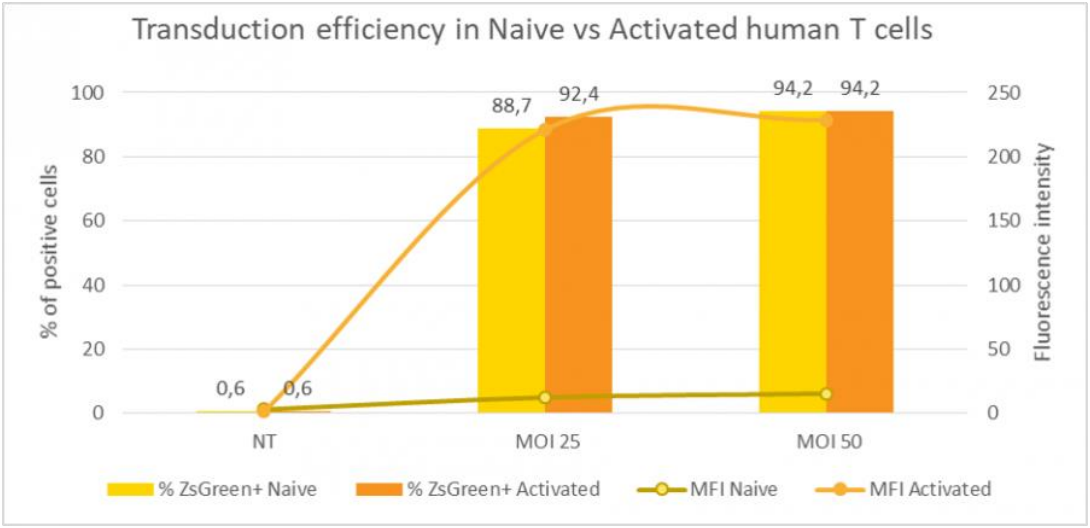
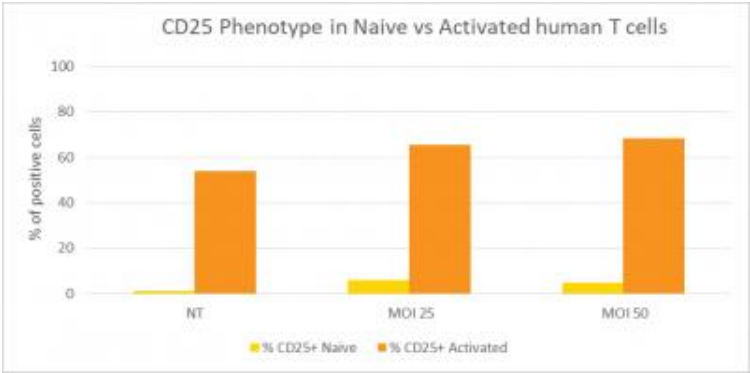
# Overcoming the challenge of T cells gene transfer with lentiviral vectors

Flash Therapeutics extensive *in vitro* transduction data show that the use of highly concentrated (1E9 TU/mL) and pure (1E8 TU/mg of proteins) lentiviral vectors increases significantly the transduction level of activated, naive and regulatory CD4+ T cells without affecting their phenotype nor viability.

## Naive and activated human total T lymphocytes




**Human naive or activated** (anti-CD3/CD28 coupled beads) T lymphocytes are transducted by a ZsGreen-expressing lentiviral vector using a range of MOI (Multiplicity of Infection) from 0 to 50 in presence of polybrene 4 µg/mL for 16h at 37°C, 5% $CO_2$.




Six days post-tranduction, the % of ZsGreen positive cells and Fluorescence Intensity are analyzed by flow cytometry.

**High CAR expression on human T cells**

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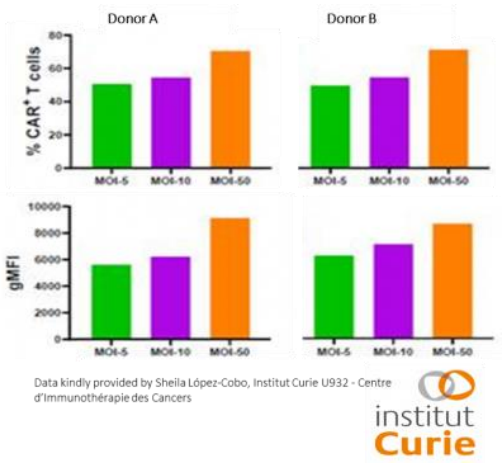
Human activated T lymphocytes harvested from 2 different donors are transduced by a CAR-expressing lentiviral vector using a range of MOI (Multiplicity of Infection) from 0 to 50 in presence of polybrene 4 µg/mL for 16h at 37°C, 5% CO2.



