Long-term Outcomes of 63 Patients with Transfusion-dependent β-Thalassemia (TDT) Followed-up to 8 Years After Treatment with betibeglogene autotemcel (beti-cel) Gene Therapy and Factors Impacting Neutrophil and Platelet Engraftment

Timothy S. Olson[1,2], Mark C. Walters[3], Janet L. Kwiatkowski[2,4], John B. Porter[5], Jennifer Schneiderman[6], Suradej Hongeng[7], Andreas E. Kulozik[8], Marina Cavazzana[9–11], Martin G. Sauer[12], Adrian J. Thrasher[13], Isabelle Thuret[14], Ashutosh Lal[3], John E.J. Rasko[15–17], Evangelia Yannaki[18], Shamshad Ali[19], Richard A. Colvin[19], Franco Locatelli[20], Alexis A. Thompson[4]

[1]Cellular Therapy and Transplant Section, Division of Oncology Children's Hospital of Philadelphia, Philadelphia, Pennsylvania; [2]Department of Pediatrics, Perelman School of Medicine of the University of Pennsylvania, Philadelphia, Pennsylvania; [3]University of California San Francisco Benioff Children's Hospital, Oakland, CA, USA; [4]Division of Hematology, Children's Hospital of Philadelphia, Philadelphia, PA, USA; [5]University College London Hospital, London, UK; [6]Ann and Robert H. Lurie Children's Hospital of Chicago, Northwestern University Feinberg School of Medicine, Chicago, IL, USA; [7]Ramathibodi Hospital, Mahidol University, Bangkok, Thailand; [8]Molecular Medicine Partnership Unit (MMPU), European Molecular Biology Laboratory (EMBL), and Department of Pediatric Oncology, Hematology, and Immunology, University of Heidelberg, Heidelberg, Germany; [9]Biotherapy Department and Clinical Investigation Center, Assistance Publique Hopitaux de Paris, Inserm, Paris, France; [10]Université de Paris, Paris, France; [11]Imagine Institute, Paris, France; [12]Department of Pediatric Hematology/Oncology and Blood Stem Cell Transplantation, Hannover Medical School, Hannover, Germany; [13]UCL Great Ormond Street Institute of Child Health and Great Ormond Street Hospital NHS Trust, London, UK; [14]Hôpital de la Timone, Marseille, France; [15]The University of Sydney, Sydney, New South Wales, Australia; [16]Centenary Institute, Camperdown, New South Wales, Australia; [17]Cell and Molecular Therapies, Royal Prince Alfred Hospital, Camperdown, New South Wales, Australia; [18]Gene and Cell Therapy Center, Hematology-HCT Unit, G. Papanikolaou Hospital, Thessaloniki, Greece; [19]bluebird bio, Inc., Somerville, MA, USA; [20]IRCCS Ospedale Pediatrico Bambino Gesù, Catholic University of the Sacred Heart, Rome, Italy.

Poster 385

INTRODUCTION

- beti-cel is a US Food and Drug Administration approved gene therapy indicated for adult and pediatric patients with β-thalassemia, who require regular red blood cell transfusions, regardless of age or genotype[1]
- beti-cel addresses the underlying cause of transfusion-dependent β-thalassemia (TDT) by adding functional copies of a modified *HBB* gene, $\beta^{A\text{-}T87Q}$, to autologous CD34+ hematopoietic stem and progenitor cells (HSPCs) through transduction with BB305 lentiviral vector (LVV)[2]
- We previously showed durable transfusion independence (TI), improvement in markers of ineffective erythropoiesis and iron overload, and a favorable benefit-risk profile in patients treated with beti-cel[3]
- Here, we report up to 8 years of efficacy and safety follow-up across phase 1/2, phase 3, and long-term follow-up (LTF) studies of patients treated with beti-cel
- Additional analyses were conducted related to:
  - Factors affecting platelet engraftment
  - The impact of granulocyte colony stimulating factor (G-CSF) on neutrophil engraftment
  - The clinical significance of elevated liver iron concentration (LIC) at baseline

OBJECTIVES

- To evaluate long-term efficacy and durability, as measured by TI, in patients treated with beti-cel
- To evaluate post-infusion engraftment kinetics, safety in patients with elevated baseline LIC, and overall long-term safety

