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Long-term efficacy and safety results of betibeglogene autotemcel gene therapy for transfusion-dependent β-thalassemia

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Abstract:
Betibeglogene autotemcel (beti-cel) gene therapy for transfusion-dependent β-thalassemia (TDT) involves autologous transplantation of hematopoietic stem and progenitor cells transduced with a modified β-globin gene to produce functional adult hemoglobin (HbAT87Q). Sixty-three participants with TDT (median [range] age: 17 [4-35] years) received beti-cel in phase 1/2 (n = 22) or phase 3 (n = 41) studies and enrolled in the long-term follow-up LTF-303 study (clinicaltrials.gov/NCT02633943; median [range] follow-up: 5.9 [2.9-10.1] years). Manufacturing refinements in phase 3 increased transduction efficiency, resulting in higher drug product vector copy number and HbAT87Q levels, which translated into higher hemoglobin and transfusion independence (TI) rates compared with phase 1/2. TI was achieved by 68.2% (15/22) of phase 1/2 participants (median weighted average Hb during TI, 10.2 g/dL) and 90.2% (37/41) of phase 3 participants (median, 11.2 g/dL) and was sustained through last follow-up. Treatment efficacy was similar across ages and TDT genotypes. Among participants achieving TI, 73% (38/52) had discontinued iron chelation at last follow-up, with no increase in liver iron concentration. Markers of ineffective erythropoiesis, including serum transferrin receptor and erythropoietin, improved with restoration of iron homeostasis. Health-related quality-of-life assessment scores showed durable improvements. No malignancies, insertional oncogenesis, or vector-derived replication-competent lentivirus were reported. These findings establish beti-cel as a durable, one-time therapy that achieves TI, restores iron balance, and improves quality of life, offering a potentially curative treatment option for people with TDT.

Conflict of interest: COI declared - see note

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Bluebird bio may provide data upon reasonable request to the following email:

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Key points

- Betibeglogene autotemcel led to sustained transfusion independence up to 10 years in people with transfusion-dependent β-thalassemia.
- This stem cell–directed gene therapy demonstrated long-term safety consistent with myeloablative autologous transplantation.

Abstract

Betibeglogene autotemcel (beti-cel) gene therapy for transfusion-dependent β-thalassemia (TDT) involves autologous transplantation of hematopoietic stem and progenitor cells transduced with a modified β-globin gene to produce functional adult hemoglobin (HbA^{T87Q}). Sixty-three participants with TDT (median [range] age: 17 [4-35] years) received beti-cel in phase 1/2 (n = 22) or phase 3 (n = 41) studies and enrolled in the long-term follow-up LTF-303 study (NCT02633943; median [range] follow-up: 5.9 [2.9-10.1] years). Manufacturing refinements in phase 3 increased transduction efficiency, resulting in higher drug product vector copy number and HbA^{T87Q} levels, which translated into higher hemoglobin and transfusion independence (TI) rates compared with phase 1/2. TI was achieved by 68.2% (15/22) of phase 1/2 participants (median weighted average Hb during TI, 10.2 g/dL) and 90.2% (37/41) of phase 3 participants (median, 11.2 g/dL) and was sustained through last follow-up. Treatment efficacy was similar across ages and TDT genotypes. Among participants achieving TI, 73% (38/52) had discontinued iron chelation at last follow-up, with no increase in liver iron concentration. Markers of ineffective erythropoiesis, including serum transferrin receptor and erythropoietin, improved with restoration of iron homeostasis. Health-related quality-of-life assessment scores showed durable improvements. No malignancies, insertional oncogenesis, or vector-derived replication-competent lentivirus were reported. These findings establish beti-cel as a durable, one-time therapy that achieves TI, restores iron

balance, and improves quality of life, offering a potentially curative treatment option for people with TDT.

Keywords: betibeglogene autotemcel, beti-cel, transfusion-dependent β-thalassemia, gene addition therapy

Introduction

People with transfusion-dependent β-thalassemia (TDT) require frequent, lifelong packed red blood cell (pRBC) transfusions to prevent premature death and other serious complications. Accumulation of iron from transfusions and increased intestinal absorption must be treated by regular chelation therapy.[1-4] While iron chelation therapy and improved monitoring have reduced morbidity and mortality associated with iron overload, underlying disease pathology and transfusion-related complications persist.[2-5] Further, people with TDT have reduced health-related quality of life (HRQOL) compared with the general population due to the physical and emotional demands of time-consuming, chronic pRBC transfusions and iron chelation, leading to suboptimal adherence to the standard of care and contributing to ongoing morbidity and/or mortality.[6,7]

Allogeneic hematopoietic stem cell transplantation (HSCT), ideally using a human leukocyte antigen– (HLA–) identical donor but possibly with an HLA-haploidentical donor, is a potentially curative treatment for β-thalassemia,[3,8-10] with optimal outcomes below the age of 14.[11] However, as HSCT is associated with the risks of graft rejection, graft-versus-host disease (GVHD), and other toxicities,[9,12-14] safer curative options are needed. Betibeglogene autotemcel (beti-cel) gene addition therapy addresses the underlying cause of TDT via autologous transplantation of hematopoietic stem and progenitor cells (HSPCs) transduced with a modified β-globin gene to produce functional adult hemoglobin (HbA) containing $\beta^{A\text{-}T87Q}$-globin (HbA^{T87Q}).[15-18]

The beti-cel treatment process in phase 1/2 and 3 studies has previously been reported, including mobilization, apheresis, myeloablation, and beti-cel infusion.[15,18-20] Beti-cel demonstrated durable transfusion independence (TI), improved markers of ineffective erythropoiesis and iron overload, a favorable risk-benefit profile, and improved HRQOL,[15,18,20,21] resulting in U.S. Food and Drug Administration approval of beti-cel for adult and pediatric patients with TDT, regardless of genotype.[22] An exploratory analysis of predictors of successful treatment outcomes in participants from the phase 3

studies has also been reported.[23] Here, we report sustained efficacy, iron homeostasis, safety, and HRQOL outcomes for participants with TDT who completed a 2-year, phase 1/2 (HGB-204: NCT01745120; HGB-205: NCT02151526) or a phase 3 study (HGB-207: NCT02906202; HGB-212: NCT03207009[24]) of beti-cel gene therapy and then entered the 13-year, long-term follow-up LTF-303 study (NCT04628585) with up to 10 years follow-up.

Methods

Study design and oversight

The non-randomized, open-label, single-dose beti-cel parent studies were initiated in 2013 (phase 1/2) and 2016 (phase 3) in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines of the International Council for Harmonisation. Following 2 years in the parent studies, participants entered LTF-303 and were followed every 6 months for 5 years after receiving drug product and then annually for a planned 15 years of total follow-up (supplemental Figure 1).

Participants

Complete eligibility criteria have been previously described[15,18] (supplemental Table 1). Briefly, in phase 1/2 studies, eligible participants had TDT without advanced organ damage and were ≤35 years of age, with transfusion dependence defined as receiving ≥8 transfusions or ≥100 mL per kilogram of body weight of pRBCs per year in the prior 2 years.[18] Eligibility in phase 3 studies was expanded to include participants ≤50 years of age with TDT. Participants in phase 3 HGB-207 had TDT due to non-β^0/β^0 genotypes, and the severe β+ mutations IVS I-110/IVS I-110 or IVS I-110/β^0 were excluded,[15] whereas in HGB-212, participants had β^0/β^0 genotypes with inclusion of the severe β+ *HBB* variant IVS I-110 G>A, which is associated with minimal residual HbA expression.[24] Across all studies, participants with evidence of severe iron overload with organ injury were ineligible (ie, advanced liver disease or cardiac T2* <10 msec by magnetic resonance imaging [MRI]). In phase 3 studies, pediatric participants with a liver iron concentration (LIC) >15 mg/g dw (dry weight) were excluded.

Gene therapy

As previously reported, participants with TDT underwent HSPC mobilization and peripheral blood collection (utilizing granulocyte colony-stimulating factor [G-CSF] and plerixafor) for drug product manufacturing.[15,18,20] The beti-cel manufacturing process was refined over the course of the parent studies.[15] In response to low hemoglobin (Hb) levels in phase 1/2 studies, the transduction medium was adapted to increase BB305 lentiviral vector (LVV) transduction efficiency, resulting in higher vector copy number (VCN) and higher percentage of cells transduced in the drug product, ultimately leading to increased total Hb post infusion.[23] Following release testing, beti-cel was dispositioned for clinical use, and participants underwent pharmacokinetic-adjusted myeloablative conditioning with busulfan over 4 days.[15,18,24] In phase 3 studies, after a minimum 48-hour washout period from busulfan, beti-cel was thawed and infused intravenously at a cell dose ≥5.0 million CD34+ cells/kg. .

