Combination of transduction enhancers with Ecotropic-MLV pseudotyped lentiviral vectors enables highly efficient transfer of chimeric antigen receptors into murine T effector and T regulatory lymphocytes

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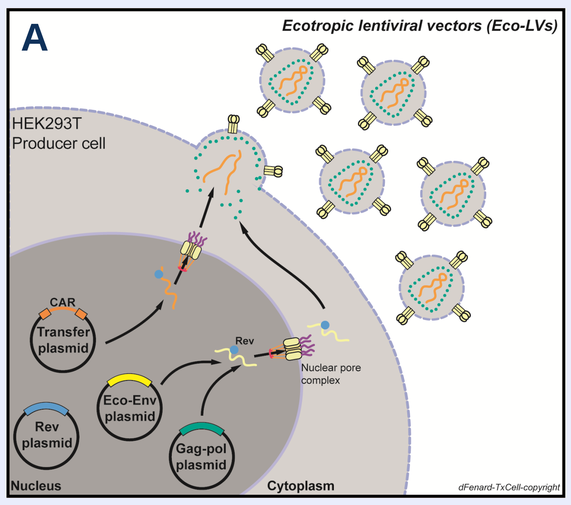
INTRODUCTION

Gene transfer of Chimeric Antigen Receptors (CARs) into T regulatory (Treg) lymphocytes using HIV-1-based lentiviral vectors (LVs) has several therapeutic applications ranging from inflammatory diseases to autoimmunity. The development of these new therapeutic approaches requires the use of murine animal models. However, the transduction of murine lymphocytes with LVs pseudotyped with the broadly used vesicular stomatitis virus envelope glycoprotein (VSV-G) is known to be inefficient. To target more specifically murine cells, it is possible to pseudotype LVs with the ecotropic murine leukemia virus envelope glycoprotein (Eco-cLV). Furthermore, numerous peptide derived-transduction enhancers have been developed to improve LV entry into target cells, a rate limiting step. We have evaluated a combination of these two technologies to improve the transduction of murine T lymphocytes.

Production optimization of Ecotropic-pseudotyped cLVs



B

	Quantity (µg)/flask T175 for VSV-G envelope	Quantity (µg)/flask T175 Eco1	Eco2	Eco3
pMDLg/pRRE	8.5	8.5	8.5	8.5
pRSV-REV	4.3	4.3	4.3	4.3
ENVELOPE	5	5	10	15
Transfer plasmid pTX021	17	17	17	17

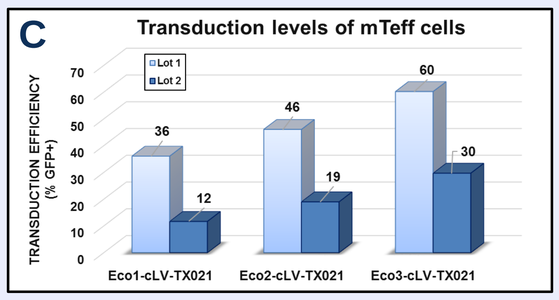


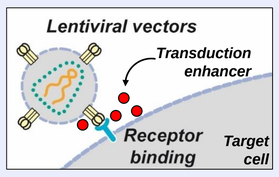
Figure 1: Production protocol of Eco-cLV-CARs. A) Representation of a HEK293T producer cell transfected with the four-plasmid lentiviral system. Lentiviral vectors (LVs) produced in the supernatant are concentrated (cLVs) by low speed centrifugation overnight and stored at -80°C. **B)** Plasmid ratio have been optimzed to produce highly infectious and concentrated ecotropic-LVs (Eco-cLVs) expressing TX021 CAR. Compared to the typical VSV-G plasmid (5µg), the quantity of ecotropic expression plasmid used was 5µg (Eco1), 10µg (Eco2) or 15µg (Eco3). **C)** To evaluate the Eco-cLV infectivity, murine T effector (mTeff) cells, isolated from C57BL/6 mice using magnetic beads, were transduced at Day 0 and cultured in presence of IL-2 and anti-CD3/CD28 beads. Briefly, viral supernatants were added to the cell suspension at a final titer of 2x10E7 TU/ml. Transduction reactions were spinoculated (1000 *g*, 90 min., 32°C) and transferred overnight at 37°C/5% CO2. Next, mTeff cells were washed and cultured in fresh complete medium. Transduction efficiencies were evaluated on two different lots by monitoring GFP expression after one week.

The quantity of ecotropic envelope during LV production is critical in order to get highly infectious lentiviral particles

mTeff cells transduction in presence of culture additives

A

Vector adhesion and fusion is a rate limiting step



ECOTROPIC surface envelope
Tropism specific for murine/rat cells
+

Transduction enhancers	Description	References
Protamine Sulfate	Highly cationic peptide	Cornetta et al. (1989) J. Virol. Methods 23:187.
Retronectin	Fragment of human fibronectin	Hanenberg et al. (1996) Nature Med. 2:876.
Vectofusin-1	Alpha-helical nanofibrillar peptide	Vermeer et al. (2017) Acta Biomater. (In press)
Protransduzin-A	Amyloid nanofibrillar peptide	Yolamanova et al. (2013) Nature Nanotechnol. 8:130.

