Automated clinical-scale retroviral transduction of T cells in a functionally closed system

Katharina Drechsel, Constanze Radek, Carolin Kolbe, Daniela Mauer, Kristina Schweizer, Nadine Mockel-Tenbrinck, Thomas Schaser, Ian C.D. Johnston, and Andrew D.M. Kaiser
Miltenyi Biotec GmbH, Bergisch Gladbach, Germany

Introduction

Genetically engineered T cells redirected against cancer show tremendous clinical potential. However, adoptive immunotherapy still faces several challenges in the complexity associated with the current clinical manufacturing methods. Most commonly used protocols for the preparation of autologous gene-modified T cells employ lenti- or gamma-retroviral vectors to obtain a stable expression of the transgene (e.g. chimeric antigen receptors). Conventionally, the protocols comprise many (open) handling steps, are labor intensive and are not adapted for commercial manufacturing. For lentiviral transduction and expansion of selected T cells, we recently released a highly automated manufacturing process based on the CliniMACS Prodigy® Platform, the T Cell Transduction (TCT) Process. Now we have further developed our process to include spinoculation and enable gamma-retroviral transduction within the single-use closed system. The transduction efficiency was further increased by adding the soluble transduction enhancer Vectofusin-1®.

Methods

1 Automated retroviral transduction on the CliniMACS Prodigy®

The complete process to generate genetically engineered T cells was performed in a single-platform, closed system, the CliniMACS Prodigy®, using the Tubing Set TS 520. Spinoculation was integrated as flexible programmable activity into the existing automated TCT Process (fig. 1). Small-scale experiments were performed with the same reagents in tissue-culture plates.

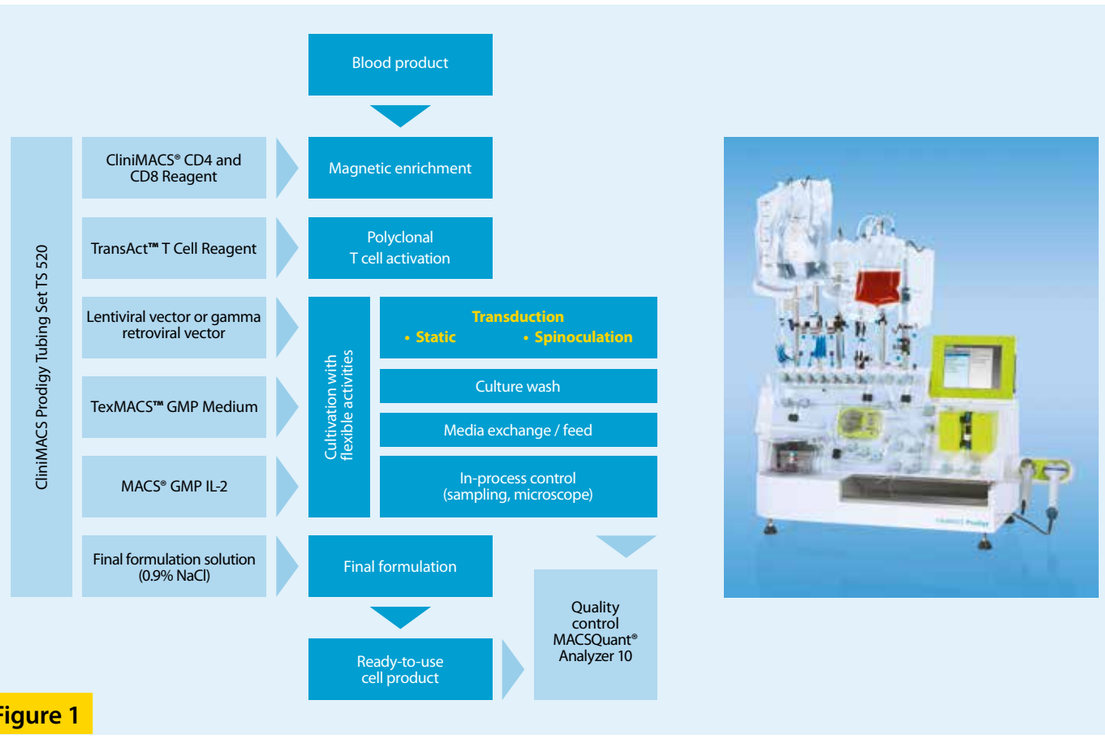

Figure 1

Results

1 Spinoculation and Vectofusin-1® increase transduction efficiencies in small scale

In small-scale experiments magnetically enriched CD4+ and CD8+ T cells were activated with TransAct™ T Cell Reagent in TexMACS™ Medium supplemented with IL-2. T cells were transduced after two days in culture with gamma-retroviral vector encoding GFP with pseudotypes RD114 at MOI 2 (fig. 2A) and GALV at MOI 1 (fig. 2B); n = 6.

Cultures were washed 6 h or 24 h after transduction and analyzed on day 7 of cultivation via flow cytometry. Transduction efficiencies could be increased by adding the soluble transduction enhancer Vectofusin-1® (10 µg/mL). Best results were obtained using the spinoculation protocol (2 h centrifugation at 400×g, 32 °C).