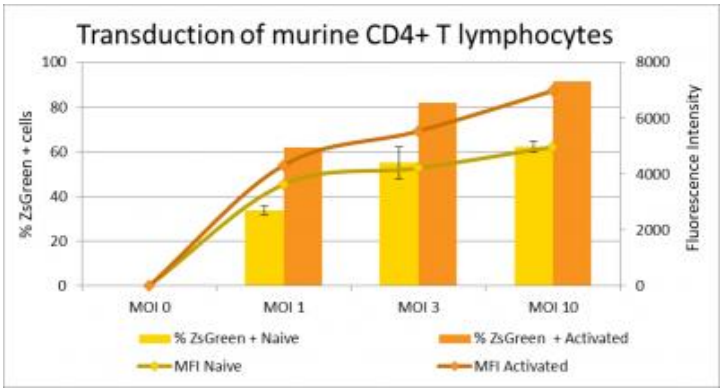

Seven days post-transduction, the cells are specifically immune–stained and the % of CAR positive cells and Fluorescence Intensity are analyzed by flow cytometry.

For both donors the transduction leads to a high CAR expression on activated human T lymphocytes.

## Naive and activated murine CD4+ T lymphocytes

Naive CD4+ 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 transducted 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 positive cells and fluorescence intensity are analyzed by flow cytometry. Cell activation (anti-CD3/CD28 coupled beads) is performed 16h after transduction as a control.

## T cells keep their phenotype and specific markers expression: Naive phenotype of T lymphocytes is maintained

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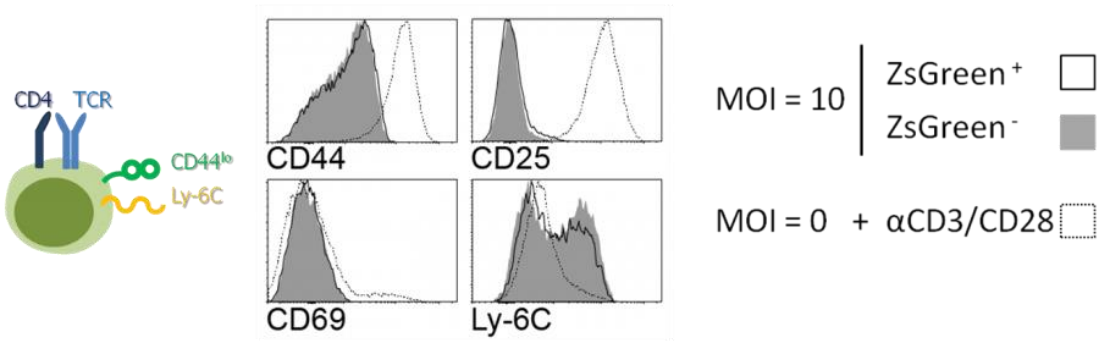
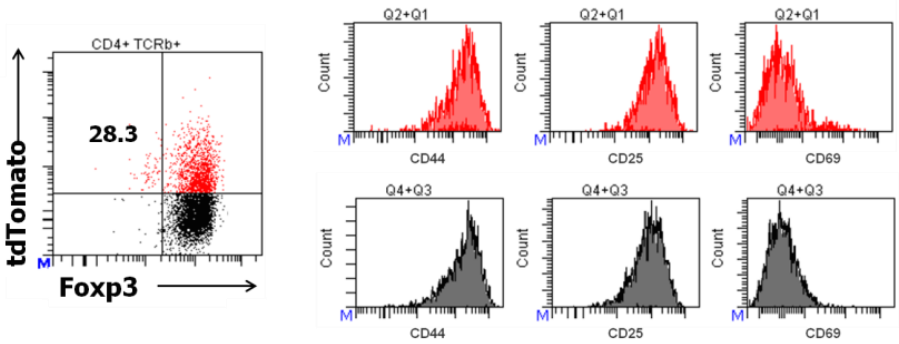



Naive CD4+ T lymphocyte are transduced by a ZsGreen-expressing lentiviral vector at MOI 10 in presence of polybrene 4µg/mL for 5h at 37°C, 5% CO2.

The naive phenotype of T cells is characterized by a low expression of CD44, which is an indicative marker for effector-memory T-cells, no expression of CD25 which is expressed specifically on stimulated T cells, nor CD69 which is an early marker of activation. Ly6 is down regulated on activated cells and it is thus found expressed on naive T cells.

Transduced T cells thus exhibit their original cell phenotype without any changes of T cells specific markers expression attesting that the composition of this pure batch of lentiviral vector does not have any impact on T cells phenotype.

