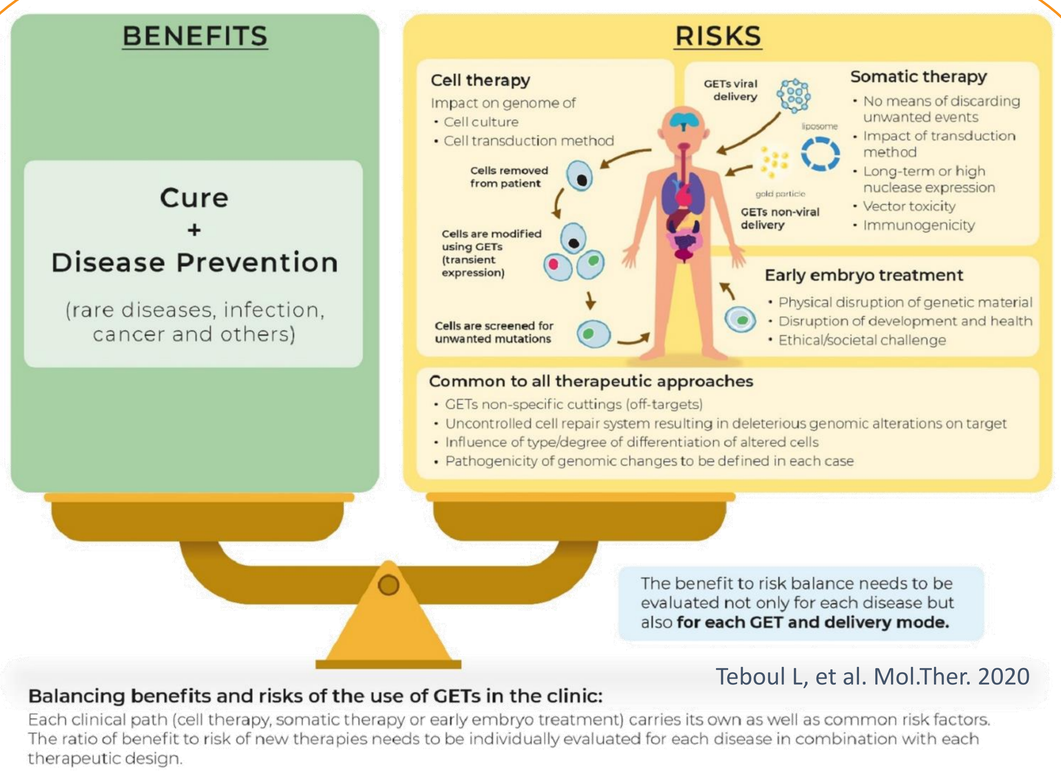
NEXT GENERATION THERAPY: INNOVATIVE AND SAFE RNA DELIVERY MEDIATED BY LENTIFLASH® PARTICLES FOR AN EFFICIENT CRISPR/CAS9-MEDIATED GENE KNOCKOUT IN HUMAN INDUCED PLURIPOTENT STEM CELLS

C. DUTHOIT[1] , J. MIANNE[2] , N. MARTIN[1] , A. ICHE[1] , R. GAYON[1], F. SAMAIN[1], J. DE-VOS[3] , P. BOUILLE[1]
[1]FLASH THERAPEUTICS, TOULOUSE, FRANCE, [2]IRMB, UNIV MONTPELLIER, INSERM, CHU MONTPELLIER, MONTPELLIER, FRANCE, [3]DEPARTMENT OF CELL AND TISSUE ENGINEERING, UNIV MONTPELLIER, CHU MONTPELLIER, MONTPELLIER, FRANCE

A. Challenges of gene editing in therapy



Teboul L, et al. Mol.Ther. 2020

Balancing benefits and risks of the use of GETs in the clinic:
Each clinical path (cell therapy, somatic therapy or early embryo treatment) carries its own as well as common risk factors. The ratio of benefit to risk of new therapies needs to be individually evaluated for each disease in combination with each therapeutic design.

Genome Editing Tools (GETs) are extremely promising in human therapy, but at least two main challenges must be considered for their use:

- efficiency in modifying the target cells
- good clinical safety

To answer the first point, the choice of **delivery method** (see **section B**) and **technologies to assess efficacy** are critical (see **section C**).

To address the second point, understanding the impacts of genome editing provides critical information to reduce risks (see **sections A and B**).

