Luspatercept improves hemoglobin levels and blood transfusion requirements in a study of patients with b-thalassemia

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Luspatercept improves hemoglobin levels and blood transfusion requirements in a study of patients with beta-thalassemia

Short title: Luspatercept in patients with beta-thalassemia

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Key Points

- A high percentage of patients with beta-thalassemia had improvement in hemoglobin or transfusion burden after receiving luspatercept.
- These findings support a randomized clinical trial to assess the efficacy and safety of luspatercept for treatment of beta-thalassemia.

Abstract

Beta-thalassemia is a hereditary disorder with limited approved treatment options; patients experience anemia and its complications, including iron overload. This study aim was to determine whether luspatercept could improve anemia and disease complications in patients with beta-thalassemia. This open-label, nonrandomized, uncontrolled study (NCT01749540 and NCT02268409) consisted of a 24-week dose-finding and expansion stage (initial stage) and a 5-year extension stage, currently ongoing. Sixty-four patients were enrolled; 33 were non-transfusion-dependent (mean hemoglobin <10.0 g/dL and <4 red blood cell [RBC] units transfused/8 weeks) and 31 were transfusion-dependent (≥4 RBC units/8 weeks). Patients received 0.2-1.25 mg/kg luspatercept subcutaneously every 21 days for ≥5 cycles in the dose-finding stage, and 0.8-1.25 mg/kg in an expansion cohort and 5-year extension. The primary endpoint was erythroid response, defined as hemoglobin increase of ≥1.5 g/dL from baseline for ≥14 consecutive days (with no RBC transfusions) for non-transfusion-dependent patients, or reduction in RBC transfusion burden of ≥20% over a 12-week period versus the 12 weeks before treatment for transfusion-dependent patients. Eighteen (58%) non-transfusion-dependent patients receiving higher dose levels of luspatercept (0.6-1.25 mg/kg) achieved mean hemoglobin increase ≥1.5 g/dL over ≥14 days versus baseline. Twenty-six (81%) with transfusion dependence achieved ≥20% reduction in RBC transfusion burden. The most common grade 1-2 adverse events were bone pain, headache, and myalgia. As of the cutoff, 33 patients remain on study. In this study, a high percentage of beta-thalassemia patients receiving luspatercept had improvements in hemoglobin or transfusion burden. These findings support a randomized clinical trial to assess efficacy and safety.

Introduction

Beta-thalassemia is a hereditary red blood cell (RBC) disorder caused by mutations in the beta-globin gene.[1] These mutations lead to oxidative stress and premature death of erythroblasts, resulting in ineffective erythropoiesis and erythroid expansion in the bone marrow.[2,3] Comorbidities, including bone deformities, splenomegaly, and leg ulcers, are the result of one or more factors associated with the disease, including ineffective erythropoiesis, anemia, and iron overload.[1]

Patients with beta-thalassemia major have a severe form of the disease and are generally transfusion-dependent. However, receiving regular RBC transfusions leads to iron overload, necessitating daily iron chelation therapy.[4] Beta-thalassemia intermedia includes less severe forms of the disease that may be initially non-transfusion-dependent, but over time may become transfusion-dependent. Although these patients are generally non-transfusion-dependent, iron overload can result from increased intestinal iron absorption due to reduced hepcidin levels.[5] Sporadic or even regular transfusions may therefore be required.[4,6,7]

Current treatment options for beta-thalassemia are mostly limited to supportive therapy. Hematopoietic stem cell transplantation is currently the only possible curative treatment, yet is only suitable for a small percentage of patients.[8] Gene therapy clinical trials for beta-thalassemia are in the early stages.[9] The use of erythropoiesis-stimulating agents, such as erythropoietin, in beta-thalassemia is not supported by controlled clinical trials.[10,11]

Members of the transforming growth factor beta (TGF-beta) superfamily of ligands, including growth differentiation factors (GDFs) and activins, have been demonstrated to act as inhibitors of late-stage erythropoiesis.[12] Luspatercept (ACE-536) is a novel recombinant fusion protein that acts as a trap for these ligands. In healthy volunteers, luspatercept was well tolerated and

associated with dose-dependent increases in hemoglobin levels.[13] RAP-536, a murine analog of luspatercept, has also been demonstrated to reduce oxidative stress and anemia in a mouse model of beta-thalassemia.[14]

The aim of this phase 2 study was to determine a tolerable and active dose level and schedule of luspatercept in adults with transfusion-dependent or non-transfusion-dependent beta-thalassemia.

Methods

Supplemental material referred to in this section can be found in the Supplemental Data file, available online.

Study oversight

The study was approved by the institutional review board or central ethics committee at each participating institution and was conducted in accordance with the Declaration of Helsinki. All patients provided written informed consent. Patients were enrolled between 11 February 2013 and 6 July 2015 at 8 sites in Italy and Greece. Detailed information on the sites where patients were recruited is shown in Supplemental Table 1.

Patients

Patients with a documented diagnosis of beta-thalassemia aged ≥18 years were eligible. Those in the dose-escalation stage had either prior splenectomy or spleen size <18 cm in the longest diameter, as measured by abdominal ultrasound.

Exclusion criteria included folate deficiency, symptomatic splenomegaly, and history of thromboembolic events; individuals with adequate folate replacement therapy could be enrolled.

Patients with any clinically significant pulmonary, cardiovascular, endocrine, neurologic, hepatic, gastrointestinal, infectious, immunologic, or genitourinary disease considered by the investigator to be not adequately controlled were also excluded.

Eligible patients were classified as either non-transfusion-dependent or transfusion-dependent. Patients not dependent on transfusions were eligible if they had a mean hemoglobin concentration of <10.0 g/dL (based on 2 or more measurements) and, if transfused, received <4 RBC units in the 8 weeks prior to study initiation. Patients with transfusion dependence were those who received an average of ≥4 RBC units every 8 weeks over the 6-month period prior to study initiation.

Study design

This phase 2, open-label, nonrandomized, uncontrolled, dose-finding study evaluated the effects of luspatercept in patients with beta-thalassemia (ClinicalTrials.gov identifiers: NCT01749540 and NCT02268409). Luspatercept was administered subcutaneously every 21 days at dose levels ranging from 0.2 to 1.25 mg/kg body weight; this starting dose was selected based on preclinical and phase 1 clinical findings, including safety margin and pharmacodynamic markers.

The study was conducted in 2 stages: the initial and extension stages (Figure 1). In the dose escalation cohorts of the initial stage, patients received up to 5 doses of luspatercept at 1 of 6 pre-specified dose levels: 0.2, 0.4, 0.6, 0.8, 1.0, and 1.25 mg/kg. There was no formal sample size calculation used in the dose-finding stage. Up to 6 individuals were enrolled in each dose level and dose cohorts were enrolled sequentially, from lowest to highest dose group. Once ≥3 patients in a dose cohort completed the study day 29 visit, response and adverse event (AE) data were reviewed by a safety review team, comprising the coordinating investigator, medical

monitor, and an independent hematologist. The safety review team made recommendations as to whether to enroll patients in the next highest dose cohort or proceed to the expansion cohort at a selected dose level.

Following the dose-escalation cohorts, an expansion cohort was enrolled. Based on recommendations of the safety review team, individuals in the expansion cohort received luspatercept at a starting dose level of 0.8 mg/kg, with dose titration up to 1.25 mg/kg allowed following completion of 2 treatment cycles. A sample of 30 evaluable patients in the expansion cohort was calculated by a priori power analysis to provide approximately 87% power (with a 1-sided significance level of 0.05) to differentiate an erythroid response rate of 30% from a minimal erythroid response rate of 10%. Patients were on study for approximately 24 weeks in the initial stage, including a 4-week screening period, a 12-week treatment period, and an 8-week follow-up period.

