New non-integrative MS2-based lentiviral particles for mRNA delivery: a safe and efficient opportunity for gene editing and gene therapy applications

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1/ Introduction

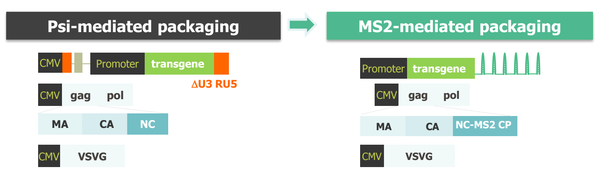
Lentiviral vectors have seen their use largely increased in clinical protocols over the past few years but safety concerns have been highlighted. The permanent genetic modification remains a focus of significant regulatory oversight and even integrase- or reverse transcriptase-deficient lentiviral vectors leads to residual integration events. Here, we describe a new chimeric lentiviral platform that allows mRNA delivery into the target cells without any genomic signature. The respective properties of the MS2 bacteriophage and the lentiviral vectors have been combined to build a non-integrative packaging system in which the wild type HIV packaging sequence is replaced by the MS2 stem-loop repeats and the MS2 coat sequence is inserted into the NucleoCapsid sequence. The resulting lentiviral particle is able to deliver a non-viral RNA into the cytoplasm of target cells, directly available for protein translation. Transduction of immortalized cells but also of T cells and HSC with these RNA lentiviral particles (RLP) shows an efficient, fast and transient expression of both reporters and functional proteins such as genome editing enzymes. Particles structure and functionality, cell transduction and characterization of such engineered cells have been compared with those obtained with an integrative lentiviral vector. Especially, by recruiting the RNA independently of dimerization and with more than four molecules per particle, RLPs allow the cotransfer of different species of RNA This new delivery system is a great candidate to take in charge the safe and clinically suitable delivery of the editing machinery, which can transiently act without inducing cellular toxicity or immunogenicity.

2/ Materials & Methods

Constructs: The dimerization-independent MS2-driven RNA packaging system using MS2-Coat-retrovirus chimeras is described in Molecular Therapy — Methods & Clinical Development (2015) 2, 15039: http://www.nature.com/articles/mtm201539. **VSV-G-pseudotyped lentiviral particles**: Productions were performed using 293T cells. The tri-transfection mix was composed of three plasmids: pENV carrying the VSVG envelope, p8.74/p8.74ΔNC-MS2CP and pLV-EF1-cDNA/pMS2-cDNA. The crude harvest was first concentrated by tangential flow ultrafiltration with polysulfone hollow-fiber cartridges. The supernatant was then diafiltered against DMEM buffer, and retentate was recovered and further concentrated on ultrafiltration disposable units. **Cell transduction:** Human naive monocytes were provided by Ambiotis. Cells were transduced by a ZsGreen expressing RLP vector, using a range of infection from 0 to 4 pg p24/cell and cultured in immature Dendritic Cells differenciation conditions. Three days post - transduction, the % of positive cells and Fluorescence Intensity , as well as specific markers expression CD11c, CD209-DCSIGN and CD86, were analyzed by flow-cytometry. Murine Bone Marrow cells were transduced by a ZsGreen expressing RLP vector, using a range of infection from 0 to 5 pg p24 /cell and cultured in immature Dendritic Cells differenciation conditions. Three days post- transduction, the % of positive cells and fluorescence intensity, specific markers expression CD11c, CD80 and CD86 were analyzed by flow cytometry. Murine naive CD4+ T lymphocytes from lymph nodes are activated using anti-CD3/28 coated beads prior to being transduced by RLP-CRE. Cells are analyzed by flow cytometry 4 days post-transduction. GFP-positive HCT116: monoclonal HCT116-GFP cells (1 GFP copy/cell) were first transduced by anti-GFP sgRNA expressing-ILV, then transduced with RLP-Cas9 (10pg P24/cell) or ILV-Cas9 (MOI20). 10 days after transduction, fluorescence was measured and quantified by FACS analysis.