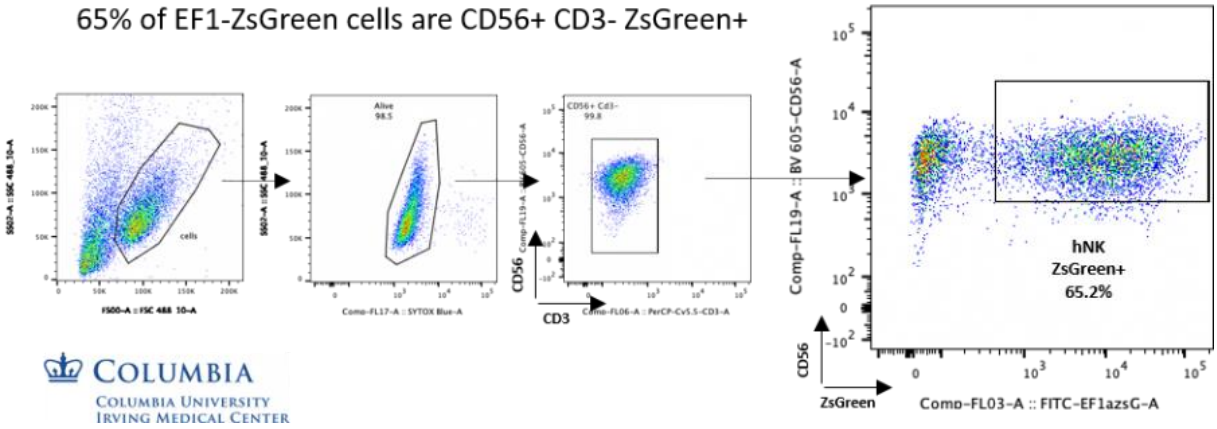
## Regulatory murine CD4+ T lymphocytes




GFP-expressing CD4+ regulatory T lymphocyte (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% $CO_2$. Five days post-transduction, the % of tdTomato positive cells and Fluorescence Intensity are analyzed by cytometry.

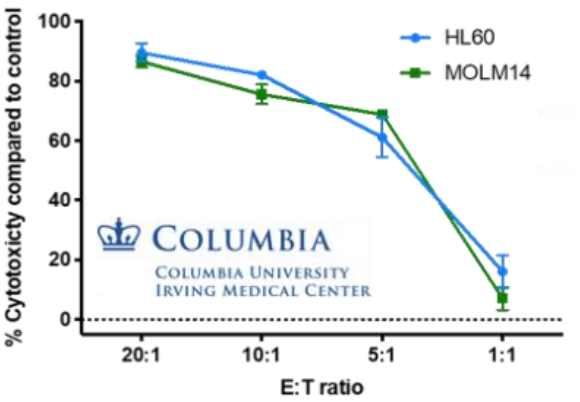
# Human NK cells

Transduced human NK cells can efficiently kill and be engrafted:




hNK CD56+ CD3- Cord blood cells are cultivated using the G-Rex® system (Wilsonwolf), in presence of IL-2, IL-15 and IL-21 and activated with artificial APCs. hNK cells are transduced twice at MOI 50 with a ZsGreen-expressing lentiviral vector. Five days post-transduction, the % of CD56+ CD3- ZsGreen positive cells is analyzed by cytometry.

Transduced hNK cells are fully functional. They can efficiently kill HL60 and MOLM14 target cells in vitro (17h incubation at various Effector: Target cells ratio)

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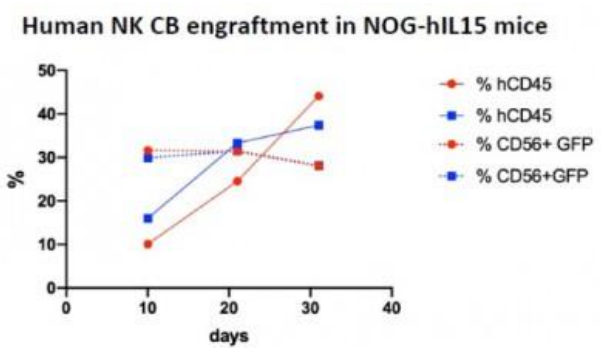


Transduced hNK cells are efficiently engrafted in vivo for up to one month post injection. PBMC are analyzed. Activated NK CD56+ CD3- Cord blood cells were transduced at MOI 50 and reactivated prior to injection into NOG-hL15 mice. Premium quality lentiviral vectors can transduce more than 65% of hNK cells, without toxicity and ensuring phenotype and cytotoxic activity. Data kindly provided by Florence Borot, Mukherjee Lab - Columbia University, ICRC.

## Human Hematopoietic Stem Cells


Human Hematopoietic Stem Cells (HSCs) are harvested from cord blood and transduced by a ZsGreen-expressing lentiviral vector using a range of MOI from 0 to 75 in presence of polybrene 4µg/mL for 16h at 37°C, 5% CO2. Seven days later, the % of ZsGreen positive cells and fluorescence intensity, as well as the expression of CD34 marker are analyzed by flow cytometry.

## Lentiviral vectors: a major technological leap

Our data bring out that the composition of lentiviral vectors in terms of titer, specific activities and purity are the success keys to ensure transduction of immune cells. This major technological leap will allow to tightly control expression of various modifiers of these cells and thus paves the way to design inventive tools and to develop cell-based cancer models.

Gene transfer using concentrated and highly purified lentiviral vectors is the best way to obtain a stable expression of the sequence of interest (cDNA, shRNA, miRNA). 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.

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