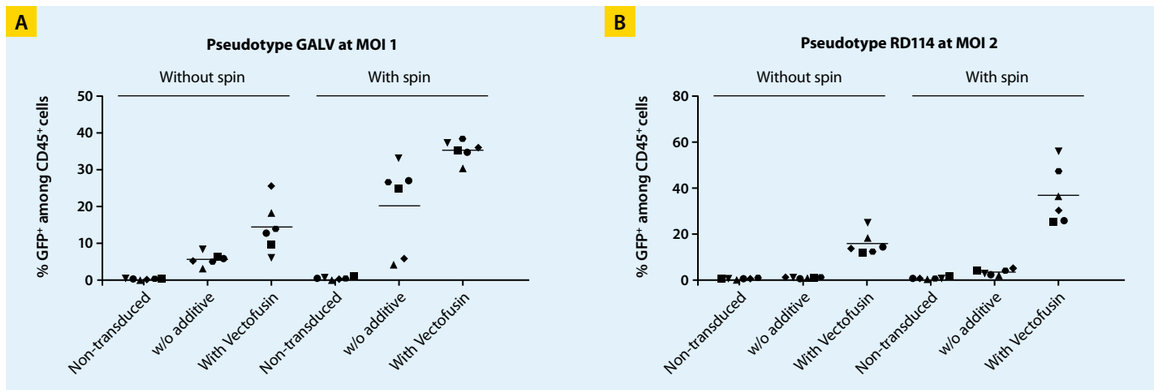

Figure 2

2 Efficient transduction on the CliniMACS Prodigy® with gamma-retroviral vectors

The spinoculation protocol was transferred to the automated system and feasibility with gamma-retroviral vectors encoding GFP was assessed. Transduction rates of enriched CD4+/CD8+ T cells transduced on day 2 with gamma-retroviral GFP vector (GALV) could be increased by adding Vectofusin-1 to the culture prior to spinoculation at 400×g for 2 h (fig. 3A, B).

