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by Maria Domenica Cappellini, John Porter, Raffaella Origa, Gian Luca Forni, Ersi Voskaridou, Frédéric Galactéros, Ali T. Taher, Jean-Benoît Arlet, Jean-Antoine Ribeil, Maciej Garbowski, Giovanna Graziadei, Chantal Brouzes, Michaela Semeraro, Abderrahmane Laadem, Dimana Miteva, Jun Zou, Victoria Sung, Tatiana Zinger, Kenneth M. Attie, and Olivier Hermine

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Sotatercept, a novel transforming growth factor beta ligand trap, improves anemia in beta-thalassemia: a phase II, open-label, dose-finding study

Maria Domenica Cappellini,[1] John Porter,[2] Raffaella Origa,[3] Gian Luca Forni,[4] Ersi Voskaridou,[5] Frédéric Galactéros,[6] Ali T. Taher,[7] Jean-Benoît Arlet,[8,9,10] Jean-Antoine Ribeil,[11] Maciej Garbowski,[2] Giovanna Graziadei,[1] Chantal Brouzes,[11] Michaela Semeraro,[11] Abderrahmane Laadem,[12] Dimana Miteva,[13] Jun Zou,[12] Victoria Sung,[14] Tatiana Zinger,[13] Kenneth M. Attie[15] and Olivier Hermine.[9,10,16]

[1]Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Department of Clinical Sciences and Community Health, University of Milan, Milan, Italy

[2]Department of Haematology, University College London, London, UK

[3]Day Hospital Talassemia, Ospedale Pediatrico Microcitemico "A.Cao", A.O. "G. Brotzu", Cagliari, Italy

[4]Centro della Microcitemia, Ospedali Galliera, Genova, Italy

[5]Thalassaemia Centre, Laikon General Hospital, Athens, Greece

[6]UMGGR, Hôpital Henri Mondor; Assistance Publique-Hôpitaux de Paris (APHP); UPEC, Créteil, France

[7]Department of Internal Medicine, American University of Beirut Medical Center, Beirut, Lebanon

[8]Department of Internal Medicine, APHP, Hôpital Européen Georges-Pompidou, Paris, France

[9]INSERM UMR1163, CNRS ERL 8254, Institut Imagine, Université Paris Descartes-Sorbonne Paris Cité, Paris, France

[10]Laboratory of Excellence GR-Ex, Paris, France

[11]Laboratory of Onco-hematology, Hôpital Necker-Enfants Malades, Paris, France

[12]Celgene Corporation, Summit, NJ, USA

[13]Celgene Corporation, Boudry, Switzerland

[14]Celgene Corporation, San Francisco, CA, USA

[15]Acceleron Pharma, Cambridge, MA, USA

[16]Department of Hematology, APHP, Hôpital Necker, Paris, France

Correspondence: Prof Maria Cappellini, e-mail: maria.cappellini@unimi.it.

Or Prof Olivier Hermine, e-mail: olivier.hermine@nck.aphp.fr.

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Abstract

β-thalassemia, a hereditary blood disorder caused by defective synthesis of hemoglobin β-globin chains, leads to ineffective erythropoiesis and chronic anemia that may require blood transfusions. Sotatercept (ACE-011) acts as a ligand trap to inhibit negative regulators of late-stage erythropoiesis in the transforming growth factor beta superfamily, correcting ineffective erythropoiesis. In this phase II, open-label, dose-finding study, 16 patients with transfusion-dependent β-thalassemia and 30 patients with non-transfusion-dependent β-thalassemia were enrolled at 7 centers in 4 countries from November 2012 to November 2014. Patients were treated with sotatercept at 0.1, 0.3, 0.5, 0.75, or 1.0 mg/kg to determine a safe and effective dose. Doses were administered by subcutaneous injection every 3 weeks. Patients were treated for ≤22 months. Response was assessed as a ≥20% reduction in transfusion burden sustained for 24 weeks in transfusion-dependent β-thalassemia patients, and an increase in hemoglobin level of ≥1.0 g/dL sustained for 12 weeks in non-transfusion-dependent β-thalassemia patients. Sotatercept was well tolerated. After a median treatment duration of 14.4 months (range 0.6-35.9), no severe life-threatening adverse events were observed; 13% of patients reported serious but manageable adverse events. The active dose of sotatercept was ≥0.3 mg/kg for non-transfusion-dependent β-thalassemia and ≥0.5 mg/kg for transfusion-dependent β-thalassemia patients. Of 30 non-transfusion-dependent β-thalassemia patients treated with ≥0.1 mg/kg sotatercept, 18 (60%) achieved a mean hemoglobin increase ≥1.0 g/dL, and 11 (37%) an increase ≥1.5 g/dL, sustained for ≥12 weeks. Four (100%) transfusion-dependent β-thalassemia patients treated with 1.0 mg/kg sotatercept achieved a transfusion-burden reduction of ≥20%. Sotatercept was effective and well tolerated in patients with β-thalassemia. Most non-transfusion-dependent β-thalassemia patients treated with higher doses achieved sustained increases in hemoglobin level. Transfusion-dependent β-thalassemia patients treated with higher doses of sotatercept achieved notable reductions in transfusion requirement.

The registration number at ClinicalTrials.gov was NCT01571635.

Introduction

β-thalassemia is a hereditary blood disorder caused by defective synthesis of the β-globin chains of hemoglobin (Hb)[1] characterized by ineffective erythropoiesis.[2-4] Mutations in the β-globin genes lead to reduced or absent β-globin chain synthesis, increasing the ratio of α-globin to non-α-globin chains. Due to the relative excess of α-globin chains, α-globin precipitates within erythroblasts as hemichromes, leading to oxidative stress, maturation arrest, membrane damage and apoptosis of late-stage erythroid precursors, and reduced red blood cell (RBC) life span.[4-6] Although erythropoiesis-stimulating agents (ESAs) have been used in patients with β-thalassemia, ineffective erythropoiesis is not corrected.[7] Use of ESAs is, therefore, not recommended for the treatment of β-thalassemia.[7,8]

β-thalassemia phenotypes vary in severity, ranging from asymptomatic thalassemia minor to non-transfusion-dependent thalassemia (NTDT) (including thalassemia intermedia and Hb E-beta thalassemia) to transfusion-dependent thalassemia (TDT) (thalassemia major). Treatment of TDT involves regular and lifelong blood transfusions leading to iron overload; long-term management of iron overload requires regular iron chelation therapy (ICT).[9,10] However, ICT is associated with significant toxicities and requires a high level of treatment adherence and monitoring that can be difficult to manage and may negatively impact patient quality of life.[11,12] Bone marrow transplantation offers potentially curative treatment,[13] but is not possible in all patients,[14] and is associated with significant morbidity and mortality.[15] Gene therapy has shown early promise but is still under investigation.[16] NTDT treatment is based on managing the long-term complications of ineffective erythropoiesis, including chronic anemia and iron overload,[17] using ICT and occasional RBC transfusion.[18,19]

Sotatercept is a ligand trap that inhibits transforming growth factor beta (TGF-β) superfamily members including growth differentiation factor 11 (GDF-11) and activin B.[20,21] GDF-11 is overexpressed in immature erythroblasts in β-thalassemia.[21] Aberrant GDF-11

production may induce expansion of erythroid progenitors and increase oxidative stress, leading to maturation arrest of late erythroid precursors and ineffective erythropoiesis.[21] Preclinical work has shown that administration of an activin receptor IIA (ActRIIA) ligand trap decreases GDF-11 concentration, reduces reactive oxidative stress levels, and promotes terminal maturation in immature erythroblasts.[21] Sotatercept is a novel recombinant fusion protein consisting of the extracellular domain of the human ActRIIA (*ACVR2A*) linked to the human immunoglobulin G1 Fc domain.[20] When administered subcutaneously, sotatercept increased Hb levels and RBC count in healthy, postmenopausal women.[22] In a phase II trial of patients with lower-risk myelodysplastic syndromes (MDS) and anemia, sotatercept reduced transfusion burden in 47% of patients with a high transfusion burden, and increased Hb levels in 58% of patients with a low transfusion burden.[23] In a β-thalassemia mouse model, RAP-011 (a murine analog of sotatercept) improved hematologic parameters including RBC count, total Hb, hematocrit, and mean corpuscular volume.[21]

The aim of this phase II study was to determine a safe, tolerable, and effective dose of sotatercept to increase Hb levels and reduce blood transfusion burden in adults with TDT and NTDT β-thalassemia.

Methods

In this phase II, open-label, dose-finding study, patients were enrolled at 7 centers in France, Greece, Italy, and the United Kingdom (listed in the *Online Supplemental Data*) from November 2012 to November 2014. The study was approved by individual institutional review boards at participating centers and, where appropriate, national health authorities, and was conducted in compliance with the Declaration of Helsinki. All patients provided written informed consent. The ClinicalTtrials.gov registration number is NCT01571635.

Inclusion criteria

Eligible patients were aged ≥18 years with a diagnosis of TDT or NTDT, and an Eastern Cooperative Oncology Group performance status of 0 to 1. Transfusion dependence was defined as receiving ≥2 RBC units every 30 days for ≥168 days prior to study enrollment, with no transfusion-free period of >45 consecutive days during this period. Mean Hb level prior to transfusion was ≤10.5 g/dL in the 168 days prior to enrollment, with the last pretransfusion Hb level preceding enrollment being ≤10.5 g/dL.

Non-transfusion dependence was defined as ≤1 episode of transfusion during the 168 days prior to enrollment; an episode of transfusion was defined as ≤4 RBC units received during the 168 days prior to enrollment. Exclusion criteria are listed in the *Online Supplemental Data*.

Study design

To determine a safe and tolerable dose of sotatercept, a dose-escalation study was carried out. Patients were initially enrolled in 2 cohorts, receiving doses of 0.1 mg/kg and 0.3 mg/kg, administered by subcutaneous injection every 3 weeks. Four dose-escalation cohorts, with doses of 0.5, 0.75, 1.0, and 1.5 mg/kg, were subsequently opened to enrollment. Details of the study design are included in the *Online Supplemental Data*.

Study end points

Primary efficacy end points were a reduction in transfusion burden of ≥20% from pretreatment levels, sustained for 24 weeks in TDT patients, and an increase in Hb level of ≥1.0 g/dL sustained for 12 weeks from mean pretreatment Hb levels in NTDT patients. Hematologic parameters, including Hb levels and RBC counts, were measured on days 1, 8, and 15 (±3 days) of each 3-week sotatercept dose period. Secondary end points included reduction in RBC transfusion burden in TDT patients, Hb level increase from baseline in NTDT patients, and safety. Exploratory end points included iron metabolism markers

(including serum ferritin [SF] and hepcidin), and clinical symptoms associated with ineffective erythropoiesis and anemia (including extramedullary hematopoiesis, leg ulcers, and bilirubin levels).

Statistical analysis

Efficacy analyses were carried out on the intent-to-treat population, which included all patients enrolled for treatment. Efficacy data are presented by assigned dose group. Safety analyses were conducted on the safety population, defined as those patients who received ≥1 dose of sotatercept. Safety data are presented prior to intrapatient dose escalation for sotatercept dose groups and presented post-intrapatient dose escalation for patients overall. The data cutoff date for this analysis was November 27, 2015. Detailed statistical methods are included in the *Online Supplemental Data*.