Efficacy and safety assessments

The proportion of participants who achieved TI (defined as a weighted average Hb ≥9 g/dL without pRBC transfusions for ≥12 months at any time after beti-cel infusion) was assessed through last follow-up. Other efficacy endpoints included VCN in peripheral blood, vector-derived HbA^{T87Q} in the peripheral blood, Hb level during TI, and annualized volume and frequency of pRBC transfusions. Measures of iron overload and dyserythropoiesis as well as therapeutic phlebotomy and iron chelation were also evaluated. Management of iron overload was at the discretion of the investigator with the stipulation that deferiprone should not be prescribed sooner than 6 months after beti-cel infusion due to the potential risk of agranulocytosis.[25] In addition, phlebotomy in lieu of chelation was recommended for participants with Hb consistently ≥11 g/dL without regular transfusions. A LIC of 5 mg Fe/g dw or higher was provided as guidance for initiating iron reduction therapy, consistent with published recommendations for managing iron overload in TDT.[1]

Achievement and kinetics of engraftment were evaluated in each parent study.[15,18,20] Safety monitoring continued following completion of parent studies through last follow-up in LTF-303, including survival, physical examination, and the number of participants with beti-cel–related adverse events (AEs), serious AEs (SAEs), malignancies, immune-related AEs (eg, autoimmune reactions, opportunistic infections), and new or worsening hematologic and nonhematologic disorders. Complete blood counts were also obtained, with surveillance for evidence of replication-competent lentivirus and insertional mutagenesis leading to persistent oligoclonality (relative frequency of ≥10% in 1 insertion site or 2 insertions sites ≥5% at 2 consecutive time points[26]). Oligoclonality is considered resolved when the participant no longer meets these criteria.

Patient-reported outcomes

HRQOL over time was assessed in participants who achieved TI using the Pediatric Quality of Life Inventory (PedsQL; range: 0-100)[27] for participants <18 years of age or the Short Form-36 Health Survey Physical Component Summary and Mental Component Summary (SF-36 PCS and MCS, respectively; range: 0-100 for each component) for participants ≥18 years of age.[28] The EQ-5D-3L visual analog scale (range: 0-100) and composite index score (range: 0-1) were also used for adult participants (supplemental Tables 2 and 3).[29] HRQOL was assessed through month 60 in LTF-303.

A questionnaire assessing β-thalassemia and patient-centric aspects of daily living (ie, ability to work or attend school, physical activity, and perceived benefit from treatment) was completed at month 36 (supplemental Table 4).

Statistical analysis

Descriptive analyses included summary tabulations with mean, standard deviation, median, interquartile range, and range for continuous variables, and percentages for categorical data. Efficacy data were summarized by genotype, age, and at specific time points (by visit up to year 5, then at year 7, 10, 15, and last follow-up). Statistical analyses included comparisons of select variables across study

phases and subgroups (eg, genotype, TI vs non-TI), Spearman correlational analyses to evaluate the relationship between VCN and HbA^{T87Q} during TI, and linear regression analyses to assess total Hb in participants who achieved TI, stratified by genotype and age subgroups and accounting for differences across study phases. Safety data were summarized for all participants by genotype, age, and during time intervals (months 0-24, 24-36, 36-48, 48-60, and 60-84; years 7-10 and 10-15).

Results

Participants

As of February 2024, all 63 participants (median [range] age: 17 [4-35] years) who had received beti-cel in a parent study enrolled in LTF-303 (median [range] follow-up: 71.2 [34.5-121.4] months) (Table 1). Two (3.2%) participants had 10 years of follow-up, and 51 (81.0%) participants had at least 5 years of follow-up (supplemental Figure 1). One participant withdrew consent for personal reasons after entering LTF-303 (39 months post beti-cel infusion) and is no longer participating in the study. This participant achieved TI and contributed data through month 36, including analyses restricted to the TI population up to the point of withdrawal.

Transduction efficiency, engraftment, and pharmacodynamics

Transduction efficiency and pharmacodynamic outcomes are presented by age group in Table 2. Seventy-one percent of adults and 87.5% of pediatric participants needed only 1 mobilization cycle to achieve the drug product dose. As previously reported, the proportion of splenectomized patients requiring 2 mobilization cycles was greater than nonsplenectomized patients.[19] However, the median number of CD34+ cells infused was similar across study phases (Table 2). All participants treated with beti-cel achieved neutrophil and platelet engraftment. Peripheral blood VCN and HbA^{T87Q} levels were stable by month 6 and durable across studies.

Ranges of VCN and HbA^{T87Q} were similar across age groups (Table 2) and higher in phase 3 than phase 1/2 (Figure 1A-B), reflecting improvements in transduction efficiency secondary to the refined

beti-cel manufacturing process. Increased VCN translated into higher Hb levels (median [range] weighted average Hb during TI: phase 1/2, 10.2 [9.1-13.1] g/dL; phase 3, 11.2 (9.8-13.9) g/dL [Wilcoxon rank-sum $P = 0.03$]). As previously reported,[15,18,30] participants who did not achieve TI had approximately five-fold fold lower VCN than those who did (median [range] 0.22 [0.09-0.38] c/dg vs 1.12 [0.11-4.88] c/dg, respectively; Figure 1C). Across all studies, the average weighted Hb during TI was significantly correlated with VCN (Spearman $\rho = 0.77$, $P < 0.001$), highlighting that higher VCN is associated with greater Hb production and achievement of TI.

After adjustment for study phase, sex, and age, participants with non-β^0/β^0 genotypes (including those with IVS-I-110/β^0 genotypes) had a 6% higher weighted average Hb during TI than participants with β^0/β^0 genotypes (gamma-log model adjusted mean ratio [AMR]: 1.06 [95% CI: 1.00-1.13]). The difference was most apparent in phase 3 studies, where median (range) weighted average Hb during TI in non-β^0/β^0 participants was 12.0 (9.8-13.4) g/dL versus 10.6 (9.8-13.9) g/dL in β^0/β^0 participants (supplemental Table 5). In a model additionally adjusting for HbA^{T87Q} levels, the genotype effect persisted: non-β^0/β^0 participants had an 8% higher weighted average Hb than β^0/β^0 participants (AMR [95% CI]: 1.09 [1.03-1.15]). These results are concordant with Figure 1D, where the non-β^0/β^0 regression line is shifted upward relative to β^0/β^0 at a given level of HbA^{T87Q}. Relative to adults, pediatric participants had significantly lower weighted-average Hb during TI (AMR [95% CI]: 0.90 [0.85-0.95]), whereas adolescents were similar to adults, consistent with age-related hematologic norms (Supplemental Table 6).[31] Sex was weakly associated with Hb in the HbA^{T87Q} model.

Achievement and maintenance of transfusion independence

Fifty-two of 63 participants achieved and sustained TI (phase 1/2: 15/22 [72.7%], supplemental Figure 2, supplemental Table 7; phase 3: 37/41 [90.2%], Figure 2, supplemental Table 5). Aside from lower VCN, no other clinically meaningful differences in baseline characteristics (age, genotype, or iron

burden) were observed in participants who did not achieve TI (supplemental Table 8). In phase 3 studies, 14/16 (87.5%) participants aged <12 years, 11/11 (100%) aged ≥12 to <18 years, and 12/14 (85.7%) aged ≥18 years achieved TI. Among genotype subgroups, 26/29 (89.7%) participants with non-β^0/β^0 genotypes and 11/12 (91.7%) participants with β^0/β^0 genotypes achieved TI. This included participants with IVS-I-110/IVS-I-110 genotypes, who were grouped with non-β^0/β^0 and demonstrated comparable rates of TI (7/8 [87.5%]). TI was achieved by 3 of 5 participants with alpha globin deletions (ie, αα/α-), a proportion comparable with the broader study population (Fisher's exact test $P = 0.21$).

One participant who no longer meets protocol-defined TI was diagnosed approximately 23 months post beti-cel infusion with wild-type human immunodeficiency virus (WT-HIV); approximately 1 month after diagnosis, they began antiretroviral treatment with tenofovir disoproxil fumarate/efavirenz/emtricitabine. They required single pRBC transfusions at 13 months post infusion due to *Vibrio parahaemolyticus* septicemia[32] and at month 21 following a 3-month decline in total Hb and HbA^{T87Q} levels. Approximately 2.5 years post infusion, transfusions were stopped for an extended period of time (approximately 4 years); however, they were resumed following a *Haemophilus influenzae* infection and Hb level <9 g/dL during year 6. Another participant (phase 3, HGB-207) required a single transfusion after a planned orthopedic surgery but still meets protocol-defined TI.

Iron homeostasis and measures of dyserythropoiesis

Participants who achieved TI had reductions in markers of ineffective erythropoiesis and iron overload (Figure 3, supplemental Figures 3 and 4). Reductions from baseline in serum ferritin at month 24 were observed across all studies (n = 52), followed by improvement in iron burden through month 60 (median [range] baseline serum ferritin, 3627.0 [784-13432] pmol/L vs month 60, 986.2 [189-12653] pmol/L). Baseline LIC was 5.2 (1.0-41.0) mg Fe/g dw versus LIC 2.2 (0.9-10.8) mg Fe/g dw at month 60. Markers of erythropoietic recovery also improved in phase 3 participants who achieved TI. Baseline serum transferrin receptor was 97.6 (17.7-235.3) nmol/L compared with 51.7 (18.4-141.1) nmol/L at last

follow-up (supplemental Table 9). Absolute reticulocyte counts and median myeloid:erythroid ratio increased through last follow-up, consistent with restored hematopoiesis and complementing erythropoietin data (supplemental Figure 5).

Among the fifteen phase 1/2 participants who achieved and sustained TI, all restarted iron chelation post infusion, and 3/15 (20.0%) received a combination of phlebotomy and chelation therapy (supplemental Table 10, supplemental Figure 6). Ten of these 15 (66.7%) have stopped chelation therapy after restarting, and all 10 remained free of chelation therapy at last follow-up. Eight of these 10 had LIC values <5 mg Fe/g dw when they stopped chelation. At last follow-up, 9/10 (90.0%) had LIC values <5 mg Fe/g dw.