Patients completing the initial stage could be enrolled in the long-term extension stage in order to receive luspatercept for up to 5 years. Those without treatment interruption could directly roll over to the extension stage at a starting dose equal to their last dose level in the initial stage. Those with treatment interruption were reassessed for eligibility and transfusion status prior to starting the extension, and received a starting dose level of 0.8 mg/kg. In this manner, patients classified as non-transfusion-dependent in the base study could be reclassified as transfusion-dependent upon entering the extension study, or vice versa, subject to transfusion status evaluation. Dose titration up to 1.25 mg/kg was allowed in the extension stage. Patients discontinuing from or completing the extension stage entered a 2-month follow-up period. Further study design and statistical information can be found in the Supplemental Methods section in the Supplemental Data file.

Study endpoints

Primary endpoint

The primary endpoint 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 non-transfusion-dependent patients, and as a reduction in RBC transfusion burden over a 12-week interval of ≥20% as compared with pre-treatment for transfusion-dependent patients.

Secondary endpoints

Secondary endpoints reported in this manuscript include hemoglobin increase of ≥1.5 g/dL from baseline for ≥12 consecutive weeks for non-transfusion-dependent patients, reduction in RBC transfusion burden of ≥33% or ≥50% for transfusion-dependent patients, time to and duration of erythroid response, and changes in liver iron concentration (LIC) measured using magnetic resonance imaging. The time to response was calculated from the day the patient received the first dose to the beginning of the first 12-week rolling period in which they satisfied the response criterion.

The safety and tolerability of luspatercept was evaluated throughout the study, including AE profiling, clinical laboratory tests, and vital signs. Additional secondary endpoint assessments beyond the scope of this manuscript include the proportion of patients requiring no RBC transfusions for ≥8 consecutive weeks, additional iron metabolism parameters, erythropoiesis parameters, bone metabolism parameters, and hemolysis parameters.

Exploratory endpoints

Exploratory endpoints included quality-of-life, which was measured using the Functional Assessment of Chronic Illness Therapy–Fatigue (FACIT-F) patient-reported outcome tool. The FACIT-F scale ranged from 0 to 52, with 52 being the best quality-of-life.[15] Other secondary

endpoints reported include improvements in leg ulcers and bone mineral density (BMD) by dual-energy X-ray absorptiometry (DEXA) scan. Leg ulcer severity was scored using a scale of 0-5, where 0 denotes fully healed and 5 denotes gangrene, as well as with measurements; however, some leg ulcers were only graded by physical examination and measurements.[16] Information on when and how the endpoints in this study were collected, including those not reported in this manuscript, can be found in Supplemental Table 2.

Statistical methods

The primary efficacy endpoint of erythroid response is summarized by percentage of responders and its 95% exact confidence interval (CI). For secondary and exploratory efficacy endpoints, the continuous measurement data including LIC, hemoglobin, duration of erythroid response, and BMD are summarized by mean change from baseline and corresponding standard deviation (SD) and 95% CI, as required. The dichotomized response measurement data including ≥33% and ≥50% RBC transfusion reduction from baseline are summarized by the percentage of the response and its 95% CI. Correlation between improvement in FACIT-F and hemoglobin change is summarized using the Pearson correlation coefficient and tested to determine the significance of this correlation. *P* values presented are based on a 2-sided test; *P* values below 0.05 are considered statistically significant. The statistical software used was SAS version 9.4 (SAS Institute, Cary, NC, USA). Changes from baseline hemoglobin values were further evaluated by mixed-effects analysis with baseline hemoglobin, investigational sites, and time; of these, sites and time were treated as random effects.

With regard to missing values when calculating the primary endpoint, a patient without adequate data for evaluation was considered a non-responder. For example, any patient without 12-week RBC transfusion data following treatment was considered a non-responder for the purpose of

calculating erythroid response. For other patient data including LIC, BMD, and quality-of-life, only data actually collected was used in the analysis; no imputation was performed.

Data sharing statement

Data requests may be submitted to Celgene at www.CelgeneClinicalDataSharing.com and must include a description of the research proposal.

Results

Patient characteristics

Sixty-four patients were enrolled between 11 February 2013 and 6 July 2015. In the initial stage, patients received luspatercept every 3 weeks for up to 5 doses (median exposure, 105 days [range, 21-107]). Thirty-five of the 64 patients were enrolled in the dose-escalation cohorts (0.2-1.25 mg/kg) and 29 were enrolled in the expansion cohort (0.8-1.25 mg/kg). At baseline, 33 patients were non-transfusion-dependent and 31 were transfusion-dependent.

In the initial stage of the study, 12 patients received luspatercept at lower dose levels of 0.2-0.4 mg/kg and 52 patients at higher dose levels of 0.6-1.25 mg/kg; all 12 lower-dose patients were non-transfusion-dependent. A total of 51 patients carried over to the long-term extension stage of the study, during which all patients received higher dose levels 0.6-1.25 mg/kg; 25 of these patients had treatment interruption between treatment stages. The median total duration of treatment for all 64 patients was 428 days (range, 21-768); treatment of 33 patients was ongoing as of the 2 September 2016 data cutoff date. Of the 18 patients who discontinued from the extension stage, 4 discontinued due to a medical reason or AE, 1 was lost to follow-up, 10 discontinued at patient request, 1 died due to a cerebrovascular accident not related to treatment, and 2 discontinued due to protocol non-compliance (Figure 1).

Of the 51 patients who continued into the extension stage, 11 had received lower dose levels in the initial stage. Thus, across both stages of the study, 63 of 64 patients received the higher dose levels of 0.6-1.25 mg/kg of luspatercept at some point during the study; 31 were non-transfusion-dependent and 32 were transfusion-dependent.

Patient demographics and baseline characteristics are shown in Table 1.[16] Median age at baseline was 38.5 years (range, 20-62) and 48% of patients were female. Among patients who were non-transfusion-dependent, the median hemoglobin level at baseline was 8.5 g/dL (range, 6.5-9.8), whereas the mean (± SD) LIC was 5.4 ± 3.8 mg/g dry weight. Among patients with transfusion dependence, the median baseline transfusion burden was 8.0 RBC units/12 weeks (range, 4-18) and the mean LIC was 5.0 ± 5.3 mg/g. In total, 43 (67%) patients had previously had a splenectomy.

Efficacy

Hemoglobin response in non-transfusion-dependent patients with beta-thalassemia

Primary endpoint

Rates of erythroid response for non-transfusion-dependent patients are shown in Table 2. Of 31 non-transfusion-dependent patients treated with luspatercept at dose levels of 0.6-1.25 mg/kg, 18 (58%) achieved a mean hemoglobin increase from baseline of ≥1.5 g/dL over 14 consecutive days (95% CI, 39.1-75.5).

Secondary endpoints

Of 31 non-transfusion-dependent patients treated with luspatercept at dose levels of 0.6-1.25 mg/kg, 22 (71%) achieved a mean hemoglobin increase from baseline of ≥1.0 g/dL over 12 weeks (95% CI, 52.0-85.8) compared with the 12 weeks before treatment; 14 (45%) non-transfusion-dependent patients achieved a mean hemoglobin increase of ≥1.5 g/dL over

12 weeks (95% CI, 27.3-64.0). Of 10 non-transfusion-dependent patients with prior splenectomy receiving luspatercept at dose levels 0.6-1.25 mg/kg, 8 (80%) achieved a mean hemoglobin increase from baseline of ≥1.5 g/dL over 12 weeks, compared with 6 of 21 patients (29%) without prior splenectomy. The relationship between splenectomy and response will be investigated further in a future trial.

Mean hemoglobin changes from baseline for those treated with luspatercept at dose levels of 0.2-0.4 mg/kg and 0.6-1.25 mg/kg in the initial stage of the study are shown in Figure 2A. Median time to hemoglobin response among non-transfusion-dependent patients in the initial stage of the study was 8 days (range, 7-30). The mean change in hemoglobin over the study duration, including the extension stage up to 18 months total duration, for all non-transfusion-dependent patients treated with dose levels 0.6-1.25 mg/kg (n = 31) is shown in Figure 2B.