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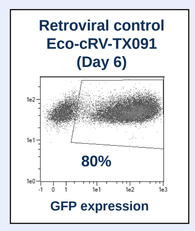

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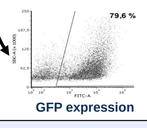
mTeff transduction (Day 3)

	Eco-cLV-TX021 2 x 10E7 TU/ml MOI 20	10E8 TU/ml MOI 100
None	39,4	52,5
RetroNectin	42,9	70,5
Vectofusin	52	77
Protransducin	79,6	79,4
Protamine Sulfate	37,4	65,4



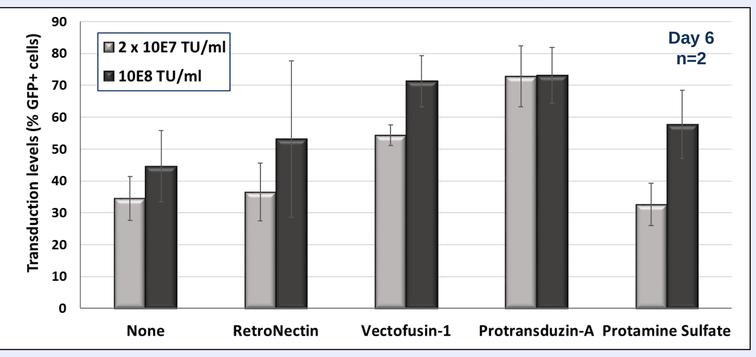
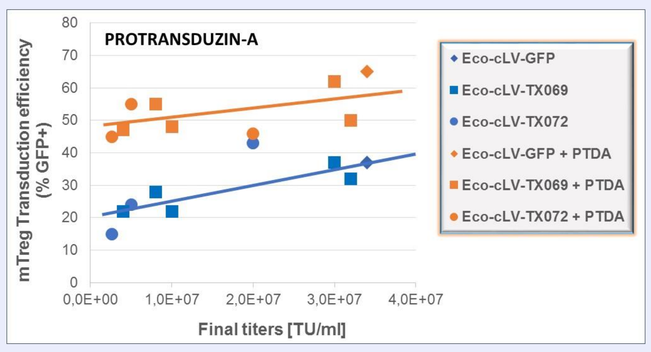


Figure 2: Murine T effector cells stably transduced with Ecotropic-pseudotyped lentiviral vectors. A) Description of different viral transduction enhancers. **B)** mTeff cells were transduced at Day 0 as described in Figure 1. Briefly, viral supernatants were preincubated few minutes with culture additives and added to the cell suspension. Transduction reactions were spinoculated and transferred overnight at 37°C/5% CO2. Next, mTeff cells were washed and cultured in fresh complete medium. Transduction efficiencies were evaluated by monitoring GFP expression after 3 to 6 days. **C)** mTeff cells were transduced overnight with a control consisting of a Moloney derived retroviral vector (Eco-cRV-TX091, 10^E7 TU/ml).

Nanofibrillar peptides, Vectofusin-1 and Protransduzin-A, strongly promote the transduction of murine Teff cells with Eco-cLV-CARs

mTreg cells transduction in presence of PTDA and VF-1

A



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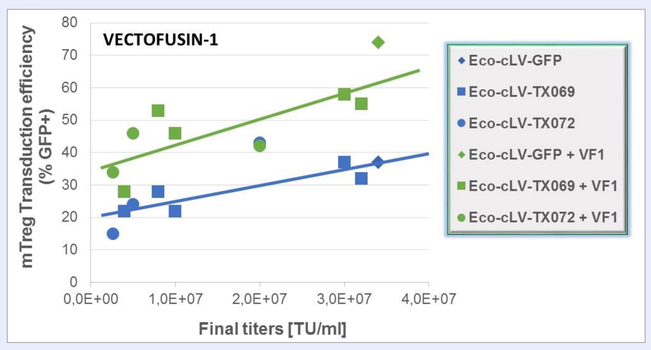

Figure 3: Improvement of mTreg transduction using Protransduzin-A or Vectofusin-1 peptides. murine Treg cells were isolated from C57BL/6 mice using magnetic beads and transduced at day 0. For that, concentrated ecotropic-pseudotyped lentiviral vectors (Eco-cLV) expressing GFP or CARs (TX069 or TX072) were pre-mixed at room temperature with transduction enhancers, either Protransduzin-A, PTDA (**A**, orange) or Vectofusin-1, VF-1 (**B**, green). Next, the Vector/peptide mix were added vol/vol on murine Tregs (2 x 10^E6 / ml) and culture plates were spinoculated (1000 *g*) for 90 min at 32°C. Finally, culture plates were transferred overnight at 37°C / 5% CO2. The final concentration of culture additives was set to 15µg/ml. After overnight transduction, Treg cells were washed and cultured in fresh medium complemented with rapamycin, cytokines and anti-CD3/CD28 beads. After one week, transduction efficiencies were evaluated by monitoring GFP expression.

