NEW NON-INTEGRATIVE MS2-BASED LENTIVIRAL PARTICLES FOR RNA DELIVERY: A SAFE AND EFFICIENT OPPORTUNITY FOR GENE EDITING APPLICATIONS AND THERAPEUTIC PERSPECTIVES

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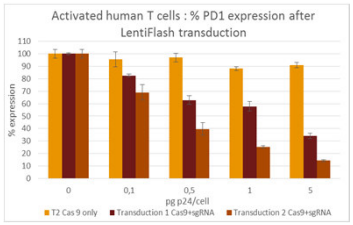
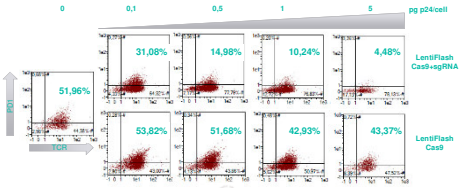
Gene editing using the CRISPR system shows great promises for gene therapy, but it must now face a number of challenges especially for the development of safe and efficient delivery tools for *in vivo*, as well as *ex vivo* gene editing. Indeed, achieving an efficient delivery in hard-to-transfect cells of clinical interest such as T cells, remains challenging and the need for new delivery tools that would allow efficient transfer on most cell types without causing any cell damages is essential for downstream therapeutic applications.

Here, we present an innovative RNA delivery vector, named LentiFlash, based on a new chimeric MS2-based lentiviral platform that allows RNA delivery into the target cells without any genomic scar. This new vector breaks with all existing systems, as the resulting lentiviral particle is able to deliver non-viral coding or non-coding RNA, at high efficiency, into the cytoplasm of any type of target cells.

HIGHLY EFFICIENT DISRUPTION OF PD-1 IN HUMAN PRIMARY T-CELLS

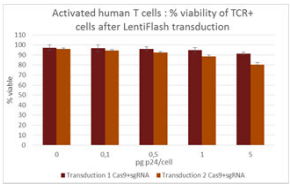
A CRISPR approach using LentiFlash RNA delivery leads to an efficient and dose-dependent knock-out of the PD-1 gene into human activated T lymphocytes, without any genome integration.

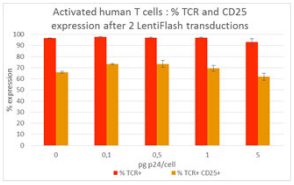




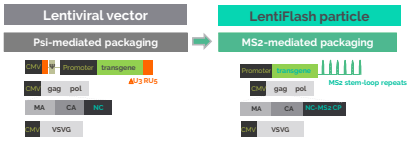
Human naive lymphocytes were provided by Ambiotis. One day after activation using CD3/CD28 beads T lymphocytes were transduced by a LentiFlash vector expressing sgRNA against the human PD-1 gene and the Cas9 or a LentiFlash vector expressing the Cas9 enzyme alone. A range of infection from 0 to 5 pg p24/cell has been used, in presence of polybrene 4µg/mL for 5h at 37°C. 5% CO_2. A second transduction has been performed in the same conditions 3 days after. Six days post transduction 1 (i.e. 3 days post transduction 2) the cells were analyzed by flow cytometry for their PD-1 expression, as well as for their viability and their surface expression of TCR and CD25.

Human T lymphocytes are efficiently transduced using highly purified and concentrated LentiFlash vectors without affecting viability and proliferation, and preserving the original phenotype of the cells.

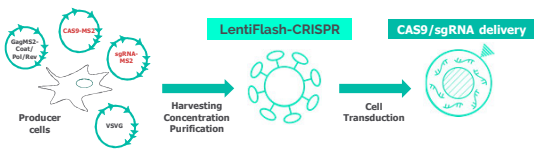




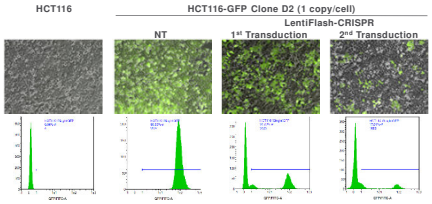
LENTIFLASH: A NEW TOOL FOR EFFICIENT CRISPR/CAS9 DELIVERY



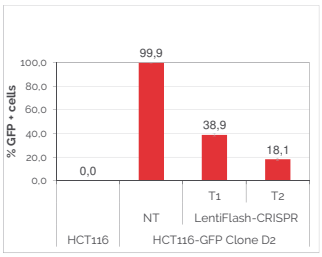
LentiFlash is a chimeric MS2-based lentiviral particle allowing to package different RNA molecules devoid of viral sequences, which results in a transient expression without genomic integration.