Results

Patients

As of November 27, 2015, 46 patients had been enrolled from November 2012 to November 2014 – 16 with TDT and 30 with NTDT (*Online Supplemental Figure S1*). Baseline demographic and disease characteristics are shown (Table 1); data are presented by assigned dose level of sotatercept prior to dose escalation. All patients received ≥2 doses of sotatercept. Median duration of treatment was 19.6 months (range, 0.6-35.9) for NTDT patients and 13.8 months (range, 1.4-27.7) for TDT patients.

Erythroid response

NTDT patients

Of the 30 patients with NTDT treated with sotatercept doses of 0.1 to 1.0 mg/kg, 18 (60%) achieved a mean Hb increase of ≥1.0 g/dL, and 11 (37%) had a mean Hb increase of ≥1.5 g/dL sustained for ≥12 weeks (Figure 1A). The highest rate of response was seen with 0.75 mg/kg sotatercept (86% [6 of 7] patients achieved an Hb increase of ≥1.5 g/dL).

Responders receiving sotatercept 0.5 mg/kg experienced the greatest maximum increase in Hb levels within 12 weeks from baseline (3.2±0.2 g/dL) (Figure 1B). No patients receiving sotatercept 0.1 mg/kg achieved a response.

Mean change in Hb levels from baseline in NTDT patients is shown (Figure 2). No significant differences in reticulocyte count and fetal hemoglobin (HbF) levels were reported for NTDT patients during the study (data not shown). The starting active dose of sotatercept in patients with NTDT, based on the observed responses, was ≥0.3 mg/kg.

Of 4 patients with NTDT receiving concomitant hydroxyurea therapy at enrollment, 3 continued to receive hydroxyurea during the study (dose range 54-84) without interruption or modification; 2 achieved a mean Hb increase of ≥1 g/dL sustained for ≥12 weeks.

TDT patients

Of 16 patients with TDT treated with sotatercept, 10 (63%) achieved transfusion burden reduction of ≥20% sustained for ≥24 weeks; 7 patients (44%) achieved a reduction of ≥33%, and 2 patients (13%), a reduction of ≥50% (Figure 3). Five of the 10 patients achieved transfusion burden reduction while maintaining stable or improved Hb levels at 12 weeks. The mean change in Hb level from baseline to the end of treatment was 0.7 g/dL in all patients with TDT. Nine patients (56%) achieved a reduction in transfusion frequency over 24 weeks, receiving fewer transfusions per 24 weeks during treatment, while maintaining stable or improved Hb levels over pretransfusion levels. Four patients (25%) also achieved a reduction in transfusion frequency over 24 weeks; however, Hb levels were lower compared with pretransfusion levels. The remaining 3 (19%) patients (2 receiving 0.75 mg/kg sotatercept, 1 receiving 0.3 mg/kg sotatercept) experienced an increase in transfusion frequency, requiring between 0.7-1.0 additional transfusions per 24 weeks, representing an increase of 6.7-17.2% from baseline. The active starting dose of sotatercept in patients with TDT, based on the observed responses, was ≥0.5 mg/kg.

Bilirubin

For NTDT patients, indirect bilirubin and total bilirubin decreased by 5-25% for patients receiving 0.3, 0.75, and 1.0 mg/kg (*Online Supplemental Figure S2A*). Among TDT patients with available bilirubin data at baseline, indirect bilirubin and total bilirubin increased by 15-80% during treatment from baseline, at all dose levels (*Online Supplemental Figure S2B*). However, the changes were not statistically significant, and there was wide variability in the level of change.

Clinical response

Extramedullary masses

The effect of sotatercept on extramedullary masses (EMM) as measured by MRI was reported for 4 patients with NTDT and 1 with TDT. The TDT patient experienced a reduction in EMM volume at 12 months posttreatment with sotatercept 0.75 mg/kg (data not shown); however, due to unequal distribution of the mass, estimation of the exact volume was not possible. One NTDT patient experienced reduction in EMM volume at 12 months posttreatment with sotatercept 0.3 mg/kg ranging from 0.4% to 59.3% across 3 separate EMM (*Online Supplemental Figure S3A*), alongside a stable increase in Hb level. Another NTDT patient treated with sotatercept 1.0 mg/kg experienced volume increases of 67% and 55% in 2 EMM, at 7 months posttreatment (*Online Supplemental Figure S3B*). This correlated with an increase in Hb levels (6 g/dL at baseline *versus* 8 g/dL at 7 months posttreatment). Two NTDT patients experienced a change in EMM volume of between −6.3% and +22.5% at 11 months posttreatment with sotatercept 1.0 mg/kg (data not shown).

Leg ulcers

Seven NTDT patients had a history of leg ulcers at baseline; the effect of sotatercept on chronic leg ulcers was reported for 1 NTDT patient. The chronic leg ulcers improved, and the dischromic area was reduced following treatment with sotatercept 0.5 mg/kg for 6 months (*Online Supplemental Figure S4*).

RBC morphology

Changes to RBC morphology were recorded for all patients during the study. Representative images of the changes observed are provided (*Online Supplemental Figure S5*). Reduction in hypochromia, anisocytosis, and poikilocytosis, and the proportion of target cells were reported, and were associated with increased Hb levels.

Serum ferritin

Among NTDT patients who responded to sotatercept treatment, mean levels of SF decreased regardless of ICT status (*Online Supplemental Figure S6 A-B*). In contrast to NTDT patients, all TDT patients received ICT. Levels of SF decreased over time in TDT patients (*Online Supplemental Figure S6C*).

Safety

Twenty-five of 46 patients (54%) experienced ≥1 treatment-related adverse event (AE); the most common treatment-related AEs in all patients were bone pain (26% [n=12]), arthralgia (15% [n=7]), back pain (11% [n=5]), asthenia/fatigue (11% [n=5]), and hypertension (11% [n=5]) (Table 2). Treatment-related AEs led to discontinuation in 8 patients (17%) (Table 2); of these 8 patients, 7 were NTD and 1 was TD.

All 46 patients experienced ≥1 treatment-emergent AE (Online Supplemental Table S1); 6 (13%) experienced ≥1 serious AE (*Online Supplemental Table S2*). Nine patients (20%) experienced ≥1 grade 3-4 treatment-emergent AE; hypertension and anemia were the most frequent (4% [n=2]) (*Online Supplemental Table S3*). Incidences of anemia were not related to treatment with sotatercept and resolved within 1 treatment cycle.

The most common treatment-emergent AEs of any grade among the 30 NTDT patients were headache (53% [n=16]), arthralgia (47% [n=14]), cough (40% [n=12]), and asthenia/fatigue (37% [n=11]) (*Online Supplemental Table S4*).

The most common treatment-emergent AEs among the 16 TDT patients were bone pain (63% [n=10]), back pain (56% [n=9]), asthenia/fatigue (56% [n=9]), headache (44% [n=7]), and arthralgia (44% [n=7]) (*Online Supplemental Table S4*). Most treatment-emergent AEs were mild and did not lead to treatment discontinuation (Table 2).

Bone pain was equally reported among sotatercept dose cohorts; mean time to first onset of bone pain was similar between dose cohorts, and the duration of bone pain was short (mean 12.0 days).

Asthenia was less frequent at higher dose levels; 13% (3 incidences/23 doses received) *versus* 5% (4 incidences/81 doses received) for TDT patients and 36% (47 incidences/132 doses received) *versus* 5% (3 incidences/67 doses received) for NTDT patients at 0.1 mg/kg and 1.0 mg/kg, respectively) (data not shown).

Observed differences between baseline and on-treatment laboratory values for liver and kidney function, including alanine aminotransferase, aspartate aminotransferase, and serum creatinine levels, were within the ranges expected for this population and were not treatment related (data not shown).

Dose delays for safety reasons were reported in 8 patients, all with NTDT.

Discussion

β-thalassemia is characterized by chronic anemia due to ineffective erythropoiesis and peripheral hemolysis. The current treatment for TDT is demanding, requiring regular blood transfusions and lifelong ICT. No standardized treatment for NTDT is available, and no treatment is currently available to improve ineffective erythropoiesis. Previous investigatory treatments for NTDT have included HbF modulators such as butyrates, azacitidine, or

hydroxyurea, with or without erythropoietin; however, results with these and other modulators of HbF have been inconsistent.[24,25]

Sotatercept, a recombinant fusion protein that acts as a ligand trap for TGF-β superfamily ligands, is hypothesized to improve late-stage erythropoiesis by reducing proliferation of early erythroid progenitors and precursors while increasing differentiation and maturation of late-stage RBC precursors.[20,21] This drug may therefore present a novel approach to restoring effective erythropoiesis in β-thalassemia.

In this phase II, open-label, dose-finding study, sotatercept exhibited a good safety profile, and was tolerated by most patients. The most frequent AEs were bone, articular or back pain, and asthenia/fatigue. Discontinuations due to AEs were rare, and the incidence of grade 3-4 AEs was low. Changes in laboratory values for liver and kidney function were not thought to be treatment-related and were in line with fluctuations seen in β-thalassemia patients without treatment. The active dose of sotatercept was ≥0.3 mg/kg for NTDT and ≥0.5 mg/kg for TDT patients. Importantly, concomitant administration of hydroxyurea did not appear to interfere with response to sotatercept, or with treatment compliance.

The majority (75%) of NTDT patients treated with higher doses (0.75-1.0 mg/kg) of sotatercept achieved sustained increases in Hb of ≥1.0 g/dL. Similarly, 66% of TDT patients treated with higher doses of sotatercept (0.75-1.0 mg/kg) achieved reductions of ≥33% in RBC transfusion requirement. Hb increase and RBC transfusion reduction correlated with increased serum exposure to sotatercept (data not shown), although responses were not proportional to sotatercept dose due to interpatient variability in serum drug exposure. The small number of patients in each dose group makes comparison between sotatercept levels difficult. Studies are ongoing to identify any differences in baseline characteristics between responders and non-responders.

Ineffective erythropoiesis in β-thalassemia is associated with increased iron absorption, and patients with TDT often require regular RBC transfusions, further increasing the risk of iron overload. Reducing ineffective erythropoiesis and transfusion burden will decrease the rate of iron loading and associated complications such as heart, liver, and endocrine disorders. In this study, reduced SF levels were observed in sotatercept-treated patients with NTDT and TDT in a dose-dependent manner, regardless of concurrent treatment with ICT. Although these results suggest that sotatercept may reduce iron absorption by reducing ineffective erythropoiesis, other mechanisms such as removal or redistribution of iron from overloaded organs may also contribute to reduced iron levels. Further studies will be required to elucidate the mechanism of action; however, reduction in iron overload with long-term use may translate into improved patient outcomes.

Sotatercept treatment was associated with a reduction in the volume of EMM in 2 patients with recorded data – 1 with NTDT and 1 with TDT – as measured by MRI. However, this change in EMM volume was not observed for all patients, and, due to technical difficulties, precise estimation of EMM volume was not always possible. Reduction in EMM did not appear to correlate with response. Further study is needed to determine the effect of sotatercept on EMM.

In rodent models, sotatercept acts on ineffective erythropoiesis to reduce production of α-globin aggregates and apoptosis of late erythroid precursors, thereby increasing the efficiency of RBC production and Hb levels.[21,26] Although Hb levels increased in NTDT patients during the study, no significant differences in HbF or reticulocyte count were reported. In a small subset of patients, normalization of RBC morphology was reported and associated with increasing Hb levels over time. These data suggest that sotatercept may increase the lifespan of RBCs in part by improving reticulocyte quality. These changes are consistent with the mode of action of sotatercept as a ligand trap for TGF-β superfamily members, including GDF-11. Binding of sotatercept to GDF-11 inhibits SMAD2/3 signaling,

reducing TGF-β superfamily ligand signaling and thereby promoting terminal differentiation of erythroblasts.[20]

There are some limitations to this study, notably the small number of patients enrolled, which limited the ability to draw comparisons between different sotatercept dose groups. The inclusion of both NTDT and TDT β-thalassemia patients also resulted in patients being grouped into smaller subgroups that further limit the scope of the study, especially as intrapatient dose escalation was allowed. The short duration of follow-up may also be a limitation, and longer-term follow-up would provide information on the long-term clinical efficacy and safety of sotatercept.

