Effective retroviral transduction of primary T cells and hematopoietic stem cells using the soluble transduction enhancer: Vectofusin-1®

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Introduction

Modification of target cells with retroviral vectors often requires the presence of a transduction-enhancing reagent. Polycationic reagents induce aggregation of vector particles and facilitate binding to target cells via electrostatic interactions, whereas bridging molecules, such as recombinant fibronectin, interact with both vector particle and cell membrane. These methods can also be combined with centrifugation to further enhance transduction performance.

Transduction performance is highly dependent upon the pseudotype used and the receptor availability on the target cell. For example, VSVG pseudotyped lentiviral vectors (LV) transduce primary T cells very effectively at low multiplicity of infection (MOI = 1), while HSCs often require 100-fold more vector. To overcome restrictions in viral vector entry to HSCs, alternative pseudotypes have been developed, which also show reduced toxicity during production. LV pseudotyped with certain of these envelope proteins (e.g. RD114, GALV, BaEV) require an enhancement reagent to effectively bind and enter HSCs and other cells such as T cells, while MV-LV pseudotyped with measles HF glycoproteins achieve good transduction rates also in the absence of enhancers.

We have assessed Vectofusin-1®, a histidine-rich, cationic amphipathic peptide, as an alternative transduction enhancer to modify both primary T cells and HSCs. Vectofusin-1 is a short peptide of 26 amino acids which can easily be synthesized to high purity for clinical use. Unlike recombinant fibronectin, it is a soluble reagent that does not have to be pre-coated on cell culture surfaces, which makes automation of transduction processes for future clinical application less cumbersome.

Results

1 Vectofusin-1® facilitates lentiviral transduction of hCD34+ cells with the novel BaEV-LV and MV-LV pseudotypes

CD34+ HSCs were magnetically isolated from cord blood and were pre-stimulated for 24 h either in StemMACS™ HSC Expansion Medium (fig. 1A) or in HSC-Brew GMP Medium containing 2% human serum albumin (HSA; fig. 1B) supplemented with recombinant human TPO, SCF, and Flt3-L. CD34+ cells were then transduced with lentiviral vectors encoding GFP at an MOI of 10 in the presence of Vectofusin-1 (fig. 1A,B) or in recombinant fibronectin-coated plates (fig. 1A). In a research protocol (fig. 1A), LV vector and Vectofusin-1 were diluted in equal volumes of serum-free medium, mixed and incubated for 5–10 minutes before addition to the target cells in StemMACS Medium containing cytokines. In a second protocol GMP-quality reagents were used (fig. 1B,C) and vectors and Vectofusin-1 were diluted in equal volumes of HSC-Brew GMP Medium without HSA, mixed, and incubated for 5–10 minutes before addition to the target cells in complete HSC-Brew GMP Medium. The transduction medium was replaced after 24 h culture. Transduction efficiency (fig. 1A,B) and viability (fig. 1C) were assessed 4 days after transduction. Two different baboon envelope constructs were used for lentiviral pseudotyping, BaEVTR and BaEVRless.[1]

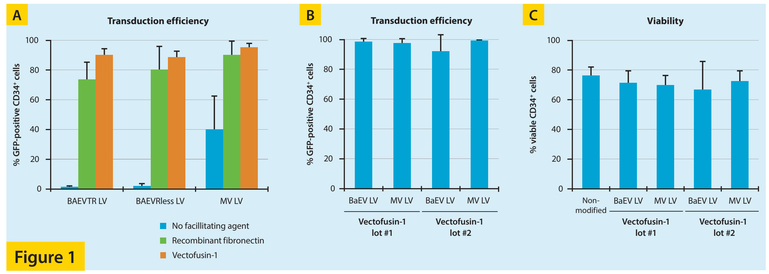

Figure 1

2 Vectofusin-1® enhances transduction of primary T cells with gamma-retroviral vectors

Human CD4+ and CD8+ T cells were magnetically enriched with CD4 and CD8 MicroBeads and were activated with TransAct™ T Cell Reagent in TexMACS™ Medium supplemented with IL-2. Two days after activation, T cells were transduced with gamma-retroviral vectors encoding GFP, pseudotyped with GALV (MOI 1; fig. 2A) and RD114 (MOI 2; 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). Highest transduction efficiencies were obtained using a spinoculation protocol (2 h centrifugation at 400×g, 32 °C).

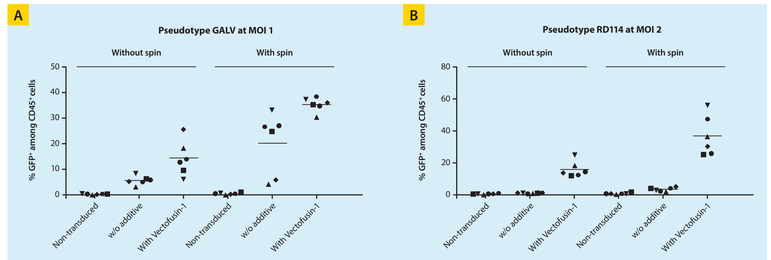

Figure 2

3 CAR T cells generated with Vectofusin-1® are functional

Primary human T cells from two donors were genetically modified using the protocol described in figure 2 using a RD114-pseudotyped viral vector encoding a chimeric antigen receptor (CAR) specific for the ErbB2 cancer target antigen[2] (MSGV-4D5-CD8-28BBZ, kindly provided by Richard Morgan, NIH, Bethesda, MD, USA). ErbB2-CAR–expressing cells were identified by flow cytometry using an ErbB2-Fc fusion protein and anti-Fc-PE conjugate (fig. 3A). Seven days following genetic modification, the T cells were incubated with target cells expressing ErbB2 (MCF-7) or lacking the antigen (SupT1) for 24 hours at an effector:target ratio of 1:2 and the supernatants were analyzed for the secretion of inflammatory cytokines (B). As a positive control, T cells were stimulated with PMA/ ionomycin. CAR T cells or cell lines alone served as negative control samples. CAR T cells generated with Vectofusin-1® were fully functional and recognized their target antigen.