LentiFlash allows to achieve more than 80% GFP knock-out in an immortalized monoclonal cell line containing one integrated GFP copy.



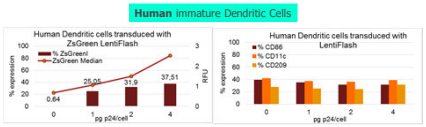
Monoclonal HCT116-GFP cells (1 GFP copy/cell) were transduced by a LentiFlash vector expressing sgRNA against the GFP and the Cas9 (10 pg p24/cell), in presence of polybrene 4µg/mL for 5h at 37°C, 5% CO2. A second transduction has been performed in the same conditions 6 days after.
14 days post transduction 2 (i.e. 20 days post transduction 1), fluorescence was measured and quantified by FACS analysis. NT : Non Transduced



THE BENEFITS OF LENTIVIRAL VECTORS WITHOUT ANY GENETIC SCAR

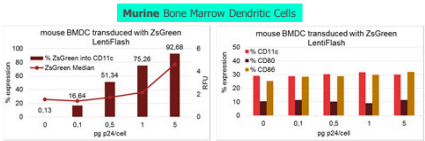
LentiFlash particle

Human immature Dendritic Cells



Human naive monocytes were provided by Ambiotis. Cells were transduced by a ZsGreen expressing LentiFlash 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 ZsGreen positive cells and Fluorescence Intensity, as well as specific markers expression CD1c, CD209-DCSIGN and CD86, were analyzed by flow cytometry.

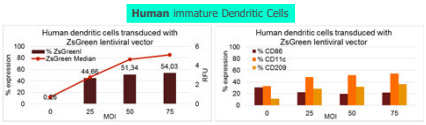
Murine Bone Marrow Dendritic Cells



Murine Bone Marrow cells were transduced by a ZsGreen expressing LentiFlash 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 ZsGreen positive cells and Fluorescence Intensity, as well as specific markers expression CD1c, CD80 and CD86, were analyzed by flow cytometry.

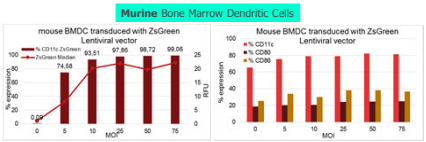
Lentiviral vector (ILV)

Human immature Dendritic Cells



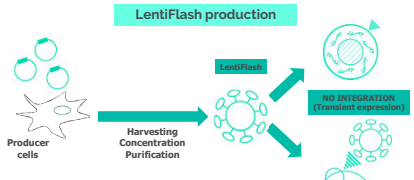
Human naive monocytes were provided by Ambiotis. Cells were transduced by a EF1-ZsGreen expressing ILV, using a range of MOI (Multiplicity of Infection) from 0 to 75 and cultured in immature Dendritic Cells differenciation conditions. Three days post-transduction, the % of ZsGreen positive cells and Fluorescence Intensity, as well as specific markers expression CD1c, CD209-DCSIGN and CD86 were analyzed by flow cytometry.

Murine Bone Marrow Dendritic Cells



Murine Bone Marrow cells were transduced by a ZsGreen expressing EF1-ZsGreen expressing ILV, using a range of MOI (Multiplicity of Infection) from 0 to 75 and cultured in immature Dendritic Cells differenciation conditions. Six days post-transduction, the % of ZsGreen positive cells and Fluorescence Intensity, as well as specific markers expression CD1c, CD80 and CD86, were analyzed by flow cytometry.

High purification level allows efficient transduction of delicate immune cells without cell phenotype modification.



LentiFlash particles can be produced using lentiviral production/purification platforms already validated in clinical settings.