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

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Introduction
Genetically engineered T cells redirected against cancer show tremendous clinical potential. However, adoptive immunotherapy has faced 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 lentiviral or gammaretroviral vectors to obtain a stable expression of the transgene of interest. Considering 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® 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. By adding the soluble transduction enhancer Vectofusin-1™, transduction efficiencies further increased.

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 Turbo Spin TS 5.5/2. 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

2 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. 2). Vectofusin-1™ did not have a negative effect on the expansion. The automated formulation and harvest in aseptic NaCl solution, an average of 115±10³ viable stable cells could be generated, with an average of 5.2±10³ viable transduced cells in total. For the characterization of the cell product, the blood product, the enriched fraction, the cultivated cells on day 5 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 2

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 a small scale CM 41.53 %. Medium supplemented with 6-2 T cells were transduced after two days in culture with gamma-retroviral vector encoding GFP with pseudotypes RD1 14 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 3

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 RD14.
- Vectofusin-1 as well as spinoculation increase transduction efficiencies and thus reduce the amount and cost of viral vector material 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.

Figure 4

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. 2). Vectofusin-1™ did not have a negative effect on the expansion. The automated formulation and harvest in aseptic NaCl solution, an average of 115±10³ viable stable cells could be generated, with an average of 5.2±10³ viable transduced cells in total. For the characterization of the cell product, the blood product, the enriched fraction, the cultivated cells on day 5 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 5

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