This study demonstrated the safety and efficacy of sotatercept in patients with β-thalassemia. Improvement in Hb levels and reduction in transfusion burden with sotatercept treatment has also been demonstrated in a phase II study of anemia patients with lower-risk MDS. [23] This suggests that sotatercept may work to decrease ineffective erythropoiesis in multiple disease states via a single underlying mode of action. Preliminary data with sotatercept led to the initiation of a similar phase II study in β-thalassemia of the related recombinant fusion protein, luspatercept. Luspatercept has more selective activity on GDF-11, and is also safe and effective in the treatment of β-thalassemia (Piga A, Perrotta S, Gamberini MR, Voskaridou E, Melpignano A, Filosa A, Caruso V, Pietrangelo A, Longo F, Tartaglione I, Borgna-Pignatti C, Zhang X, Laadem A, Sherman M, and Attie KM, manuscript submitted) and MDS.[27] Luspatercept is comprised of the modified extracellular domain of human ActRIIB linked to the human IgG Fc domain,[26,28] and has a similar mode of action to sotatercept but does not bind to other members of the TGF-β superfamily, such as activin A.[20,26] A double-blind, randomized, placebo-controlled phase III trial of luspatercept in patients who require regular RBC transfusions due to β-thalassemia has recently completed recruitment (BELIEVE; NCT02604433). Luspatercept is also being studied in a phase III trial of patients with very low-, low- and intermediate-risk MDS (MEDALIST; NCT02631070).

While the decision was made not to advance trials of sotatercept in β-thalassemia due to binding of sotatercept to activin A, sotatercept represents the first drug developed in its class, functioning as a TGF-β superfamily inhibitor to correct ineffective erythropoiesis. TGF-β superfamily inhibition may provide an alternative or complementary treatment option for patients with β-thalassemia.

Authorship

Contribution: A.L., J.A.R., J.B.A., O.H., M.D.C., and J.P. contributed to the study concept and design; all authors participated in the acquisition, analysis, or interpretation of data; M.D.C., O.H., A.L., and T.Z. drafted the manuscript; all authors critically revised the manuscript for important intellectual content; J.Z. performed the statistical analyses; A.L. and K.M.A. obtained funding; A.L. and T.Z. provided administrative, technical, or material support; T.Z. and D.M. supervised the study; M.D.C. 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; all authors had full access to all of the data in the study and are fully responsible for content and editorial decisions for this manuscript.

Conflict-of-interest disclosure: M.D.C. has served on advisory boards at Celgene Corporation, Novartis, and Sanofi/Genzyme. J.P. has received research funding from Novartis, and has served on advisory boards at Agios Pharmaceuticals, Bluebird Bio, Celgene Corporation, and Novartis. R.O. has received honoraria from Apopharma and Novartis; and has served on advisory boards at Bluebird Bio. G.L.F. has received research funding from Novartis and Shire; has been a consultant for Roche; has received travel expenses from Novartis; and is on the data safety monitoring board of Apopharma. F.G. has received grants from Addmedica and Novartis. A.T.T. has received research funding from Celgene Corporation, La Jolla Pharmaceuticals, Novartis Pharmaceuticals, and Roche; and has received honoraria from Novartis Pharmaceuticals. J.A.R. has received grants from Addmedica and Vitalaire; and has been a consultant for Bluebird Bio, Cydan, and Novartis.

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References

1. Galanello R, Origa R. Beta-thalassemia. Orphanet J Rare Dis. 2010;5:11.
2. Malamos B, Belcher EH, Gyftaki E, Binopoulos D. Simultaneous studies with Fe59 and Cr51 in congenital haemolytic anaemias. Nucl Med (Stuttg). 1961;2:1-20.
3. Weatherall DJ, Clegg JB. The thalassemia syndromes. 2nd ed. Oxford, England: Blackwell Scientific Publications; 1972.
4. Arlet JB, Dussiot M, Moura IC, Hermine O, Courtois G. Novel players in β-thalassemia dyserythropoiesis and new therapeutic strategies. Curr Opin Hematol. 2016;23(3):181-188.
5. Rund D, Rachmilewitz E. Beta-thalassemia. N Engl J Med. 2005;353(11):1135-1146.
6. Melchiori L, Gardenghi S, Rivella S. Beta-thalassemia: HiJAKing ineffective erythropoiesis and iron overload. Adv Hematol, 2010;2010:938640.
7. Fibach E, Rachmilewitz EA. Does erythropoietin have a role in the treatment of β-hemoglobinopathies? Hematol Oncol Clin North Am. 2014;28(2):249-263.
8. Gardenghi S, Grady RW, Rivella S. Anemia, ineffective erythropoiesis, and hepcidin: interacting factors in abnormal iron metabolism leading to iron overload in β-thalassemia. Hematol Oncol Clin North Am. 2010;24(6):1089-1107.

9. Cappellini MD, Cohen A, Porter J, Taher A, Viprakasit V. Guidelines for the management of TD thalassemia (TDT). 3rd ed. Nicosia, Cyprus: Thalassemia International Federation; 2014.
10. Rund D. Thalassemia 2016: modern medicine battles an ancient disease. Am J Hematol. 2016;91(1):15-21.
11. Haghpanah S, Nasirabadi S, Ghaffarpasand F, et al. Quality of life among Iranian patients with beta-thalassemia major using the SF-36 questionnaire. Sao Paulo Med J. 2013;131(3):166-172.
12. Dahlui M, Hishamshah MI, Rahman AJ, Aljunid SM. Quality of life in transfusion-dependent thalassemia patients on desferrioxamine treatment. Singapore Med J. 2009;50(8):794-799.
13. Baronciani D, Angelucci E, Potschger U, et al. Hematopoietic stem cell transplantation in thalassemia: a report from the European Society for Blood and Bone Marrow Transplantation Hemoglobinopathy Registry, 2000–2010. Bone Marrow Transplant. 2016;51(4):536-541.
14. Qari MH, Wali Y, Albagshi MH, et al. Regional consensus opinion for the management of beta thalassemia major in the Arabian Gulf area. Orphanet J Rare Dis. 2013;8:143.
15. Angelucci E, Matthes-Martin S, Baronciani D, et al. Hematopoietic stem cell transplantation in thalassemia major and sickle cell disease: indications and management recommendations from an international expert panel. Haematologica. 2014;99(5):811-820.
16. Negre O, Eggimann AV, Beuzard Y, et al. Gene therapy of the β-hemoglobinopathies by lentiviral transfer of the β(A(T87Q))-globingene. Hum Gene Ther. 2016;27(2):148-165.
17. Saliba AN, Taher AT. Morbidities in non-transfusion-dependent thalassemia. Ann N Y Acad Sci. 2016;1368(1):82-94.

18. Taher A, Vichinsky E, Musallam K, Cappellini MD, Viprakasit V. Blood transfusion. In: Weatherall D, ed. Guidelines for the management of non transfusion dependent thalassaemia (NTDT). Nicosia, Cyprus: Thalassaemia International Federation; 2013.
19. Rachmilewitz EA, Giardina PJ. How I treat thalassemia. Blood. 2011;118(13):3479-3488.
20. Carrancio S, Markovics J, Wong P, et al. An activin receptor IIA ligand trap promotes erythropoiesis resulting in a rapid induction of red blood cells and haemoglobin. Br J Haematol. 2014;165(6):870-882.
21. Dussiot M, Maciel TT, Fricot A, et al. An activin receptor IIA ligand trap corrects ineffective erythropoiesis in β-thalassemia. Nat Med. 2014;20(4):398-407.
22. Ruckle J, Jacobs M, Kramer W, et al. Single-dose, randomized, double-blind, placebo-controlled study of ACE-011 (ActRIIA-IgG1) in postmenopausal women. J Bone Miner Res. 2009;24(4):744-752.
23. Komrokji R, Garcia-Manero G, Ades L, et al. Sotatercept with long-term extension for the treatment of anemia in patients with lower-risk myelodysplastic syndromes: a phase 2, dose-ranging trial. Lancet Haematol. 2018;5(2):e63-e72.
24. Porter J, Garbowski M. Novel erythropoiesis stimulating agents in thalassemia. European Hematology Association Education Programme. 2015;9:311-320.
25. Taher AT, Musallam KM, Karimi M, et al. Overview on practices in thalassemia intermedia management aiming for lowering complication rates across a region of endemicity: the OPTIMAL CARE study. Blood. 2010;115(10):1886-1892.
26. Suragani RN, Cadena SM, Cawley SM, et al. Transforming growth factor-β superfamily ligand trap ACE-536 corrects anemia by promoting late-stage erythropoiesis. Nat Med. 2014;20(4):408-414.
27. Platzbecker U, Germing U, Götze KS, et al. Luspatercept for the treatment of anaemia in patients with lower-risk myelodysplastic syndromes (PACE-MDS): a multicentre, open-label, phase 2 dose-finding study with long-term extension study. Lancet Oncol. 2017;18(10):1338-1347.

28. Attie KM, Allison MJ, McClure T, et al. A phase 1 study of ACE-536, a regulator of erythroid differentiation, in healthy volunteers. Am J Hematol. 2014;89(7):766-770.

Table 1. Baseline characteristics for NTDT and TDT patients treated with sotatercept by assigned dose level.

Characteristic	Sotatercept dose					
	0.1 mg/kg (n=8)	0.3 mg/kg (n=9)	0.5 mg/kg (n=8)	0.75 mg/kg (n=12)	1.0 mg/kg (n=9)	Overall (N=46)
NTDT patients	**n=6**	**n=6**	**n=6**	**n=7**	**n=5**	**n=30**
Age, years						
Median	39.5	47.0	39.0	43.0	41.0	42.0
Range	32.0-53.0	37.0-55.0	34.0-54.0	29.0-65.0	19.0-52.0	19.0-65.0
Female, n (%)	3 (50)	3 (50)	3 (50)	6 (86)	1 (20)	16 (53)
Weight, kg						
Median	54.0	58.0	64.2	56.0	62.0	58.9
Range	48.0-85.0	53.6-65.0	41.0-75.0	47.6-75.0	56.0-67.0	41.0-85.0
Hb, g/dL						
Median	8.8	8.5	8.5	8.7	7.6	8.4
Range	5.9-10.7	6.0-9.5	6.4-9.3	7.1-9.6	6.6-9.4	5.9-10.7
Mean±SD	8.6±1.68	8.3±1.26	8.2±1.13	8.5±0.89	7.7±1.04	8.3±1.18
MCV, fL						
Mean	76.2	75.6	75.5	63.2	78.6	73.3

