Mobilization and Apheresis Experience in Patients Receiving One-Time Beti-cel Gene Therapy for Transfusion-Dependent β-Thalassemia: A Pooled Analysis

Jennifer Schneiderman,[1*] David Friedman,[2] Christina Anderson,[3**] Ge Tao,[3] Shamshad Ali,[3] Alexandria Petrusich,[4**] Himal L. Thakar,[3] Richard A. Colvin,[3] Evangelia Yannaki[5]

[1]Ann & Robert H. Lurie Children's Hospital of Chicago, Northwestern University Feinberg School of Medicine, Chicago, IL; [2]Children's Hospital of Philadelphia, Philadelphia, PA; [3]bluebird bio, Inc., Somerville, MA; [4]Prime Medicine, Inc., Cambridge, MA; [5]G. Papanikolaou General Hospital, Thessaloniki, Greece

*Presenting author. **Former employee of bluebird bio.*

Poster 304

INTRODUCTION

- Betibeglogene autotemcel (beti-cel) gene therapy is approved for the treatment of adult and pediatric patients with transfusion-dependent β-thalassemia (TDT)[1]
- Beti-cel gene therapy addresses the underlying cause of TDT by adding functional copies of a modified version of the β-globin gene to autologous CD34+ hematopoietic stem cells (HSCs) via a third-generation, self-inactivating lentiviral vector (LVV), BB305[2]
- Following one-time beti-cel therapy, HbA^{T87Q}, functional adult hemoglobin (Hb) containing an amino acid substitution (T→Q) at position 87, is produced in red blood cells (RBCs).[2] HbA^{T87Q} has similar oxygen binding affinity to HbA[3]
- In beti-cel clinical trials, HSC mobilization with granulocyte colony-stimulating factor (G-CSF) and plerixafor has been used to achieve sufficient yields of CD34+ cells for gene therapy in patients with TDT (phase 1/2: HGB-204 [NCT01745120] and HGB-205 [NCT02151526]; phase 3: HGB-207 [NCT02906202] and HGB-212 [NCT03207009])[2,4]

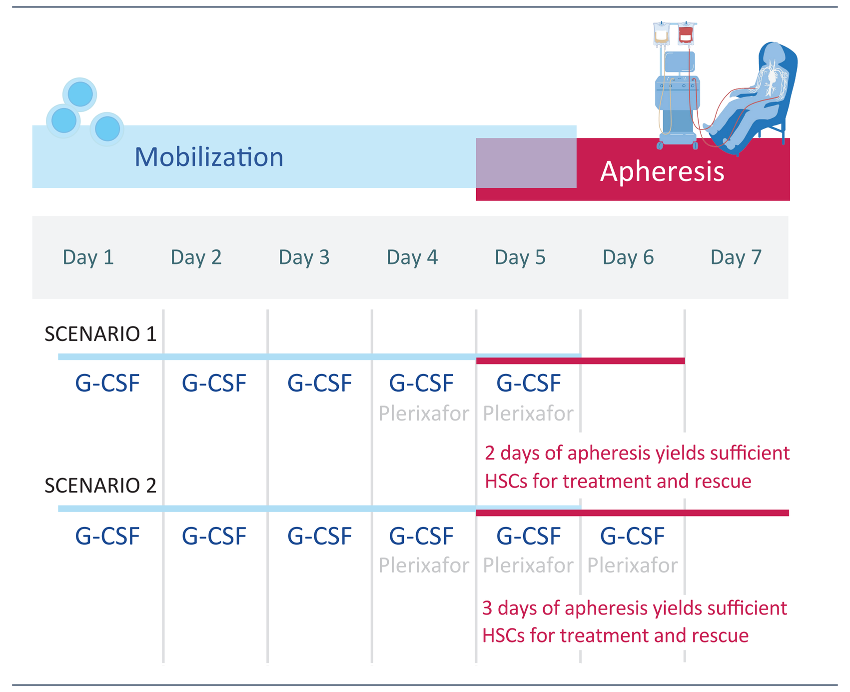
OBJECTIVE

- To evaluate pooled data on the mobilization and collection of HSCs from the intent-to-treat populations in HGB-204, HGB-205, HGB-207, and HGB-212