Transfusion burden reduction in transfused patients with beta-thalassemia

Primary endpoint

Thirty-two patients with transfusion dependence were treated with luspatercept at dose levels of 0.6-1.25 mg/kg during the study. Of these, 26 (81%) achieved a transfusion burden reduction of ≥20% over any 12 weeks on study compared with the 12 weeks prior to baseline (95% CI, 63.6-92.8); these data are presented in Table 2.

Secondary endpoints

RBC transfusion burden reduction of ≥33% was achieved in 23 (72%) patients (95% CI, 53.3-86.3), and ≥50% reduction was achieved in 20 (63%; 95% CI, 43.7-78.9). Of 21 transfusion-dependent patients with prior splenectomy, 14 (67%) achieved a transfusion burden reduction of ≥20% over a fixed 12-week period versus baseline, compared with 4 of 11 patients (36%) without prior splenectomy.

Transfusion burden reduction relative to pre-treatment for all transfused patients who had a baseline transfusion burden of ≥2 RBC units and received 0.6-1.25 mg/kg luspatercept is shown in Figure 3. For transfused patients, the mean transfusion burden over the best recorded 12-week responses on treatment was 3.0 units (SD ± 3.76), compared with 7.6 units (SD ± 3.56) at baseline. The pre-transfusion hemoglobin levels did not vary substantially on treatment as compared to pre-treatment, and ranged between 9 and 10 g/dL.

Liver iron concentration

Secondary endpoint

Of 15 patients with non-transfusion-dependent beta-thalassemia with baseline LIC ≥3 mg/g dry weight who were treated for ≥4 months, 5 (33%) achieved a decrease in LIC ≥2 mg/g dry weight (95% CI, 11.8-61.6) (Figure 4A). The mean LIC for non-transfusion-dependent patients at the end of the initial stage of treatment was −0.36 mg/g dry weight (SD ± 1.59) compared with 5.38 mg/g (SD ± 3.79) at baseline. Of 9 patients with transfusion dependence with baseline LIC ≥3 mg/g dry weight who were treated for ≥4 months, 5 (56%) achieved a decrease in LIC ≥2 mg/g dry weight (95% CI, 21.2-86.3) (Figure 4B). The mean LIC for transfusion-dependent patients at the end of initial stage of treatment was −0.27 mg/g dry weight (SD ± 1.64) compared with 5.03 mg/g (SD ± 5.32) at baseline. All LIC responders were receiving ongoing concomitant iron chelation therapy.

Exploratory endpoints

Leg ulcers

Six patients had from 1 to 6 leg ulcers over the malleoli at baseline. Each of the 6 patients had complete healing of at least 1 ulcer, with a total of 9 ulcers fully healed, 2 partially healed, and 3

not improved. The median time to complete healing was 10 weeks (range, 6-33). An example of ulcer healing is illustrated in Figure 5.

Bone mineral density

Those receiving luspatercept were assessed for changes in BMD. On baseline DEXA scans, mean (± SD) BMD Z-score was −0.74 ± 1.21 for total hip (n = 25) and −1.61 ± 0.88 for lumbar spine (n = 27). After 24 weeks of treatment at dose levels ≥0.6 mg/kg, the mean percentage change in BMD was +5.3 ± 15.8% for total hip (*P* = 0.23; n = 14) and +3.1 ± 6.6% for lumbar spine (*P* = 0.07; n = 17).

Quality-of-life

Quality-of-life changes were measured using the FACIT-F patient-reported outcome tool.[15] Improvements in FACIT-F score in non-transfusion-dependent patients was significantly correlated with the mean 12-week change in hemoglobin (r = 0.64; *P* = 0.002; n = 21). Of the non-transfusion-dependent patients with a baseline FACIT-F deficit (<44 points) compared with normative data,[17] 7 of 9 (78%) improved by ≥3 points at 24 weeks. The majority of these patients (86%) also had an improvement in mean hemoglobin of ≥1.0 g/dL over a 12-week period. As expected, we did not observe any significant changes in transfusion-dependent patients.

Adverse events

AEs assessed as treatment-related are shown in Table 3. Grade 1-2 AEs were common, mainly during the first 8 weeks, with a clear trend to decrease. The most frequent were bone pain, headache, myalgia, arthralgia, musculoskeletal pain, back pain, and injection site pain. Grade 3 AEs considered treatment related were uncommon: 6 reported in 5 (8%) patients: 3 reports of grade 3 bone pain, 2 reports of grade 3 asthenia, and 1 of grade 3 headache. No treatment-

related grade 4 AEs or serious AEs were reported. One patient with a history of cardiac disease died due to cardiac arrest considered unrelated to treatment; no treatment-related deaths were reported. Four of 64 (6%) patients discontinued treatment due to AEs. AEs were more frequent during the first 8 weeks, with a trend to decrease over time. Dose-delay and full AE information can be found in found in the Supplemental Results section and Supplemental Table 3, respectively, in the Supplemental Data file.

Results pertaining to luspatercept pharmacokinetics are located within the Supplemental Results section in the Supplemental Data file.

Discussion

In this phase 2 study of patients with beta-thalassemia, the primary finding was the achievement of protocol-defined erythroid response, which occurred in 71% of non-transfusion-dependent patients and 81% of transfusion-dependent patients receiving higher doses of luspatercept. Population pharmacokinetic modeling suggests that luspatercept 1.0 mg/kg is an appropriate starting dose for further studies in patients with beta-thalassemia, with titration up to 1.25 mg/kg if required to achieve optimal exposure.[18]

Luspatercept is a novel recombinant fusion protein containing a modified activin receptor type IIB (ActRIIB, ACVR2B) that acts as a ligand trap to block inhibitors of late-stage erythropoiesis.[12] In this manner, luspatercept functions as an erythroid maturation agent to restore RBC production and ameliorate anemia. To date, most AEs reported in patients with beta-thalassemia receiving luspatercept have been grade 1 or 2, and few patients reported grade 3-4 AEs. In this study the most frequent treatment-related AE, bone pain, was more commonly observed in patients with transfusion dependence and may be due in part to the

withdrawal of transfusions in response to treatment. Collection of longer-term AE data is ongoing in the extension stage of the study.

In this study, most patients with low FACIT-F quality-of-life fatigue scores at baseline had improved scores following luspatercept treatment. Most patients with low baseline fatigue scores who achieved an erythroid response had improvements in FACIT-F score during the study. Although the FACIT-F questionnaire focuses on the symptoms of anemia, transfusion dependent patients were also assessed and no significant change in score was observed post treatment. This could be expected for patients who were asymptomatic at baseline with hemoglobin levels maintained on treatment.

Iron overload affects patients with beta-thalassemia regardless of whether they are transfusion-dependent or non-transfusion-dependent. In non-transfusion-dependent beta-thalassemia patients, abnormal regulation of intestinal absorption leads to iron overload, whereas in patients with transfusion dependence, iron overload occurs mostly as a result of frequent RBC transfusions.[3,5] Despite the availability of iron chelation therapies, the mean baseline LIC determined by magnetic resonance imaging was found to be abnormal in both non-transfusion-dependent and transfusion-dependent patients, with a mean concentration of 5.4 and 5.0 mg/g dry weight, respectively. Luspatercept treatment was associated with clinically significant decreases in LIC, particularly in patients who had baseline LIC ≥3 mg/g dry weight and who were receiving concomitant iron chelation therapy; the relative contributions of luspatercept and iron chelation therapy cannot be resolved.

