Predictors of Biologic Efficacy With Lovotibeglogene Autotemcel (Lovo-cel) Gene Therapy in Patients With Sickle Cell Disease

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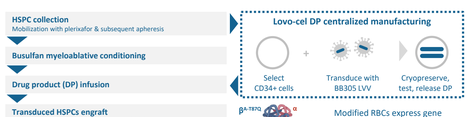
Poster 302

INTRODUCTION

- Lovotibeglogene autotemcel (lovo-cel) is a one-time cell and gene therapy for the treatment of sickle cell disease (SCD) that adds functional copies of normal adult β-globin gene with a single amino acid change ($\beta^{A\text{-}T87Q}$)[1] into a patient's own hematopoietic stem and progenitor cells (HSPCs)
- Anti-sickling hemoglobin (Hb), HbA^{T87Q}, has near-identical oxygen affinity to wild-type HbA[2] and results in reduced sickled red blood cells (RBCs), decreased hemolysis, and a reduction in vaso-occlusive events with improved Hb levels



This study explores the relative influence of genotype and pharmacodynamics on patient outcomes post lovo-cel treatment.

LW, lentiviral vector.

METHODS

Study design

- This post hoc, exploratory analysis used data from 47 patients who received lovo-cel as of February 13, 2023, using the mobilization and manufacturing process applied in HGB-206 (NCT02140554) Group C and HGB-210 (NCT04293185)
- Details of the trial design and clinical outcomes have been previously published,[3] with updated results included in Poster 301

Table 1. α-globin genotypes in the study population

	Total, n=47
α-globin genotype, n (%)	
αα/αα	32 (68.1)
αα/-α$^{3.7}$	13 (27.7)
-α$^{3.7}$/-α$^{3.7}$	2 (4.3)[a]

[a]Adult patient with -α$^{3.7}$/-α$^{3.7}$ genotype was excluded from analyses unless otherwise noted due to transfusion dependence post lovo-cel.

Analyses

- A pairwise Spearman correlation analysis was conducted to understand relationships between postinfusion transduction efficiency metrics and selected hematological and hemolysis indices
- A stepwise multiple regression was conducted with interaction terms to dissect the role of α-globin genotype in the relationship between transduction and Hb
- A cross-validated random forest (RF) regression was designed to identify DP attributes predictive of peripheral blood (PB) vector copy number (VCN) using 15 DP characteristics. Missing data were imputed using the k-nearest neighbor algorithm (k=5), and hyperparameters were tuned to minimize the root-mean-square error

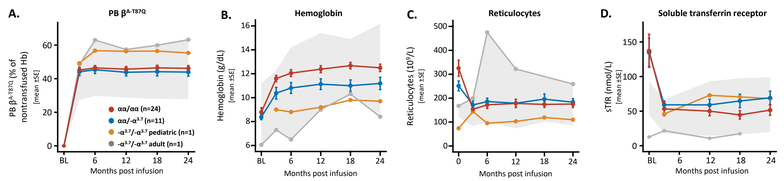
Pharmacodynamic assays developed to characterize lovo-cel

PB VCN	% LVV+ colonies	βA-T87Q	βA-T87Q / RBC	Sickling
qPCR	*qPCR*	*RP-HPLC*	*Single-cell western*	*Imaging flow cytometry*
Average number of BB305 vector copies in PB cells	Percentage of transduced BFU-E colonies cultured from PB	Percentage of β^{T87Q} protein from a PB sample of all globin chains	Percentage of RBCs in the PB that contain β^{T87Q}	AUC of the percentage of cells that are sickled at 2% O_2 over time
1.5 c/dg (0.4, 3.7)	**64% of colonies** (33.2, 90.1)	**45% HbA^{T87Q}** (27.3, 58.5)	**86% of RBCs** (68.1, 94.6)	**84% × hour** (52.6, 134.4)

Methods used for each assay are indicated by italics in blue headers. Values denote the median (min, max) across lovo-cel–treated patients, taken from the assay-level medians of all pharmacodynamic assessments on or after month 6; n=37.
AUC, area under the curve; BFU-E, burst-forming unit-erythroid; c/dg, copies/diploid genome; HbA^{T87Q}, anti-sickling hemoglobin; HbS, sickle hemoglobin; LVV, lentiviral vector; PB, peripheral blood; qPCR, quantitative polymerase chain reaction; RBC, red blood cell; VCN, vector copy number; RP-HPLC, reversed-phase high-performance liquid chromatography.

