Automation of hematopoietic stem cell transduction: Results of a head-to-head comparison between a manual and an automated procedure

Ute Bissels[1], Ian Johnston[1], Sarah Reinartz[1], Dominik Brams[1], Faidra Aivazidou[1], Dariusz Krenz[1], Inga von Bomhard[1], Sebastian Knöbel[1], Andreas Bosio[1], and Eleni Papanikolaou[1,2]
[1]Miltenyi Biotec B.V. & Co. KG , Bergisch Gladbach, Germany | [2]Laboratory of Biology, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece

Introduction

Hematopoietic stem cells (HSCs) are an important source for cell-based therapies and can be genetically modified to treat blood disorders such as primary immunodeficiencies and hemoglobinopathies. Currently, clinical protocols for the transduction of CD34⁺ cells comprise many open handling steps and are error prone and labor intensive. As a result, these protocols are difficult to upscale and generate high variability. These drawbacks overall pose a higher risk for the formulation of the final cell product, which can be compensated only by intense personnel training. In order to address these issues that hamper the wider application of gene therapies, automated solutions need to be developed that could significantly maximize the potential of these approaches by simultaneously providing a platform of predictable outcomes and a system to perform good manufacturing practice (GMP)-compliant processes. To this end, Miltenyi Biotec had previously developed the CliniMACS Prodigy® as a closed, GMP-compliant system for T cell transduction. Here we present results of large-scale lentiviral transductions of hematopoietic stem cells performed on the CliniMACS Prodigy in terms of efficiency of the procedures and viability of the end product, compared to the "classical" manual transductions that are executed with open handling steps.

Results

1 HSC transduction process on the CliniMACS Prodigy®

We developed a procedure for cultivation and transduction of CD34⁺ cells using the integrated cell-processing platform CliniMACS Prodigy®. CD34⁺ cells were separated from mobilized leukapheresis and automatically processed within a closed, single-use tubing set. Processing steps included pre-stimulation, cultivation, lentiviral transduction, and harvesting of the gene-modified CD34⁺ cells. A specific software application – the hematopoietic stem cell engineering (HSCE) process – was developed that offers the user the possibility of adapting specific parameters, e.g., culture volume, shaker types, and number of transduction hits (fig. 1).

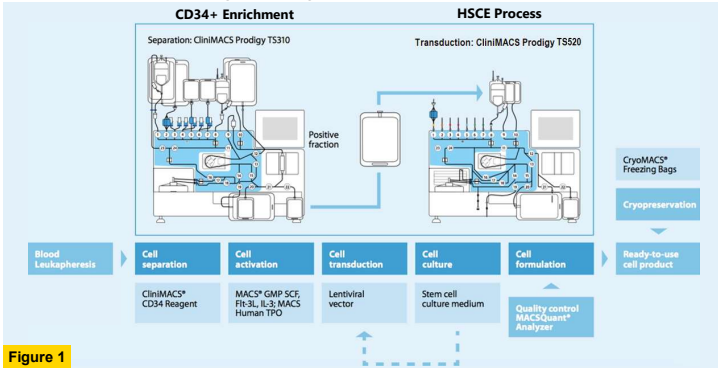

Figure 1

2 Large scale lentiviral transduction in comparison to manual processes (cell recovery)

After pre-activation of HSCs with cytokines on the CliniMACS Prodigy or in manual experiments (24-well plates), the CD34⁺ cells were transduced with lentiviral vector encoding GFP at an MOI of 30 or 100. Afterwards, transduced cells were harvested and the CD34⁺ cell product was analyzed. Recovery of the CD34⁺ cells (fig. 2) cultured on the CliniMACS Prodigy amounted to 106% on average and showed a significantly lower variability (SD: 12.7) compared to the manual process (SD: 33.5).

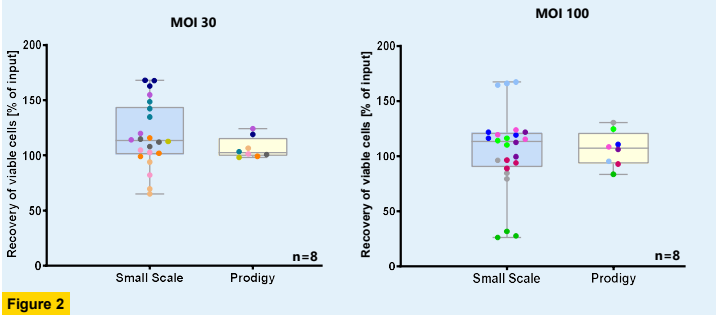

Figure 2

3 Large scale lentiviral transduction in comparison to manual processes (transduction efficiency and VCN)

For quality control of the cell product, the cells were applied to different downstream assays: (1) liquid culture until day 5, (2) CFU assay, and (3) vector copy number (VCN) analysis. Using the low, non-saturating MOI of 30, the transduction efficiency measured by flow cytometry on day 5 was on average 39% for the CliniMACS Prodigy and 26% for the manual process (fig. 3). An MOI of 100 resulted in an average transduction efficiency of 62% for the CliniMACS Prodigy and 60% for the manual process.