Erythroid expansion due to ineffective erythropoiesis in beta-thalassemia can lead to skeletal deformities and decreased BMD,[19] which improved with luspatercept treatment in mouse models of the disease.[14] The patients in this study had low mean baseline total hip and lumbar

spine BMD Z-scores, with increases during treatment with luspatercept that were not found to be statistically significant in this study.

Severe anemia, ineffective erythropoiesis, iron overload, and hypercoagulable state are all potential risk factors for developing leg ulcers in beta-thalassemia, particularly in non-transfused patients.[20] Of the 6 patients with leg ulcers, which were often long-standing and resistant to other therapies, all had complete healing of 1 or more leg ulcers, which may be due to drug effects on anemia, ineffective erythropoiesis, and/or iron overload, as well as TGF-beta superfamily signaling pathways; the mechanism is the subject of further ongoing research.

Limitations

The study has several limitations. First, the study had a single-group, open-label design without a control group for comparison. However, the lower-dose groups were considered sub-therapeutic. Second, the study had a relatively small number of patients treated at different dose levels. Higher dose groups with similar responses were pooled for this reason. Third, many patients had normal baseline BMD; the small numbers of patients with low BMD limited the analysis and interpretation of the effects of luspatercept. Fourth, the notable decreases in LIC were confounded by patients receiving concomitant chronic treatment with iron chelation therapy. Nonetheless, decreases in LIC may be due in part to the decrease of transfusion burden and/or increase in iron utilization related to luspatercept treatment.

Conclusions

In this open-label, nonrandomized, uncontrolled dose-finding study of patients with beta-thalassemia, a high percentage had improvement in hemoglobin or transfusion burden following use of luspatercept. These findings support a randomized clinical trial to assess efficacy and safety.

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Authorship

Contribution: A. Piga had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Concept and design: A. Piga, K.M.A., A.L., and M.L.S.

Acquisition, analysis, or interpretation of data: A. Piga, K.M.A., C.B.-P., V.C., A.F., M.R.G., A.L., F.L., A.M., S.P., A. Pietrangelo, M.L.S., I.T., E.V., and X.Z.

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Administrative, technical, or material support: C.B.-P.

Study supervision: A. Piga, K.M.A., and M.L.S.

Other: K.M.A. (medical monitor).

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Table 1. Patient demographics and baseline characteristics

	Initial luspatercept dose groups		Total (N = 64)
	0.2-0.4 mg/kg (n = 12)	0.6-1.25 mg/kg (n = 52)	
Age, median (range), years	33.5 (22-53)	41.0 (20-62)	38.5 (20-62)
Female, n (%)	5 (42)	26 (50)	31 (48)
Splenectomy, n (%)	10 (83)	33 (64)	43 (67)
Non-transfusion-dependent patients, n	12	21	33
Hemoglobin, median (range), g/dL	8.5 (7.0-9.6)	8.5 (6.5-9.8)	8.5 (6.5-9.8)
LIC, mean (SD), mg/g dry weight	5.1 (2.9)	5.6 (4.3)	5.4 (3.8)
Transfusion-dependent patients, n	0	31	31
RBC transfusion burden, median (range), units/12 weeks*	NA	8.0 (4-18)	8.0 (4-18)
LIC, mean (SD), mg/g dry weight†	NA	5.0 (5.3)	5.0 (5.3)
BMD			
Total hip, mean (SD), Z-score	−1.1 (0.90)	−0.66 (1.27)	−0.74 (1.21)
Lumbar spine, mean (SD), Z-score	−1.62 (0.58)	−1.61 (0.95)	−1.61 (0.88)
FACIT-F score, median (range)‡	NA	41.5 (25-48)	41.5 (25-48)

BMD, bone mineral density; FACIT-F, Functional Assessment of Chronic Illness Therapy–Fatigue; LIC, liver iron concentration; Q1-Q3, interquartile range; NA, not applicable; RBC, red blood cell; SD, standard deviation.

*Baseline RBC transfusion burden is defined as the total number of RBC units transfused in the 12 weeks prior to first dose of luspatercept (and confirmed over 6 months prior to treatment).

†LIC data were not available at baseline for 1 of the 31 transfusion-dependent patients.

‡FACIT-F score was assessed in 26 of the 64 patients, none of which received 0.2-0.4 mg/kg luspatercept. For scoring assessment, a scale of 0 to 52 is used, with 0 meaning worst quality-of-life and 52 meaning best. A score <44 points is considered to be a FACIT-F deficit.[16]

Table 2. Response rates based on various erythroid response criteria in patients with beta-thalassemia treated with luspatercept 0.6-1.25 mg/kg in the initial and extension stages

	0.6-1.25 mg/kg luspatercept (N = 63)*	95% CI
Non-transfusion-dependent patients, n/N (%)	n = 31†	
Mean hemoglobin increase ≥1.5 g/dL for 14 days	**18/31 (58)**	**39.1-75.5**
Mean hemoglobin increase ≥1.0 g/dL for 12 weeks	22/31 (71)	52.0-85.8
Mean hemoglobin increase ≥1.5 g/dL for 12 weeks	14/31 (45)	27.3-64.0
Decrease in LIC ≥2 mg/g dry weight‡	5/15 (33)	11.8-61.6
Transfusion-dependent patients, n/N (%)	n = 32†	
≥20% transfusion burden reduction	**26/32 (81)**	**63.6-92.8**
≥33% transfusion burden reduction	23/32 (72)	53.3-86.3
≥50% transfusion burden reduction	20/32 (63)	43.7-78.9
Decrease in LIC ≥2 mg/g dry weight‡	5/9 (56)	21.2-86.3

Results shown are the numbers of patients achieving any of the specified endpoints over any unbroken 12-week period on study compared with baseline; only patients receiving dose levels 0.6-1.25 mg/kg luspatercept at any time during the study are shown (63/64 total patients).
Primary endpoint data are shown in bolded text; secondary endpoint data are shown in non-bolded text.
CI, confidence interval; LIC, liver iron concentration.
*One of the 64 patients initially enrolled is not included in the table as the patient did not receive luspatercept ≥0.6 mg/kg during the study.
†One patient classified as non-transfusion-dependent during the initial stage of the study was reclassified as transfusion-dependent prior to entering the extension stage; and is counted as transfusion-dependent in the table.
‡Includes patients with baseline LIC ≥3 mg/g dry weight who were treated for ≥4 months.

Table 3. Adverse events considered related to treatment occurring during the study

Preferred term,* n (%)	Related grade 1-2 TEAEs in ≥2 patients (N = 64)	Related grade 3 TEAEs† (N = 64)
Bone pain	21 (33)	3 (5)
Headache	16 (25)	1 (2)
Myalgia	13 (20)	0
Arthralgia	12 (19)	0
Musculoskeletal pain	10 (16)	0
Back pain	7 (11)	0
Injection site pain	7 (11)	0
Asthenia	6 (9)	2 (3)
Erythroblastosis	3 (5)	0
Injection site erythema	3 (5)	0
Musculoskeletal chest pain	3 (5)	0
Neck pain	3 (5)	0
Pain in jaw	3 (5)	0
Pyrexia	3 (5)	0
Chills	2 (3)	0
Influenza	2 (3)	0
Injection site pruritus	2 (3)	0
Macules	2 (3)	0
Muscle spasms	2 (3)	0
Nausea	2 (3)	0
Pain in extremity	2 (3)	0
Paresthesia	2 (3)	0

Maximum severity reported per patient per preferred term.
TEAE, treatment-emergent adverse event.
*Classified according to the Medical Dictionary for Regulatory Activities (MedDRA). Results shown are the numbers of patients reporting the specified TEAEs.
†No grade 4 adverse events considered related to luspatercept treatment were reported.