METHODS

- Beti-cel trials were single-arm, open-label, single-dose studies that enrolled pediatric and adult patients with TDT, with or without prior splenectomy
- In this post hoc, pooled analysis, the ages of patients by study were 12-35 years in HGB-204, 16-19 years in HGB-205, 4-34 years in HGB-207, and 4-33 years in HGB-212
- Patients underwent HSC mobilization with G-CSF (5 or 10 µg/kg/day in patients without or with a spleen, respectively) and plerixafor (0.24 mg/kg/day), followed by CD34+ cell collection by apheresis[2,4] (**Figure 1**)
- The target number of cells to be collected via apheresis was ≥10×10⁶ CD34+ cells/kg in HGB-204 and HGB-205 and ≥12×10⁶ CD34+ cells/kg in HGB-207 and HGB-212, irrespective of age or genotype, for a minimum drug product dose of 5x10⁶ CD34+ cells/kg
- The number of mobilization cycles needed to achieve cell collection target was analyzed; data for various baseline indexes are summarized descriptively based on number of mobilization cycles
- In an exploratory analysis, Fisher's exact test was used to assess the relationship between splenectomy status and the number of mobilization cycles

Figure 1. Mobilization and apheresis schedule[a,b]



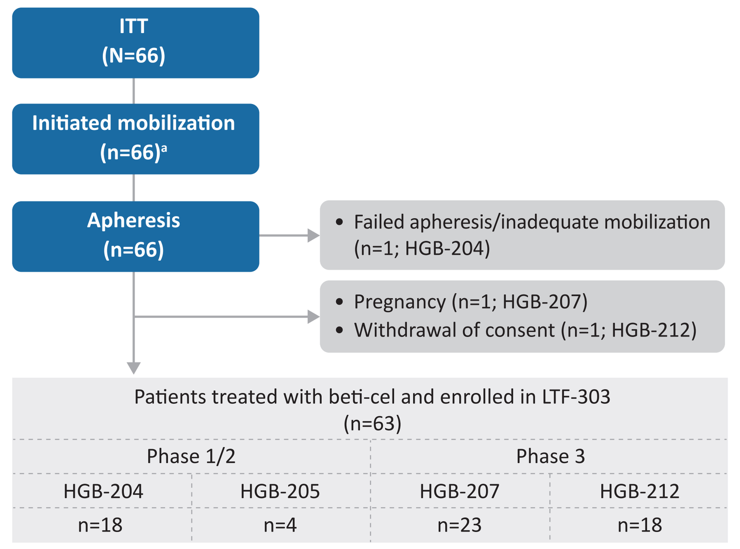
[a]G-CSF dose in splenectomized patients was 5 µg/kg/day and 10 µg/kg/day in nonsplenectomized patients; plerixafor dose was 0.24 mg/kg. [b]Apheresis generally occurred on mobilization days 5 and 6, and if a third day of collection was needed, G-CSF and plerixafor dosing was extended to day 6.
G-CSF, granulocyte colony-stimulating factor; HSC, hematopoietic stem cell.

RESULTS

Baseline characteristics and follow-up

- Across the 4 studies, 66 patients initiated mobilization (splenectomized, n=18; nonsplenectomized, n=48; **Figure 2**; **Table 1**)

Figure 2. Enrollment in beti-cel studies



[a]Splenectomized, n=18; nonsplenectomized, n=48.
ITT, intent-to-treat.

Table 1. Demographic and clinical characteristics

Characteristic	Pooled population N=66	Splenectomized n=18	Nonsplenectomized n=48
Age at consent, median (min, max), y	17.5 (4, 35)	21.0 (11, 34)	15.0 (4, 35)
Sex, n (%)			
Female	37 (56.1)	10 (55.6)	27 (56.3)
Male	29 (43.9)	8 (44.4)	21 (43.8)
Genotype, n (%)			
β^0/β^0	21 (31.8)	5 (27.8)	16 (33.3)
Non-β^0/β^0	44 (66.7)	12 (66.7)	32 (66.7)
Other[a]	1 (1.5)	1 (5.6)	0
Weight at initiation of mobilization,[b] median (min, max), kg	49.9 (14.8, 88.1)	60.1 (43.4, 88.1)	40.5 (14.8, 80.4)
pRBC transfusion volume for 2 y before enrollment, median (min, max),[c] mL/kg	190.4 (75, 289)	160.2 (119, 276)	195.0 (75, 289)
Age at initiation of regular transfusions,[d] median (min, max), mo	36.0 (1, 312)	48.0 (1, 192)	36.0 (2, 312)

[a]Other genotype reported as HBB:c.92+1G>T and unknown. [b]Baseline weight was included if unavailable at initiation of mobilization. [c]Pooled population (n=65), splenectomized (n=17), nonsplenectomized (n=48). [d]Pooled population (n=64), splenectomized (n=17), nonsplenectomized (n=47).
pRBC, packed red blood cells.

