Maximizing safety and efficacy in hematopoietic stem cell gene therapy

Poster ID: P0425

Aurora Giommetti[1], Christin Laura Bosbach[1], Valeriya Olevska[1], Julia Dzionek[1], Stephanie Soltenborn[1], Felix Hebbeker[1] Svenja Oberbörsch[1], Rimas Orentas[2], Andreas Bosio[1] and Eleni Papanikolaou[1]
[1] Miltenyi Biotec B.V. & Co.KG, Bergish Gladbach, Germany | [2] Miltenyi Biotec North America, Gaithersburg, MD, USA

Scan the QR code to download the poster (PDF):

Introduction

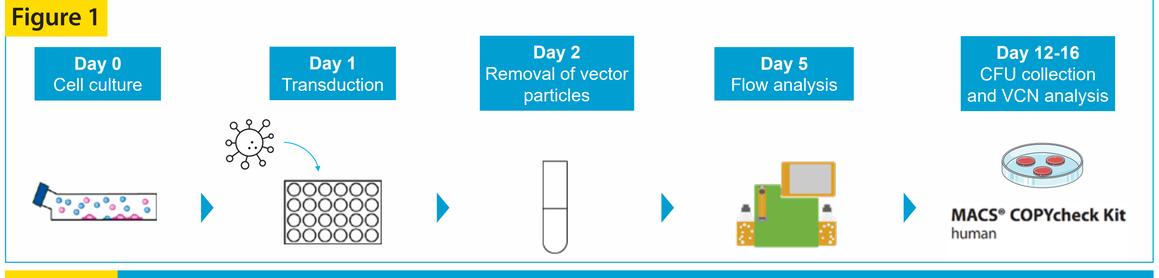
Hematopoietic stem cell (HSC) gene therapy has been recently approved for beta-thalassemia, sickle cell disease and adrenal leukodystrophy, specifically Lyfgenia™, Casgevy® and Skysona™. However, in the case of sickle cell disease, the challenges of high cost and scalability, as well as increased safety concerns regarding therapy-associated leukemic events indicate a need for improved and standardized manufacturing steps that allow for large scale implementation of gene therapy interventions to a degree that addresses the population suffering from the long-term sequelae of this disease. Automation is the key to achieve these goals and, to this end, the CliniMACS Prodigy® as an automated, closed, GMP-compliant platform for HSC transduction is an ideal candidate to streamline cell processing. This device had previously demonstrated promising results in terms of cell viability, transduction efficiency and vector copy number (VCN) for HSC transduction, when a GFP encoding lentiviral vector[1] was utilized. In the current study, we intend to provide further insights on the performance of the CliniMACS Prodigy for HSC transduction by employing a transmembrane protein-encoding lentiviral vector, followed by assessment of the transduction efficiency, CFU analysis and characterization and VCN determination.

Methods

1 Workflow for small scale lentiviral transduction

The manual transduction process consists of a series of open-handling steps starting from fresh or frozen CD34+ enriched cells from CliniMACS Prodigy that are put in culture at day 0. After one day, 1x10^6 cells are seeded in a 24-well plate and transduced with the CD19 CAR lentiviral vector at MOI 30. A washing step with PBS is required after 24h to remove the viral particles from the cells which are then analyzed by flow cytometry at day 5 to assess the transduction efficiency. Meanwhile, before and after transduction, CFU assays are performed and will be counted and characterized at day 12-16. Additionally, we collected single CFUs to carry out the VCN analysis (Fig.1).