Figure Legends

Figure 1. Study design and cohorts. AE, adverse event; NTD, non-transfusion-dependent; TD, transfusion-dependent. The analytic sample size for the primary study endpoint was 63 patients (31 NTD and 32 TD); this included all patients who received 0.6-1.25 mg/kg luspatercept in either the initial or extension stages of the study. Doses below 0.6 mg/kg luspatercept were not considered efficacious. *Patients assigned to the dose-escalation cohorts received luspatercept at dose levels 0.2-1.25 mg/kg; patients were assigned to the following dose levels: 0.2 mg/kg (n = 6 patients; all NTD), 0.4 mg/kg (n = 6 patients; all NTD), 0.6 mg/kg (n = 5 NTD patients, n = 1 TD patient), 0.8 mg/kg (n = 3 NTD patients, n = 3 TD patients), 1.0 mg/kg (n = 2 NTD patients, n = 4 TD patients), and 1.25 mg/kg (n = 1 NTD patient, n = 4 TD patients). †Patients assigned to the expansion cohort and the extension stage received luspatercept at dose levels 0.8-1.25 mg/kg; patients were assigned to the expansion cohort following review of AE and efficacy data for patients in the dose-escalation cohorts. ‡Patients entering the extension with treatment interruption were those who had finished the initial stage of treatment and had completed the end of study visit at least 28 days before receiving the first dose in the extension. §Patients enter the 2-month follow-up after discontinuing from or completing the extension stage; due to the treatment period of 60 months, no patients have yet entered the follow-up period.

Figure 2. Mean (± 95% confidence interval) change in hemoglobin level relative to baseline. (A) Non-transfusion-dependent patients with beta-thalassemia treated with luspatercept 0.2-0.4 mg/kg compared with 0.6-1.25 mg/kg during the initial stage of the study. (B) All non-transfusion-dependent patients with beta-thalassemia treated with luspatercept 0.6-1.25 mg/kg during the initial and/or extension stages of the study.

Figure 3. Transfusion burden reduction versus baseline for patients with beta-thalassemia treated with luspatercept (n = 32). RBC, red blood cell. (A) Percentage change in RBC transfusion burden over a continuous 12-week period post-baseline versus the 12-week baseline period. Each bar represents 1 patient; the best recorded continuous 12-week period of transfusion burden reduction post-baseline was used to calculate the percentage change for each patient. (B) Absolute change in RBC units on study versus baseline. Each circle represents 1 patient's baseline RBC transfusion burden, as absolute number of transfused units in a 12-week period; each line represents the best recorded transfusion burden reduction for the patient over a continuous 12-week period post-baseline. For both panels, only patients with a baseline transfusion burden of ≥2 RBC units and 12-week post-baseline transfusion data are shown.

Figure 4. Liver iron concentration change in (A) non-transfusion-dependent beta-thalassemia patients and (B) transfusion-dependent beta-thalassemia patients. dw, dry weight; LIC, liver iron concentration. Each filled circle represents 1 patient's baseline LIC; each line represents the best recorded change in LIC post-baseline. Only patients with ≥4 months post-baseline LIC are included. Patients noted to have received iron chelation therapy could have received it in the 84 days prior to treatment as well as on study.

Figure 5. Leg ulcer healing in a patient with non-transfusion-dependent beta-thalassemia treated with luspatercept. The patient received 0.4 mg/kg luspatercept in the initial stage of the study and up to 1.25 mg/kg luspatercept in the extension stage. The image example shown is representative of an ulcer that healed within 6 weeks.