- Total cell collection target was achieved after 1 mobilization cycle in 52/66 (78.8%) patients and after 2 cycles in 13/66 (19.7%)
  - One patient (HGB-204) with a β^E/β^0 genotype and a prior splenectomy was withdrawn for inadequate apheresis after 1 cycle with G-CSF and plerixafor; this patient experienced leukocytosis and increased lymphocyte counts after baseline that were deemed potentially clinically significant
  - Among phase 3 patients, total cell collection target was achieved after 1 mobilization cycle in 34/43 (79.1%) patients and after 2 cycles in 9/43 (20.9%)
- The majority of patients (14/16 [87.5%] aged <12 years, 15/17 [88.2%] ≥12 to <18 years, and 23/33 [69.7%] ≥18 years) achieved total cell collection target after 1 mobilization cycle
- A significantly greater proportion of splenectomized patients (8/18 [44.4%]) had 2 mobilization cycles vs nonsplenectomized patients (5/48 [10.4%]; *P*=0.0042; **Table 2**)

Table 2. Details of mobilization cycles and apheresis procedures

Characteristic	Pooled population N=66	Splenectomized n=18	Nonsplenectomized n=48
Number of mobilization and apheresis cycles per patient used for DP, n (%)			
1	53 (80.3)	10 (55.6)	43 (89.6)
2	13 (19.7)[a]	8 (44.4)	5 (10.4)
		P=0.0042	
Number of apheresis collection days per mobilization cycle, median (min, max)[b]	**2.0 (1.0, 3.0)**	**2.5 (1.5, 3.0)**	**2.0 (1.0, 3.0)**
Proportion of patients who had apheresis collections by cycle and collection day,[c] n (%)			
Mobilization and apheresis cycle 1			
Collection day 1	66 (100)	18 (100)	48 (100)
Collection day 2	56 (84.8)[d]	18 (100)[d]	38 (79.2)
Collection day 3[e]	18 (27.3)	11 (61.1)	7 (14.6)
Mobilization and apheresis cycle 2			
Collection day 1	13 (100), n=13	8 (100), n=8	5 (100), n=5
Collection day 2	8 (61.5), n=13	7 (87.5), n=8	1 (20.0), n=5
Whole blood volume processed per cycle and collection day, median (min, max), L			
Cycle 1	21.4 (3.5, 57.6), N=66	33.2 (18.1, 57.6), n=18	17.2 (3.5, 42.0), n=48
Collection day 1	11.3 (3.5, 31.5), n=65	13.9 (7.9, 31.5), n=17[f]	10.1 (3.5, 24.6), n=48
Collection day 2	10.6 (2.5, 19.3), n=56	13.3 (6.0, 19.3), n=18	9.0 (2.5, 18.4), n=38
Collection day 3[e]	11.4 (5.2, 19.0), n=18	12.5 (6.3, 19.0), n=11	10.8 (5.2, 14.1), n=7
Cycle 2	20.9 (5.6, 40.6), n=13	29.1 (15.4, 40.6), n=8	7.0 (5.6, 17.6), n=5
Collection day 1	13.1 (5.6, 22.2), n=13	15.3 (12.4, 22.2), n=8	7.0 (5.6, 8.9), n=5
Collection day 2	15.0 (8.5, 20.2), n=8	15.0 (8.5, 20.2), n=7	9.1, n=1
Total blood volumes processed,[g] median (min, max), × (-fold of a patient's total blood volume)			
Cycle 1	6.32 (2.58, 12.16), n=43	9.09 (5.09, 11.71), n=8	6.10 (2.58, 12.16), n=35
Collection day 1	3.33 (2.28, 6.85), n=43	3.56 (2.39, 4.12), n=8	3.33 (2.28, 6.85), n=35
Collection day 2	3.25 (1.00, 4.61), n=35	3.29 (1.89, 4.11), n=8	3.18 (1.00, 4.61), n=27
Collection day 3[e]	3.06 (1.90, 4.44), n=8	2.70 (1.90, 3.87), n=6	4.06 (3.67, 4.44), n=2
Cycle 2	7.05 (1.61, 9.07), n=9	7.22 (6.41, 8.13), n=5	2.96 (1.61, 9.07), n=4
Collection day 1	3.95 (1.61, 4.52), n=9	4.01 (3.00, 4.29), n=5	2.96 (1.61, 4.52), n=4
Collection day 2	3.63 (2.93, 4.54), n=6	3.41 (2.93, 4.00), n=5	4.54, n=1