METHODS

- Patients with TDT underwent HSPC collection after mobilization with G-CSF and plerixafor, followed by pharmacokinetic-adjusted myeloablative busulfan conditioning and beti-cel infusion
- Patients were followed for 2 years in the parent phase 1/2 (HGB-204, NCT01745120; HGB-205, NCT02151526) and phase 3 (HGB-207, NCT02906202; HGB-212, NCT03207009) studies
  - Enrollment and treatment are complete
- Patients then entered the 13-year ongoing long-term follow-up study, LTF-303 (NCT02633943)
- The primary efficacy outcome, transfusion independence (TI), was defined as weighted average hemoglobin (Hb) ≥9 g/dL without packed red blood cell transfusions for ≥12 months
- Patient- and drug-specific factors were analyzed for their association with platelet engraftment
- The relationship between G-CSF use and neutrophil engraftment was analyzed
  - G-CSF was not recommended for 21 days after beti-cel infusion in phase 3 studies without approval from a medical monitor
  - After day 21 post-infusion, G-CSF could be used according to institutional guidelines
- Patients with elevated LIC (≥15 mg/g at baseline) underwent biopsy for liver disease assessment and were monitored for hepatic adverse events (AEs) and changes in LIC throughout the study

RESULTS

Baseline characteristics and follow-up

- As of July 2022, 63 patients who received beti-cel infusion have been followed for a median (min–max) of 52.0 (20.1–101.7) months across the 4 parent studies and LTF (**Table 1**)

Table 1. Baseline characteristics and follow-up for patients treated in beti-cel studies

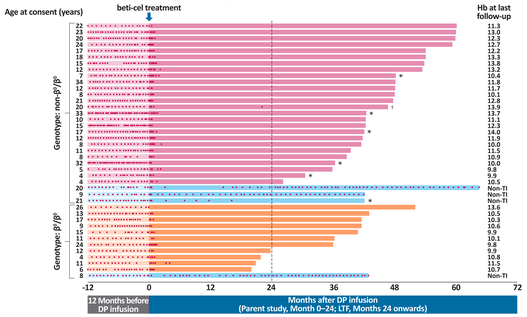
	Phase 1/2 (N=22)	Phase 3 (N=41)
Genotype, n (%)		
Non-β^0/β^0	14 (64)	29 (71)
β^0/β^0	8 (36)	12 (29)
Age at consent, median (min–max), years	20 (12–35)	13 (4–34)
Liver iron concentration, median (min–max), mg Fe/g dw	7.1 (0.4–26.4)	4.9 (1.0–41.0)
Cardiac T2*, median (min–max), msec	34 (10–54)	37 (15–75)
Splenectomy, n (%)	9 (41)	7 (17)
Fertility preservation[a], n (%)	13 (59)	30 (73)
Duration of follow-up, median (min–max), months	85.0 (67.9–101.7)	42.4 (20.1–64.6)

[a]Fertility preservation was an optional procedure.
Fe/g dw, iron content per gram dry weight.

Achievement and maintenance of TI

- 52 patients achieved TI: 15/22 (68.2%) in phase 1/2 and 37/41 (90.2%) in phase 3
- The manufacturing process was refined between phase 1/2 and phase 3; beti-cel approval is based on the drug product used in phase 3 studies
- One-time beticel treatment in phase 3 studies was followed by durable TI with a median follow-up of 42.4 months (**Figure 1**)
  - These results are consistent across age groups and genotypes
- Three patients who achieved TI required packed red blood cell (pRBC) transfusions for acute events (for surgery, phase 3, n=1; for anemia in the setting of cat scratch disease, phase 1/2, n=1; for bloody diarrhea and influenza, phase 1/2, n=1 [late breaking data])
  - All three patients continue to benefit from treatment and do not require chronic pRBC transfusions to manage β-thalassemia

Figure 1. Transfusion status in phase 3 patients



Numbers at the ends of lanes represent unsupported total Hb (g/dL) at last follow-up. Red dots depict transfusion episodes. Vertical dashed black line denotes completion of parent study and rollover to LTF-303. There were 15 patients with HbE/β^0 genotype enrolled in all studies; 6 were enrolled in phase 3 studies; all 15 achieved TI. Data as of July 2022. TI defined as weighted average Hb ≥9 g/dL without pRBC transfusions for ≥12 months.
*Indicates 6 IVS-I-110 homozygous or IVS-1-110/β^0 genotype. †After a planned orthopedic surgery, the patient had blood loss, which required 1 pRBC transfusion. At last follow-up, median Hb among phase 3 patients who achieved TI was 11.1 g/dL (range 9.5–13.6 g/dL).
DP, drug product; Hb, hemoglobin; pRBC, packed red blood cell; TI, transfusion independence.

