All-in-one Delivery Using LentiFlash®, a chimeric RNA Delivery Technology Designed for Clinical Applications

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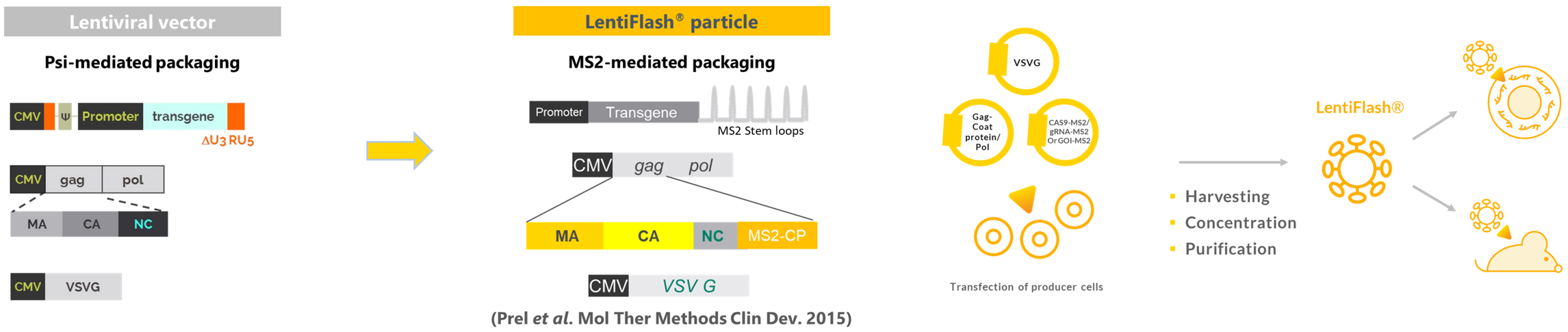
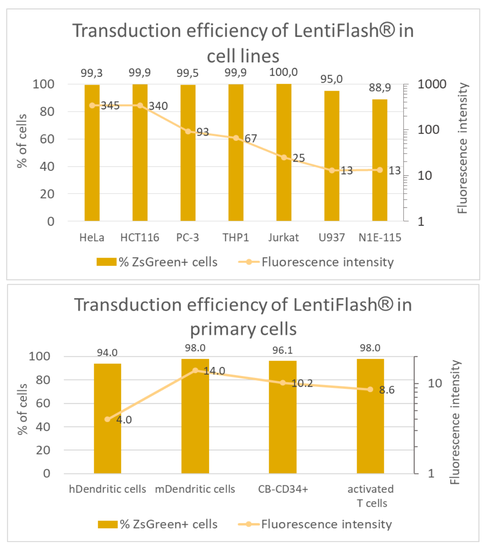
PRODUCTION OF HIGHLY PURIFIED AND CONCENTRATED LENTIFLASH®



(Prel *et al.* Mol Ther Methods Clin Dev. 2015)