RESULTS

Figure 1. Biologic efficacy of lovo-cel in SCD

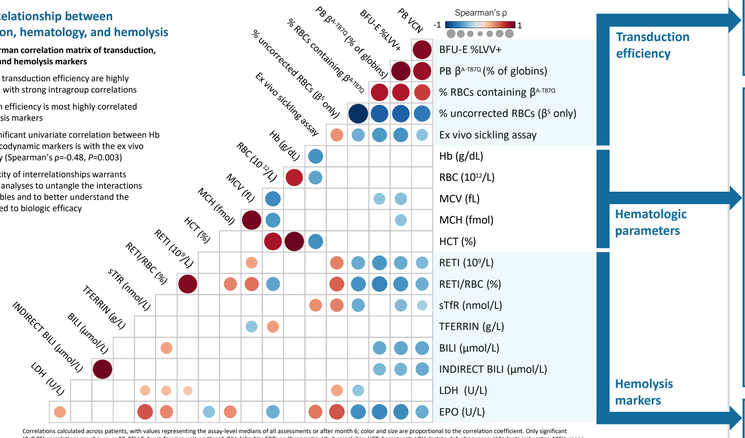


Longitudinal biologic efficacy in lovo-cel–treated patients over 24 months post infusion. (A) PB $\beta^{A\text{-}T87Q}$ levels are stable within 6 months post lovo-cel treatment. Treatment with lovo-cel is associated with increased (B) hemoglobin and decreased markers of hemolysis [eg, (C) reticulocytes and (D) sTFR]; hematologic indices likewise stabilize by 6 months post lovo-cel.
- Patients with single α-globin gene deletion genotypes exhibit decreased Hb compared with patients with αα/αα genotypes
- Even with consistent improvement across patients, there is variance in hematologic values post lovo-cel

n=37 evaluable patients; n varies by time point based on available data. For sTfR: αα/αα, n=17; αα/-α3.7, n=9. Six-month data for sTfR not shown due to limited data availability. Gray shading denotes subjects with min and max levels of each marker ≥6 months (median) post treatment.
BL, baseline; Hb, hemoglobin; PB, peripheral blood; SCD, sickle cell disease; SE, standard error; sTfR, soluble transferrin receptor.

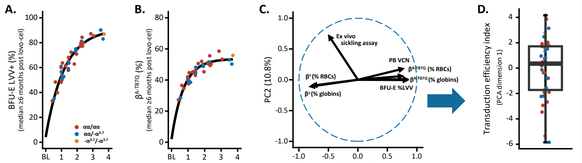
Figure 2. Relationship between transduction, hematology, and hemolysis

Pairwise Spearman correlation matrix of transduction, hematologic, and hemolysis markers
- Measures of transduction efficiency are highly interrelated, with strong intragroup correlations
- Transduction efficiency is most highly correlated with hemolysis markers
- The only significant univariate correlation between Hb and pharmacodynamic markers is with the ex vivo sickling assay (Spearman's ρ=-0.48, P=0.003)
- The complexity of interrelationships warrants multivariate analyses to untangle the interactions among variables and to better understand the factors related to biologic efficacy



Correlations calculated across patients, with values representing the assay-level medians of all assessments or after month 6; color and size are proportional to the correlation coefficient. Only significant (P<0.05) correlations are shown; n=37. BFU-E, burst-forming unit-erythroid; BILI, bilirubin; EPO, erythropoietin; Hb, hemoglobin; HCT, hematocrit; LDH, lactate dehydrogenase; LVV, lentiviral vector; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume; PB, peripheral blood; RBC, red blood cell; RETI, reticulocytes; sTfR, soluble transferrin receptor; TFERRIN, transferrin; VCN, vector copy number.

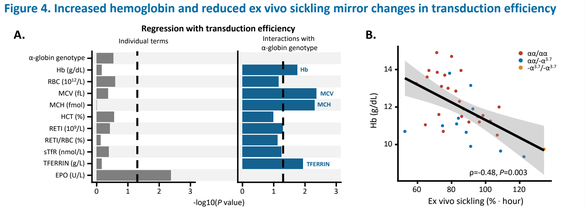
Figure 3. Pharmacodynamic parameters are highly interrelated and can be reduced to a single transduction efficiency index



Correlations between pharmacodynamic parameters. As shown in Figure 2, there are several assays to assess postinfusion transduction efficiency that have been developed to characterize lovo-cel, with all the pairwise correlations across the assays being significant. (A) Increasing PB VCN results in increased %LVV+ colonies in the PB. (B) PB VCN positively correlates with production of gene therapy–derived β-globin. (C) PCA of PD parameters shows that PB VCN and $\beta^{A\text{-}T87Q}$ (% of globins and % of RBCs) are highly correlated and inversely correlated with β^S levels. While highly correlated with the other assays, the PCA indicates that the ex vivo sickling assay contributes some distinct information, captured by PC2 (10.4% of the variance). PCA of PD markers captures >75% of variance in a single dimension, making it feasible to reduce all parameters to single variable (D), referred to as the transduction efficiency index.