Of the 37 phase 3 participants who achieved TI, 23 (62.2%) restarted iron chelation post infusion and 9/37 (24.3%) underwent phlebotomy. Fourteen of 23 (60.9%) stopped chelation therapy after restarting and remained free of chelation therapy at last follow-up. Including the 14/37 phase 3 participants who never restarted iron chelation, a total of 28 (75.7%) were not receiving chelation therapy at last follow-up (8 continued to receive phlebotomy). Among the 14 who restarted and then stopped chelation, 11 had LIC values <5 mg Fe/g dw when they stopped chelation. At last follow-up, 12/14 (85.7%) had LIC values <5 mg Fe/g dw. Among the 28 participants no longer receiving chelation therapy at last follow-up, 22 (78.6%) had LIC <5 mg Fe/g dw.

Most participants who achieved TI and were off chelation therapy at last follow-up also demonstrated continued and progressive reductions in serum ferritin (supplemental Figure 4). Across studies, median (range) LIC in participants receiving phlebotomy at last follow-up was 3.44 (0.81-30.0) mg Fe/g dw.

Across studies, 8 participants achieved and maintained TI with Hb <10 g/dL at last follow-up. These participants had lower VCN and higher iron burden (median [range] LIC: 4.0 [1.9-10.2] mg Fe/g

dw) than other participants who achieved TI (3.0 [0.8-30.0] mg Fe/g dw; supplemental Table 8), suggesting suboptimal transduction efficiency may contribute to lower Hb levels despite achieving TI. All 8 restarted chelation therapy at some point post infusion, 4 have since discontinued, and 2 of these 4 had LIC values <5 mg Fe/g dw when they stopped chelation and at last follow-up. The other 2 participants had LIC values of 10.8 and 14.4 mg Fe/g dw when they stopped chelation and 9.7 and 7.6 mg Fe/g dw at last follow-up, respectively.

Patient-reported outcomes

Among participants who achieved TI and had available HRQOL data, early improvements were reported across age groups and measures, except for the mean EQ-5D-3L composite index score, which generally remained stable throughout the study. Overall, improvements were sustained through month 60 (supplemental Table 11). Clinically meaningful (mean score increase >2) improvements were observed in adults for SF-36 MCS scores starting at month 24 (mean [SD], +2.5 [9.4]; n = 12) (Figure 4A) and for SF-36 PCS at month 36 (+2.4 [4.2]; n = 11) (Figure 4B). Scores for both components were maintained above the normative population mean (50) at month 60. For pediatric participants, the mean PedsQL total score demonstrated clinically meaningful improvement at month 36 (+12.2 [10.2]; n = 15), and scores remained above the normative population mean (81) at month 60 (n = 8) (Figure 4C).

Testimonial questionnaires were also administered to assess β-thalassemia and patient-centric aspects of daily living. Among those who achieved TI (including the participant with WT-HIV who later lost TI), participants reported significant improvements in ability to seek employment or be employed from baseline (66.7% [12/18]) to month 36 (90.9% [10/11]; supplemental Table 4). There was a reduction in school absences from baseline (84.0% [21/25]) to month 36 (42.9% [6/14]), and all 6 participants who still had absences at month 36 reported fewer absences due to management of thalassemia. Additionally, 53.8% (14/26) no longer required management of disease symptoms, and

77.8% (21/27) reported improvement in physical activity. When asked if they had experienced an overall benefit from beti-cel transplant, all (100%; [26/26]) reported that they had.

Safety

No participants enrolled in the phase 1/2 or phase 3 studies died. AEs considered possibly related to beti-cel by the investigator have been previously reported through 24 months.[15,18,20,24] Between 24 and 36 months post beti-cel infusion, 1 participant developed mild prolonged thrombocytopenia and was noted to have antibodies against glycoprotein IIb/IIIa, suggestive of immune thrombocytopenia. This participant did not require treatment. Another participant was diagnosed with hepatic focal nodular hyperplasia with an increase in size and number of hepatic nodules between 36 and 48 months post beti-cel infusion. Both AEs were considered by the investigator to be possibly related to the treatment received. No other beti-cel–related AEs were reported beyond 48 months (Table 3).

Three participants (phase 1 [HGB-204], n = 2; phase 3 [HGB-207], n = 1) had persistent oligoclonality at last follow-up (supplemental Figure 7). One additional participant (phase 1 [HGB-204]), had previous persistent oligoclonality that no longer meets the criteria. Total infused CD34+ cells for these participants ranged from 5.2×10^6 cells/kg to 13.0×10^6 cells/kg, and drug product VCN was 0.8 copies per diploid genome (c/dg) to 5.3 c/dg. All 4 achieved neutrophil and platelet engraftment and sustained TI through last follow-up. No association was observed between persistent oligoclonality and drug product transduction efficiency, CD34 dose, peripheral blood VCN at 6 months, or average weighted Hb during TI, although interpretation is limited by the small number of affected participants. Analysis of integration sites for these participants is presented in supplemental Figure 7 and integration sites by study phase are summarized in supplemental Figure 8. There has been no clinical indication to investigate for clonal hematopoiesis among these participants. No hematologic malignancies, insertional oncogenesis, or vector-derived replication-competent lentivirus were observed.

Discussion

This analysis of the beti-cel clinical program highlights long-term safety and durability with beti-cel gene addition therapy, with follow-up of at least 5 years for 51 participants and 10 years for 2 participants. The proportion of participants who achieved and sustained TI increased from 68.2% (15/22) in phase 1/2 studies to 90.2% (37/41) in phase 3. Across studies, peripheral blood VCN and HbA^{T87Q} levels were stable by month 6, durable through last follow-up, and were higher in phase 3 than phase 1/2, owing to the optimized beti-cel drug product manufacturing. As previously reported, optimized manufacturing also led to high TI rates and clinically meaningful Hb levels in participants with severe genotypes (ie, β^0/β^0 and IVS-I-110 in homozygous or compound heterozygous combination with β^0).[20,21] Key metrics, including transduction efficiency, pharmacodynamics, and TI rate, were also similar across age subgroups.

Together with these findings, results of a previously reported post hoc multivariate analysis suggest that the manufacturing process for beti-cel used in phase 3 trials consistently results in efficient transduction (often exceeding 80% LVV+ cells), which is strongly associated with achieving TI.[30] In these prior analyses, the percentage of LVV+ cells was the best predictor of clinical outcomes, and achieving ≥62% transduced cells increased the likelihood of achieving TI.[30] In this study, participants who did not achieve TI had lower VCN than those who achieved TI, with no other clinically meaningful differences in baseline characteristics, underscoring that transduction efficiency, rather than intrinsic patient factors, drives long-term efficacy. Observed differences in weighted average Hb likely reflect differences in transduction efficiency. Consistent with this interpretation, the generally lower Hb observed with β^o/β^o genotypes likely reflects the complete absence of endogenous HbA and greater reliance on transduced cells for total Hb production. Thus, achieving higher transduction efficiency may be particularly important for this subgroup. Notably, following optimized beti-cel manufacturing, TI rates were

comparable across genotypes, supporting the potential for manufacturing improvements to mitigate genotype-related disparities in clinical outcomes.

Among participants who achieved and maintained TI, an initial increase in iron level due to hypertransfusion and supportive transfusions for engraftment was followed by effective restoration of iron homeostasis and reduced iron management burden. Seventy-three percent of participants who achieved TI across the beti-cel studies were no longer receiving iron chelation therapy, and 82% of these participants had LIC <5 mg Fe/g dw at last follow-up. The slow and gradual restoration of iron homeostasis in this study is consistent with similar observations following allogeneic hematopoietic stem cell transplantation.[13] In beti-cel–treated participants, non-LVV+ cells that do not express HbA^{T87Q} may continue to have ineffective erythropoiesis. However, despite this and the lack of a uniform approach for iron management (ie, oral chelation and/or phlebotomy) in this study, net effects in the LIC and serum ferritin data presented here trend towards normalizing iron homeostasis. Differences in iron clearance kinetics between phase 1/2 and 3 may reflect variability in iron management or underlying hematopoietic recovery, though this was not directly assessed. Among phase 3 participants who achieved and maintained TI, markers of ineffective erythropoiesis, including serum transferrin receptor, erythropoietin, and reticulocyte count, showed trends consistent with restored hematopoiesis with no indications of ineffective erythropoiesis. One participant who lost TI after WT-HIV infection and initiation of antiretroviral therapy experienced declines in total Hb and HbA^{T87Q}, consistent with exacerbation of underlying bone marrow insufficiency during periods of additional stress.

No hematologic malignancies or new or worsening hematological or nonhematological disorders occurred following beti-cel treatment. Mild prolonged thrombocytopenia suggestive of immune thrombocytopenia was reported in 1 patient, but no treatment was required. Few AEs were considered related to beti-cel >2 years post infusion. Liver focal nodular hyperplasia is a known, typically indolent, and nonprogressive complication associated with myeloablative conditioning regimens, as previously

reported after HSCT in iron-overloaded patients.[33] While hematologic malignancies have been reported in lovotibeglogene autotemcel trials using the same BB305 LVV, no cases of insertional oncogenesis have been observed in any trial to date.[34,35] The 4 participants with oligoclonality will be closely monitored to better understand the natural history of this clonally restricted hematopoiesis over time. It is not clear if this phenomenon is a stochastic founder effect of a limited number of repopulating HSPCs or if other uncharacterized drivers of proliferation are contributing.[36] A longer period of follow-up is needed to define mechanistic underpinnings of oligoclonality and its natural history.