Safety

- 19% (12/63) of patients experienced ≥1 adverse event (AE) that was considered related or possibly related to beti-cel by the investigator
- The most common beti-cel related AEs were abdominal pain and thrombocytopenia (**Table 2**)
- Veno-occlusive liver disease (serious and nonserious) was reported in 11% (7/63) of patients
- All 5 patients who experienced serious VOD received defibrotide and recovered
- No malignancies, insertional oncogenesis, vector-derived replication competent lentivirus, or clonal predominance was observed

Table 2. AEs occurring in ≥2 patients

	N=63 N (%)
AEs considered possibly related or related to beti-cel	
Abdominal pain	5 (8)
Thrombocytopenia	3 (5)
Serious AEs	
VOD	5 (8)
Pyrexia	5 (8)
Neutropenia	3 (5)
Thrombocytopenia	3 (5)
Sepsis	3 (5)
Appendicitis	2 (3)
Febrile neutropenia	2 (3)
Major depression	2 (3)
Stomatitis	2 (3)

The following AEs assessed as possibly related or related to beti-cel by the investigator occurred in 1 patient (1.6%): immune thrombocytopenia, leukopenia, neutropenia, tachycardia, dysplasia, non-cardiac chest pain, pain in extremity, focal nodular hyperplasia, dyspnea, and hot flush.
Sepsis events included neutropenic sepsis, bacterial sepsis and sepsis not otherwise specified. Data as of July 2022.
AE, adverse event. VOD, veno-occlusive liver disease.

Factors affecting platelet engraftment post infusion

- Using univariate Cox proportional hazard model, 5 factors; splenectomy status, age, preconditioning body weight, and pre-conditioning leukocyte and platelet counts, were found to significantly affect platelet engraftment (p<0.05) (**Table 3**)
- Hazard ratios were defined as the ratio of rates corresponding to platelet engraftment by two distinct levels of a subgroup of interest

Table 3. Factors analyzed for association with platelet engraftment

Factor	Hazard Ratio (95% CI)	p-value
Splenectomy (Yes vs No)	3.556 (1.892, 6.683)	**<0.0001**
Age at ICF (years)	1.030 (1.000, 1.062)	0.0518
Age at ICF group 2 (≥18 vs <18)	1.731 (1.035, 2.897)	**0.0366**
Genotype (non-β^0/β^0 vs β^0/β^0)	0.684 (0.393, 1.190)	0.1788
Day of neutrophil engraftment	0.962 (0.921, 1.004)	0.0786
Baseline annualized pRBC transfusion number (n/year)	0.991 (0.941, 1.043)	0.7297
Pre-conditioning body weight (kg)	1.017 (1.001, 1.034)	**0.0349**
Pre-conditioning LIC (mg/g)	0.996 (0.963, 1.030)	0.8190
Pre-conditioning cardiac T2* (msec)	0.985 (0.963, 1.008)	0.2013
Pre-conditioning serum ferritin (pmol/L)	1.000 (1.000, 1.000)	0.9614
Pre-conditioning leukocytes count (10^9/L)	1.139 (1.039, 1.248)	**0.0056**
Pre-conditioning hematocrit (%)	1.048 (0.984, 1.117)	0.1433
Pre-conditioning Hb (g/dL)	1.166 (0.971, 1.400)	0.0994
Pre-conditioning platelet count (10^9/L)	1.004 (1.002, 1.006)	**<0.0001**
Pre-conditioning platelet count group (10^9/L) (≥150 vs. <150)	2.279 (0.898, 5.784)	0.0831
G-CSF use from Day 1 to Day 21 (Yes vs No)	0.822 (0.462, 1.461)	0.5041
Busulfan estimated average AUC (min*µmol/L)	1.000 (1.000, 1.000)	0.5609
Busulfan frequency (Q6H vs. Q24H)	0.917 (0.531, 1.585)	0.7571
DP cell dose (10^6/kg)	1.003 (0.960, 1.048)	0.8859
DP VCN (c/dg)	0.994 (0.843, 1.171)	0.9391
DP %LVV+ Cells	1.002 (0.992, 1.013)	0.6515

Median platelet engraftment time in phase 3: 46 days (range 20–94); in phase 1/2: 36 days (range 19–191).
AUC, area under the curve; c, copy; CI, confidence interval; dg, diploid genome; DP, drug product; G-CSF, granulocyte colony-stimulating factor; Hb, hemoglobin; ICF, informed consent form; LIC, liver iron concentration; LVV, lentiviral vector; pRBC, packed red blood cell; Q6H, every six hours; Q24H, every 24 hours; VCN, vector copy number.