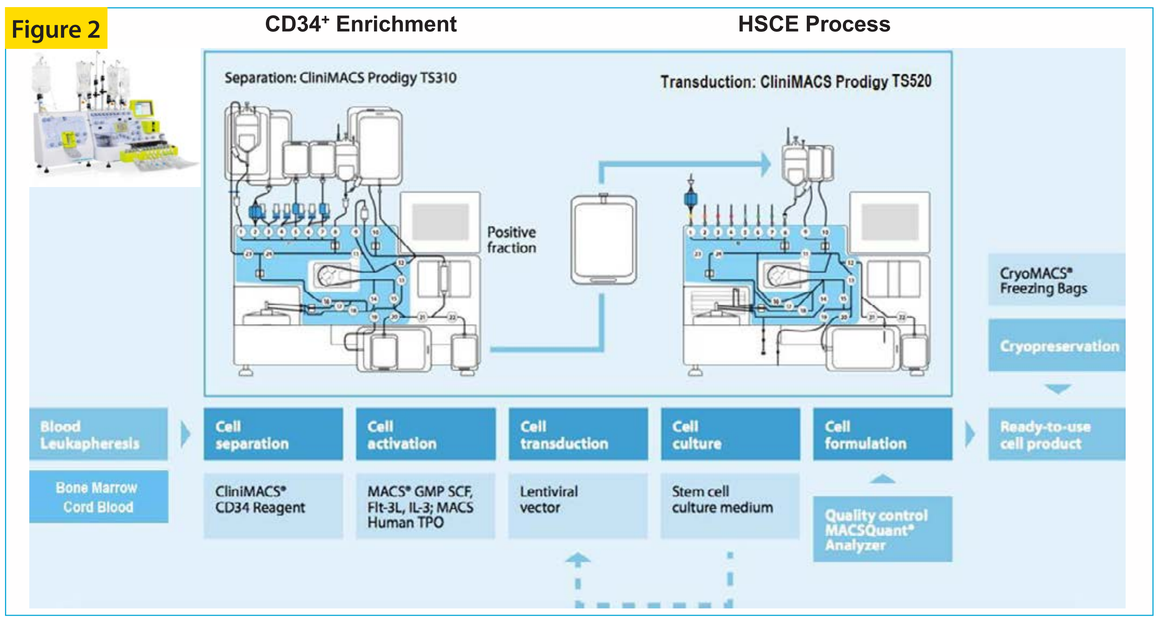
Figure 1



2 Large scale HSC Engineering process on the CliniMACS Prodigy

For the generation of genetically modified HSCs, the Hematopoietic Stem Cell Engineering (HSCE) software on the CliniMACS Prodigy was applied. Within a closed, single-use tubing set (TS), CD34+ cells undergo two separate steps: (i) CD34+ cell enrichment; and (ii) HSC engineering consisting of cultivation, viral transduction and harvesting of the gene-modified product.

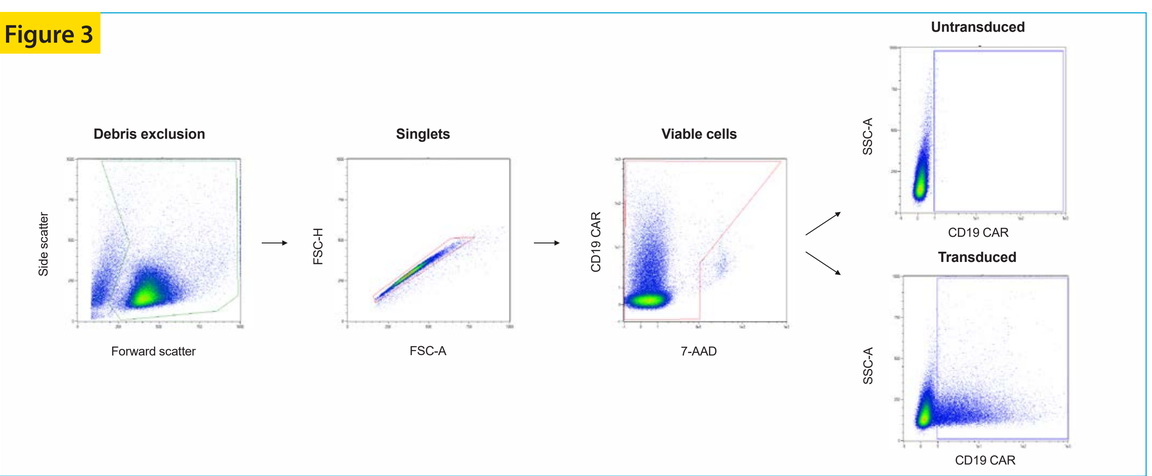
Figure 2



3 Gating strategy

The gating strategy shown in figure 3 was applied to evaluate the recovery, viability, and transduction efficiency of HSCs. The analysis was performed with the MACSQuantify software™.