People with TDT who receive traditional treatment aimed at preventing severe anemia and managing iron overload have considerable physical and emotional impairment and substantial productivity loss due to absenteeism from work and school.[37] Participants (across all age groups) who achieved TI in this study had clinically meaningful improvements in HRQOL with sustained benefits observed up to 48 months, demonstrating how beti-cel treatment can largely alleviate time loss due to undergoing transfusions, managing iron overload, and treatment-related logistics.

Conclusion

This study presents the longest follow-up evaluation of LVV gene addition therapy in β-thalassemia. Beti-cel achieved durable TI and sustained Hb production across genotypes and ages, with restoration of iron homeostasis and meaningful improvements in quality of life. The safety profile remains consistent with that of myeloablative autologous transplantation, with no cases of malignancy or insertional oncogenesis observed. Future analyses through year 15 of LTF-303 will provide additional insights into late effects, including dyserythropoiesis, iron status, growth, end-organ function, and fertility. These findings support beti-cel as a one-time therapy offering durable clinical benefit and the potential for cure in transfusion-dependent β-thalassemia.

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Authorship

Contribution:

J.L.K., A.A.T., M.C., M.C.W., and F.L. were involved in design and conceptualization of the study with bluebird bio, Inc. All of the authors generated, collected, and had access to the clinical data and confirmed that the study was conducted in accordance with the protocol. The authors and the sponsor, bluebird bio, Inc., interpreted the data. All of the authors reviewed and approved the manuscript for publication and vouched for the accuracy and completeness of the data. Statistical analysis was provided by S.A.

Conflict-of-interest disclosure:

J.L.K. is a consultant for Agios, BioMarin, bluebird bio, Inc., Bristol Myers Squibb, Celgene, CRISPR/Vertex, Imara, Forma, Chiesi, Silence Therapeutics and has received research funding from Apopharma, Bioverativ, bluebird bio, Inc., CRISPR/Vertex, Imara, Forma, Editas, and Sangamo.

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J.S. participated in an advisory committee for bluebird bio, Inc.

I.T. participated in clinical trials for bluebird bio, Inc.

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M.C. is a consultant for and an equity holder of Smart Immune.

S.H. Nothing to disclose.

T.S.O. is a consultant for bluebird bio, Inc.

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J.E.J.R. is a shareholder of RareCyte and Woke Pharmaceuticals; has provided supply of material (MTA) or consultancy or honoraria for RareCyte, Novartis, bluebird bio, Inc., Spark Therapeutics, Inc., Cynata, Pfizer, and CRISPR Tx; cofounded AAVec Bio; and is a non-executive director for Kennerton Capital.

J.B.K. is a consultant for bluebird bio, Inc., Novartis, Novo Nordisk, Pfizer, and Vertex.

S.A. and C.P. are former employees of and hold stock options in bluebird bio, Inc.

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M.C.W. is a member of an advisory board and current equity holder of stock options for Ensoma, Inc., and a steering committee member for Vertex Pharmaceuticals.

F.L. has received honoraria from and/or has been a member of the speakers bureau for Novartis, Jazz Pharmaceuticals, Medac, Sobi, Miltenyi, bluebird bio, Inc., Amgen, Neovii, and BMS, and has participated in an advisory board for Vertex.

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References

1. Farmakis D, Porter J, Taher A, Domenica Cappellini M, Angastiniotis M, Eleftheriou A. 2021 Thalassaemia International Federation guidelines for the management of transfusion-dependent thalassemia. *Hemasphere*. 2022;6(8):e732. doi:10.1097/hs9.0000000000000732

2. Taher AT, Cappellini MD. How I manage medical complications of β-thalassemia in adults. *Blood*. 2018;132(17):1781-1791. doi:10.1182/blood-2018-06-818187

3. Taher AT, Musallam KM, Cappellini MD. β-thalassemias. *N Engl J Med*. 2021;384(8):727-743. doi:10.1056/NEJMra2021838

4. Tubman VN, Fung EB, Vogiatzi M, et al. Guidelines for the standard monitoring of patients with thalassemia: Report of the thalassemia longitudinal cohort. *J Pediatr Hematol Oncol*. 2015;37(3):e162-e169. doi:10.1097/mph.0000000000000307

5. Bonifazi F, Conte R, Baiardi P, et al. Pattern of complications and burden of disease in patients affected by beta thalassemia major. *Curr Med Res Opin*. 2017;33(8):1525-1533. doi:10.1080/03007995.2017.1326890

6. Paramore C, Levine L, Bagshaw E, Ouyang C, Kudlac A, Larkin M. Patient- and caregiver-reported burden of transfusion-dependent β-thalassemia measured using a digital application. *Patient*. 2021;14(2):197-208. doi:10.1007/s40271-020-00473-0

7. Tuysuz G, Tayfun F. Health-related quality of life and its predictors among transfusion-dependent thalassemia patients. *J Pediatr Hematol Oncol*. 2017;39(5):332-336. doi:10.1097/mph.0000000000000790

8. Farmakis D, Giakoumis A, Angastiniotis M, Eleftheriou A. The changing epidemiology of the ageing thalassaemia populations: a position statement of the Thalassaemia International Federation. *Eur J Haematol*. 2020;105(1):16-23. doi:10.1111/ejh.13410

9. Lucarelli G, Isgrò A, Sodani P, Gaziev J. Hematopoietic stem cell transplantation in thalassemia and sickle cell anemia. *Cold Spring Harb Perspect Med*. 2012;2(5):a011825. doi:10.1101/cshperspect.a011825

10. Porter J. Beyond transfusion therapy: new therapies in thalassemia including drugs, alternate donor transplant, and gene therapy. *Hematology Am Soc Hematol Educ Program*. 2018;2018(1):361-370. doi:10.1182/asheducation-2018.1.361

11. 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. doi:10.3324/haematol.2013.099747

12. Baronciani D, Angelucci E, Potschger U, et al. Hemopoietic 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. doi:10.1038/bmt.2015.293

13. Chaudhury S, Ayas M, Rosen C, et al. A multicenter retrospective analysis stressing the importance of long-term follow-up after hematopoietic cell transplantation for β-thalassemia. *Biol Blood Marrow Transplant*. 2017;23(10):1695-1700. doi:10.1016/j.bbmt.2017.06.004

14. Di Bartolomeo P, Santarone S, Di Bartolomeo E, et al. Long-term results of survival in patients with thalassemia major treated with bone marrow transplantation. *Am J Hematol*. 2008;83(7):528-530. doi:10.1002/ajh.21175

15. Locatelli F, Thompson AA, Kwiatkowski JL, et al. Betibeglogene autotemcel gene therapy for non-β(0)/β(0) genotype β-thalassemia. *N Engl J Med*. 2022;386(5):415-427. doi:10.1056/NEJMoa2113206

16. Negre O, Bartholomae C, Beuzard Y, et al. Preclinical evaluation of efficacy and safety of an improved lentiviral vector for the treatment of β-thalassemia and sickle cell disease. *Curr Gene Ther*. 2015;15(1):64-81. doi:10.2174/1566523214666141127095336

17. Negre O, Eggimann AV, Beuzard Y, et al. Gene therapy of the β-hemoglobinopathies by lentiviral transfer of the β(A(T87Q))-globin gene. *Hum Gene Ther*. 2016;27(2):148-165. doi:10.1089/hum.2016.007

18. Thompson AA, Walters MC, Kwiatkowski J, et al. Gene therapy in patients with transfusion-dependent β-thalassemia. *N Engl J Med*. 2018;378(16):1479-1493. doi:10.1056/NEJMoa1705342

19. Schneiderman JS, Friedman D, Anderson C, et al. Mobilization and Apheresis Experience in Patients Receiving One-Time Beti-cel Gene Therapy for Transfusion-Dependent β-Thalassemia: A Pooled Analysis. *Transplant Cell Ther*. 2024;30(2):S233-S234. doi:10.1016/j.jtct.2023.12.305

20. Kwiatkowski JL, Walters MC, Hongeng S, et al. Betibeglogene autotemcel gene therapy in patients with transfusion-dependent, severe genotype β-thalassaemia (HGB-212): a non-randomised, multicentre, single-arm, open-label, single-dose, phase 3 trial. *Lancet*. 2024;404(10468):2175-2186. doi:10.1016/s0140-6736(24)01884-1

21. Thompson AA, Kwiatkowski JL, Schneiderman J, et al. Betibeglogene autotemcel (beti-cel) gene addition therapy results in durable hemoglobin A (HbA) production with up to 10 years of follow-up in participants with transfusion-dependent β-thalassemia. Presented at: 66th American Society of Hematology Annual Meeting; December 7-10, 2024; San Diego, California. Poster 2194.