LENTIFLASH®: A NEW TECHNOLOGY FOR TRANSIENT AND SAFE RNA DELIVERY

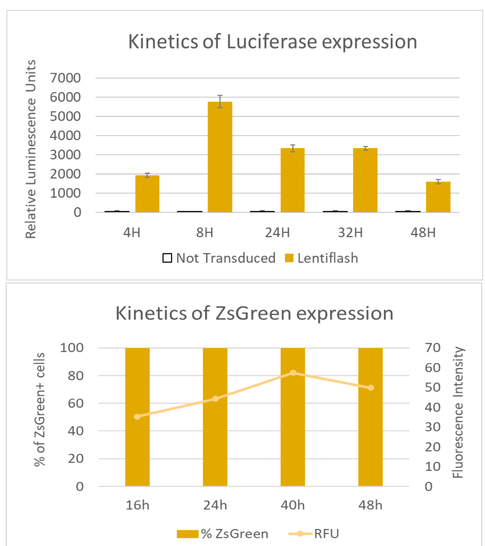
Close to 100% transduction efficiency



Transduction efficiency

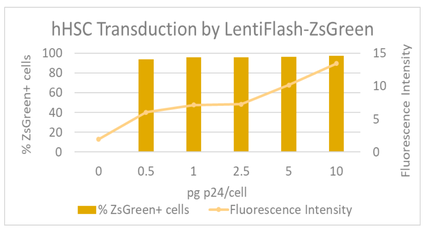
LentiFlash® is a cutting-edge technology for RNA delivery. It overcomes challenges raised by DNA delivery as RNA is directly delivered, and transiently expressed, into the cytoplasm.
Hence, transduced cells are free of viral RNA which is a great advantage for therapeutic purposes using T cells or HSCs.
It's also capable of delivering multiple RNA species, such as different coding RNAs and/or Cas9 mRNA + sgRNAs.

Transient and tailored expression



Expression duration

Expression duration depends on the half-life of the protein encoded by the delivered RNA.

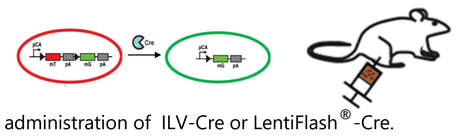


Tailored expression

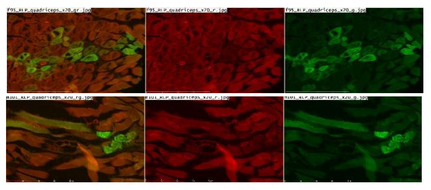
The dose of LentiFlash® can be tailored to fit the desired expression level.

Efficient *in vivo* delivery

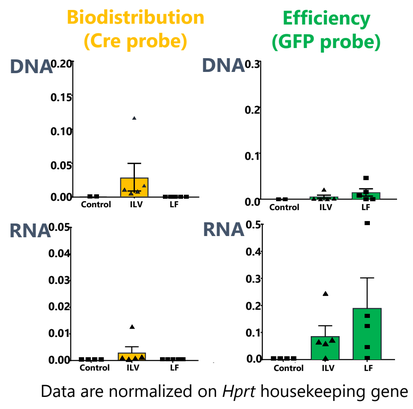
mT/mG reporter mice model



Local intra-muscular administration of ILV-Cre or LentiFlash® -Cre.
Analysis 2 weeks post injection



- Quadriceps histology with GFP immuno-staining shows an efficient local Cre activity, with no excision in other organs.



Data are normalized on *Hprt* housekeeping gene

- DNA and RNA analysis through ddPCR from the same sample.

As expected, Cre recombinase is only detected with ILV.

GFP reporter is detected at a higher level after LentiFlash® injection compared to ILV.

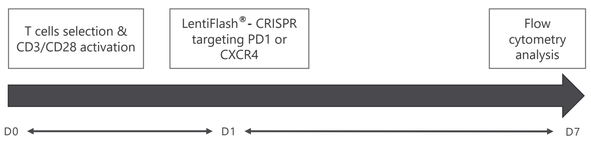
Collaboration with PHENOMIN-Institut Clinique de la Souris (ICS)

Increased *in vivo* efficiency compared to integrative vectors

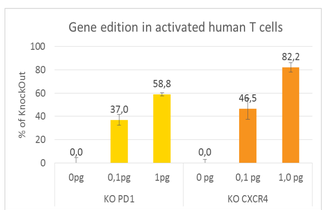
Higher deletion efficiency with the LentiFlash® than with the integrative lentiviral vector. No residual expression of the Cre recombinase is detected.