Characteristic	Sotatercept dose					
	0.1 mg/kg (n=8)	**0.3 mg/kg (n=9)**	**0.5 mg/kg (n=8)**	**0.75 mg/kg (n=12)**	**1.0 mg/kg (n=9)**	**Overall (N=46)**
Median	75.8	75.8	68.4	61.1	80.3	72.1
Range	67.7-83.8	61.6-90.5	62.6-103.4	54.6-80.4	70.6-89.3	54.6-103.4
TDT patients	**n=2**	**n=3**	**n=2**	**n=5**	**n=4**	**n=16**
Age, years						
Median	41.5	34.0	35.0	45.0	39.5	35.5
Range	34.0-49.0	23.0-39.0	34.0-36.0	33.0-51.0	27.0-46.0	23.0-51.0
Female, n (%)	1 (50)	1 (33)	0 (0)	2 (40)	2 (50)	6 (38)
Weight, kg						
Median	49.4	65.7	70.7	58.0	63.0	60.1
Range	46.9-51.8	52.0-71.0	60.5-80.8	48.5-83.0	53.5-85.9	46.9-85.9
β-thalassemia major, n (%)	2 (100)	1 (33)	2 (100)	3 (60)	4 (100)	12 (75)
β-thalassemia intermedia[*], n (%)	0	2 (67)	0	2 (40)	0	4 (25)
Transfusion burden, RBC units/24 weeks[†]	15, 33	14, 18, 33	30, 30	8, 18, 18, 30, 35	18, 18, 18, 24	-

Characteristic	Sotatercept dose					
	0.1 mg/kg (n=8)	0.3 mg/kg (n=9)	0.5 mg/kg (n=8)	0.75 mg/kg (n=12)	1.0 mg/kg (n=9)	Overall (N=46)
Hb, g/dL						
Median	9.5	8.6	9.8	8.9	9.6	9.3
Range	9.3-9.7	8.5-10.6	9.4-10.1	8.0-10.0	8.6-10.9	8.0-10.9

NTDT: non-transfusion-dependent β-thalassemia; TDT: transfusion-dependent β-thalassemia; Hb: hemoglobin; SD: standard deviation; MCV: mean corpuscular volume; RBC: red blood cell. [*]Patients with β-thalassemia intermedia gene mutations who met the transfusion burden requirement were classified as having TDT. [†]Values presented for transfusion burden for individual TDT patients.

Table 2. Incidence of treatment-related AEs of any grade occurring in sotatercept-treated patients in any dose cohort.

AEs, n (%)	Sotatercept dose (data presented prior to intrapatient dose escalation)					Overall (data presented post-intrapatient dose escalation) (N=46)
	0.1 mg/kg (n=8)	0.3 mg/kg (n=9)	0.5 mg/kg (n=8)	0.75 mg/kg (n=12)	1.0 mg/kg (n=9)	
Patients with ≥1 treatment-emergent AE	8 (100)	9 (100)	8 (100)	12 (100)	9 (100)	46 (100)
Patients with ≥1 treatment-related AE[*]	2 (25)	2 (22)	4 (50)	8 (67)	6 (67)	25 (54)
Bone pain	2 (25)	2 (22)	2 (25)	1 (8)	4 (44)	12 (26)
Arthralgia	0	0	1 (13)	3 (25)	1 (11)	7 (15)
Back pain	0	0	1 (13)	2 (17)	2 (22)	5 (11)
Asthenia/fatigue	0	0	1 (13)	3 (25)	1 (11)	5 (11)
Hypertension	0	0	1 (13)	2 (17)	1 (11)	5 (11)
Pain in extremity	0	0	1 (13)	2 (17)	0	3 (7)
Patients with treatment-emergent AEs leading to discontinuation[†]	2 (25)	0	1 (13)	3 (25)	2 (22)	8 (17)

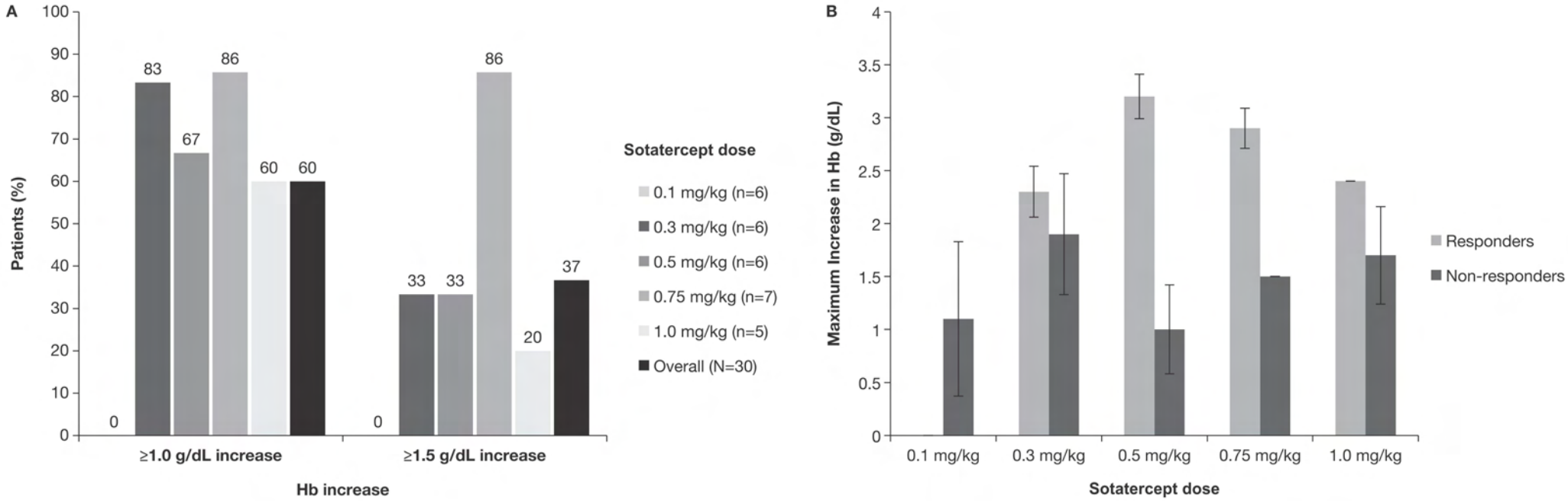
AEs, n (%)	Sotatercept dose (data presented prior to intrapatient dose escalation)					Overall (data presented post-intrapatient dose escalation) (N=46)
	0.1 mg/kg (n=8)	0.3 mg/kg (n=9)	0.5 mg/kg (n=8)	0.75 mg/kg (n=12)	1.0 mg/kg (n=9)	
Treatment-emergent AEs leading to discontinuation[‡]						
Hypertension	0	0	0	2 (17)	0	2 (4)
Superficial thrombophlebitis	1 (13)	0	0	0	0	1 (2)
Injection site erythema	0	0	0	1 (8)	0	1 (2)
Pyrexia	0	0	0	0	1 (11)	1 (2)
Extramedullary hematopoiesis	0	0	0	0	1 (11)	1 (2)
Ventricular extrasystoles	0	0	1 (13)	0	0	1 (2)
Hypersensitivity	0	0	0	1 (8)	0	1 (2)
Bone pain	1 (13)	0	0	0	0	1 (2)

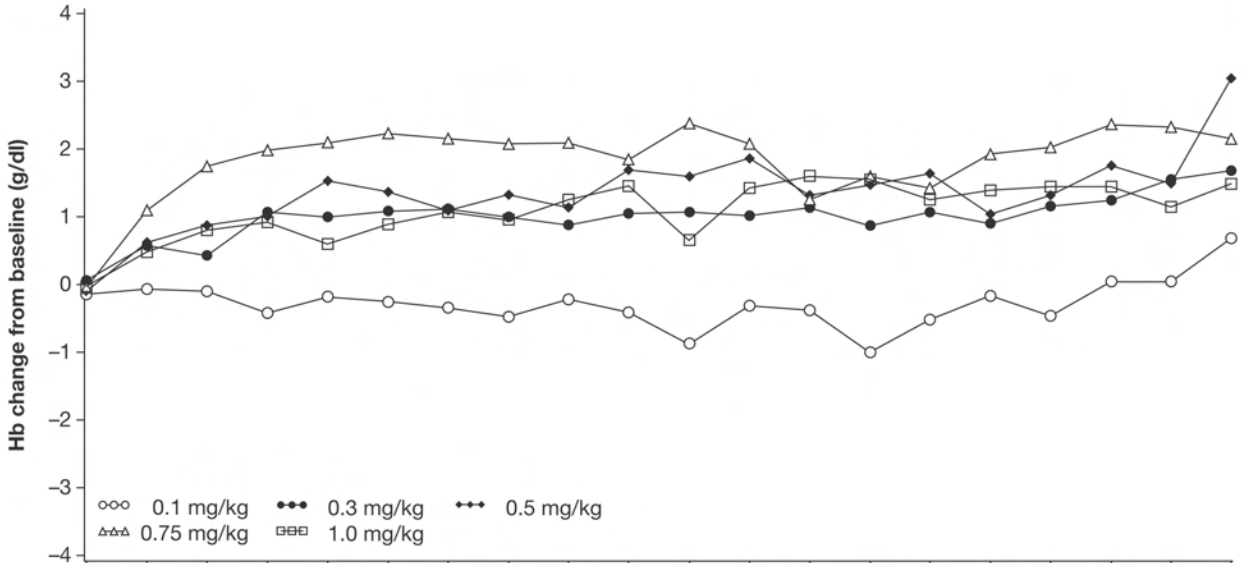
AEs in each dose cohort are presented prior to intrapatient dose escalation. Total AEs are presented post-intrapatient dose escalation. AE: adverse event. [*]AEs occurring in ≥5% of sotatercept-treated patients. [†]AEs occurring in any sotatercept-treated patient. [‡] One patient had both injection site erythema and hypersensitivity as AEs leading to treatment discontinuation.

Figure 1. Response to sotatercept treatment in patients with non-transfusion-dependent β-thalassemia (NTDT). (A) Percentage of sotatercept-treated NTDT patients achieving a mean hemoglobin (Hb) level increase from baseline of ≥1.0 g/dL and ≥1.5 g/dL sustained for ≥12 weeks by assigned dose group. (B) Average maximum increase in Hb levels within 12 weeks from baseline levels in responders *versus* non-responders by dose group. Responders were those patients achieving a ≥1.0 g/dL increase in Hb levels sustained for ≥12 weeks. Error bars show standard deviation of the mean.

Figure 2. Mean change in hemoglobin (Hb) levels from baseline up to day 400 in patients with non-transfusion-dependent β-thalassemia (NTDT) treated with different doses of sotatercept. Data are presented by assigned dose level, prior to intrapatient dose escalation.