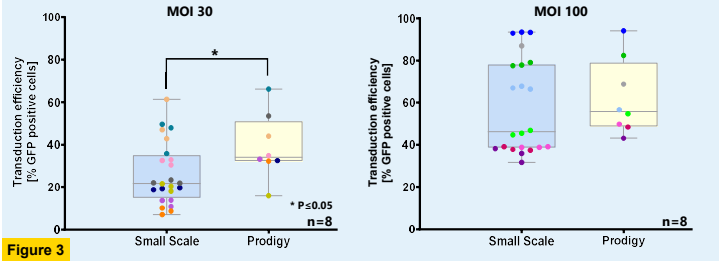

Figure 3

Analysis of the CFU assay confirmed the higher transduction efficiency of the CliniMACS Prodigy process (fig. 4A) with a significant increase from an average of 30% (manual) to 57% (CliniMACS Prodigy) at an MOI of 30 (fig. 4B). At the MOI of 100, the transduction efficiency was on average 70% for the CliniMACS Prodigy and 60% for the manual process (fig. 4B). In terms of total colony count, the number of CFU colonies generated from the CliniMACS Prodigy was 80 vs 65 for the manual process when an MOI of 30 was utilized, and an average of 40 from the CliniMACS Prodigy vs 38 for the manual process when an MOI of 100 was utilized (data not shown). Furthermore, the average VCN was 1.9 and 2.2 (MOI 30) and 3.4 and 3.2 (MOI 100) for cells from the manual procedure and the CliniMACS Prodigy, respectively (fig. 5).

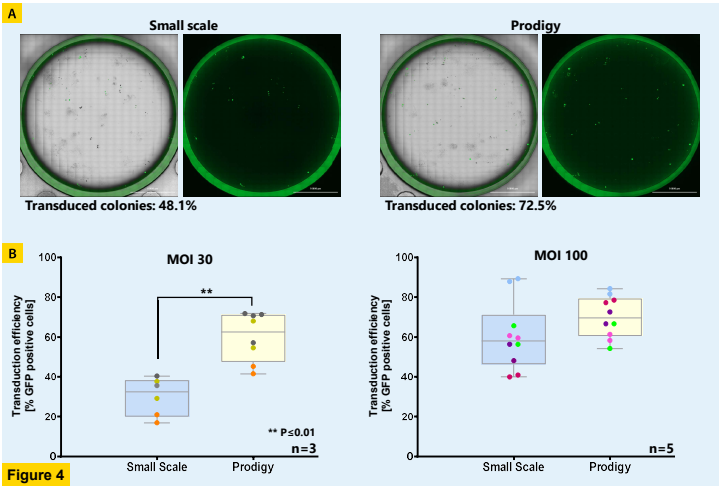

Figure 4

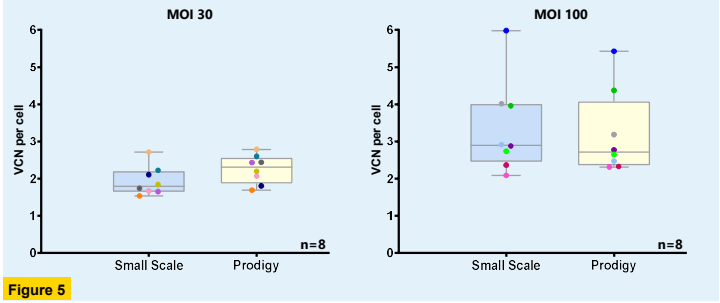

Figure 5

Conclusion

- With regard to the recovery of CD34⁺ cells after transduction, the process of the CliniMACS Prodigy® resulted in a significantly lower variability compared to the manual process and generates higher transduction rates at low, non-saturating MOIs.
- Automated cell processing and genetic manipulation of HSCs can be performed efficiently in a closed system with minimal user interaction.