[a]One patient from HGB-212 (1/66; 1.5%) required 3 mobilization cycles, but cells from only 2 of these cycles were used for DP manufacturing. [b]Values represent per patient median number of apheresis collection days, including patients who had 1 mobilization cycle and patients who had 2. [c]Cell collection for DP was done within 48 hours and shipped to the manufacturing site. No more than 2 consecutive apheresis procedure products may be sent for each transduction. Each transduction produces an individual DP lot. Each mobilization cycle may include up to 3 apheresis procedure days. Apheresis procedure products can also be used for rescue cells and may come from either a full or partial day of collection. Note that apheresis collections intended for DP manufacture on a given day may be split to remove cells for rescue (≥1.5×10⁶ CD34+ cells/kg, but <4.0×10⁶ CD34+ cells/kg) only if ≥25×10⁶ CD34+ cells/kg are procured or an aggregate of ≥16×10⁶ CD34+ cells/kg are collected through subsequent days of apheresis.[5] [d]Includes the patient from HGB-204 with a β^E/β^0 genotype and a prior splenectomy who was withdrawn for inadequate apheresis after 1 cycle with G-CSF and plerixafor. This patient did not receive DP. [e]CD34+ cells collected were only used for rescue and not for DP manufacture. [f]One patient is missing their day 1 blood processed volume. [g]Patient's total blood volume was available for 43 patients.
DP, drug product; G-CSF, granulocyte colony-stimulating factor.

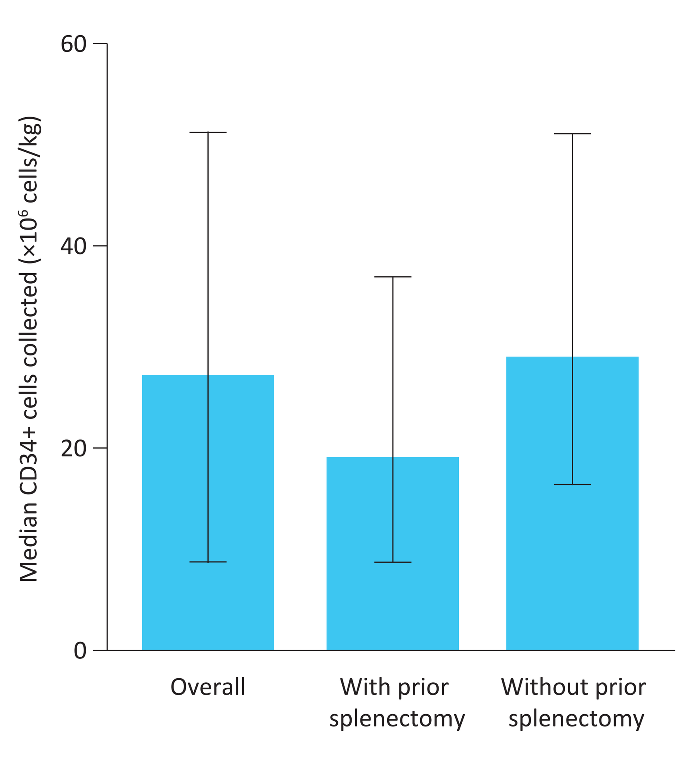
- Median (min, max) CD34+ cell yield collected for beti-cel manufacturing was 19.44 (8.9, 36.9) ×10⁶ cells/kg in splenectomized patients and 29.46 (16.9, 51.1) ×10⁶ cells/kg in nonsplenectomized patients (**Table 3**; **Figure 3**)