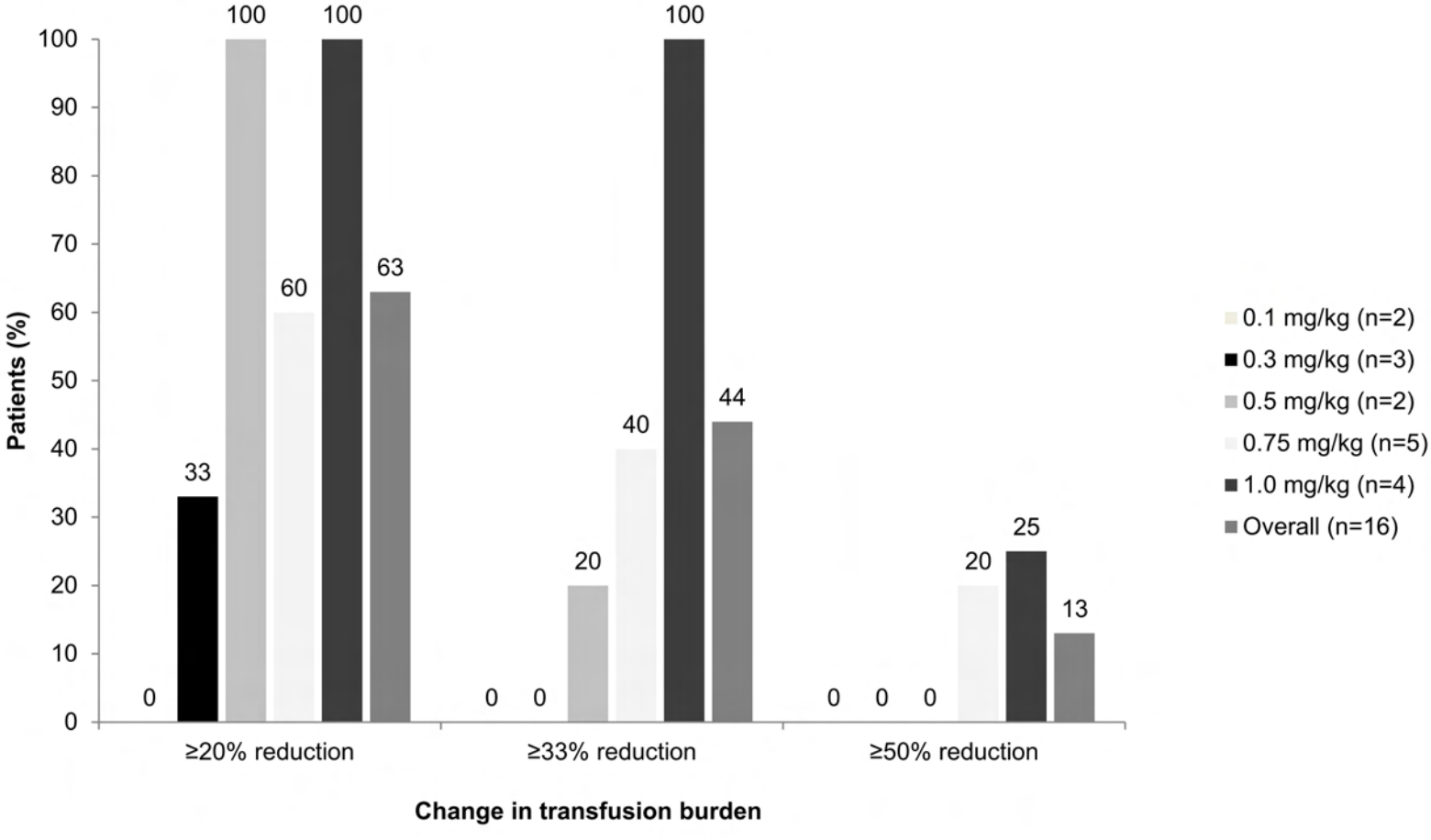
Figure 3. Percentage of transfusion-dependent β-thalassemia (TDT) patients treated with different doses of sotatercept that achieved a reduction in transfusion burden of ≥20%, ≥33%, and ≥50% sustained for ≥24 weeks.


A
Patients (%)
100
90
80
70
60
50
40
30
20
10
0
83
67
86
60
60
0
33
33
86
20
37
≥1.0 g/dL increase
≥1.5 g/dL increase
Hb increase
Sotatercept dose
0.1 mg/kg (n=6)
0.3 mg/kg (n=6)
0.5 mg/kg (n=6)
0.75 mg/kg (n=7)
1.0 mg/kg (n=5)
Overall (N=30)
B
Maximum Increase in Hb (g/dL)
4
3.5
3
2.5
2
1.5
1
0.5
0
0.1 mg/kg
0.3 mg/kg
0.5 mg/kg
0.75 mg/kg
1.0 mg/kg
Sotatercept dose
Responders
Non-responders



Number of patients

0.1 mg/kg	6	6	6	5	5	3	3	3	3	3	3	3	3	3	3	2	2	2	2	1
0.3 mg/kg	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	5	5	4	4
0.5 mg/kg	6	6	6	6	5	5	5	5	5	4	4	4	4	4	4	4	4	3	3	2
0.75 mg/kg	7	7	7	7	6	6	6	6	6	6	5	5	3	3	3	3	3	3	3	3
1.0 mg/kg	5	5	5	5	5	4	4	3	3	3	3	3	3	3	3	2	2	2	2	2


Patients (%)
100
90
80
70
60
50
40
30
20
10
0
0
33
100
60
100
63
0
0
20
40
100
44
0
0
0
20
25
13
≥20% reduction
≥33% reduction
≥50% reduction
Change in transfusion burden
0.1 mg/kg (n=2)
0.3 mg/kg (n=3)
0.5 mg/kg (n=2)
0.75 mg/kg (n=5)
1.0 mg/kg (n=4)
Overall (n=16)

Supplemental Data

Methods

Exclusion criteria

Exclusion criteria included major cardiac problems, erythropoiesis-stimulating agent use ≤28 days prior to enrollment, history of grade ≥3 thromboembolic events, and initiation of, or change in, hydroxyurea treatment ≤1 year prior to enrollment.

Study design

Patients with transfusion-dependent β-thalassemia (TDT) and patients with non-transfusion-dependent β-thalassemia (NTDT) were assigned alternately to receive either 0.1 mg/kg or 0.3 mg/kg sotatercept (Figure S1), with the first TDT patient enrolled receiving 0.1 mg/kg and the first NTDT patient enrolled receiving 0.3 mg/kg. Sotatercept was provided as a lyophilized powder in labeled, rubber-stoppered glass vials, which was reconstituted with water for injection prior to administration. Each dose was administered by subcutaneous injection in the upper arm, abdomen, or thigh once every 21 days. Higher dose levels were opened to enrollment sequentially if ≤1 of 6 patients experienced a dose-limiting toxicity (DLT) ≥21 days after the last patient in that cohort received his or her first dose of sotatercept. A potential recommended dose (PRD), defined as the highest dose level at which ≤1 of 6 patients experienced a DLT, was to be determined based on the first 3 doses of sotatercept administered. Once the PRD was established, an additional 10 patients were planned to be enrolled at the PRD level (Figure S1). If the previous dose level studied exceeded the PRD, doses were reduced as required. The actual recommended dose was to be defined and assessed after review of the safety and efficacy data. A DLT was defined as any 1 or more of the following: grade ≥3 hypertension (according to National Cancer Institute Common Terminology Criteria for Adverse Events [NCI-CTCAE], version 4.0 available at

https://ctep.cancer.gov/protocoldevelopment/electronic_applications/ctc.htm), hemoglobin (Hb) >14 g/dL (within 7 days posttransfusion) sustained for 1 week, or any NCI-CTCAE version 4.0 grade ≥3 toxicity related to sotatercept.

Patients were scheduled to participate in the study for approximately 9 months, up to a maximum of 27 months. Patients were enrolled to receive up to 6 doses of sotatercept with the treatment period planned to last approximately 4 to 5 months, up to a maximum of 22 months, dependent on dose delays. Patients receiving 6 doses of sotatercept, who showed signs of efficacy with no dose delay or a <12-week delay between each dose, could continue treatment for ≤22 months (subject to investigator discretion). Upon completion of the main treatment phase, any patient who demonstrated clinical benefit, as assessed by the investigator, could continue receiving treatment under the “long-term treatment period”, up to a maximum of 3 years. The posttreatment follow-up period was 112 days from the last dose of sotatercept. Dose delays were defined as a dose not administered >4 days from the planned dosing day due to Hb >12.5 g/dL, and/or hematocrit >40%, and/or grade ≥2 hypertension. Patients who discontinued prior to receiving at least 6 doses of sotatercept continued into the posttreatment follow-up period. Patients who did not exhibit any sign of efficacy at the primary assigned dose level and who completed at least 3 doses could be dose-escalated to the highest open dose level. Patients who showed intermittent signs of efficacy at the primary assigned dose level (including reduction in transfusion burden or intermittent Hb increase ≥1 g/dL compared with mean pretreatment Hb values but with mean on-treatment increase <1 g/dL over the previous 3 doses) could be dose-escalated to the next higher open dose level. Patients who showed signs of efficacy at the primary assigned dose level but lost the response during the treatment period could be dose-escalated to the next higher open dose level. Intrapatient dose escalation was planned to be carried out once over the whole treatment period. However, after safety and efficacy data review, the steering committee could allow dose escalation following special

intrapatient dose-escalation requests such as, but not limited to, patients who had been dose-escalated once but lost the response to treatment.

During the study treatment period and the follow-up period, concomitant blood transfusions could be given if the patient's Hb fell below the mean Hb value calculated based on the patient's transfusion history record 168 days prior to study day 1. All transfusion decisions were at investigator discretion and per local practice. In order to see a rapid effect of sotatercept without potential confounding caused by red blood cell (RBC) transfusions, the first dose of sotatercept should have been administered 7-17 days after the last transfusion prior to patient enrollment (study day 1). For all subsequent doses, if the next planned RBC transfusion was to be administered on the same day as sotatercept, sotatercept administration should have occurred at least 24 hours after the RBC transfusion.

Statistical analyses

Demographics and baseline characteristics were analyzed by assigned dose level for the safety population; baseline values were the last values collected on or before the start of study therapy. Dose reductions/interruptions were summarized by cohort and assigned dose level, including patients experiencing ≥1 dose reduction/interruption and time to first dose reduction/interruption.

All efficacy analyses were presented by assigned dose level of sotatercept. Descriptive statistics such as mean, minimum, median, maximum, and sample size were used to describe transfusion burden at baseline and during treatment. Changes in transfusion burden from baseline to on-treatment Hb values prior to each RBC unit transfusion were summarized for

transfusions received prior to sotatercept treatment and during sotatercept treatment, if applicable.

Transfusion burden reduction from baseline to treatment was analyzed by dose level, based on the RBC transfusion-dependent population. Transfusion burden at baseline was defined as the total number of RBC units transfused within 168 days (24 weeks) prior to the first dose of study therapy. Transfusion burden during treatment was defined as the total number of RBC units transfused during the treatment divided by the treatment duration and multiplied by 168 days. The result was a 168-day transfusion burden average.

Hb levels were analyzed for both TDT and NTDT patients. Baseline Hb level was determined as the last Hb level measured prior to the first dose of sotatercept. Mean pretreatment Hb level was calculated from all Hb levels collected for a period of 168 days prior to study day 1. The proportion of patients with erythroid response, defined as transfusion-free with a Hb increase from baseline of ≥1.0 or ≥1.5 g/dL, was measured over a continuous 12-week rolling interval during the treatment period.

Safety analyses were performed on the safety population. Adverse events (AEs) were coded using the Medical Dictionary for Regulatory Activities (MedDRA). Treatment-emergent AEs were summarized by worst severity grade, system organ class, and preferred term. Treatment-emergent AEs leading to death or treatment discontinuation, those classified by the NCI CTCAE, version 4.0 as grade ≥3, those related to investigational product, and serious treatment-emergent AEs were summarized separately.

Clinical laboratory results were summarized descriptively by treatment group. Clinically significant hematologic and non-hematologic laboratory abnormalities were listed and summarized according to the NCI CTCAE, version 4.0, by treatment group.

Sample size and patient allocation

This was a dose-finding study; sample size depended on the dose levels evaluated. Up to a maximum of 65 patients were planned for enrollment into the study, including approximately 10 patients who were planned for enrollment into an extended cohort to evaluate the safety and efficacy end points at the PRD level.

A unique multi-digit patient identification number was manually assigned by site staff to each patient entering the treatment period. The two starting doses, 0.1 mg/kg and 0.3 mg/kg, were opened for enrollment in parallel, with TDT and NTDT patients assigned alternately to each dose level.