Figure 3



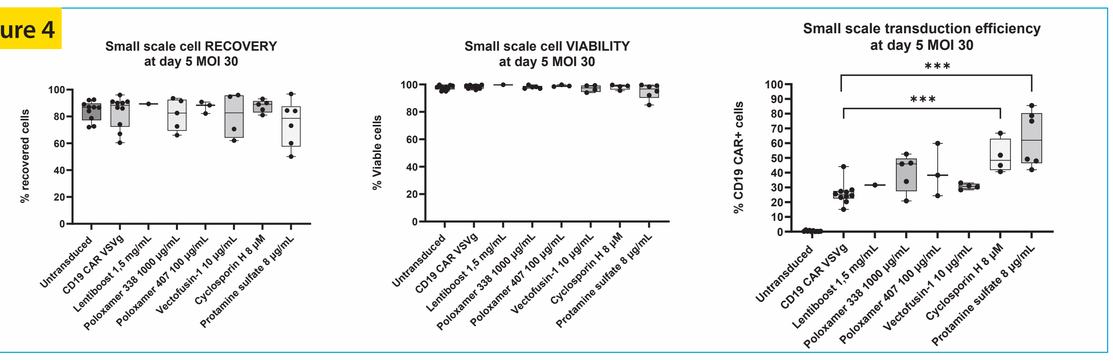
References: 1. Bissels, U. *et al, Mol.Ther.* 28; 4, 2019: 385-385.

Funding: Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or the European Research Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

Results

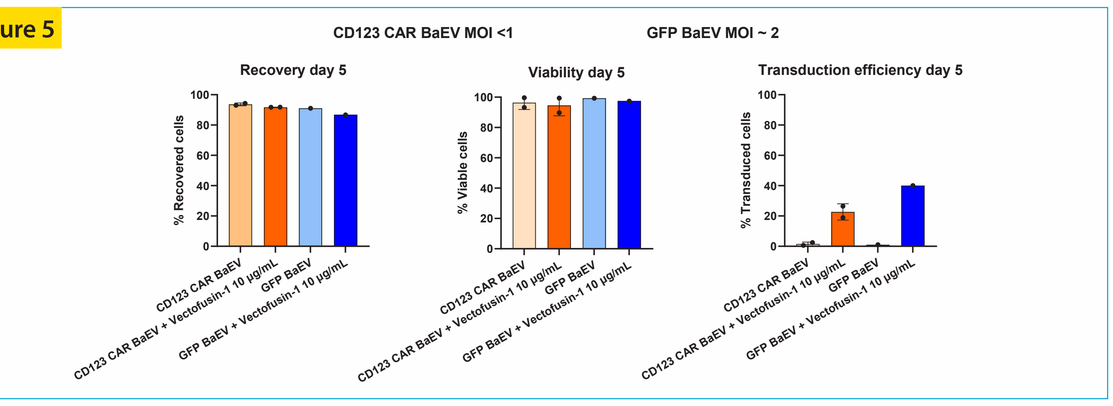
A crucial obstacle for successful clinical application is the limited transduction rate of hematopoietic stem cells. To overcome this challenge, we analyzed the activity of several transduction enhancers on important parameters such as cell recovery, viability and transduction efficiency. To assess their effects, we transduced CD34+ HSC isolated from mobilized leukapheresis with a VSVg pseudotyped CD19 CAR lentiviral vector at MOI 30. With the addition of either poloxamer 388 or vectofusin-1®, FACS analysis of liquid culture at day 5 revealed a similar percentage of CD19 CAR expressing cells compared to the transduction with CAR vector alone. In contrast, cyclosporin H and protamine sulfate treatments significantly increased the amount of CD19 CAR+ HSCs compared to the condition without transduction enhancer. In terms of recovery and viability, the transduction and the TEs treatments do not significantly influence the percentage of recovered and viable CD34+ cells (Fig. 4).

Figure 4



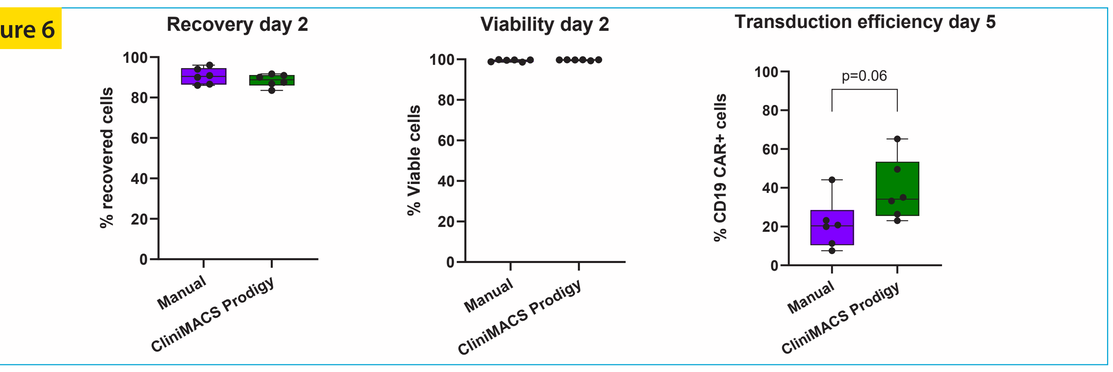
To further investigate the activity of vectofusin-1 as transduction enhancer on HSCs, we repeated the small scale experiments testing Baboon pseudotyped lentiviral vectors (CD123 CAR BaEV and GFP BaEV) at low MOI. Promising results have shown that with the addition of vectofusin-1 the transduction efficiency increased with both vectors tested (Fig.5).