22. ZYNTEGLO (betibeglogene autotemcel). Prescribing information. bluebird bio, Inc.; 2022.

23. Walters MC, Kwiatkowski JL, Porter JB, et al. Long term outcomes of 63 patients with transfusion-dependent β-thalassemia (TDT) followed up to 7 years post-treatment with betibeglogene autotemcel (beti-cel) gene therapy and exploratory analysis of predictors of successful treatment outcomes in phase 3 trials. *Blood*. 2022;140(Supplement 1):5355-5357. doi:10.1182/blood-2022-162400

24. Lal A, Locatelli F, Kwiatkowski JL, et al. Northstar-3: interim results from a phase 3 study evaluating lentiglobin gene therapy in patients with transfusion-dependent β-thalassemia and either a β0 or ivs-i-110 mutation at both alleles of the HBB gene. *Blood*. 2019;134(Supplement_1):815-815. doi:10.1182/blood-2019-128482

25. FERRIPROX (deferiprone). Prescribing information. Chiesi USA, Inc.; 2021.

26. U.S. Food and Drug Administration. Long Term Follow-Up After Administration of Human Gene Therapy Products: Guidance for Industry. Rockville, MD. 2020. Accessed 2 September 2025, https://www.fda.gov/media/113768/download.

27. Varni JW, Burwinkle TM, Seid M, Skarr D. The PedsQL 4.0 as a pediatric population health measure: feasibility, reliability, and validity. *Ambul Pediatr*. 2003;3(6):329-41. doi:10.1367/1539-4409(2003)003<0329:tpaapp>2.0.co;2

28. Sobota A, Yamashita R, Xu Y, et al. Quality of life in thalassemia: a comparison of SF-36 results from the thalassemia longitudinal cohort to reported literature and the US norms. *Am J Hematol*. 2011;86(1):92-5. doi:10.1002/ajh.21896

29. EuroQol Research Foundation. EQ-5D-3L User Guide. 2018. Accessed 1 December 2024, https://euroqol.org/information-and-support/documentation/user-guides/.

30. Whitney D, Shestopalov I, Fincker M, et al. Drug product attributes predict clinical efficacy in betibeglogene autotemcel gene therapy for β-thalassemia. *Mol Ther Methods Clin Dev*. 2023;31:101155. doi:10.1016/j.omtm.2023.101155

31. Braat S, Fielding KL, Han J, et al. Haemoglobin thresholds to define anaemia from age 6 months to 65 years: estimates from international data sources. *Lancet Haematol*. 2024;11(4):e253-e264. doi:10.1016/s2352-3026(24)00030-9

32. Hongeng S, Anurathapan U, Songdej D, et al. Wild-type HIV infection after treatment with lentiviral gene therapy for β-thalassemia. *Blood Adv*. 2021;5(13):2701-2706. doi:10.1182/bloodadvances.2020003680

33. Cattoni A, Rovelli A, Prunotto G, et al. Hepatic focal nodular hyperplasia after pediatric hematopoietic stem cell transplantation: the impact of hormonal replacement therapy and iron overload. *Pediatr Blood Cancer*. 2020;67(4):e28137. doi:10.1002/pbc.28137

34. Goyal S, Tisdale J, Schmidt M, et al. Acute Myeloid Leukemia Case after Gene Therapy for Sickle Cell Disease. *N Engl J Med*. 2022;386(2):138-147. doi:10.1056/NEJMoa2109167

35. Hsieh MM, Bonner M, Pierciey FJ, et al. Myelodysplastic syndrome unrelated to lentiviral vector in a patient treated with gene therapy for sickle cell disease. *Blood Adv*. 2020;4(9):2058-2063. doi:10.1182/bloodadvances.2019001330

36. Spencer Chapman M, Cull AH, Ciuculescu MF, et al. Clonal selection of hematopoietic stem cells after gene therapy for sickle cell disease. *Nat Med*. 2023;29(12):3175-3183. doi:10.1038/s41591-023-02636-6

37. Aydinok Y, Purushotham S, Yucel A, et al. Systematic literature review of the indirect costs and humanistic burden of β-thalassemia. *Ther Adv Hematol*. 2024;15:20406207241270872. doi:10.1177/20406207241270872

Tables

Table 1. Participant demographics and characteristics (transfusion population)

Parameter	Phase 1/2		Phase 3		All 4 studies (N = 63)	Participants with HRQOL data (n = 59)
	HGB-204 (N = 18)	HGB-205 (N = 4)	HGB-207 (N = 23)	HGB-212 (N = 18)		
Sex, n (%)						
Female	13 (72.2)	2 (50.0)	12 (52.2)	8 (44.4)	35 (55.6)	33 (56)
Male	5 (27.8)	2 (50.0)	11 (47.8)	10 (55.6)	28 (44.4)	26 (44)
Age at consent, n (%)						
Adult, ≥18 years	15 (83.3)	2 (50.0)	9 (39.1)	5 (27.8)	31 (49.2)	
Adolescent, ≥12 to <18 years	3 (16.7)	2 (50.0)	6 (26.1)	5 (27.88)	16 (25.4)	–
Pediatric, <12 years	0	0	8 (34.8)	8 (44.44)	16 (25.4)	
Race/ethnic group, n (%)						
Asian	14 (77.8)	2 (50.0)	13 (56.5)	7 (38.9)	36 (57.1)	34 (58)
White	4 (22.2)	2 (50.0)	8 (34.8)	10 (55.6)	24 (38.1)	22 (37)
Other/not reported	0	0	2 (8.7)	1 (5.6)	3 (4.7)	3 (5)
Genotype, n (%)						
Non-β^0/β^0 [a]	10 (55.6)	4 (100)	23 (100)	6 (33.3)	43 (68.3)	
β^0/β^0	8 (44.4)	0	0	12 (66.7)	20 (31.7)	–
αα/α-	3 (16.7)	0	1 (4.3)	1 (5.6)	5 (7.9%)	
Age at consent, median (range), years	20 (12-35)	17.5 (16-19)	15 (4-34)	12.5 (4-33)	17 (4-35)	17 (4-35)
Age at first transfusion, median (range), months	10 (0-132) [b]	21 (1-84)	12 (3-84)	8 (0-132)	10 (0-132)	–
pRBC transfusion volume ≤2 years before enrollment, median (range), mL/kg of body weight/year	169.1 (124.4-273.2)	181.9 (138.8-197.3)	207.9 (142.1-274.4)	185.2 (74.6-267.8)	190.0 (74.6-276.1)	–
pRBC transfusions/year ≤2 years before enrollment, median (range)	13.8 (10.0-17.5)	12.5 (10.5-13.0)	16.0 (11.5-37.0)	17.0 (11.0-39.5)	15.0 (10.0-39.5)	–
Weighted average nadir Hb level before transfusion, median (range), g/dL [c]	9.3 (7.0-10.1)	9.4 (8.1-10.8)	9.6 (7.5-11.0)	9.5 (8.2-10.7)	9.5 (7.0-11.0)	–
Splenectomy, n (%)	6 (33.3)	3 (75.0)	4 (17.4)	16 (25.4)	29 (46.0)	–
Iron status, median (range)						
LIC, mg Fe/g dw	5.7 (0.4-26.4)	11.2 (3.9-14.0)	5.3 (1.0-41.0)	3.5 (1.2-13.2)	5.3 (0.4-41.0)	

Cardiac T2*, msec	35 (10-54)	33 (29-46)	36.7 (21-57)	37 (15-75)	36.1 (10-75)	–
Ferritin, pmol/L	3147 (748-8629)[d]	4185 (2139-7097)	4438 (784-22517)	3275 (1279-8874)	3519 (748-22517)[e]	

dw, dry weight; Hb, hemoglobin; HRQOL, health-related quality of life; LIC, liver iron concentration; pRBC, packed red blood cell.
[a]Includes non-β^0/β^0 participants phenotypically similar to β^0/β^0 (IVS-1-110 homozygous or IVS-1-110/β^0 genotype).
[b]n = 15.
[c]The nadir Hb level is defined as the most recent Hb level before each pRBC transfusion, on the day of transfusion or within 3 days before the transfusion. The weighted average nadir Hb level at baseline is the weighted average of the nadir Hb level values in the 2-year period before enrollment.
[d]n = 17.
[e]n = 62.

Table 2. Transduction efficiency and pharmacodynamic endpoints (transfusion population)

	Phase 1/2 (N = 22)			Phase 3 (N = 41)		
	Adult ≥18 y (n = 17)	**Adolescent ≥12 to <18 y (n = 5)**	**Pediatric <12 y (n = 0)**	**Adult ≥18 y (n = 14)**	**Adolescent ≥12 to <18 y (n = 11)**	**Pediatric <12 y (n = 16)**
Number of mobilization cycles, n (%)[a]						
1 cycle	13 (82.4)	5 (100)	–	9 (64.3)	9 (81.8)	14 (87.5)
2 cycles[b]	4 (23.5)	0	–	5 (35.7)[a]	2 (18.2)	2 (12.5)
CD34+ cells infused, median (range), $\times 10^6$ cells/kg	8.1 (5.2-14.0)	12.0 (6.2-18.1)	–	8.9 (5.2-13.6)	7.4 (5.0-19.4)	10.2 (6.1-42.1)
DP cells transduced, median (range), %	32 (18-58)	25 (17-34)	–	77.5 (53-90)	82.0 (34-90)	72.2 (34-94)
Day of neutrophil engraftment, median (range)[c]	19 (14-30)	17 (16-27)	–	21 (13-27)	26 (16-38)	26 (17-39)
Day of platelet engraftment, median (range)	36 (19-191)	55 (20-65)	–	43 (21-58)	50 (25-84)	51 (20-94)
Month 6 PB VCN, median (range), c/dg	0.4 (0.1-1.6)	0.5 (0.1-4.7)	–	1.4 (0.2-3.4)	1.9 (0.2-4.5)	0.8 (0.2-3.3)
Month 6 HbA^{T87Q}, median (range), g/dL	4.7 (0.4-8.9)	5.5 (1.0-9.6)	–	9.4 (3.4-12.0)	8.7 (3.8-10.6)	8.0 (5.2-10.5)

DP, drug product; G-CSF, granulocyte colony-stimulating factor; HbA^{T87Q}, functional adult hemoglobin containing $\beta^{A\text{-}T87Q}$-globin; HSC, hematopoietic stem cell; PB peripheral blood; VCN, vector copy number.