Table 3. CD34+ cell yield

Characteristic	Pooled population N=66	Splenectomized n=18	Nonsplenectomized n=48
PB CD34+ cell count during mobilization and apheresis, median (min, max), cells/µL			
Day 1 of collection	105.70 (26.6, 505.5)	82.00 (36.1, 388.0)	156.90 (26.6, 505.5)
Day 2 of collection	95.35 (10.0, 505.5)	68.00 (10.0, 242.0)	135.00 (24.0, 505.5)
Number of CD34+ cells collected, median (min, max), ×10⁶ cells/kg	**27.10 (8.9, 51.1) n=43**	**19.44 (8.9, 36.9) n=8**	**29.46 (16.9, 51.1) n=35**
For beti-cel manufacturing	18.92 (0.1, 49.1) n=66	14.46 (0.1, 34.4) n=18	20.45 (9.2, 49.1) n=48
For rescue	4.00 (0.0,[a] 55.0) n=65	3.80 (0.0, 7.8) n=17	5.00 (1.5, 55.0) n=48
Number of CD34+ cells collected by Hb at start of mobilization, median (min, max), ×10⁶ cells/kg			
Patients with Hb ≥11 g/dL	18.80 (0.1, 49.1) n=43	12.96 (0.1, 33.3) n=12	22.30 (9.5, 49.1) n=31
Patients with Hb <11 g/dL	19.04 (9.2, 34.4) n=23	22.19 (14.1, 34.4) n=6	17.60 (9.2, 33.9) n=17

[a]0.0 represents the patient with inadequate apheresis after 1 cycle with G-CSF and plerixafor.
G-CSF, granulocyte colony-stimulating factor; Hb, hemoglobin; PB, peripheral blood.

Figure 3. CD34+ cell yield after mobilization by splenectomy status



Data are presented as median (min, max).

- Among patients with available values, baseline median liver iron concentration, Hb, total white blood cells (WBCs), neutrophil:lymphocyte ratio in blood, and myeloid:erythroid ratio in bone marrow were similar between patients who had 1 mobilization cycle and those who had 2 (**Table 4**)
  - However, as expected, median WBCs, reticulocytes, and platelet counts at baseline were higher in splenectomized patients
- Following hypertransfusion for 60 days, median (min, max) Hb on day 1 of G-CSF was 11.80 g/dL (8.8, 14.2) in splenectomized patients and 11.70 g/dL (8.3, 15.2) in nonsplenectomized patients
- Despite the lower G-CSF dose of 5 µg/kg/day required for splenectomized patients (vs 10 µg/kg/day for nonsplenectomized patients), their WBCs and reticulocytes were higher during mobilization and apheresis (data not shown)

Table 4. Baseline characteristics by number of mobilization cycles

Baseline characteristic at study entry, median (min, max)	Pooled population			Patients with 1 mobilization cycle			Patients with 2 mobilization cycles		
		Splenectomized			Splenectomized			Splenectomized	
	All	Yes	No	All	Yes	No	All	Yes	No
Liver iron concentration, mg Fe/g of dry weight	5.3 (0.4, 41.0) n=63	6.6 (0.4, 41.0) n=16	5.3 (1.0, 26.4) n=47	5.5 (1.0, 26.4) n=50	9.8 (1.9, 16.8) n=8	5.2 (1.0, 26.4) n=42	2.4 (0.4, 41.0) n=13	2.4 (0.4, 41.0) n=8	6.3 (1.2, 9.7) n=5
Hb, g/dL	10.6 (8.6, 14.0) N=66	10.7 (8.7, 14.0) n=18	10.5 (8.6, 13.9) n=48	10.3 (8.6, 14.0) n=53	10.7 (9.1, 14.0) n=10	10.3 (8.6, 13.9) n=43	11.1 (8.7, 12.5) n=13	10.7 (8.7, 12.0) n=8	11.2 (9.8, 12.5) n=5
Total white blood cells, ×10⁹ cells/L	8.1 (3.1, 34.2) N=66	12.2 (7.1, 34.2) n=18	7.4 (3.1, 15.1) n=48	8.2 (3.1, 34.2) n=53	13.5 (7.5, 34.2) n=10	7.6 (3.1, 15.1) n=43	7.6 (4.5, 14.1) n=13	11.1 (7.1, 14.1) n=8	6.8 (4.5, 7.6) n=5
Reticulocytes, ×10⁹ cells/L	35.2 (0.3, 235) n=62	52.6 (10.8, 235) n=15	29.0 (0.5, 205) n=47	32.9 (0.5, 205) n=50	49.4 (16.2, 186) n=8	32.4 (0.5, 205) n=42	41.1 (10.8, 235) n=12	58.5 (10.8, 235) n=7	21 (12, 43) n=5
Platelets, ×10⁹ cells/L	310 (104, 933) N=66	553 (282, 933) n=18	264 (104, 588) n=48	307 (104, 933) n=53	578 (282, 933) n=10	272 (104, 588) n=43	398 (113, 863) n=13	535 (366, 863) n=8	183 (113, 275) n=5
Neutrophil:lymphocyte ratio	1.3 (0.3, 5.8) N=66	1.1 (0.3, 1.8) n=18	1.5 (0.6, 5.8) n=48	1.3 (0.3, 5.8) n=53	0.9 (0.3, 1.5) n=10	1.5 (0.6, 5.8) n=43	1.3 (0.7, 2.0) n=13	1.3 (0.7, 1.8) n=8	1.3 (1.1, 2.0) n=5
Myeloid:erythroid ratio[a]	0.4 (0.1, 2.8) n=33	0.4 (0.1, 0.6) n=6	0.4 (0.1, 2.8) n=27	0.4 (0.1, 2.8) n=24	0.5 n=1	0.4 (0.1, 2.8) n=23	0.3 (0.1, 0.9) n=9	0.4 (0.1, 0.6) n=5	0.3 (0.2, 0.9) n=4