Study locations

Hôpital Henri Mondor, Creteil, France
Hôpital Necker-Enfants Malades, Paris, France
Laikon General Hospital, Athens, Greece
Universita degli Studi Cagliari, Cagliari, Italy
Ospedale Galliera, Genoa, Italy
Fondazione IRCCS Ca Granda Ospedale Maggiore Policlinico, Milan, Italy
University College London Cancer Institute, London, United Kingdom

IRB sites and approval dates

Name of IRB	Address	Date of Approval
CPP Ile-de-France II Ethics Committee	149, rue de Sevres 75743 Paris France Cedex 15 Carre Necker- Porte N2	Sep 27, 2017
London Central Research Ethics Committee	Health Research Authority Ground Floor, Skipton House 80 London Rd. London SE1 6LH United Kingdom	Aug 02, 2017
Regional Ethics Committee of Liguria	Largo Rosanna Benzi, 10 16132 Genoa Italy	Aug 10, 2017
Ethics Committee - Milano Area 2	Via Francesco Sforza, 28 20122 Milano Italy	Jul 05, 2017
Independent Ethics Committee of Azienda Ospedaliera Universitaria Di Cagliari	Via Ospedale, 54 09124 Cagliari Italy	Nov 27, 2017
National Ethics Committee	284 Mesogeion Ave. 15562 Cholargos Athens Greece	Aug 02, 2017
Scientific Council of LAIKO General Hospital of Athens	17 Ag. Thoma str. 11527 Athens Greece	Aug 10, 2017

Table S1. Incidence of treatment-emergent AEs of any grade; AEs occurring in ≥5% of sotatercept-treated patients reported overall.

AEs, n (%)	Sotatercept dose (data presented prior to intrapatient dose escalation)					Overall (data presented post-intrapatient dose escalation) (N=46)
	0.1 mg/kg (n=8)	0.3 mg/kg (n=9)	0.5 mg/kg (n=8)	0.75 mg/kg (n=12)	1.0 mg/kg (n=9)	
Patients with ≥1 AE	8 (100)	9 (100)	8 (100)	12 (100)	9 (100)	46 (100)
Arthralgia	3 (38)	3 (33)	6 (75)	6 (50)	1 (11)	21 (46)
Asthenia/fatigue	4 (50)	1 (11)	5 (63)	6 (50)	3 (33)	20 (44)
Headache	0	4 (44)	4 (50)	9 (75)	1 (11)	23 (50)
Bone pain	3 (38)	3 (33)	4 (50)	3 (25)	4 (44)	18 (39)
Back pain	2 (25)	2 (22)	3 (38)	3 (25)	3 (33)	16 (35)
Cough	1 (13)	2 (22)	4 (50)	3 (25)	2 (22)	16 (35)
Pyrexia	1 (13)	5 (56)	1 (13)	3 (25)	4 (44)	15 (33)
Oropharyngeal pain	3 (38)	1 (11)	2 (25)	3 (25)	1 (11)	14 (30)
Pain in extremity	3 (38)	0	3 (38)	2 (17)	2 (22)	12 (26)
Hypertension	0	0	3 (38)	3 (25)	2 (22)	12 (26)
Dizziness	0	1 (11)	3 (38)	2 (17)	0	11 (24)

AEs, n (%)	Sotatercept dose (data presented prior to intrapatient dose escalation)					Overall (data presented post-intrapatient dose escalation) (N=46)
	0.1 mg/kg (n=8)	0.3 mg/kg (n=9)	0.5 mg/kg (n=8)	0.75 mg/kg (n=12)	1.0 mg/kg (n=9)	
Nausea	0	1 (11)	3 (38)	3 (25)	1 (11)	10 (22)
Myalgia	0	2 (22)	3 (38)	2 (17)	1 (11)	10 (22)
Upper abdominal pain	2 (25)	3 (33)	1 (13)	3 (25)	0	9 (20)
Influenza-like illness	1 (13)	3 (33)	2 (25)	1 (8)	0	8 (17)
Rhinitis	1 (13)	1 (11)	4 (50)	1 (8)	0	8 (17)
Neck pain	0	0	2 (25)	4 (33)	0	8 (17)
Upper respiratory tract infection	0	2 (22)	3 (38)	1 (8)	1 (11)	7 (15)
Epistaxis	0	0	0	2 (17)	3 (33)	7 (15)
Diarrhea	0	1 (11)	0	2 (17)	0	6 (13)
Musculoskeletal pain	0	2 (22)	1 (13)	2 (17)	0	6 (13)
Toothache	0	1 (11)	1 (13)	1 (8)	1 (11)	6 (13)
Increased alanine aminotransferase	1 (13)	1 (11)	1 (13)	1 (8)	1 (11)	5 (11)
Nasopharyngitis	1 (13)	0	2 (25)	1 (8)	0	5 (11)
Sinusitis	0	1 (11)	2 (25)	0	1 (11)	5 (11)

AEs, n (%)	Sotatercept dose (data presented prior to intrapatient dose escalation)					Overall (data presented post-intrapatient dose escalation) (N=46)
	0.1 mg/kg (n=8)	0.3 mg/kg (n=9)	0.5 mg/kg (n=8)	0.75 mg/kg (n=12)	1.0 mg/kg (n=9)	
Hypotension	2 (25)	0	2 (25)	0	0	5 (11)
Influenza	0	2 (22)	1 (13)	1 (8)	0	5 (11)
Amenorrhea	0	1 (11)	0	2 (17)	0	5 (11)
Lower abdominal pain	1 (13)	1 (11)	0	2 (17)	0	4 (9)
Oral herpes	1 (13)	2 (22)	0	0	0	4 (9)
Nasal congestion	0	2 (22)	0	1 (8)	0	4 (9)
Teething	0	2 (22)	1 (13)	0	0	4 (9)
Tinnitus	0	1 (11)	0	1 (8)	1 (11)	4 (9)
Musculoskeletal chest pain	0	0	0	2 (17)	0	4 (9)
Musculoskeletal stiffness	0	0	1 (13)	1 (8)	0	4 (9)
Pharyngitis	1 (13)	0	0	0	1 (11)	4 (9)
Vomiting	0	0	1 (13)	1 (8)	0	4 (9)
Dysuria	0	1 (11)	1 (13)	1 (8)	0	3 (7)
Dyspepsia	0	1 (11)	0	1 (8)	1 (11)	3 (7)

AEs, n (%)	Sotatercept dose (data presented prior to intrapatient dose escalation)					Overall (data presented post-intrapatient dose escalation) (N=46)
	0.1 mg/kg (n=8)	0.3 mg/kg (n=9)	0.5 mg/kg (n=8)	0.75 mg/kg (n=12)	1.0 mg/kg (n=9)	
Increased aspartate aminotransferase	0	1 (11)	0	1 (8)	1 (11)	3 (7)
Tachycardia	0	2 (22)	0	0	1 (11)	3 (7)
Abdominal pain	0	0	1 (13)	1 (8)	0	3 (7)
Escherichia urinary tract infection	0	0	0	0	2 (22)	3 (7)
Sciatica	0	1 (11)	0	1 (8)	0	3 (7)
Somnolence	1 (13)	0	0	1 (8)	0	3 (7)
Local swelling	1 (13)	0	1 (13)	0	0	3 (7)
Skin ulcer	1 (13)	0	1 (13)	0	0	3 (7)
Fall	0	1 (11)	1 (13)	0	0	3 (7)
Posttraumatic pain	0	0	2 (25)	0	0	3 (7)
Nephrolithiasis	0	1 (11)	0	0	1 (11)	3 (7)
Extramedullary hemopoiesis	0	0	0	0	2 (22)	3 (7)
Seasonal allergy	0	0	0	0	1 (11)	3 (7)
Decreased appetite	0	0	1 (13)	1 (8)	0	3 (7)

AEs in each dose cohort are presented prior to intrapatient dose escalation. Total AEs are presented post-intrapatient dose escalation. AE: adverse event.

Table S2. Incidence of serious AEs among sotatercept-treated patients.

Serious AEs, n (%)	Sotatercept dose (data presented prior to intrapatient dose escalation)					Overall (data presented post-intrapatient dose escalation) (N=46)
	0.1 mg/kg (n=8)	0.3 mg/kg (n=9)	0.5 mg/kg (n=8)	0.75 mg/kg (n=12)	1.0 mg/kg (n=9)	
Patients with ≥1 serious AE	2 (25)	1 (11)	1 (13)	1 (8)	1 (11)	6 (13)
Bacterial prostatitis	0	0	0	1 (8)	0	1 (2)
Pharyngotonsillitis	0	1 (11)	0	0	0	1 (2)
Subcutaneous abscess	0	1 (11)	0	0	0	1 (2)
Pyrexia	0	1 (11)	0	0	0	1 (2)
Lumbar vertebral fracture	0	0	1 (13)	0	0	1 (2)
Bone pain	1 (13)	0	0	0	0	1 (2)
Syncope	0	0	0	0	1 (11)	1 (2)
Superficial thrombophlebitis	1 (13)	0	0	0	0	1 (2)

AEs in each dose cohort are presented prior to intrapatient dose escalation. Total AEs are presented post-intrapatient dose escalation. AE: adverse event.

Table S3. Incidence of grade 3-4 treatment-emergent AEs among sotatercept-treated patients.

Grade 3-4 treatment-emergent AEs, n (%)	Sotatercept dose (data presented prior to intrapatient dose escalation)					Overall (data presented post-intrapatient dose escalation) (N=46)
	0.1 mg/kg (n=8)	0.3 mg/kg (n=9)	0.5 mg/kg (n=8)	0.75 mg/kg (n=12)	1.0 mg/kg (n=9)	
Patients with ≥1 grade 3-4 AE	1 (13)	1 (11)	1 (13)	4 (33)	2 (22)	9 (20)
Hypertension	0	0	0	2 (17)	0	2 (4)
Anemia	1 (13)	0	0	1 (8)	0	2 (4)
Bone pain	1 (13)	0	0	0	0	1 (2)
Alloimmunization	0	0	0	0	1 (11)	1 (2)
Asthenia/fatigue	0	0	0	1 (8)	0	1 (2)
Ventricular extrasystoles	0	0	1 (13)	0	0	1 (2)
Bacterial prostatitis	0	0	0	1 (8)	0	1 (2)
Hemolytic transfusion reaction	0	0	0	0	1 (11)	1 (2)
Increased fetal hemoglobin	0	0	0	0	1 (11)	1 (2)
Syncope	0	0	0	0	1 (11)	1 (2)
Amenorrhea	0	1 (11)	0	0	0	1 (2)

AEs in each dose cohort are presented prior to intrapatient dose escalation. Total AEs are presented post-intrapatient dose escalation. AE: adverse event.

Table S4. Incidence of treatment-emergent AEs in NTDT and TDT patients treated with sotatercept; treatment-emergent AEs occurring in ≥5% patients overall.