[a]Patients underwent HSC mobilization with G-CSF for 5-6 days (5 or 10 µg/kg/day in patients without or with a spleen, respectively) and plerixafor (0.24 mg/kg/day) for 2-3 days starting day 4, followed by CD34+ cell collection by apheresis for 2-3 days starting on day 5.

[b]One participant needed 3 mobilization cycles, but cells from only 2 of these were used for DP manufacturing.

[c]Neutrophil engraftment was defined as the first of 3 consecutive days with an absolute neutrophil count ≥500 cells/mm^3 and platelet engraftment as the first of 3 consecutive platelet counts ≥20,000 cells/mm^3 at least 7 days after the last platelet transfusion.

Table 3. Beti-cel–related and serious AEs >2 years post beti-cel infusion

Preferred term[a]	**Participants, n (%) (N = 63)**
Possibly beti-cel related[b]	
Liver focal nodular hyperplasia[c]	1 (1.6)
Immune thrombocytopenia	1 (1.6)
Serious AEs	
Bacillus bacteremia	1 (1.6)
Cholelithiasis	1 (1.6)
Diabetic ketoacidosis	1 (1.6)
Ectopic pregnancy	1 (1.6)
Epstein-Barr virus infection	1 (1.6)
Fetal death	1 (1.6)
Gallbladder enlargement	1 (1.6)
Gallbladder polyps	1 (1.6)
Gastritis	1 (1.6)
Gonadotropin deficiency	1 (1.6)
Influenza	1 (1.6)
Major depression	1 (1.6)
Neutropenia	1 (1.6)
Placental polyp	1 (1.6)
Pulmonary embolism	1 (1.6)
Pyrexia	1 (1.6)

AE, adverse event

[a]Medical Dictionary for Regulatory Activities (MedDRA) version 19.0 or later.

[b]The study treatment and the AE were reasonably related in time, and the AE could be explained equally well by causes other than exposure to the study treatment product.

[c]Reported between 24 and 36 months post beti-cel infusion; an increase in size and number of hepatic nodules was reported for this participant between 36 and 48 months post beti-cel infusion.

Figure Legends

Figure 1. Pharmacodynamic measures and their relationship to Hb and transfusion independence following beti-cel.
(A) Median (Q1, Q3) PB VCN through long-term follow-up, stratified by study phase. (B) Median (Q1, Q3) total Hb (solid line) and HbA^{T87Q} levels (dashed line) over time by study phase. (C) Relationship between HbA^{T87Q} and PB VCN at last follow-up across all participants (Spearman ρ = 0.79, $P < 0.001$), with a fitted curve derived from a monotonic transformation to accurately capture the plateau in HbA^{T87Q} at higher VCN. Participants who achieved TI are shown in purple and those who did not are shown in yellow; study phase is indicated by shape (triangle, phase 1/2; circle, phase 3). (D) Linear fit of HbA^{T87Q} versus total Hb in participants who achieved TI, stratified by genotype subgroup (green, β^0/β^0; orange, non-β^0/β^0). Colors are consistent across panels A & B: blue, phase 1/2; red, phase 3. Shaded regions in panels C and D represent 95% confidence intervals.
Hb, hemoglobin; HbA^{T87Q}, functional adult hemoglobin containing $\beta^{A\text{-}T87Q}$-globin; PB, peripheral blood; TI, transfusion independence; Q1, quartile 1; Q3, quartile 3; VCN, vector copy number.

Figure 2. Longitudinal transfusion status and Hb response in phase 3 participants by genotype and age.
Each horizontal lane represents an individual participant, grouped by (A) genotype and (B) age category. Red dots indicate transfusion episodes. Numbers displayed at end of lanes represent unsupported total Hb (g/dL) at last follow-up. The vertical dashed line denotes completion of parent study and rollover to LTF-303. Transfusion independence defined as weighted average Hb ≥9 g/dL without pRBC transfusions for ≥12 months.
DP, drug product; Hb, hemoglobin; pRBC, packed red blood cell; TI, transfusion independent.
*Identifies 8 non-β^0/β^0 participants phenotypically similar to β^0/β^0 (IVS 1-110 homozygous or IVS 1-110/β^0 genotype).
†After a planned orthopedic surgery, the participant had blood loss that required 1 pRBC transfusion.
‡Identifies αα/α- participants.

Figure 3. Iron burden measures over time and relative change in participants who achieved TI.
(A) Median (Q1, Q3) serum ferritin over time from baseline through month 60 in participants who achieved TI. (B) Median (Q1, Q3) LIC over time from baseline through month 60. Gray shading represents the reference range for each measure. (C) Change in LIC from month 12 to last follow-up and (D) change in serum ferritin from baseline to last follow-up, shown as individual participant values. Colors are consistent across all panels: blue, phase 1/2; red, phase 3.
dw, dry weight; LIC, liver iron concentration; Q1, quartile 1; Q3, quartile 3; TI, transfusion independence.

Figure 4. Health-related quality of life scores over time after beti-cel in participants who achieved TI.
(A) SF-36 Mental Component Summary scores in adults through month 60. (B) SF-36 Physical Component Summary scores in adults through month 60. (C) PedsQL total scores in pediatric participants through month 60. All values shown as mean ± SD. Data are stratified by baseline status relative to the population norm: participants with scores better than the norm (red) and worse than the norm (gray). All participants who achieved TI are also shown together in blue. Horizontal dashed lines indicate population normative means; shaded regions represent standard error. MCID is noted for each measure.
MCID, minimal clinically important difference; PedsQL, Pediatric Quality of Life Inventory; SD, standard deviation; SP-36, Short Form-36; TI, transfusion independent.

[a]1 participant with physical component summary scores worse than the population norm at baseline reported a recent fracture prior to data capture at month 36.