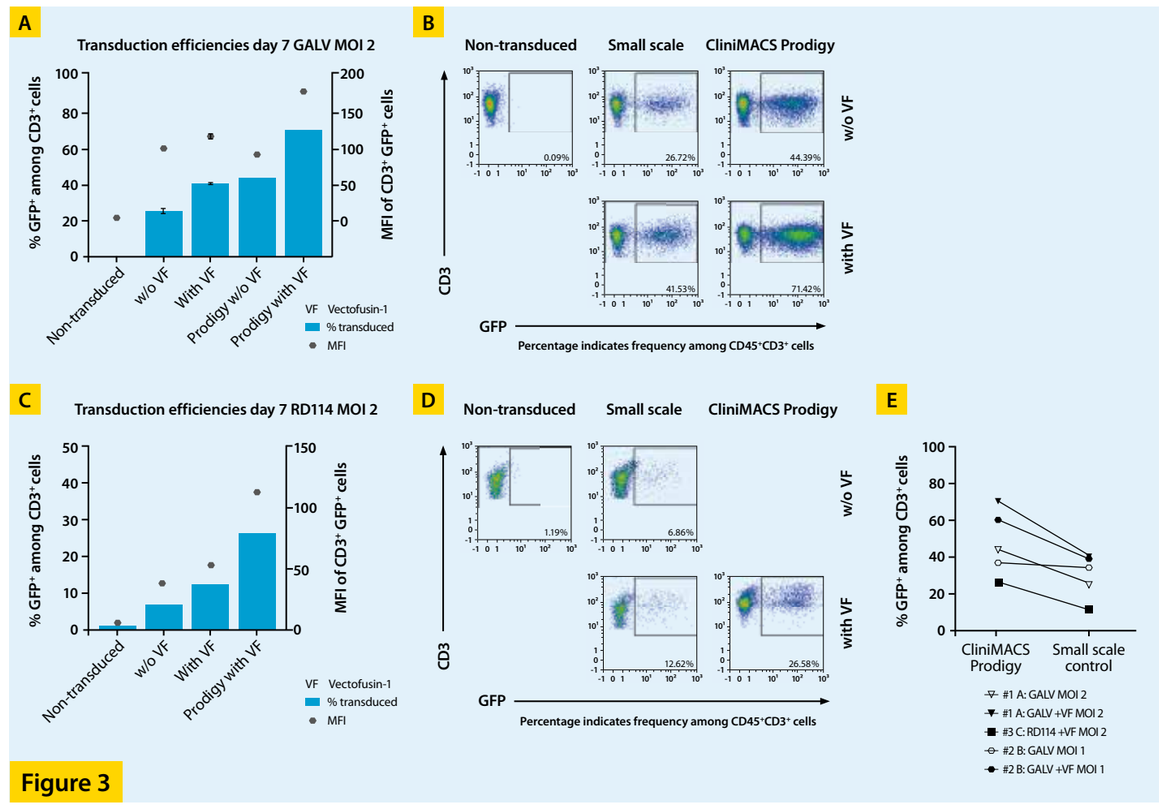

Figure 3

3 Expansion and characterization of cell product

After transduction on day 2 in the CliniMACS Prodigy, the T cells stimulated with TransAct T Cell Reagent were further expanded in the CliniMACS Prodigy using the automated feeding and media exchange activities of the TCT Process (fig. 4). Vectofusin-1 did not have a negative effect on the expansion. After the automated formulation and harvest in isotonic NaCl solution, an average of 1.159×10⁹ viable cells could be generated, with an average of 5.121×10⁸ viable transduced cells in total. For the characterization of the cell product, the blood product, the enriched fraction, the cultivated cells on day 7, and the final product were analyzed by flow cytometry for their cell composition (fig. 5A). The increase in the percentage of CD8+ T cells shows that the cultivation conditions favored expansion of these cells. The majority of T cells had the central memory T cell phenotype (fig. 5B).

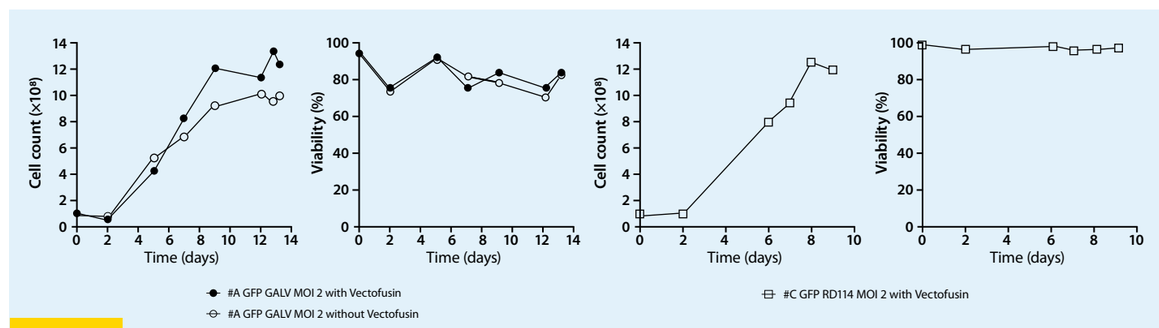

Figure 4

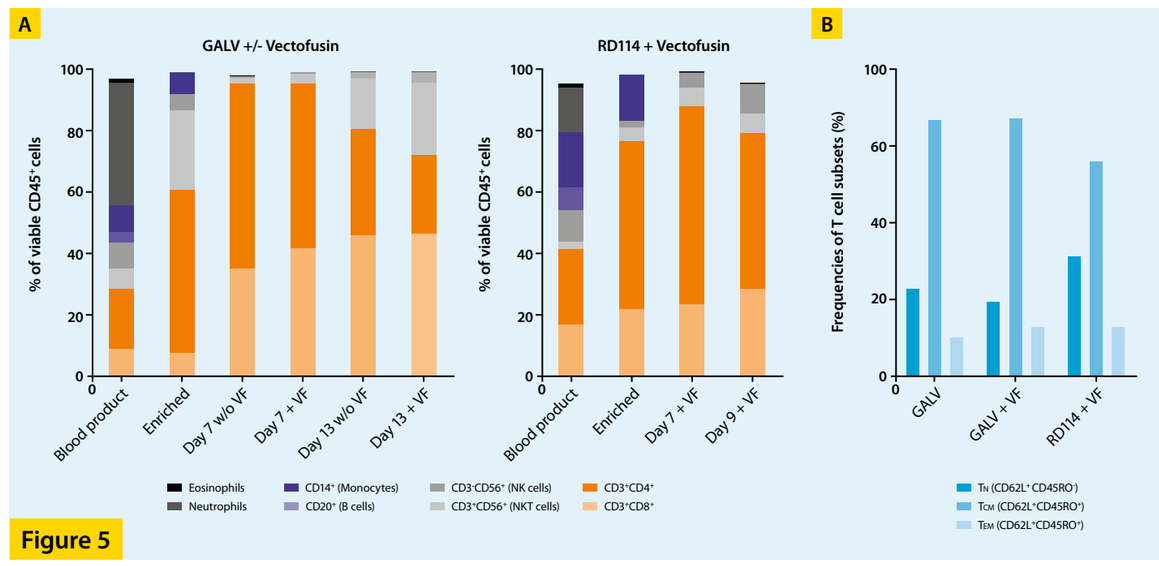

Figure 5

Conclusion

- To enable gamma-retroviral transduction, spinoculation has been implemented into the T Cell Transduction Process on the CliniMACS Prodigy for automated enrichment, activation, transduction, and expansion of T cells.
- Feasibility of the generation of clinically relevant numbers of gamma-retrovirally modified T cells could be shown with the pseudotypes GALV and RD114.
- Vectofusin-1 as well as spinoculation increase transduction efficiencies and thus reduce the amount and cost of viral vector needed per manufacturing run.
- The flexibility and ease of use associated with the TCT process and the CliniMACS Prodigy Platform will enable the development of cellular therapies for the treatment of large patient groups and make economic commercial-scale manufacturing possible.

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