Figure 1

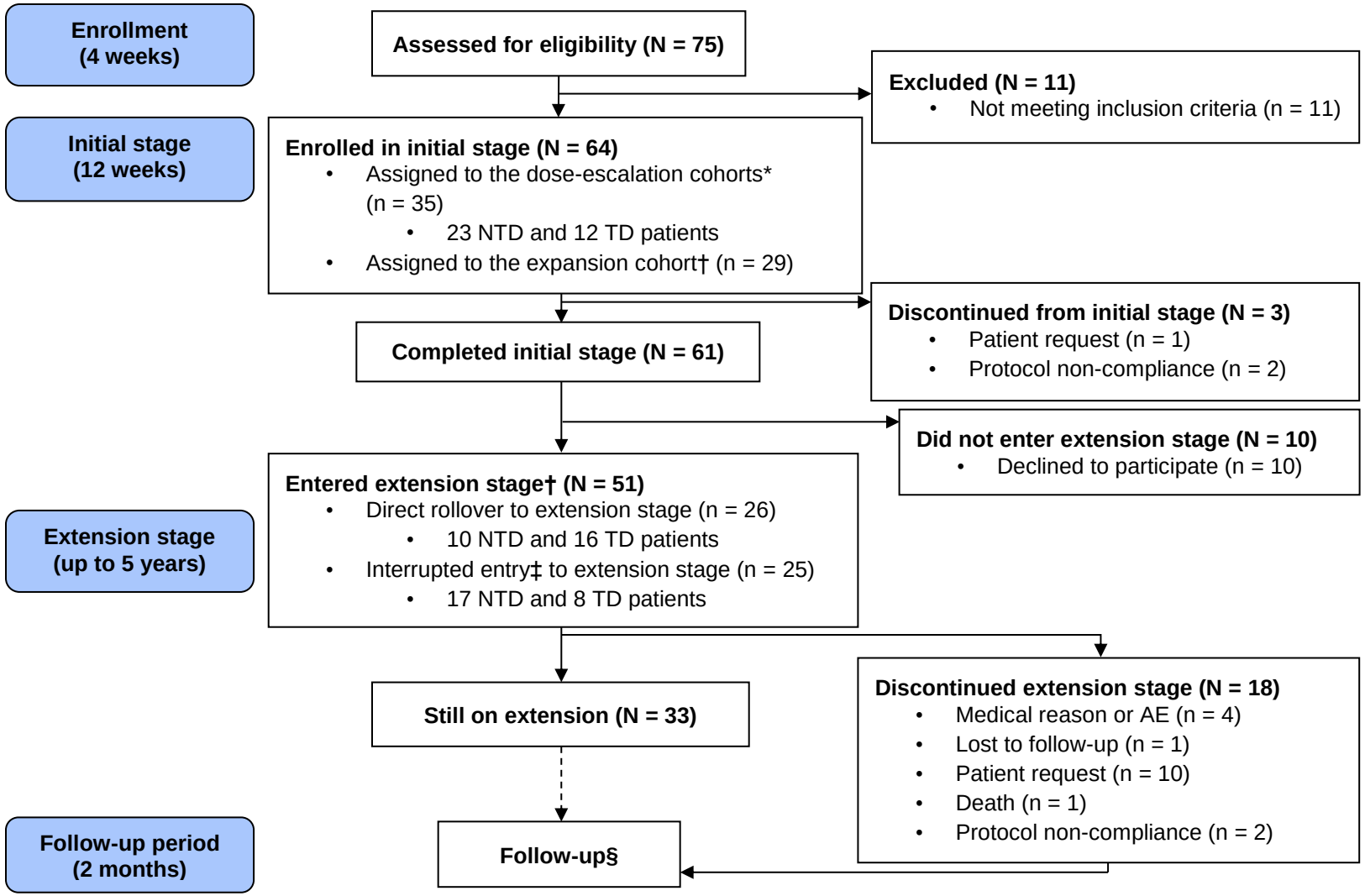
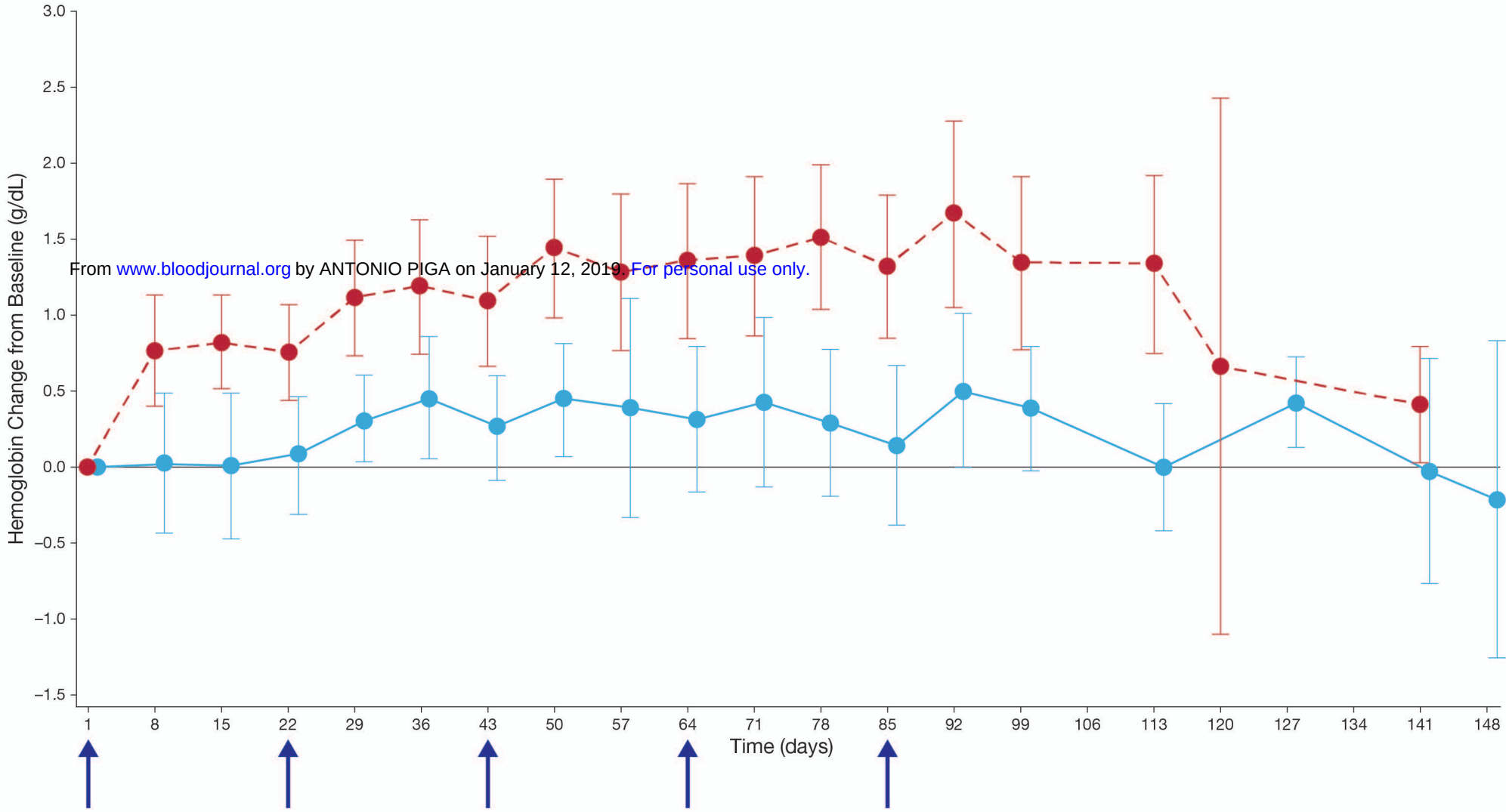
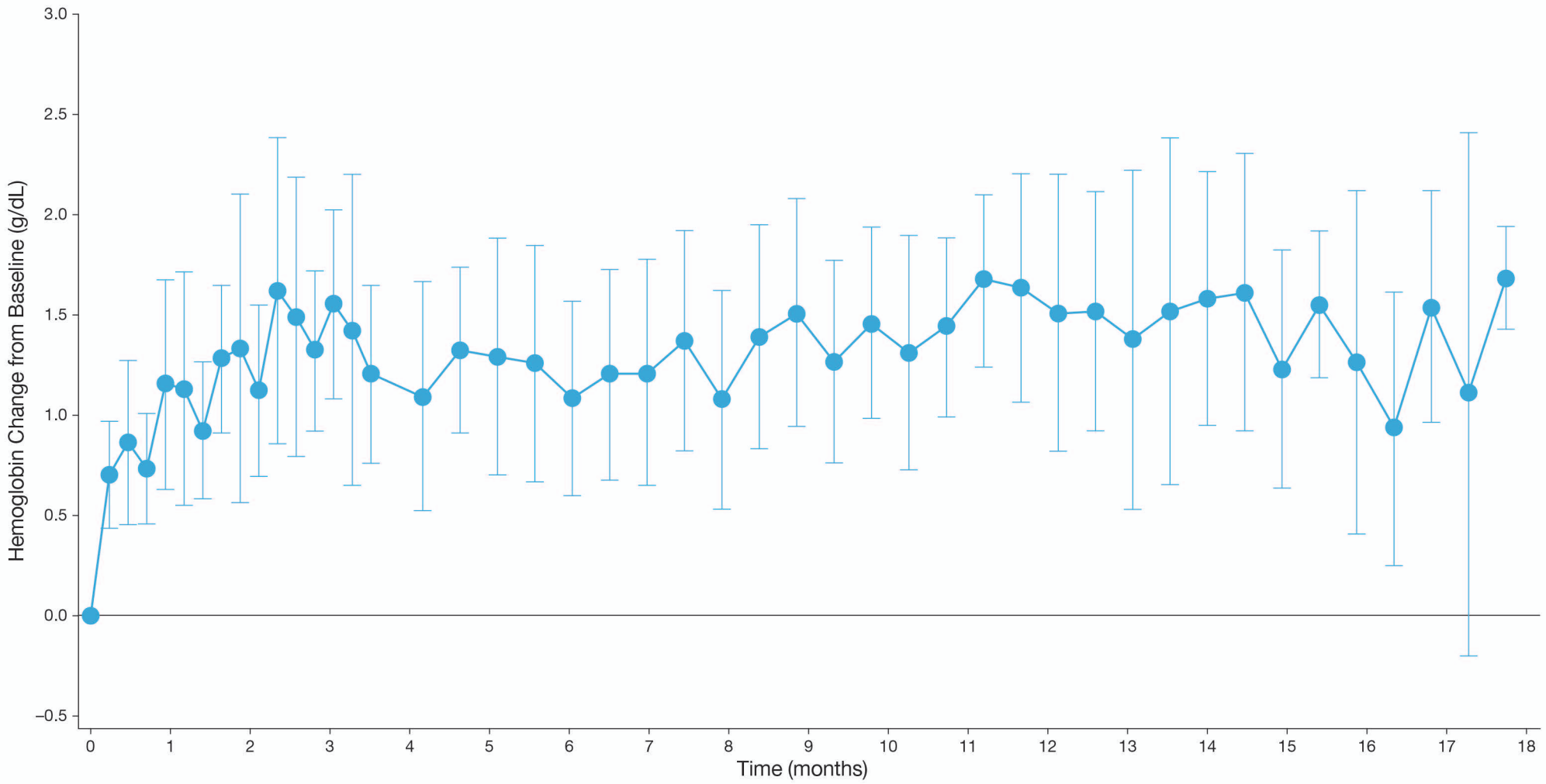

Enrollment (4 weeks)
Assessed for eligibility (N = 75)
Excluded (N = 11)
• Not meeting inclusion criteria (n = 11)
Initial stage (12 weeks)
Enrolled in initial stage (N = 64)
• Assigned to the dose-escalation cohorts* (n = 35)
• 23 NTD and 12 TD patients
• Assigned to the expansion cohort† (n = 29)
Discontinued from initial stage (N = 3)
• Patient request (n = 1)
• Protocol non-compliance (n = 2)
Completed initial stage (N = 61)
Did not enter extension stage (N = 10)
• Declined to participate (n = 10)
Extension stage (up to 5 years)
Entered extension stage† (N = 51)
• Direct rollover to extension stage (n = 26)
• 10 NTD and 16 TD patients
• Interrupted entry‡ to extension stage (n = 25)
• 17 NTD and 8 TD patients
Still on extension (N = 33)
Discontinued extension stage (N = 18)
• Medical reason or AE (n = 4)
• Lost to follow-up (n = 1)
• Patient request (n = 10)
• Death (n = 1)
• Protocol non-compliance (n = 2)
Follow-up period (2 months)
Follow-up§

