same in transfusion burden during weeks 13–24 and weeks 37–48, respectively. In this study, 22 of 32 (68.8%) patients achieved a reduction in transfusion burden of $\geq 33\%$ during any 12-week interval, and 12 of 29 (41.4%) TD patients for both weeks 13–24 and weeks 37–48. Similar trends were seen with the proportion of patients who achieved a reduction in transfusion burden of $\geq 50\%$ from baseline during the same time points, suggesting that the treatment effect may be stronger in this population. However, because of the difference in study design (randomized *versus* nonrandomized trial) and sample size, caution should be taken before drawing conclusions.

Luspatercept inhibits ligands of the TGF- β superfamily and promotes late-stage erythroid maturation.²⁷ In *in vitro* murine models, luspatercept reduced reactive oxygen species caused by Smad2/3-pathway overactivation, resulting in decreased apoptosis of RBC precursors.²⁸ In low-risk myelodysplastic syndromes, luspatercept increased the mean ratio of reticulocytes:sTfR1. This result indicates that erythroid maturation is improved because the ratio of reticulocytes:sTfR1 approximates the ratio of late-stage erythropoiesis (reticulocytes) within total erythropoiesis (sTfR1); furthermore, this ratio does not change in nonresponding patients.²⁹ We postulate that this mechanism of action may also underpin the efficacy of luspatercept in β -thalassemia. In addition, in patients with β -thalassemia receiving luspatercept, a lower hepcidin level together with reduced serum ferritin levels has been observed,³⁰ suggesting that a proportion of iron stores are mobilized, some of which is incorporated into new hemoglobin. This

mechanism could explain how hemoglobin levels increase in patients treated with luspatercept.

Analysis of the levels of several key markers of ineffective erythropoiesis during the study also showed temporal trends indicative of a long-term benefit of luspatercept. In NTD responders, serum EPO, reticulocytes, and sTfR1 levels did not rise significantly, consistent with the preclinical results that indicate an improvement in RBC maturation without a trend to erythropoietic expansion, though it should be noted that erythroid expansion seemed to occur in nonresponders irrespective of transfusion dependence status. Furthermore, serum ferritin levels showed a consistent downward trend in NTD and TD patients who achieved an erythroid response. Luspatercept is a ligand trap that acts as an erythroid maturation agent to restore RBC production and ameliorate anemia.³⁰ Decreases in markers of ineffective erythropoiesis, such as serum EPO, were observed in NTD erythroid responders. While differences in these markers were apparent between TD responders and nonresponders, interpretation is complicated because of the potential confounding effect of ongoing transfusions. In contrast, the impact of luspatercept treatment on markers of iron accumulation, notably serum ferritin levels and LIC, was most evident in TD erythroid responders; decreases in LIC were greatest in TD patients with baseline LIC ≥ 3 mg/g dw. These observations are consistent with the mechanism of action of luspatercept as a stimulator of erythroid maturation that increases hemoglobin levels in NTD patients and reduces the need for transfusions in TD patients. Furthermore, it can be speculated that luspatercept has a direct effect on iron metabolism independently of its effect on erythropoiesis, because of the decrease in serum ferritin levels, despite an increase in erythropoiesis as measured by sTfR1 in nonresponding patients. However, this is unlikely, as consistent effects would have been observed on serum ferritin, liver iron, and cardiac iron as well.

Quality of life is significantly impacted in patients with β -thalassemia irrespective of their transfusion burden status.¹⁴ Increased mean FACIT-F scores, indicating a decrease in fatigue symptoms, were observed in NTD patients and patients with lower baseline levels of fatigue (FACIT-F score <44). Improvements in FACIT-F scores were positively correlated with increased mean hemoglobin levels.

In NTD patients, mean 6MWD increased by 50m. While there have been no formal studies published on clinically meaningful improvement in 6MWD for patients with β -thalassemia, a clinically meaningful improvement of 14–44m has been reported for patients with cardiopulmonary and other diseases.^{31–34}

These results suggest that the positive impact of luspatercept on long-term erythroid response translates to improved quality of life.