Figure 1

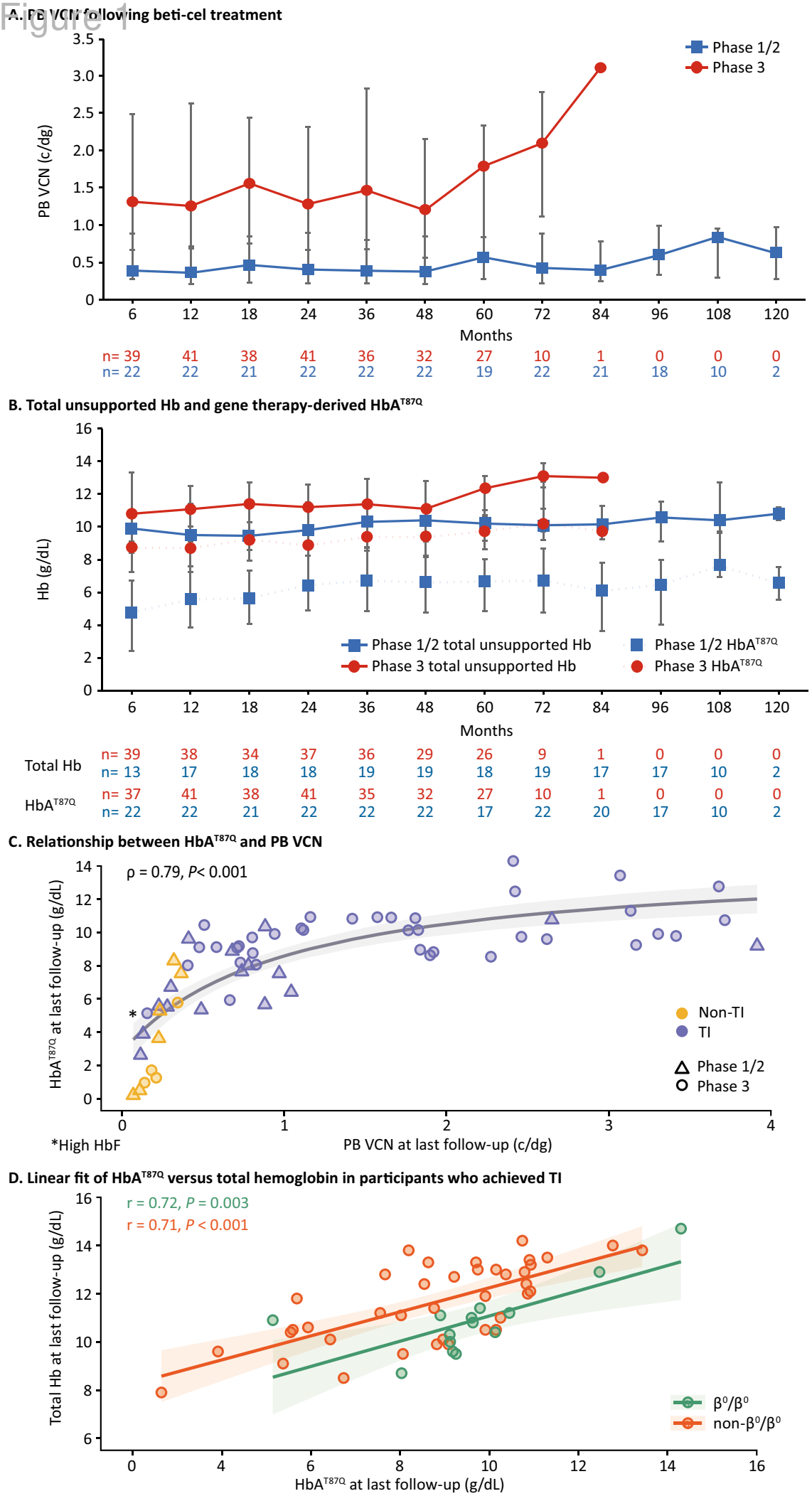
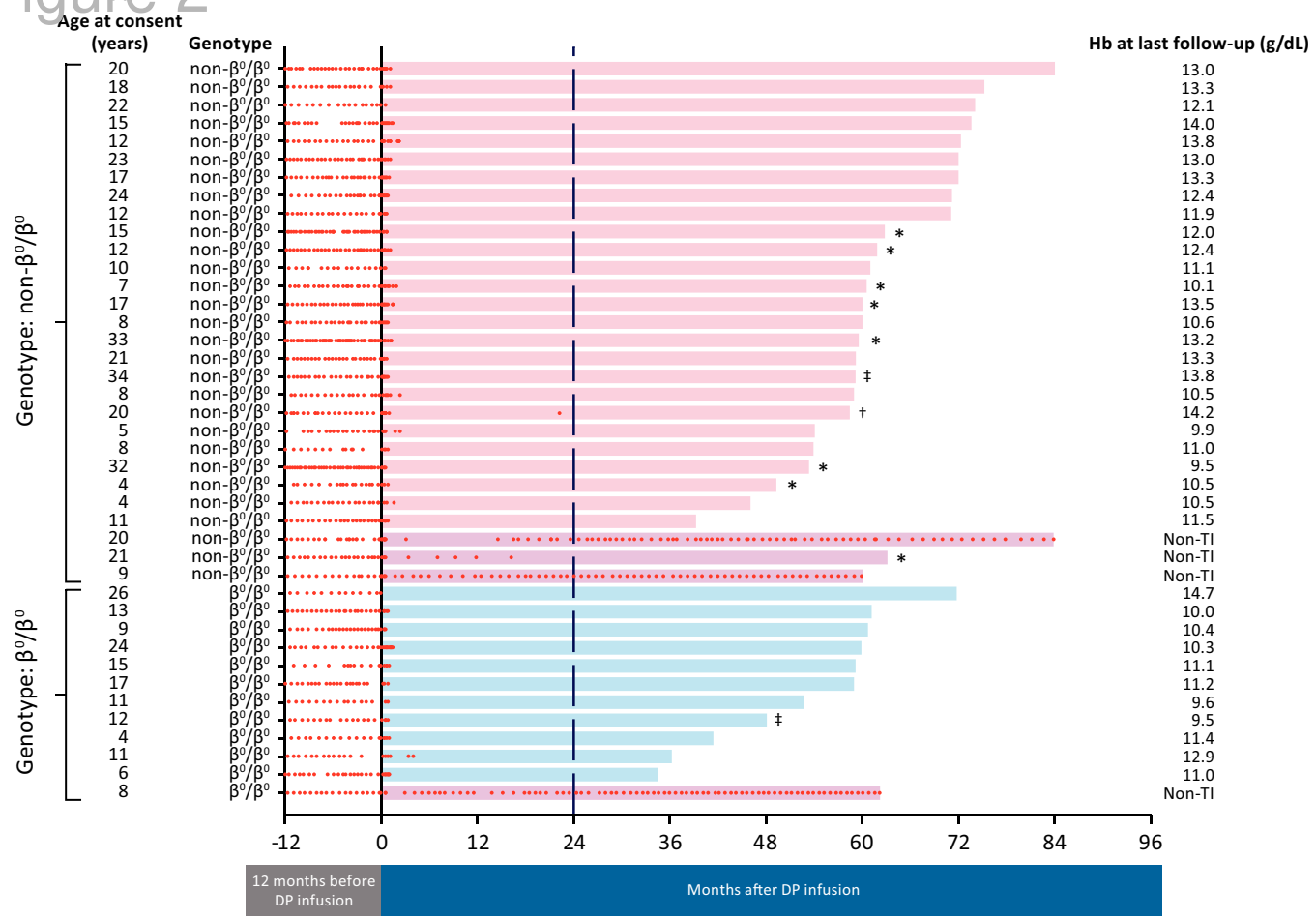