Figure 2A



No. of patients																					
Luspatercept 0.2–0.4 mg/kg	12	12	12	12	12	12	12	12	12	11	11	12	10	9	12	0	9	0	3	0	4	5
Luspatercept 0.6–1.25 mg/kg	21	21	21	21	21	21	21	18	19	20	20	19	19	17	18	0	13	4			5	

Figure 2B



No. of patients	31	*	*	*	*	*	*	*	*	*	*	*	*	*	23	23	25	19	20	25	22	22	22	21	19	19	19	21	21	16	16	14	13	13	11	12	12	11	8	8	9	8	5	5	4

Figure 3A

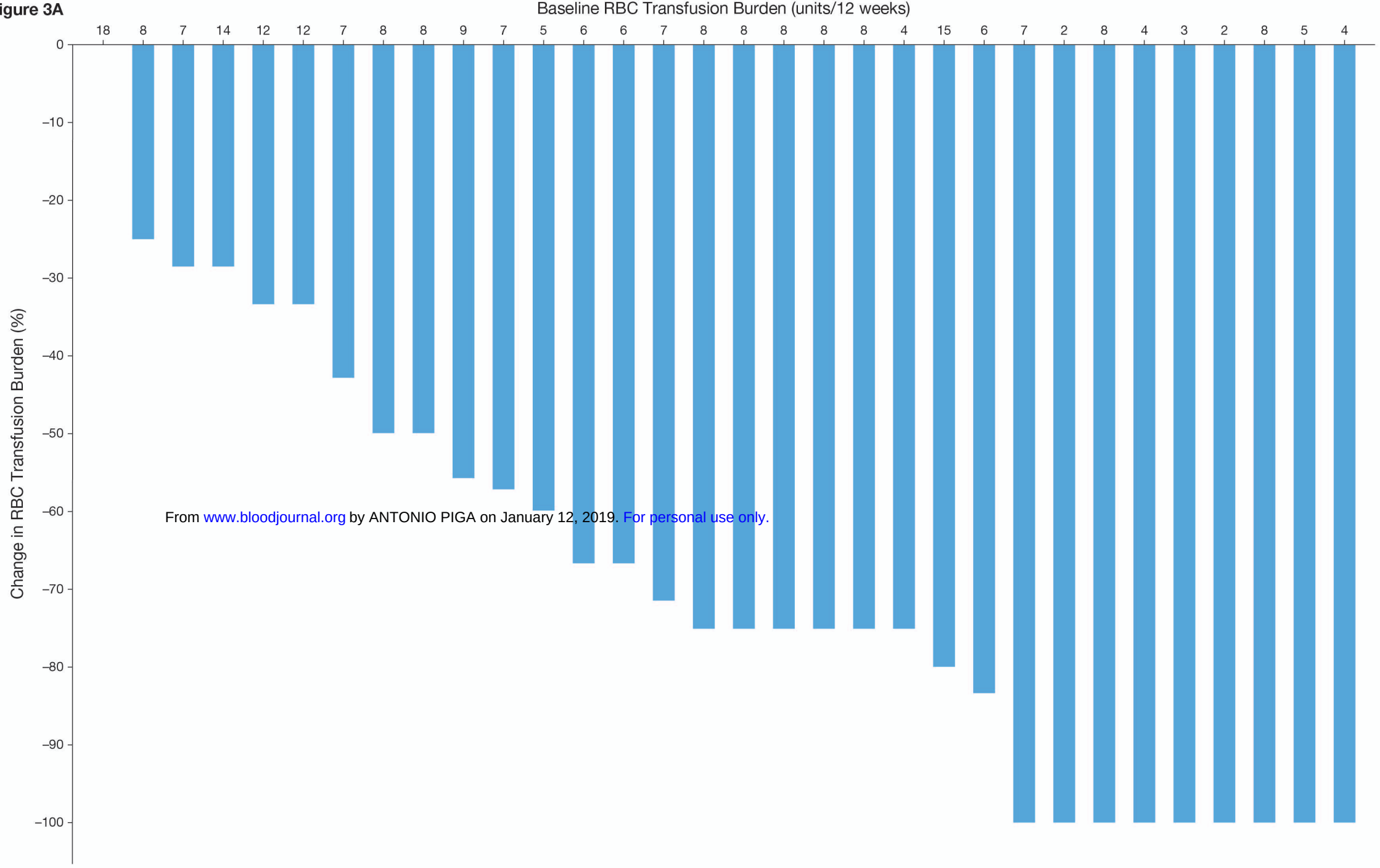


Figure 3B