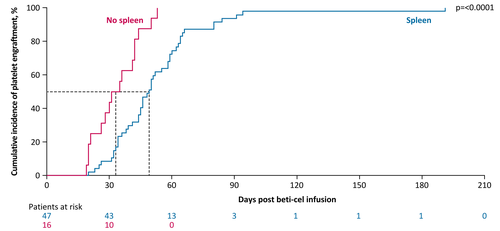
- Among these 5 significant factors, splenectomy has the largest effect and is the most clinically meaningful factor for platelet engraftment
- Patients with splenectomy are 3.556 times more likely to have platelet engraftment at a given time and thus a shorter time to platelet engraftment compared with patients without splenectomy (**Figure 2**)
- The other 4 factors are significantly associated with splenectomy status, thus they may appear to be significant due to the confounding effect (**Table 4**)
- Drug product characteristics did not influence platelet engraftment

Table 4. Association of splenectomy status with other factors affecting platelet engraftment[†]

Variables	Platelet count coefficient (p-value)	Leukocyte count coefficient (p-value)	Age at ICF coefficient (p-value)	Body weight (kg) coefficient (p-value)
Splenectomy (Yes vs No)	0.81 (<0.0001)	0.42 (0.0005)	0.37 (0.0030)	0.46 (0.0001)

[†]Coefficient was derived using univariate Cox proportional hazard model.
ICF, informed consent form.

Figure 2. Cumulative incidence of platelet engraftment by splenectomy status



- Most patients (44/63 [69.8%]) achieved platelet engraftment after hospital discharge
- 9 patients experienced bleeding events after hospital discharge, 2 of which were serious
  - Serious AE of contusion occurred on Day 505; patient had achieved platelet engraftment on Day 44
  - Serious AE of epistaxis occurred on Day 69; patient achieved platelet engraftment on Day 80
  - Among the 7 patients with non-serious bleeding events, 6 experienced bleeding events after platelet engraftment and 1 patient experienced bleeding events on the day of platelet engraftment
  - Most bleeding events happened long after platelet engraftment and were not likely related to delayed platelet engraftment

G-CSF use and neutrophil engraftment

- 24% (10/41) of patients in phase 3 and 27% (17/63) of all patients received G-CSF within 21 days of beti-cel infusion
- G-CSF does not appear to affect time to neutrophil engraftment (**Table 5**)
- While all patients achieved neutrophil engraftment after beti-cel infusion in the clinical studies, 7% (3/41) of phase 3 patients and 6% (4/63) of all patients remained dependent on G-CSF beyond Day 43, one through Day 77 and one through Day 156
- There was no association between G-CSF and time to platelet engraftment

Table 5. G-CSF use during days 1-21 following beti-cel infusion and time to neutrophil engraftment

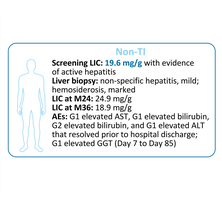
	Phase 3 N=41		All patients N=63	
	No G-CSF use (Day 1–21) N=31	G-CSF use (Day 1–21) N=10	No G-CSF use (Day 1–21) N=46	G-CSF use (Day 1–21) N=17
Neutrophil engraftment achieved, n (%)	31 (100)	10 (100)	46 (100)	17 (100)
Time to neutrophil engraftment median (min, max), days	26.0 (13, 39)	23.0 (16, 36)	23.5 (13, 39)	21.0 (14, 36)

Neutrophil engraftment defined as absolute neutrophil count ≥500 cells/mL for three days.
G-CSF, granulocyte colony stimulating factor; max, maximum; min, minimum.

