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Predictive Immunotherapy Models: Overcoming the challenge of T cells gene transfer

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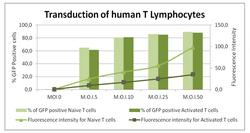
Have you ever dreamt you could transduce 60% of naive and 90% of activated T lymphocytes ?

No more sorting of your cells : adoptive immunotherapy made easier

in vitro

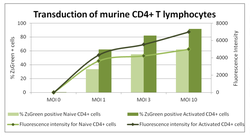
As with human T cells, murine CD4+ T lymphocytes (naive, activated or regulatory subpopulations) are efficiently transduced using highly purified and concentrated lentiviral vectors without affecting viability and proliferation, and preserving the original phenotype of the cells.

Naive and activated **human** total T lymphocytes



Human naive or activated (CD3/CD28) T lymphocytes are transduced by a GFP-expressing lentiviral vector using a range of MOI (Multiplicity of Infection) from 0 to 50 in presence of polybrene 4µg/mL for 5h at 37°C, 5% CO_2.
Five days post-transduction, the % of GFP positive cells and Fluorescence Intensity are analyzed by flow cytometry.

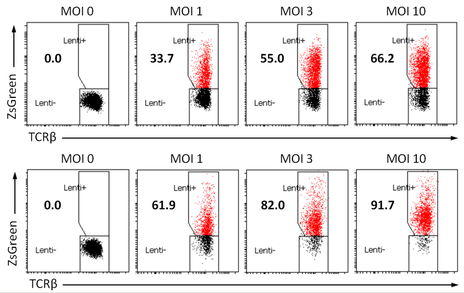
Naive and activated **murine** CD4+ T lymphocytes

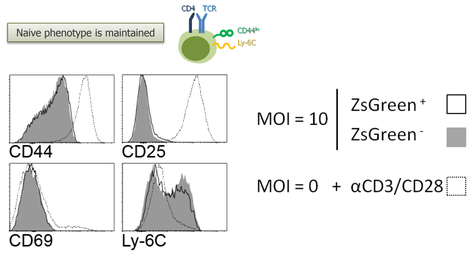


Naive CD4+ T lymphocytes are obtained from lymph nodes by negative selection (CD8b, CD45R (B220), CD11b (Mac1), Ter-119, and CD16/CD32). Purified CD4 cells are then labelled with antibodies against Lineage markers (CD8a, NK1.1, gdTCR and CD25) and with an anti-CD44 antibody. Naive CD4+ T lymphocytes are FACS-sorted as CD44lo Lin- cells and transduced by a ZsGreen-expressing lentiviral vector using a range of MOI from 0 to 30 in presence of polybrene 4µg/mL for 5h at 37°C, 5% CO2.
Four days later, the % of ZsGreen+ cells and Fluorescence Intensity are analyzed by flow cytometry. Cell activation (anti -CD3/CD28 coupled beads) is performed 16h after transduction as a control.

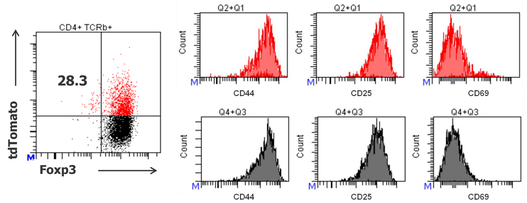
Naive

Activated





murine CD4+ **regulatory** T lymphocytes



GFP-expressing CD4+ regulatory T lymphocytes (Tregs) are obtained from FoxP3-GFP mice. Tregs are FACS-sorted as GFP positive cells, then transduced by a tdTomato-expressing lentiviral vector at MOI 10 in presence of polybrene 4µg/mL for 5h at 37°C, 5% CO2.
Five days post-transduction, the % of tdTomato positive cells and Fluorescence Intensity are analyzed by cytometry.

in vivo

Murine CD4+ T lymphocytes efficiently transduced using highly purified and concentrated lentiviral vectors can be used directly for adoptive immunotherapy studies, without any subsequent sorting and with the assurance the transduced cells maintain their original phenotype.
Any populations of T lymphocytes efficiently modified with lentiviral vectors are then the best tool for adoptive immunotherapy studies

1- **Naive murine** CD4+ CD44lo Lin- T lymphocytes purification

CD45.1 donor mice

2- **Naive murine** CD4+ CD44lo Lin- T lymphocytes transduction

ZsGreen-expressing lentiviral vector

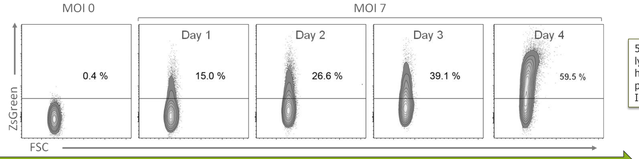
MOI 7
+ polybrene 4µg/mL

3-I*n vitro* control culture of transduced naive CD4+ T lymphocytes

3- Adoptive transfer of 10^6 **transduced** naive CD4+ T lymphocytes

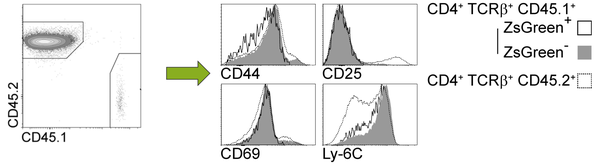
CD45.2 recipient mice

4- Kinetic of *in vitro* ZsGreen expression: Flow cytometry analyses of the % of ZsGreen positive cells from day1 to day4 post-transduction.



5- Seven days post adoptive transfer, lymph nodes and spleens are harvested and the % of ZsGreen positive cells and Fluorescence Intensity are analyzed by cytometry.

Day 0 — Day 1 — Day 4 — Day 8



- Our data shows that we can achieved effective transgene expression into naive and activated murine CD4+ T lymphocytes.
- These T lymphocytes exhibit an original cell phenotype without any changes of T lymphocytes specific markers expression.
- These genetically modified murine T lymphocytes can be easily followed in vivo after adoptive transfer thanks to fluorescent reporters.

Gene transfer using **concentrated** and **highly purified** lentiviral vectors is the best way to get a stable expression of the sequence of interest (cDNA, shRNA, miRNA, CRISPR/Cas9).
Compared to transfection or standard lentiviral vectors, they allow time, money, and energy saving, providing a **single tool from *in vitro* to *in vivo* applications**.
This major technological leap will allow to tightly control expression of various cells modifiers; it thus paves the way to design original tools and develops cell-based cancer models.