[a]Bone marrow.
Hb, hemoglobin.

Safety

- Overall, 51/66 (77.3%) patients experienced ≥1 adverse event (AE) attributed to mobilization/apheresis (**Table 5**), the most common of which were hypocalcemia and thrombocytopenia
- Grade ≥3 AEs occurred in 22.2% (4/18) of splenectomized patients vs 6.3% (3/48) of nonsplenectomized
- Serious AEs were reported in only 1 patient (thrombocytopenia and hypokalemia; this patient had a prior splenectomy)

Table 5. Adverse events attributed to mobilization and apheresis (occurring in >5% of patients)

	Pooled population N=66	Splenectomized n=18	Nonsplenectomized n=48
Patients with AEs attributed to mobilization/apheresis, n (%)	**51 (77.3)**	**16 (88.9)**	**35 (72.9)**
AEs in >1 patient			
Hypocalcemia	13 (19.7)	3 (16.7)	10 (20.8)
Thrombocytopenia	12 (18.2)	5 (27.8)	7 (14.6)
Bone pain	10 (15.2)	1 (5.6)	9 (18.8)
Headache	9 (13.6)	3 (16.7)	6 (12.5)
Back pain	6 (9.1)	1 (5.6)	5 (10.4)
Peripheral sensory neuropathy	6 (9.1)	4 (22.2)	2 (4.2)
Nausea	5 (7.6)	1 (5.6)	4 (8.3)
Dizziness	4 (6.1)	3 (16.7)	1 (2.1)
Leukocytosis[a]	4 (6.1)	2 (11.1)	2 (4.2)
Patients with grade ≥3 AEs attributed to mobilization/apheresis, n (%)	**7 (10.6)**	**4 (22.2)**	**3 (6.3)**
Thrombocytopenia	5 (7.6)	3 (16.7)	2 (4.2)
Leukocytosis[a]	2 (3.0)	1 (5.6)	1 (2.1)
Pain	1 (1.5)	1 (5.6)	0
Hypocalcemia	1 (1.5)	1 (5.6)	0
Hypokalemia	1 (1.5)	0	1 (2.1)
Patients with SAEs attributed to mobilization/apheresis, n (%)	**1 (1.5)**	**1 (5.6)**	**0**
Thrombocytopenia	1 (1.5)	1 (5.6)	0
Hypokalemia	1 (1.5)	1 (5.6)	0

[a]Leukocytosis occurred in 2 patients in HGB-207: 1 patient had a maximum leukocyte count of 104.12×10⁹ cells/L and 1 patient had a maximum leukocyte count of 118.05×10⁹ cells/L.
AE, adverse event; SAE, serious adverse event.

CONCLUSIONS

- The mobilization and apheresis protocols used in beti-cel clinical trials for the treatment of patients with TDT were well tolerated and effective, with most patients only requiring 1 mobilization cycle
- Protocols described here achieved target CD34+ cell yields for HSC transduction, with a majority of patients in the pooled trial population undergoing only 1 mobilization cycle
- The proportion of splenectomized patients who had 2 mobilization cycles was significantly greater compared with nonsplenectomized patients
- The mobilization and apheresis protocols were well tolerated, with hypocalcemia and thrombocytopenia being the most common AEs attributed to mobilization and apheresis
- These analyses can inform real-world practice to achieve optimal CD34+ cell yields for successful beti-cel drug product manufacturing and treatment

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DISCLOSURES

JS: Membership on board of directors or advisory committees at bluebird bio; consultancy for Therakos Mallinckrodt. **CA, AP:** Former employees of and current equity holders in bluebird bio. **GT, SA, HLT, RAC:** Current employees of and equity holders in bluebird bio. **DF, EY:** Nothing to disclose.

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