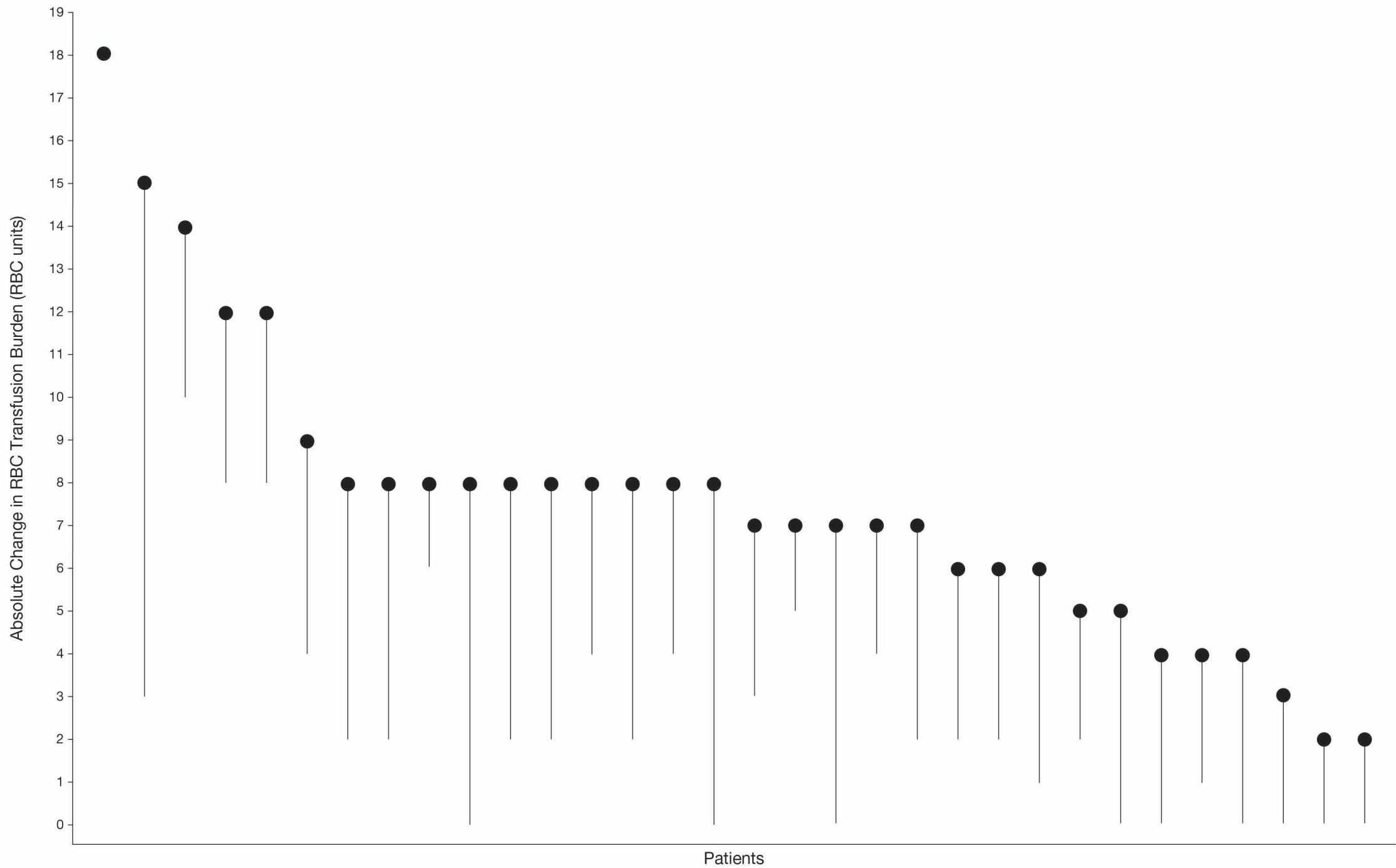


Figure 4A

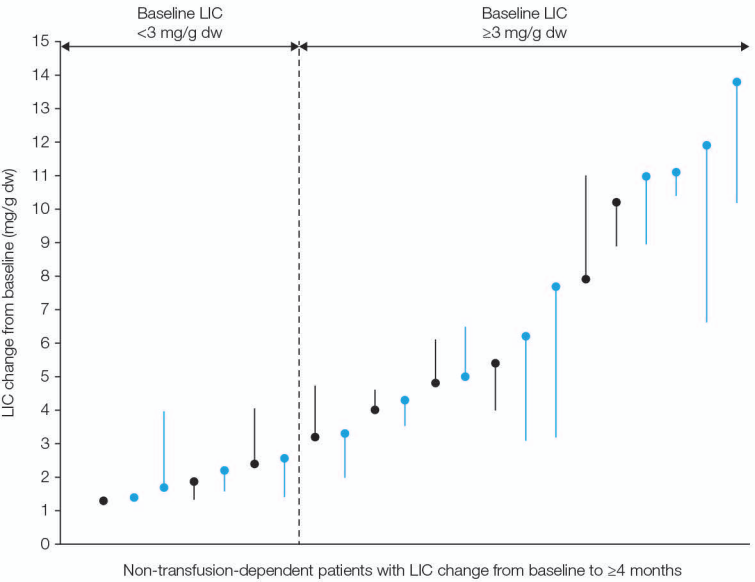


Figure 4B

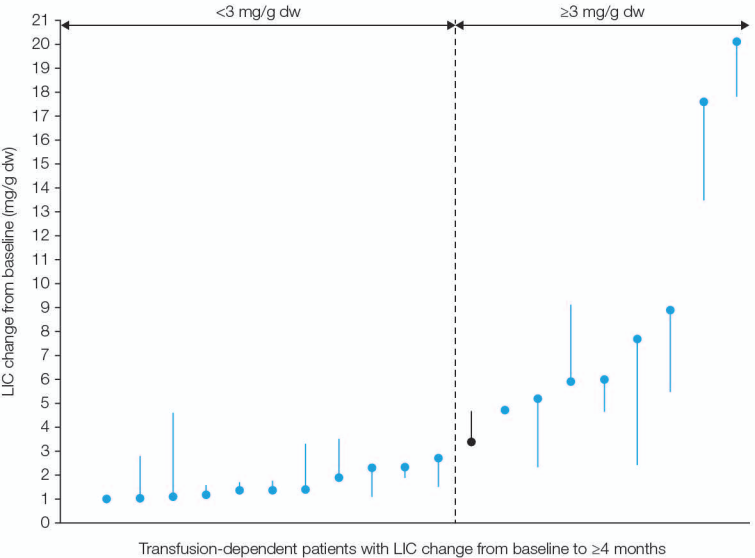
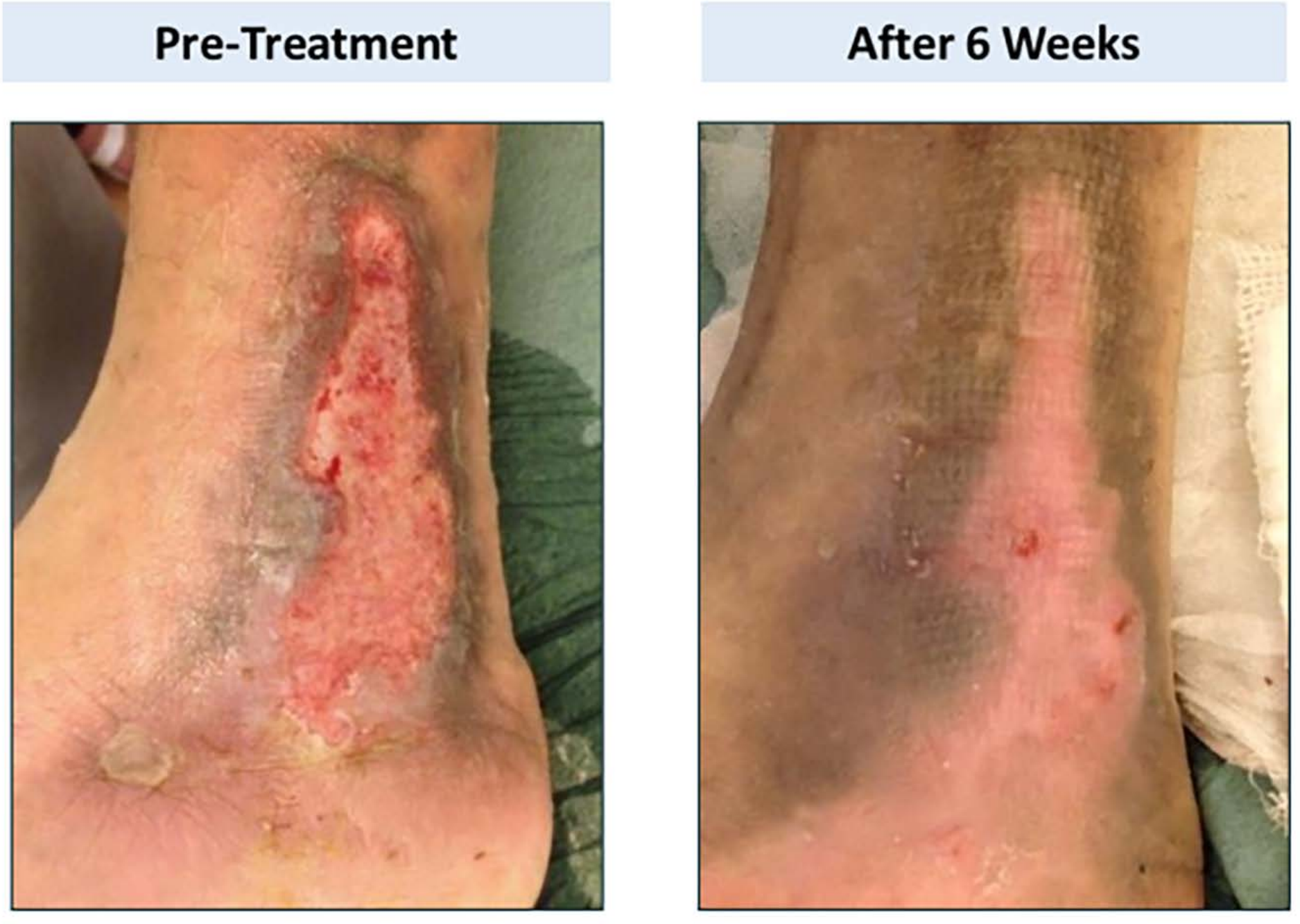


Figure 5



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Luspatercept improves hemoglobin levels and blood transfusion requirements in a study of patients with beta-thalassemia

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