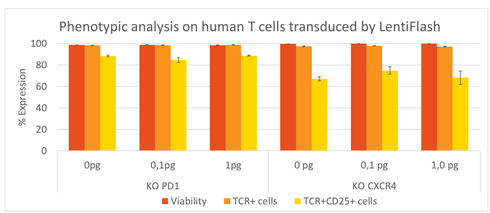
LENTIFLASH®: A NEW TECHNOLOGY FOR KO APPLICATIONS

In human primary T cells with a sgRNA and Cas9



Activated T cells were transduced by LentiFlash® vector (0,1 and 1 pg of p24/cell) expressing Cas9 and a sgRNA targeting human PD1 or CXCR4 genes.





Human T lymphocytes display high KO efficiency using highly purified and concentrated LentiFlash® vectors without affecting viability nor proliferation, and preserving the original cell phenotype.

LentiFlash® manages to deliver multiple RNA species into all cell types, such as different coding RNA or Cas9 mRNA + sgRNA.

In normal human keratinocytes (NHK) with a sgRNA and Cas9

NHK-GFP were transduced by LentiFlash® particles (5pg of p24/cell) expressing Cas9 and a sgRNA targeting the GFP sequence. One week later, cells were analyzed by flow cytometry.

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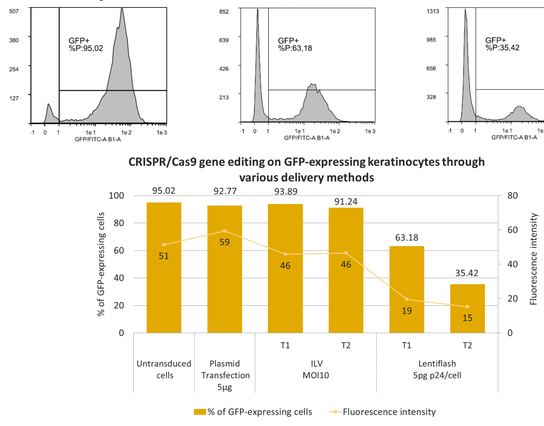


CRISPR/Cas9 gene editing on GFP-expressing keratinocytes through various delivery methods

LentiFlash® allows to achieve very high KO efficiency in NHK, contrary to transfection or integrative lentivectors, without the need of antibiotic selection.

Since LentiFlash® is a RNA and not a DNA delivery tool, the lifespan of delivered CRISPR/Cas9 system is very short, reducing the off-target risks.

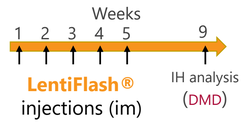
The use of highly purified and concentrated LentiFlash® permits to preserve the cell phenotype and maintain the differentiation capabilities of cells towards reconstructed skin models.

In addition to LentiFlash® transduction efficiency, the KO efficacy depends on sgRNA inherent potency (Yuen et al, NAR, 2017), genomic environment, type and state of the targeted cells (activated or not, quiescent or not).

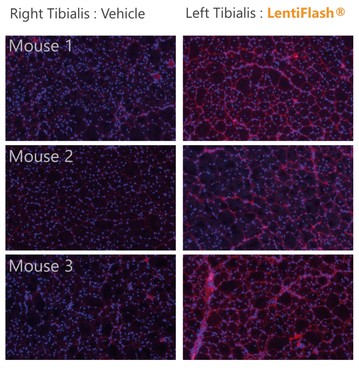
LENTIFLASH®: A NEW TECHNOLOGY FOR EXON SKIPPING APPLICATIONS

Exon skipping *in vivo* with two sgRNAs and Cas9

Mice DMD model with a STOP codon in exon 23 of the dystrophin (*Mdx*) gene



Left tibialis: sgRNAs/Cas9
Right tibialis: vehicle



Duchenne disease model:

- No cure
- Current pre-clinical strategies use AAVs (DNA delivery) to deliver gene editing systems
- Restoration of DMD activity using LentiFlash®

Collaboration with PHENOMIN-Institut Clinique de la Souris (ICS)

This proof of concept shows that LentiFlash® is capable of delivering not only one sgRNA, but two sgRNAs, in addition to the Cas9.

This is a highly promising therapeutic strategy to repair the defective dystrophin gene.