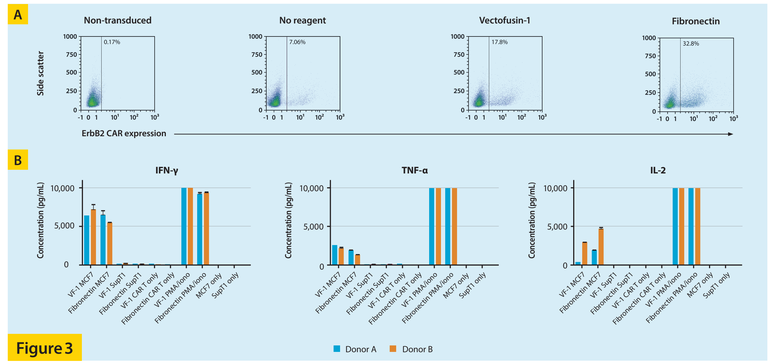

Figure 3

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

Full automation of clinical-scale production of genetically engineered T cells is challenging when solid-phase transduction enhancers such as recombinant fibronectin are used. We therefore assessed transduction enhancement in the presence of Vectofusin-1 in a single-platform, closed system, the CliniMACS Prodigy® (fig. 4C), using the single-use tubing set CliniMACS Prodigy TS 520. The spinoculation transduction protocol was integrated as a flexible programmable activity into the existing automated lentiviral TCT Process. The feasibility of automated transduction was assessed using gamma-retroviral vectors encoding GFP and compared with small-scale experiments performed with identical reagents in tissue culture plates. 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. 4A, B). Using the conditions previously optimized in small scale for RD114 pseudotyped vectors with Vectofusin-1 and spinoculation also resulted in good transduction efficiencies in the CliniMACS Prodigy (fig. 4D, E). Transductions with GALV and RD114 pseudotypes performed on the CliniMACS Prodigy yielded results comparable to the respective small-scale controls (fig. 4F). 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. Vectofusin-1 did not have a negative effect on the expansion and after the automated formulation and harvest in isotonic NaCl solution, an average of 1.16×10⁹ viable T cells (mainly central memory phenotype) could be generated, with an average of 5.12×10⁸ viable transduced cells in total (data not shown).

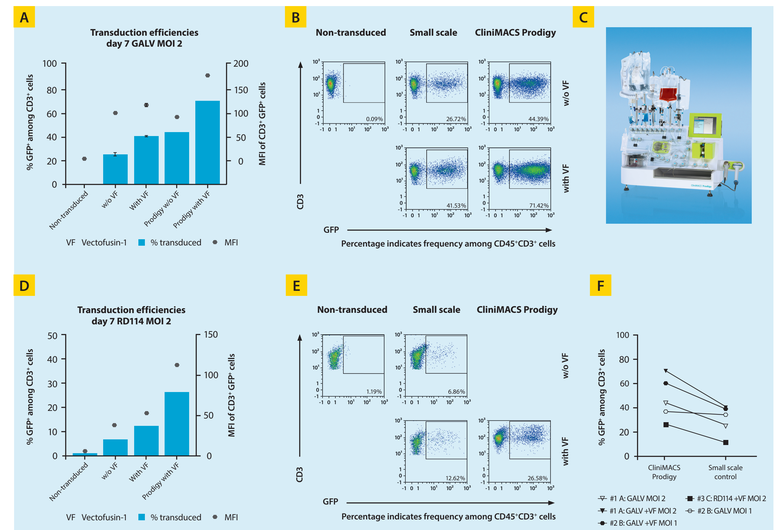

Figure 4

Conclusion and outlook

- Vectofusin-1 is a soluble transduction enhancer that is not dependent on immobilization and is therefore fully compatible with automated workflows such as the T Cell Transduction Process on the CliniMACS Prodigy.
- Vectofusin-1 enhances transduction of human CD34+ cells with lentiviral vectors displaying novel pseudotypes (BaEV, MV).
- Vectofusin-1 enhances T cell transduction with gamma-retroviral vectors (GALV, RD114 pseudotypes).
- CAR T cells generated using Vectofusin-1 are functional.
- Vectofusin-1 increases transduction efficiencies and thus reduces the amount and cost of viral vector needed per experiment.
- To enable automated manufacture of genetically modified T cells, a gamma-retroviral T cell transduction step incorporating Vectofusin-1 has been implemented into the T Cell Transduction Process on the CliniMACS Prodigy for automated enrichment, activation, transduction, and expansion of T cells.

References
1. Girard, A. *et al.* (2014) Blood 124: 1221–1231.
2. Zhao, Y. *et al.* (2009) J. Immunol. 183: 5563–5574.

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