The long-term safety profile of luspatercept in patients with β -thalassemia is generally consistent with that previously reported.¹⁹ While the rates of the most common treatment-related any grade TEAEs (bone pain, headache, and myalgia) with longer term treatment exceeded those previously reported, rates of grade 3 or 4 TEAEs were comparable to those previously reported and no new safety signals were detected. Because the Reblozyl[®] prescribing information from the European Medicines Agency (EMA)³⁵ and Food and Drug Administration (FDA)³⁶ provides no warnings for cardiac-related events, we believe that the sole case of asystole that occurred during this study is an isolated incident. However, because arrhythmias are a frequent and growing problem in adult patients with thalassemia, more reports of its coincidence with luspatercept treatment might be expected.

These results support the long-term safety of luspatercept in patients with β -thalassemia. In contrast to sickle cell disease, hydroxyurea is not commonly used in practice for the long-term management of patients with β -thalassemia. The efficacy of hydroxyurea is driven by the type of genetic defects and the quality of conservative care. In a phase 3 trial, adverse events experienced by patients with TD β -thalassemia treated with hydroxyurea for 6 months included headache (2 of 30 patients), thrombocytopenia (1 of 30), leukopenia (1 of 30), hyperpigmentation (1 of 30), urinary tract infection (2 of 30), nausea (1 of 30), vomiting (1 of 30), and abdominal pain (1 of 30).³⁷ The short-term safety profile of hydroxyurea appears to be favorable to luspatercept; however, the increases in hemoglobin are generally less than those for luspatercept³⁸; likewise, hydroxyurea benefit is not as durable. Furthermore, long-term randomized trials of hydroxyurea in β -thalassemia are lacking.

The limitations of this study have been reported previously.¹⁹ In addition, there is a potential impact of the reclassification of patient transfusion status upon entry to the extension study on the results presented here, though this happened rarely and the effect on outcomes, if any, is marginal. This phase 2 study also had a relatively small number of patients, as it concatenated different study phases with different aims (dose finding for the base study and long-term safety and efficacy for the extension study). As in most long-term follow-up studies, the number of patients decreased markedly at later time points, mainly because of a demanding extension study protocol. Overall, there were high rates of discontinuation of the study, predominantly because of nonmedical reasons, such as the demanding protocol for the extension study, with medical reasons only accounting for the discontinuation of 4 of 51 (7.8%) patients. Significantly, 18 of 51 (35.5%) patients remain enrolled in the extension study, the results of which will be the subject of a future report. This study also had no control group, although a broad range of doses in the dose-ranging part of study were tested. Finally, the decrease in LIC observed was confounded by concurrent administration of ICT in some patients.

Conclusion

In conclusion, a long-term assessment of outcomes in patients with β -thalassemia treated with luspatercept shows that luspatercept treatment is associated with sustained increases in hemoglobin levels in NTD patients and a sustained reduction in transfusion burden in TD patients. Markers of ineffective erythropoiesis and iron overload improved in NTD patients who responded to luspatercept treatment, as did quality of life, supporting the role of luspatercept as an erythroid maturation agent with enhanced iron metabolism. Luspatercept was well tolerated over the extended period of observation in the 5-year extension phase of this phase 2 study.

Declarations

Ethics approval and consent to participate

The study was approved by the institutional review board or central ethics committee at each participating institution and was conducted according to the Declaration of Helsinki. All patients provided written informed consent.

Consent for publication

All authors provided consent to submit this report for publication.

Author contributions

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Paolo Ricchi: Investigation; Writing – review & editing.

Vincenzo Caruso: Investigation; Writing – review & editing.

Antonello Pietrangelo: Investigation; Writing – review & editing.

Xiaosha Zhang: Formal analysis; Writing – review & editing.

Jeevan K. Shetty: Writing – review & editing.

Kenneth M. Attie: Conceptualization; Writing – review & editing.

Immacolata Tartaglione: Investigation; Writing – review & editing.

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Competing interests

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Availability of data and materials

Bristol-Myers Squibb policy on data sharing may be found at <https://www.bms.com/researchers-and-partners/independent-research/data-sharing-request-process.html>.

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Supplemental material

Supplemental material for this article is available online.

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