AEs, n (%)	TDT (n=16)	NTDT (n=30)	Overall (N=46)
Patients with ≥1 AE	16 (100)	30 (100)	46 (100)
Headache	7 (44)	16 (53)	23 (50)
Arthralgia	7 (44)	14 (47)	21 (46)
Asthenia/fatigue	9 (56)	11 (37)	20 (44)
Bone pain	10 (63)	8 (27)	18 (39)
Back pain	9 (56)	7 (23)	16 (34)
Cough	4 (25)	12 (40)	16 (35)
Pyrexia	5 (31)	10 (33)	15 (33)
Oropharyngeal pain	4 (25)	10 (33)	14 (30)
Pain in extremity	5 (31)	7 (23)	12 (26)
Hypertension	4 (25)	8 (27)	12 (26)
Dizziness	4 (25)	7 (23)	11 (24)
Nausea	4 (25)	6 (20)	10 (22)
Myalgia	4 (25)	6 (20)	10 (22)

AEs, n (%)	TDT (n=16)	NTDT (n=30)	Overall (N=46)
Upper abdominal pain	4 (25)	5 (17)	9 (20)
Influenza-like illness	3 (19)	5 (17)	8 (17)
Rhinitis	2 (13)	6 (20)	8 (17)
Neck pain	1 (6)	7 (23)	8 (17)
Upper respiratory tract infection	1 (6)	6 (20)	7 (15)
Epistaxis	3 (19)	4 (13)	7 (15)
Musculoskeletal pain	2 (13)	4 (13)	6 (13)
Diarrhea	1 (6)	5 (17)	6 (13)
Toothache	4 (25)	2 (7)	6 (13)
Influenza	2 (13)	3 (10)	5 (11)
Nasopharyngitis	2 (13)	3 (10)	5 (11)
Increased alanine aminotransferase	2 (13)	3 (10)	5 (11)
Hypotension	1 (6)	4 (13)	5 (11)
Sinusitis	3 (19)	2 (7)	5 (11)
Amenorrhea	1 (6)	4 (13)	5 (11)
Teething	2 (13)	2 (7)	4 (9)

AEs, n (%)	TDT (n=16)	NTDT (n=30)	Overall (N=46)
Nasal congestion	3 (19)	1 (3)	4 (9)
Lower abdominal pain	0	4 (13)	4 (9)
Tinnitus	1 (6)	3 (10)	4 (9)
Oral herpes	2 (13)	2 (7)	4 (9)
Vomiting	0	4 (13)	4 (9)
Pharyngitis	3 (19)	1 (3)	4 (9)
Musculoskeletal chest pain	1 (6)	3 (10)	4 (9)
Musculoskeletal stiffness	0	4 (13)	4 (9)
Tachycardia	1 (6)	2 (7)	3 (7)
Dysuria	1 (6)	2 (7)	3 (7)
Increased aspartate aminotransferase	1 (6)	2 (7)	3 (7)
Dyspepsia	2 (13)	1 (3)	3 (7)
Escherichia urinary tract infection	0	3 (10)	3 (7)
Local swelling	0	3 (10)	3 (7)
Sciatica	0	3 (10)	3 (7)

AEs, n (%)	TDT (n=16)	NTDT (n=30)	Overall (N=46)
Somnolence	2 (13)	1 (3)	3 (7)
Abdominal pain	0	3 (10)	3 (7)
Nephrolithiasis	1 (6)	2 (7)	3 (7)
Extramedullary hemopoiesis	2 (13)	1 (3)	3 (7)
Seasonal allergy	1 (6)	2 (7)	3 (7)
Decreased appetite	1 (6)	2 (7)	3 (7)
Fall	0	3 (10)	3 (7)
Posttraumatic pain	1 (6)	2 (7)	3 (7)
Skin ulcer	0	3 (10)	3 (7)

Data presented post-intrapatient dose escalation. AE: adverse event; NTDT: non-transfusion-dependent β-thalassemia; TDT: transfusion-dependent β-thalassemia.

Figure S1. Study design.

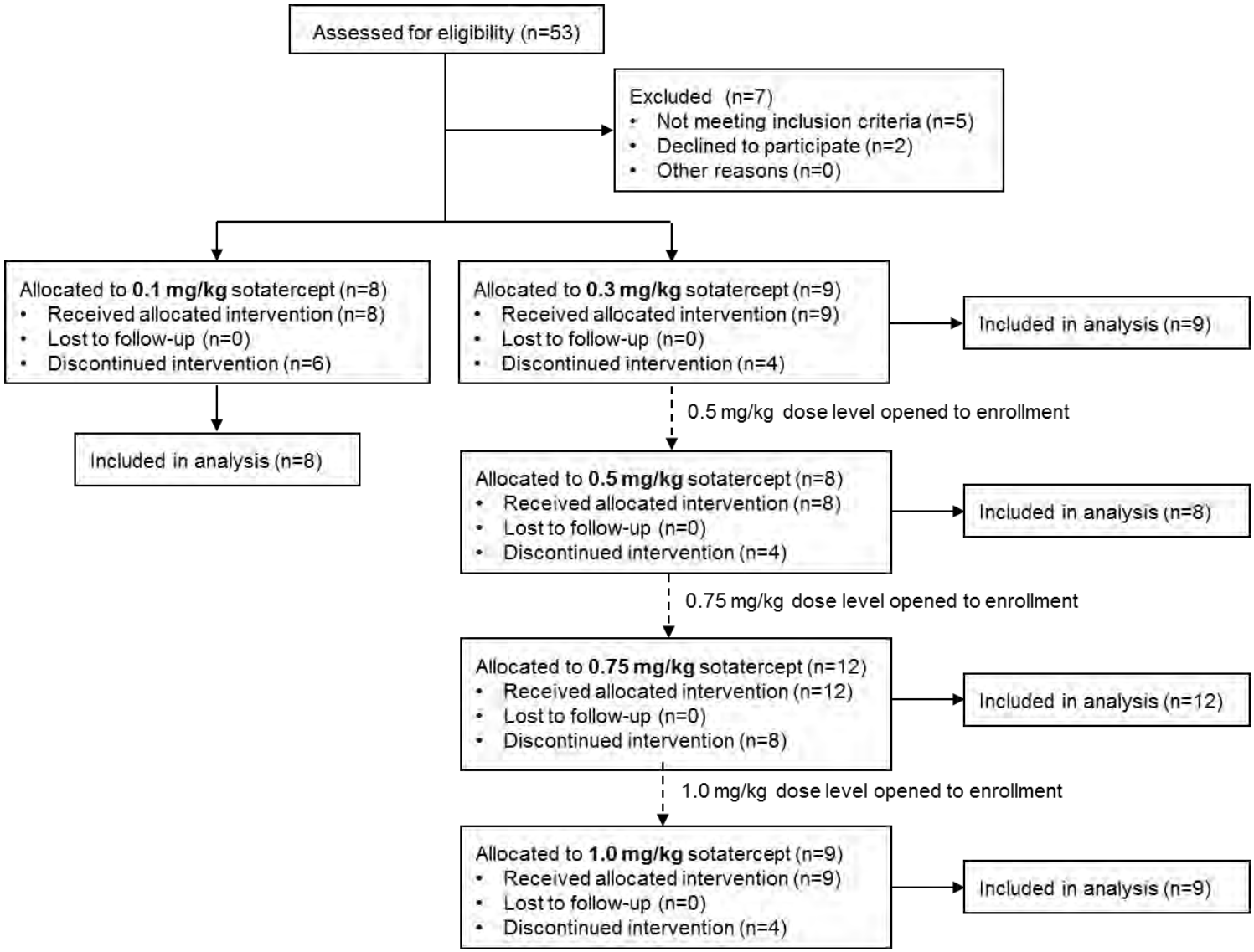


Figure S2. Mean (SD) change in levels of total bilirubin and indirect bilirubin from baseline during treatment with sotatercept. (A) Mean (SD) change in patients with non-transfusion-dependent β-thalassemia. (B) Mean (SD) change in patients with transfusion-dependent β-thalassemia. Data post-intrapatient dose escalation not included. SD: standard deviation.

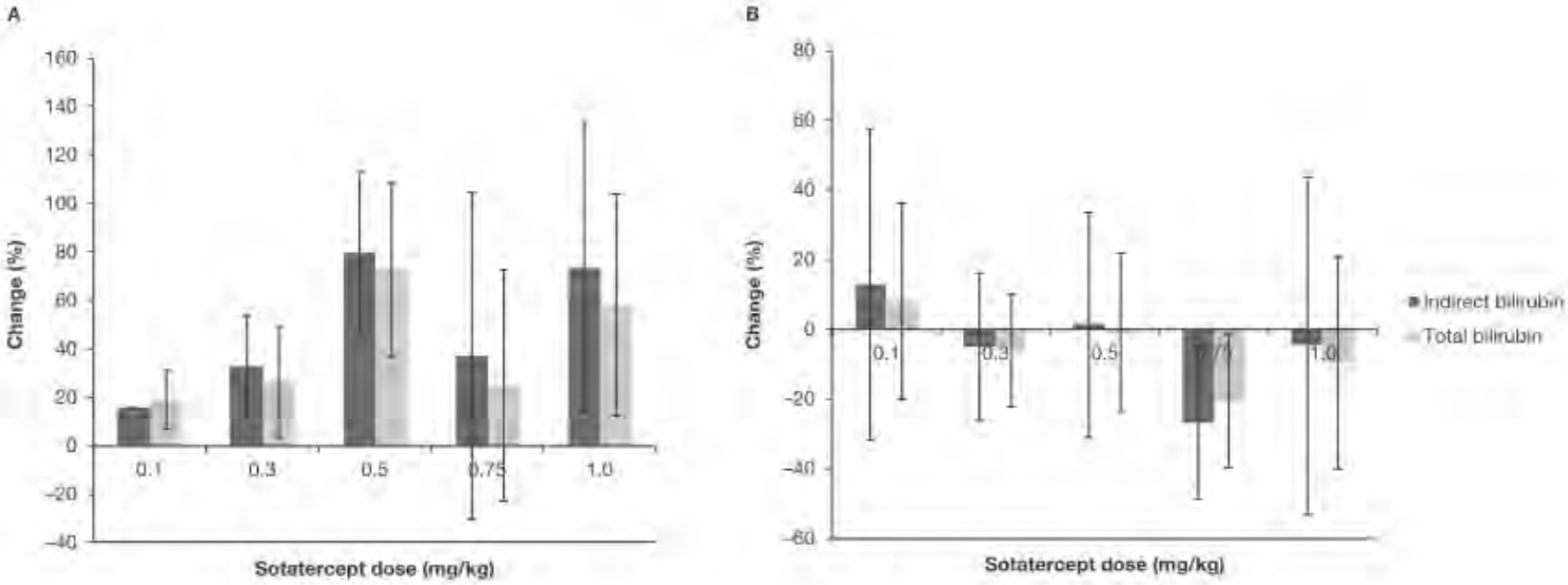


Figure S3. Change in extramedullary masses (EMM) in patients with non-transfusion-dependent β-thalassemia (NTDT). (A) EMM at baseline *(left*) and at 12 months posttreatment (*right*) in a patient enrolled to 0.3 mg/kg sotatercept. (B) EMM at baseline (*left*) and at 7 months posttreatment (*right*) in a patient enrolled to 1.0 mg/kg sotatercept. Hb: hemoglobin; MCV: mean corpuscular volume; RBC: red blood cell.