The use of transduction enhancers strongly promote mTreg transduction with Ecotropic-pseudotyped lentiviral vectors

mTreg cells expansion and viability post-transduction

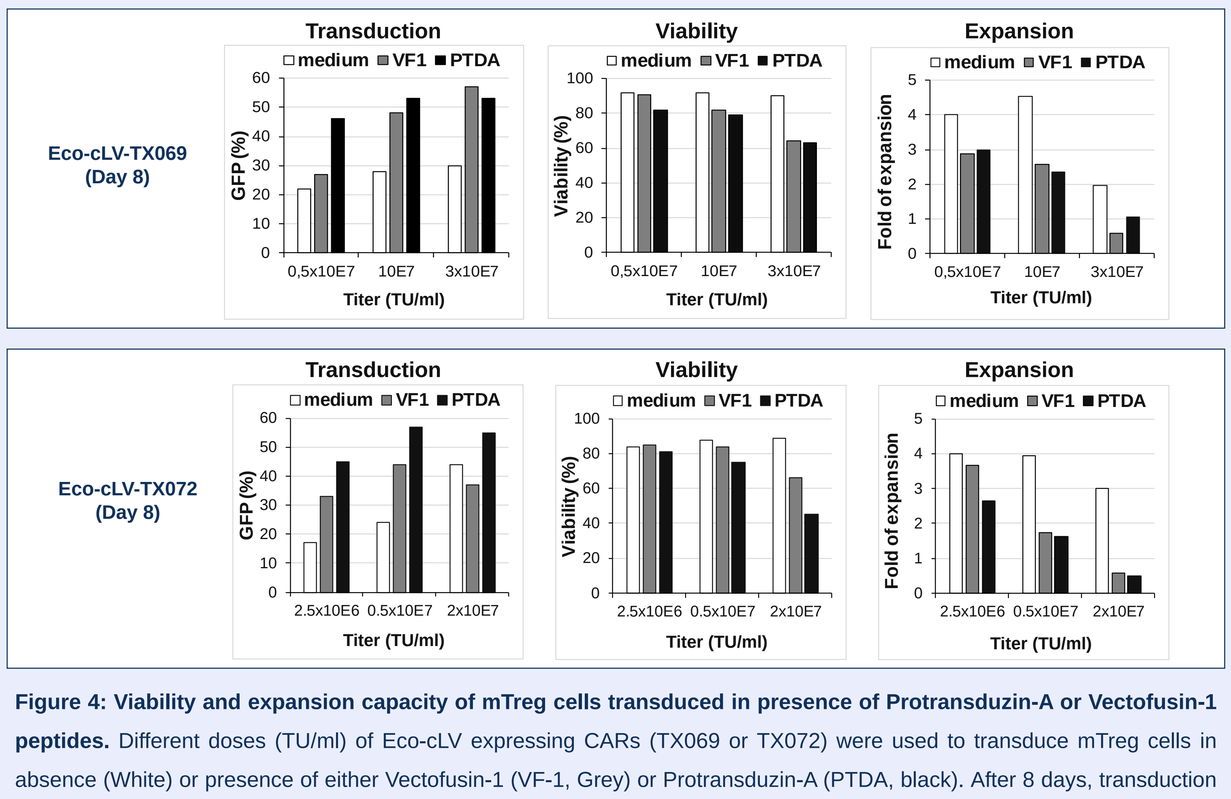

Figure 4: Viability and expansion capacity of mTreg cells transduced in presence of Protransduzin-A or Vectofusin-1 peptides. Different doses (TU/ml) of Eco-cLV expressing CARs (TX069 or TX072) were used to transduce mTreg cells in absence (White) or presence of either Vectofusin-1 (VF-1, Grey) or Protransduzin-A (PTDA, black). After 8 days, transduction efficiencies (% of GFP+ cells), the viability (cell counts) and the fold of expansion have been evaluated.

The viability and the expansion of mTreg cells are correct for transduction conditions using less than 10E7 TU/ml of ecotropic cLV.

CONCLUSION & DISCUSSION

Among the family of transduction enhancers tested, Vectofusin-1® (also called LAH4-A4 peptide) and Protransduzin-A® (also called EF-C peptide) showed a very significant improvement of murine T cell transduction (from 50 to 80%) with Eco-LVs. Under optimal transduction conditions, the viability of the target cells was not affected by the culture additives and the expansion of the transduced cells was correct using viral titers below 10E7 TU/ml. In conclusion, the strong increase of lentiviral transduction into murine Teff or Treg lymphocytes, by 1) Ecotropic pseudotyping, 2) the use of low-speed concentrated Eco-cLV and 3) the use of transduction enhancers, allowed us to bypass the strong lentiviral restrictions described in murine cells. It opens the way to the implementation of robust protocols for CAR lentiviral gene transfer into murine animal models.

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