n=37. (C) The dashed circle (radius=1) represents the maximum possible contribution of variables to the principal components. Variables closer to the circle's edge strongly influence the component axes.
BFU-E, burst-forming unit-erythroid; BL, baseline; c/dg, copies/diploid genome; LVV, lentiviral vector; PB, peripheral blood; PC1, principal component 1 (% variance captured); PC2, principal component 2 (% variance captured); PCA, principal component analysis; PD, pharmacodynamic; RBC, red blood cell; VCN, vector copy number.

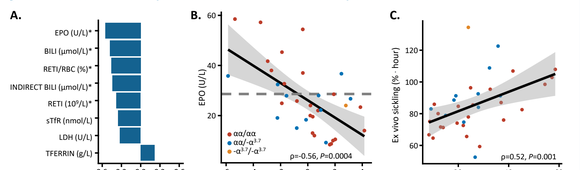
Figure 4. Increased hemoglobin and reduced ex vivo sickling mirror changes in transduction efficiency



Correlations between hematologic indices and pharmacodynamics. (A) Stepwise multiple regression model (adjusted R^2=0.69, P=0.001) including both individual terms (gray) and interaction terms for α-globin genotype (blue) demonstrates that hematologic indices (ie, Hb, HCT) are significantly correlated with transduction efficiency (ie, PC1) in a genotype-specific manner. (B) Subjects with increased levels of Hb exhibited significantly decreased sickling in an ex vivo assay (P=0.003).

(A) Dotted line denotes P=0.05; n=36; excluding -α$^{3.7}$/-α$^{3.7}$ patients. Four data points imputed with k-nearest neighbors algorithm (k=5). (B) Gray shaded area denotes 95% confidence interval.
EPO, erythropoietin; Hb, hemoglobin; HCT, hematocrit; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume; PB, peripheral blood; PC1, principal component 1; RBC, red blood cell; RETI, reticulocytes; sTfR, soluble transferrin receptor; TFERRIN, transferrin.

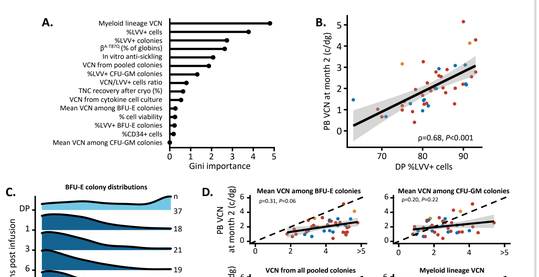
Figure 5. Reduction in hemolysis is associated with the efficiency of transduction



Correlations between hemolysis markers and pharmacodynamics. (A) The transduction efficiency index is inversely correlated with hemolysis markers, including significant relationships with EPO, bilirubin, and the reticulocyte/RBC ratio. (B) Increased transduction index is associated with decreased EPO levels. (B) Decreased EPO corresponds with a significant decrease in ex vivo sickling (P=0.001).
- All subjects with evaluable EPO levels and HbA <70% at baseline (n=11) had a reduction in EPO (median decrease=56%; min, max=29%, 93%)
- Together, the relationship between decreased EPO, decreased sickling, and increased total RBCs (**Figure 2**) suggests an increased RBC lifespan[4] associated with transduction efficiency

(A) *P<0.05; n=37. (B) Dotted line denotes upper limit of normal range for EPO. (B, C) Gray shaded area denotes 95% confidence interval. BILI, bilirubin; EPO, erythropoietin; HbA, adult hemoglobin; LDH, lactate dehydrogenase; RBC, red blood cell; RETI, reticulocytes; sTfR, soluble transferrin receptor; TFERRIN, transferrin.

