Molecular Therapy
Methods & Clinical Development
Commentary

Optimizing lentiviral genomic integrations to cure beta-thalassemia: The least required for success?

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Gene addition by *ex vivo* lentiviral transduction of a curative beta-globin gene into hematopoietic stem cells of patients suffering from blood transfusion-dependent beta-thalassemia (TDT) has successfully treated several patients.[1–3] In particular, the vector BB305 was used in phase 3 clinical trials (HGB-207 and HGB-212) to transduce hematopoietic stem cells of patients with TDT.[4] The drug product (betibeglogene autotemcel or beti-cel; also known as Zynteglo) was then infused to treat 41 patients.[1,3] Of these, 37 patients (90%) attained transfusion independence (as defined as an average hemoglobin level of $\geq$9 g/dL).[5] Based on these results, in 2022, the FDA approved Zynteglo as the first cell-based gene therapy to treat adult and pediatric patients with TDT.

The report by Dr. Whitney and colleagues investigated several manufacturing parameters for their potential to predict clinical efficacy, indicated as blood transfusion independence.[5] They observed a significant correlation between peripheral blood vector copy number (VCN) and gene-therapy-derived therapeutic protein expression. The main finding is that patients showing peripheral VCN of ~0.75 copies per diploid genome (c/dg) achieved blood transfusion independence. In contrast, patients with peripheral blood VCN <0.75 c/dg had variable outcomes, with only some achieving transfusion independence.

Using $\geq$0.75 c/dg as a surrogate biomarker for transfusion independence, the analysis indicated that the best predictive features were drug product quality attributes and not manufacturing parameters. For instance, while the cell concentration during transduction did not impact the phenotypic outcome, lentiviral vector positive (LVV+) cells in the drug product (%LVV+ cells) showed a significant correlation with the $\geq$0.75 c/dg threshold. The %LVV+ cell parameter indicates the percentage of cells in the drug product with at least one genomic transgene integration, as measured by PCR using individual flow-sorted cells. Based on this correlation, it is evident that at least 50% of the cells of the drug product had to be transduced to reach transfusion independence and that the best results were achieved when most of the cells were transduced. In this case, a VCN higher than one was required to target most cells. Overall, this analysis highlights some critical parameters that could be utilized in future trials to predict the chances of success for each patient: VCN and the percentage of LVV+ cells in the drug product (Figure 1).

In addition, they find a good correlation between the VCNs in the drug products (before infusion) and peripheral blood VCNs 6 months post-infusion. However, VCNs are likely higher when comparing the cells before infusion and after long-term engraftment. Investigating the correlation between the initial and post-infusion VCNs would be very helpful. This could be utilized to predict if the *ex vivo* transduction will deliver a product with long-term VCNs in the curative range.

The caveats in extending these parameters to other trials relate to the characteristics of the vectors utilized and the transduction protocol. First, other vectors may express different levels of the curative beta-globin gene at single genomic integration. If this is the case, a vector expressing higher beta-globin gene levels may require fewer VCNs and LVV+ cells to achieve transfusion independence. Second, protocols to transduce hematopoietic stem cells may provide different results comparing the VCNs in the drug product vs. peripheral blood. For instance, many transduction protocols now utilize enhancers, such as poloxamers and prostaglandin E2, to increase VCNs.[3,6] However, the increased transduction levels achieved by these enhancers are higher when comparing the VCNs in the drug product before and after infusion.[3,6,7] Therefore, different transduction protocols will need to be re-evaluated considering these and other variables.

To evaluate the expression level of BB305 at single genomic integration, Dr. Whitney and colleagues deduced this value by the results observed in non-β0/β0 patients. Based on the estimate that the average endogenous hemoglobin in non-β0/β0 patients was 2.5 g/dL, they indicated that 0.75 VCN BB305 was sufficient to generate 6.5 g/dL curative hemoglobin, allowing patients to reach the curative levels of $\geq$9 g/dL. In a separate analysis, they observed that 5 to generate" 50% of the beta-globin chains made in healthy erythroid cells. This indicates that one copy of BB305, in normal cells, can generate ~40% of the beta-globin chains made by an endogenous beta-globin gene. This suggests that one copy of BB305 in healthy cells may make less than 6.5 g/dL hemoglobin.