Outcomes in patients with LIC ≥15mg/g at baseline

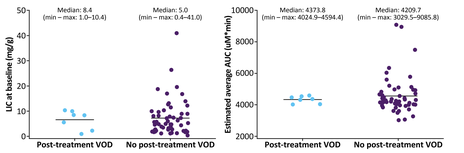
- In phase 3 studies, 4 patients (all adults and from HGB-207 study) treated with beti-cel entered the study with screening LIC ≥15 mg/g
- Following screening, all 4 patients underwent liver biopsy which confirmed that they did not meet exclusion criteria for advanced liver disease
- 3 of these patients achieved TI (**Figure 3**)
- Liver-related AEs for all 4 patients were transaminase related and no veno-occlusive liver disease (VOD) was reported (**Figure 4**)
- Additionally, 2 patients from phase 1/2 (HGB-204) had a screening LIC ≥15mg/g
  - The first patient had LIC of 26.4, 18.2, and 4.1 mg/g at baseline, M24 and M48, respectively
  - The second patient had LIC of 17.0, 15.0, and 11.2 mg/g at baseline, M24, and M48, respectively
  - Both underwent liver biopsy and advanced liver disease was excluded
  - Both achieved TI and neither experienced liver-related AEs or VOD

Figure 3. Patients with elevated baseline LIC in phase 3 study



ALT, alanine transaminase; AST, aspartate transaminase; G, grade; GGT, gamma-glutamyl transferase; LIC, liver iron concentration; M, month; TI, transfusion independence.

Figure 4. LIC at baseline and estimated AUC by incidence of VOD



Average AUC by patient using observed and derived AUC, which is defined using the average and defined AUC per dose, multiplied by observed dose when AUC is missing. The horizontal lines indicate the mean incidence of serious and non-serious VOD.
g, gram; LIC, liver iron concentration; max, maximum; min, minimum; VOD, veno-occlusive liver disease.

CONCLUSIONS

- All patients in beti-cel clinical program achieved neutrophil and platelet engraftment
- The current analysis suggests splenectomy as the most clinically relevant factor for platelet engraftment
- The time to achievement of neutrophil engraftments did not differ between patients who received G-CSF and those who did not
- The presence of elevated LIC at baseline was not associated with substantial liver disease or incidence of VOD; 5 of 6 patients with elevated LIC (≥15 mg/g) achieved TI
- Additional analyses on factors impacting engraftment timing and on baseline LIC levels may help inform real-world beti-cel treatment decisions and clinician expectations for patient management
- Patients with TDT, regardless of age or genotype, experienced durable TI through up to 8 years following beti-cel infusion, which was associated with a favorable safety profile consistent with that of myeloablative autologous transplantation

REFERENCES

1. Zynteglo [package insert]. Somerville, MA. 2022.
2. Locatelli F et al. *N Engl J Med* 2022; 386:415–427.
3. Schneiderman J et al. Tandem TCT Meetings, Philadelphia, 2022.

ACKNOWLEDGMENTS

All authors contributed to and approved the poster; writing and editorial assistance were provided by Synergy Medical Communications, funded by bluebird bio.

DISCLOSURES

TSO reports consulting fees from bluebird bio. **MCW** reports receiving consulting fees from AllCells, Biolabs, Inc., Ensoma, Inc., and Vertex. **JLK** reports receiving consulting fees from Agios, bluebird bio, Inc., Bristol Myers Squibb, Celgene, Chiesi, Forma, Biomarin, Regeneron, Imara, and Silence Therapeutics; and research funding from bluebird bio, Inc., Appopharma, Bioverativ, Vertex, Agios, Forma, Imara, and Sangamo. **JBP** reports research funding from Silence Therapeutics; membership of advisory committees for Agios Pharmaceuticals, Inc., bluebird bio, Inc., Celgene; and membership of a steering committee for Vifor Pharm. **AL** reports receiving research funding from bluebird bio, Inc., Celgene, Novartis, and Protagonist Therapeutics. **JEJR** reports personal fees from IMAGO Biosciences, Gilead, AbbVie, Pfizer, CSL Behring, Woke Pharmaceuticals, Takeda, and the Office of the Gene Technology Regulator, Australian Government, personal fees and other from Rarecyte, other from Genea, grant support from Spark, bluebird bio, Inc., Cyclerion, and Novartis, personal fees and non-financial support, and other from the International Society for Cell & Gene Therapy outside the submitted work. **SA** is an employee of bluebird bio, Inc. **RC** is an employee of and holds equity in bluebird bio, Inc. **FL** reports receiving consultancy fees from Amgen and Novimmune; speakers fees from bluebird bio, Inc. **AAT** reports receiving consulting fees from Agios, Beam, bluebird bio, Inc., Bristol Myers Squibb, and CRISPR/Vertex; research funding from Bavaltia, Biomarin, bluebird bio, Inc., Bristol Myers Squibb, CRISPR/Vertex, and Editas; and equity from Global Blood Therapeutic. All other authors have nothing to disclose.

These studies were sponsored by bluebird bio