Figure 5



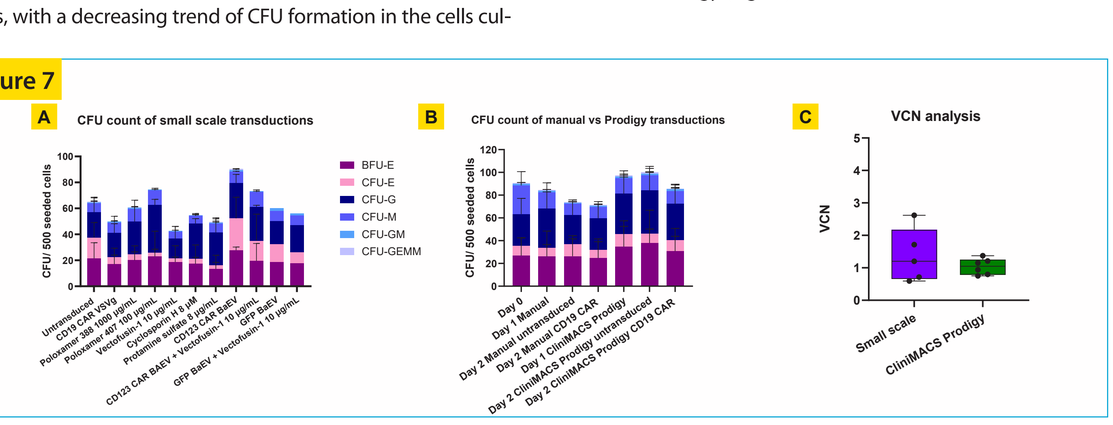
Following the same experimental design as in the open steps, transduction experiments on the CliniMACS Prodigy were performed in parallel. The transduction with CD19 CAR vector at MOI 30 resulted in comparable cell recovery and viability between the manual and the automated workflows, however the transduction efficiency is higher on the Prodigy compared to the small scale setting (Fig. 6).

Figure 6



In order to investigate the effects of transduction and of different cultivation conditions on stem cell clonogenic capacity, we tested the cells potential to differentiate into various hemopoietic lineages on day 12-16 post-transduction by standard colony forming unit (CFU) assay. From the small scale experiments, there are variations in the CFU count among the conditions, but no significant differences between conditions treated with the same vector were detected (Fig.7A). From the transductions in open steps and in parallel on the Prodigy, based on our preliminary results, we observed fluctuations in the total number of CFUs, with a decreasing trend of CFU formation in the cells cultivated in open steps, but overall no significant differences were detecting in the CFU count and characterization among the conditions (Fig. 7B). Moreover, individual CFUs were picked from the culture to analyze the vector copy number (VCN) per cell via qPCR using the MACS® COPYcheck Kit. Figure 7C illustrates the VCN of CD19 CAR+ colonies, considering as cut-off the values of CFU derived from untransduced sample. Preliminary analysis performed excluding outlier values shows no significant difference in the VCN between the open steps and the CliniMACS Prodigy (Fig.7C).

Figure 7



Conclusion and outlook

- The addition of transduction enhancers increases the transduction efficiency of CD34+ cells with a VSVg pseudotyped CD19 CAR lentiviral vector in small scale experiments.
- The transduction of CD34+ cells with CD19 CAR vector results in an higher transduction efficiency on the CliniMACS Prodigy compared to the manual setting, corroborating our previous results.
- VCN analysis is currently ongoing
- Aiming at maximizing the safety of lentiviral transduction of HSCs, the next steps will focus on the generation of a cell model that potentially predicts the transformation of HSCs into leukemic cells by evaluating their differentiation potential when cultivated under extreme conditions of stress.
- *In vivo* studies: NSG mice to test engraftment of unmodified and transduced CD34+ cells processed in small scale and on the CliniMACS Prodigy.