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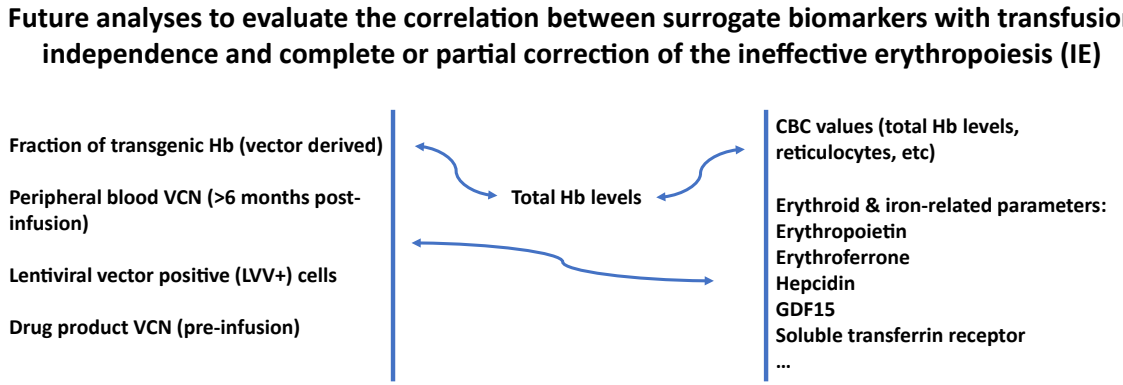

Figure 1. Suggested analyses to evaluate the relationship between the best surrogate biomarker parameters, transfusion independence, and ineffective erythropoiesis

Obviously, the differences observed in thalassemic and healthy cells are dictated by the relative abundance of alpha- and beta-globin chains in these two conditions. Therefore, the use of BB305 and similar vectors may be curative in patients with beta-thalassemic when there are relatively less competing endogenous beta-globin chains to generate a hemoglobin tetramer. Therefore, the minimal curative VCN may be higher in conditions with no reduction of endogenous beta-globin synthesis, like in sickle cell anemia.

The authors also claim that the patients' genotype did not correlate with transfusion independence. Out of the 27 patients that reached transfusion independence, 37 of the 41 patients had β0/β0, non-β0/β0, IVS-I-110 homozygous, or IVS-I-110/β0 mutations. Therefore, it is assumed that only four patients were non-β0/non-β0.

Table 1. Correlation between gene therapy parameters and ineffective erythropoiesis

Correlation analysis between surrogate biomarkers and transfusion independence (≥9 g/dL)[a]
Best parameters:
Peripheral blood VCN
Lentiviral vector positive (LVV+) cells
Additional desirable analysis[b]
Relationship between drug product VCN and peripheral blood VCN (>6 months post-infusion)

[a]Summary of the best surrogate biomarkers and transfusion independence described in this manuscript.
[b]Desirable additional correlation for present and future trials.

However, this statement should be taken cautiously, as too few non-β0/non-β0 patients were included to evaluate the correlation between VCN and genotype on transfusion independence, as suggested by a previous study.[8]

Moreover, although the relationship between hemoglobin levels and complete correction of the beta-thalassemic phenotype was not the goal of this manuscript, in future studies, parameters such as VCN, LVV+ cells, and the proportion of transgenic beta-globin chains should be evaluated against markers that can identify residual ineffective erythropoiesis. For instance, residual ineffective erythropoiesis could be assessed by collecting detailed complete blood count (CBC) values as well as erythroid and iron-related parameters, such as erythropoietin, erythroferrone, hepcidin, GDF15, and soluble transferrin receptor, to name a few (Table 1).[9,10] It would be desirable that the scientific community would reach a consensus not only on the requirement to allow safe and meaningful clinical trials but also to collect data to assess the long-term efficacy (or limitations) of these and future approaches.

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DECLARATION OF INTERESTS

S.R. is a scientific advisory board member of Ionis Pharmaceuticals, Meira GTx, Vifor, and Disc Medicine. Present–last 5 years: S.R. has been or is a consultant for GSK, BMS, Incyte, Cambridge Healthcare Res, Celgene Corporation, Catenion, First Manhattan Co., FORMA Therapeutics, Ghost Tree Capital, Keros Therapeutics, Noble Insight, Protagonist Therapeutics, Sanofi Aventis US, Slingshot Insight, Spexis AG, Techspert.io, BVF Partners L.P., Rallybio, LLC, venBio Select LLC, ExpertConnect LLC, and LifeSci Capital.

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