Figure 2



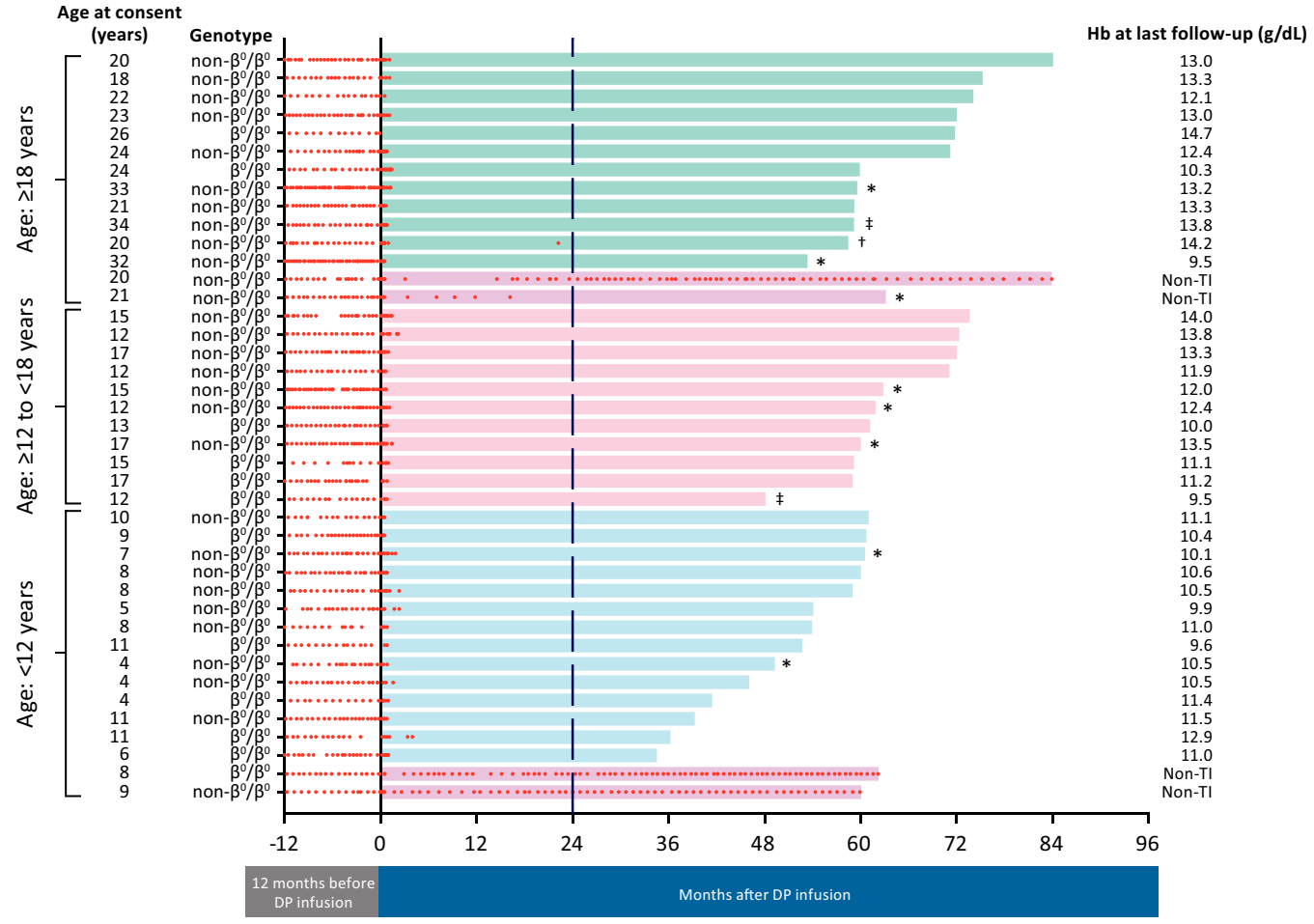


Figure 3

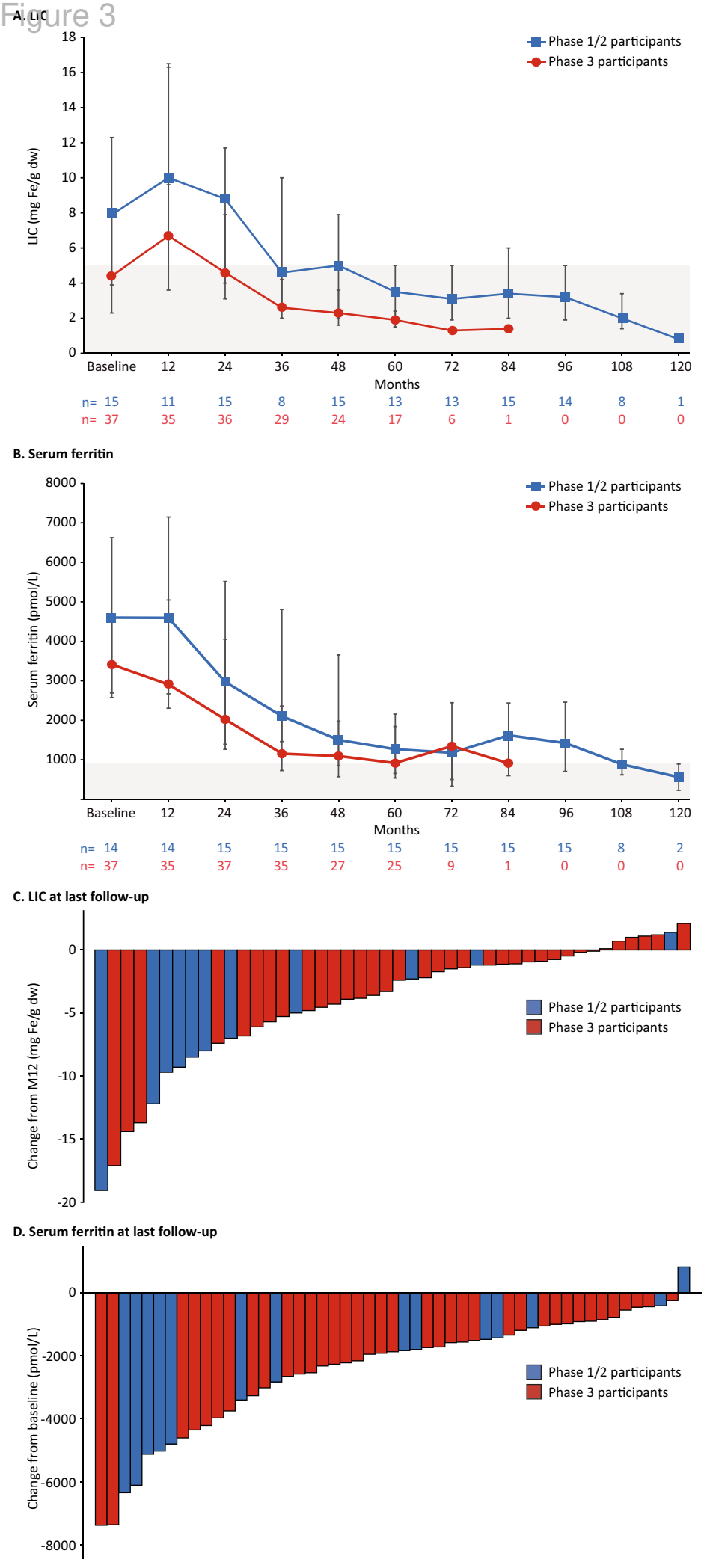
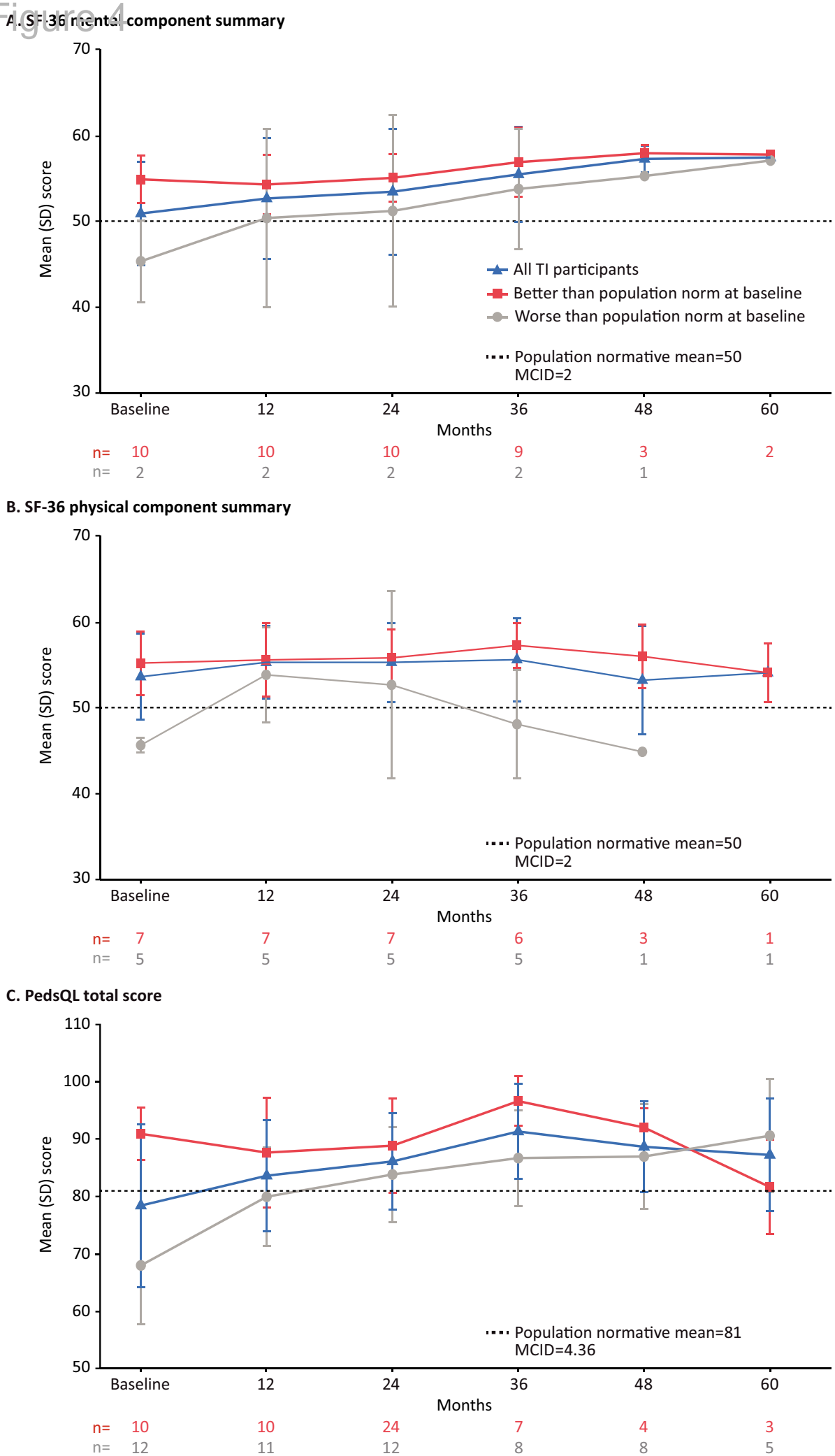


Figure 4



Long-Term Efficacy and Safety of Betibeglogene Autotemcel Gene Therapy for Transfusion-Dependent β-Thalassemia (TDT)

Context of Research

Betibeglogene autotemcel (beti-cel) gene therapy for transfusion-dependent β-thalassemia (TDT) involves autologous transplantation of hematopoietic stem and progenitor cells transduced with a modified β-globin gene to produce functional adult hemoglobin

ClinicalTrials.gov ID NCT02633943

Phase 1/2
68% TI

Increased transduction efficiency

Phase 3
90% TI

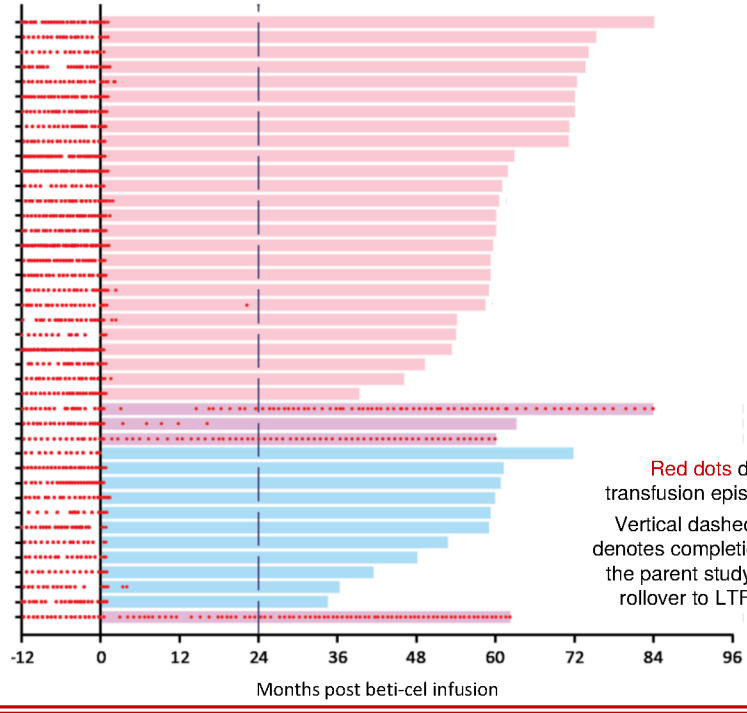
Participants who achieved **transfusion independence (TI)** had:

- Reduced ineffective erythropoiesis
- Reduced iron overload
- Improved health-related quality-of-life scores

76% of phase 3 participants who achieved TI were **off chelation therapy at last follow-up**

Outcomes

Sustainable transfusion independence with up to 10 years follow-up



Conclusion: In patients with transfusion-dependent β-thalassemia, betibeglogene autotemcel gene therapy led to sustained transfusion independence and normal or near-normal hemoglobin levels, with a favorable long-term safety profile.

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blood Visual Abstract