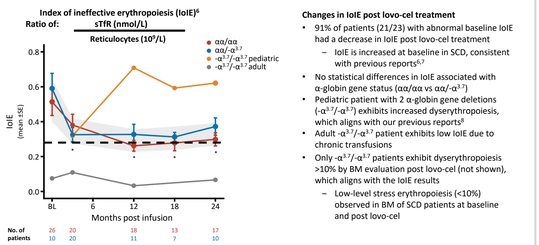
Figure 6. DP attributes predict pharmacodynamic responses



Relationship between PB VCN post lovo-cel and DP attributes. (A) Cross-validated RF regression model to predict PB VCN at 2 months post lovo-cel (RMSE=0.78, R^2=0.28). PB VCN is the easiest transduction efficiency assay to perform and thus was chosen for DP attribute RF. (B) The percentage of LVV+ cells in the DP is the most highly correlated release assay with PB VCN. Similar results were observed with betibeglogene autotemcel (beti-cel).[5] Increased transduction of the DP leads to an increase in PB VCN after treatment with lovo-cel. (C) VCN distribution is consistently established in the engrafted cell population within 1 month post treatment with lovo-cel. The VCN distribution in the DP includes clones with much higher VCN than observed in the PB, suggesting that a subset of the more highly transduced cells in the DP do not engraft. (D) VCN measured in the myeloid lineage most closely predicts the PB VCN post lovo-cel treatment, in agreement with the RF prediction.

Analyses include adult -α$^{3.7}$/-α$^{3.7}$ subject. (A) n=43; VCN from unscheduled visits at day 58, 65, and 75 were included for 3 subjects. (B, D) Gray shaded area denotes 95% confidence interval. (C) n=number of subjects assayed at each time point. (D) Dotted line = theoretical 1:1 relationship between DP VCN and PB VCN. BFU-E, burst-forming unit-erythroid; c/dg, copies/diploid genome; CFU-GM, colony-forming unit–granulocyte, macrophage; DP, drug product; LVV, lentiviral vector; PB, peripheral blood; RF, random forest; RMSE, root-mean-square error; TNC, total nucleated cell; VCN, vector copy number.

Figure 7. Ineffective erythropoiesis is normalized post lovo-cel

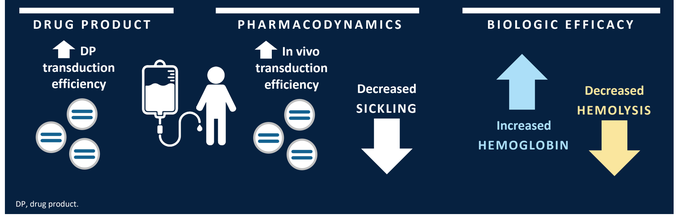


Changes in IoIE post lovo-cel treatment
- 91% of patients (21/23) with abnormal baseline IoIE had a decrease in IoIE post lovo-cel treatment
  - IoIE is increased at baseline in SCD, consistent with previous reports[6,7]
- No statistical differences in IoIE associated with α-globin gene status (αα/αα vs αα/-α$^{3.7}$)
- Pediatric patient with 2 α-globin gene deletions (-α$^{3.7}$/-α$^{3.7}$) exhibits increased dyserythropoiesis, which aligns with our previous reports[8]
- Adult -α$^{3.7}$/-α$^{3.7}$ patient exhibits low IoIE due to chronic transfusions
- Only -α$^{3.7}$/-α$^{3.7}$ patients exhibit dyserythropoiesis >10% by BM evaluation post lovo-cel (not shown), which aligns with the IoIE results
  - Low-level stress erythropoiesis (<10%) observed in BM of SCD patients at baseline and post lovo-cel

*P<0.01 compared with BL (ANOVA followed by post hoc Tukey HSD); gray=95% confidence interval; month 24 from adult subject with -α$^{3.7}$/-α$^{3.7}$ deletions derived from month 18 sTfR and month 24 reticulocyte count; dotted line denotes upper limit of normal for IoIE.[6] ANOVA, analysis of variance; BL, baseline; BM, bone marrow; HSD, honestly significant difference; IoIE, index of ineffective erythropoiesis; SCD, sickle cell disease; SE, standard error; sTfR, soluble transferrin receptor.

CONCLUSIONS

- In clinical studies of lovo-cel, the improvement in Hb and reduction of hemolysis is significantly correlated with postinfusion transduction efficiency
- Transduction efficiency, as measured by peripheral blood vector copy number (PB VCN), is an important predictor of biologic efficacy and correlates with lovo-cel drug product (DP) attributes
- DP transduction efficiency and the resulting PB VCN predict biologic efficacy of lovo-cel
- Ex vivo sickling is the strongest correlate with total Hb post treatment and suggests that biologic efficacy is the result of an increase in RBC lifespan[4]
- Ineffective erythropoiesis, as measured by the ratio of soluble transferrin receptor and absolute reticulocyte count,[6] approached normal levels following treatment with lovo-cel except in patients with 2 α-globin gene deletions



DP, drug product.

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DISCLOSURES

MAK, IS, LC, HJ, MF, HE, AC, FJP: Current employees of and equity holders in bluebird bio.