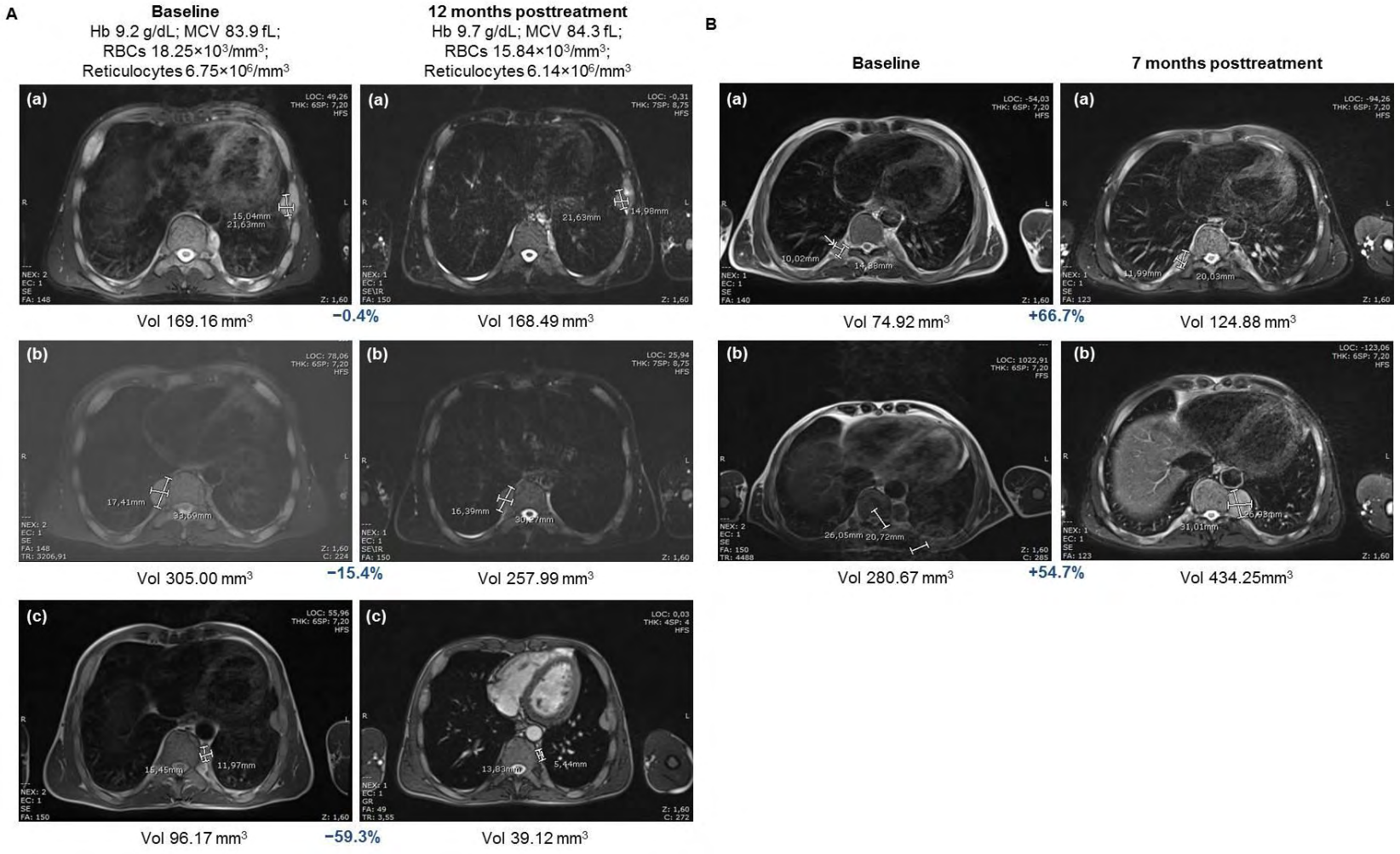


Figure S4. **Improvement in leg ulcers after 6 months treatment with sotatercept 0.5 mg/kg in a patient with non-transfusion-dependent β-thalassemia (NTDT).** The 51-year-old, female patient underwent a skin graft at 4.5 months posttreatment.

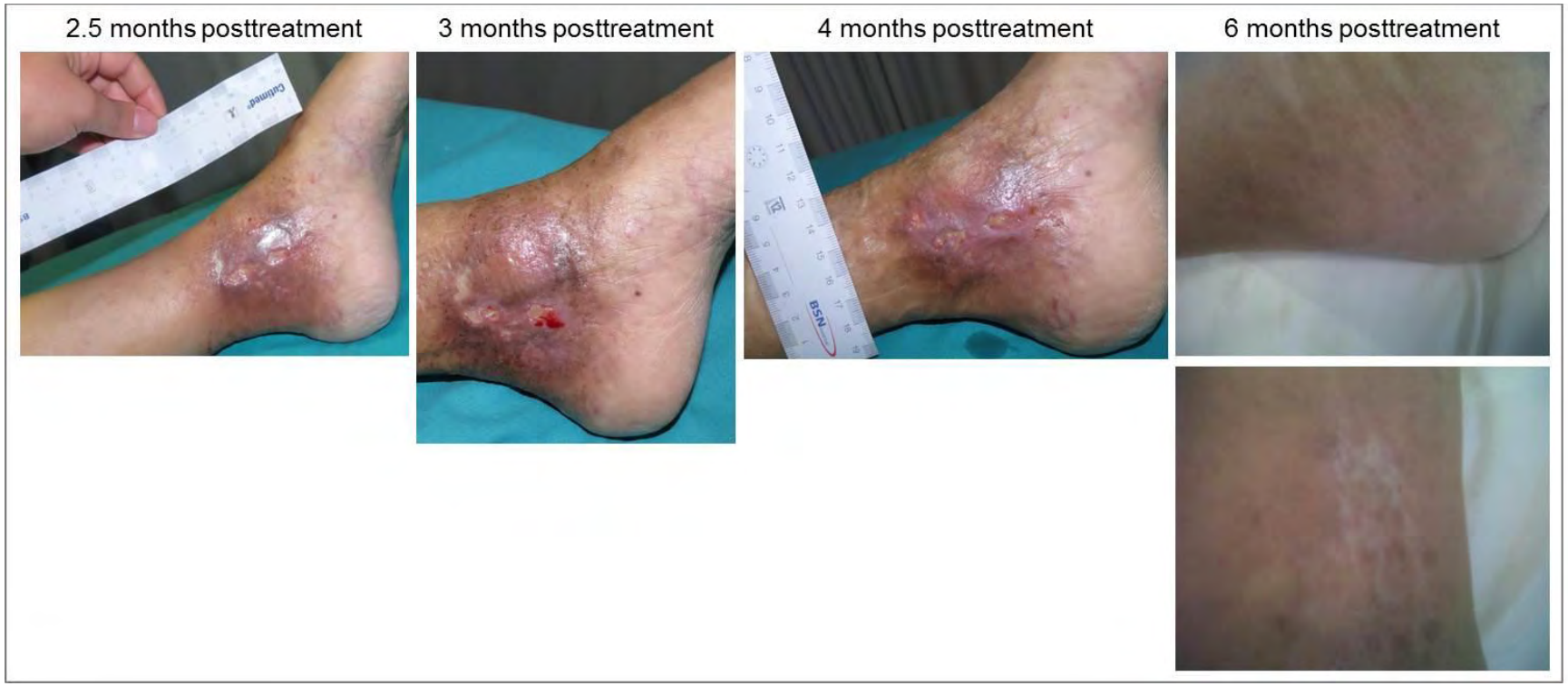


Figure S5. Change in red blood cell (RBC) morphology. (A) A 37-year-old, male patient with non-transfusion-dependent β-thalassemia (NTDT) treated with sotatercept 0.3 mg/kg (dose escalation to 0.5 mg/kg at 11 months). (B) A 54-year-old, male patient with NTDT treated with sotatercept 0.5 mg/kg. (C) A 65-year-old, female patient with NTDT treated with sotatercept 0.75 mg/kg. Hb: hemoglobin.

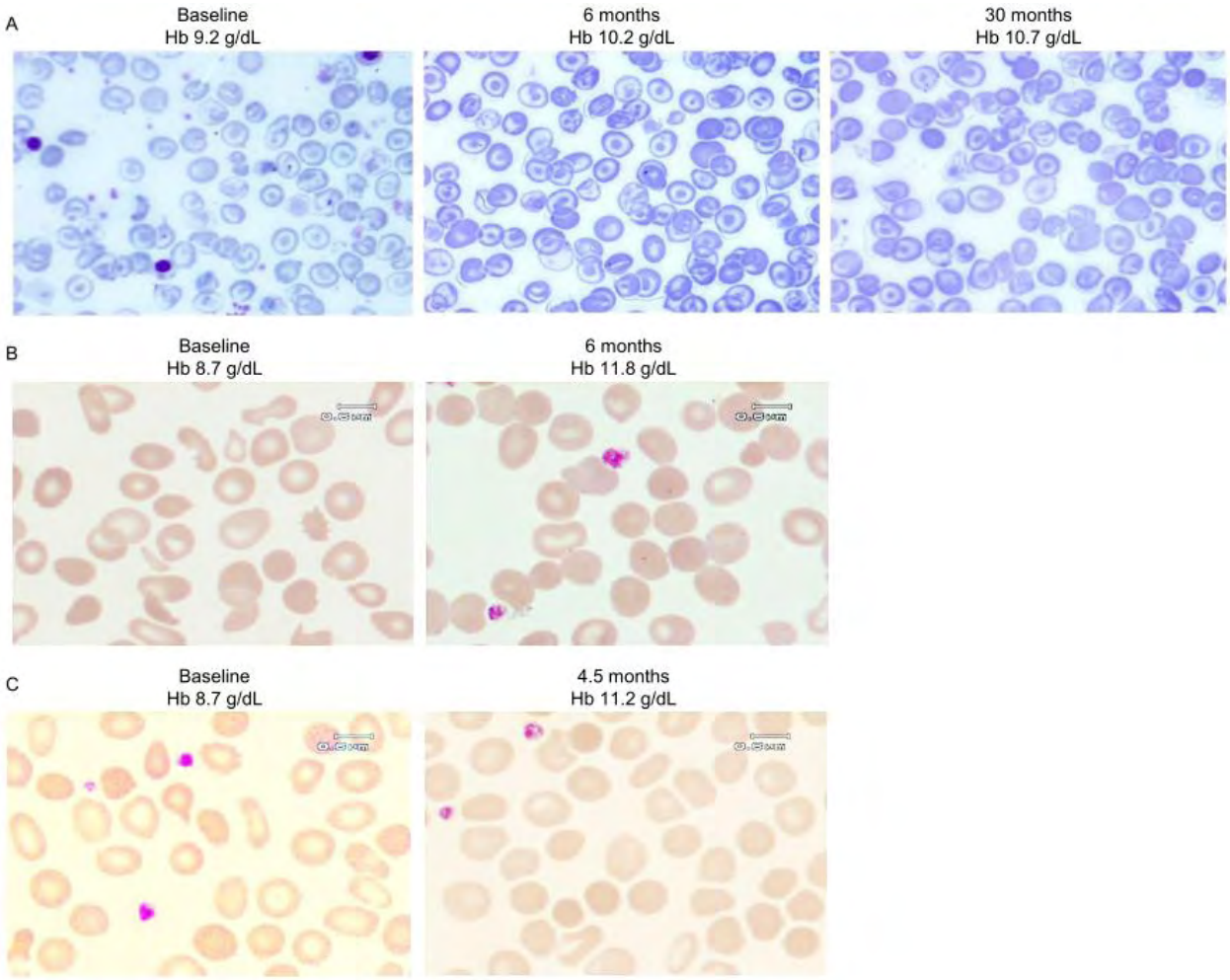


Figure S6. Mean absolute serum ferritin (SF) levels. (A) Patients with non-transfusion-dependent β-thalassemia (NTDT) receiving iron chelation therapy (ICT); (B) Patients with NTDT not receiving ICT; (C) Patients with transfusion-dependent β-thalassemia (TDT) receiving ICT. Data post-intrapatient dose escalation not included.