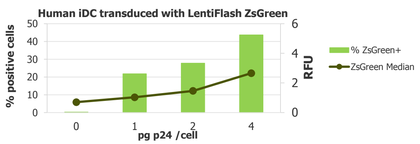
3/ Results

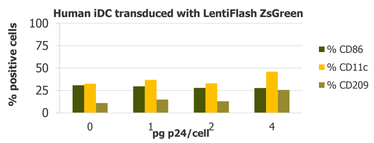
3-1/ Description



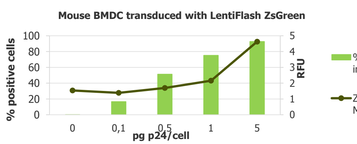
3-2/ RNA delivery efficiency

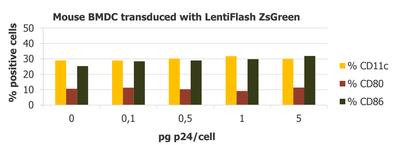
- Human immature Dendritic Cells





- Murine Bone Marrow Dendritic Cells



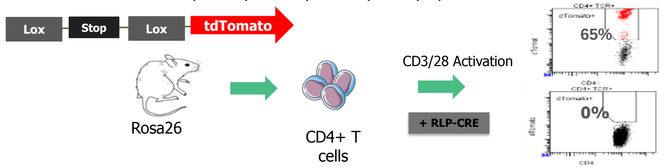


RLPs lead to EFFICIENT & TRANSIENT gene expression without phenotype modification in **dendritic cells**

RLP is **a versatile and efficient cell transfer system** for RNAs delivery:
- *ex vivo* in dendritic & T cells
- *in vivo* in mice.

- T cells

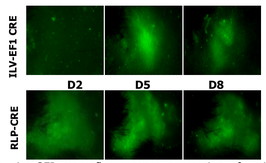
Naive CD4+ T lymphocytes are activated using anti-CD3/28 coated beads prior being transduced by RLP-CRE. Cells are analyzed by flow cytometry 4 days post-transduction.



RLPs lead to efficient & TRANSIENT gene expression without phenotype modification in T **cells**

- *in vivo*

Local injection of Cre vectors into the skin (i.d.) of ROSA26-GFP reporter mice

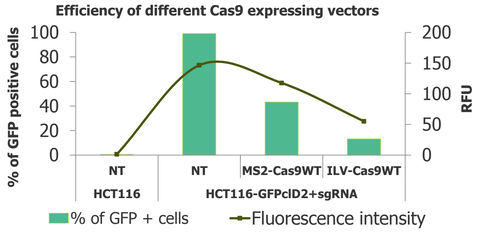


In vivo GFP macrofluorescence expression of mice injected in the back skin with either rLV-EF1-Cre (up) or RLP-Cre (bottom). Pictures are representative of several animals.

RLPs lead to efficient CRE-mediated recombination in **skin**

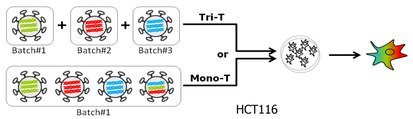
RLP leads to a functional expression of genome engineering enzymes such as CRE recombinase and Cas9 *in vitro* & *in vivo*

3-3/ Gene-editing

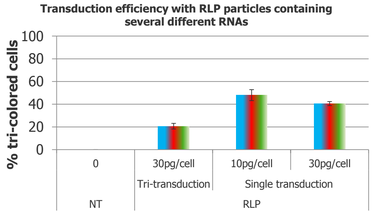


RLPs lead to efficient Cas9 expression and gene silencing

3-4/ Multi-transduction



HCT116 cells were submitted either to a Tri-Transduction (Tri-T) with the three different batches of LentiFlash: RLP-ZsGreen + RLP-mCherry + RLP-mtBFP (10pg P24/cell for each batch), or to a Mono-Transduction (Mono-T) with only one batch of LentiFlash coproduced with the ZsGreen + mCherry + mtBFP constructs (10pg or 30pg P24/cell). The same experiment has been performed using ILVs.
24h after transduction, fluorescence was measured and quantified by FACS analysis. Data is mean +/- SD (n=3).



RLPs lead to efficient multiple co-expression into cells

RLP allows co-transfering with different species of RNA for:
- gene-editing methods: TALEN, Zn Finger or CRISPR/Cas9
- immunotherapy approaches: Ag, CARs, TCRs