B. LentiFlash® for RNA delivery

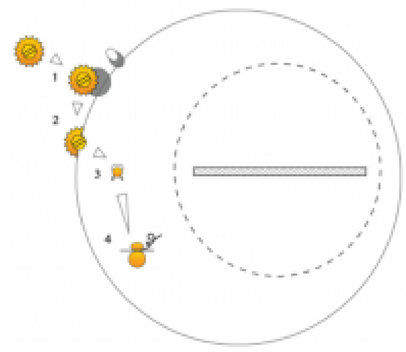
The mode of delivery is one of the main factors that can be tackled to reduce **the risk of toxicity** and to provide **efficient cells targeting**.

Compared to DNA therapies, **RNA therapies** are more versatile, while reducing the risk of genomic alterations because they avoid recombination events in the host genome.

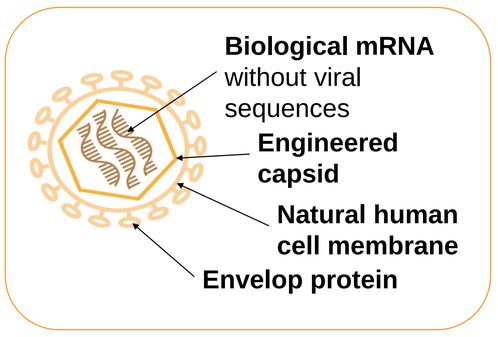
As a game-changing RNA carrier, LentiFlash®, efficiently and safely delivers **multiple biological RNA species** into the cell cytoplasm.

What is LentiFlash® ?

- A chimeric system combining properties of bacteriophages. and lentiviral particle
- Direct **RNA delivery** into the cytoplasm
- Available either for translation or nuclear import **without reverse transcription**.
- A proprietary technology.



- **Biological RNA** delivery technology
  → RNAs devoid of lentiviral sequences (~~LTR, Ψ, RRE~~)
  → Non-integrative technology → No insertional mutagenesis



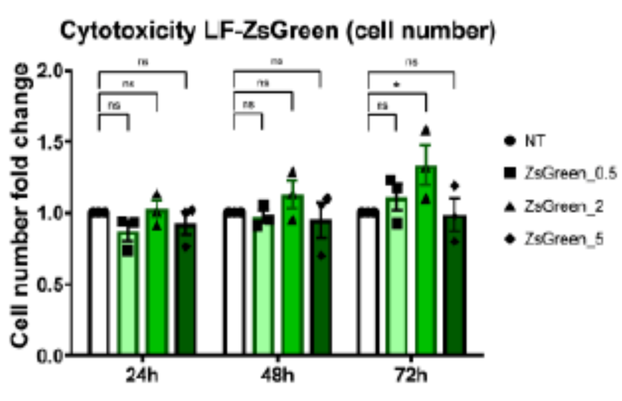
- **High entry efficiency** into proliferating and non-dividing cells
  → VSV-G pseudotyping
  → **New pseudotyping** to target specific cells
- Rapid bioavailability of RNAs
  → High and **rapid transient expression**
- **No pre-existing immunity** in humans
  → Lentiviral proteins, human cell membrane, pseudo-typing with VSV-G, to which humans are barely exposed
- Lentiviral platform already **validated for clinical settings**

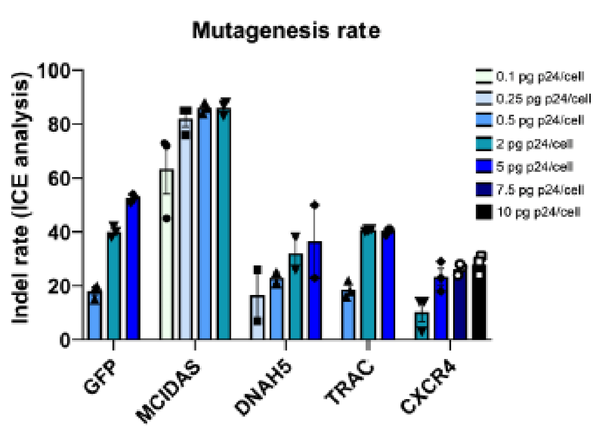
APPLICATIONS

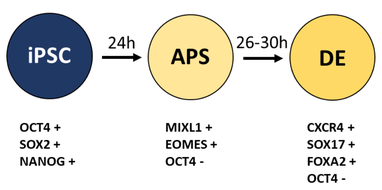
Gene editing | Regenerative medicine | Vaccination

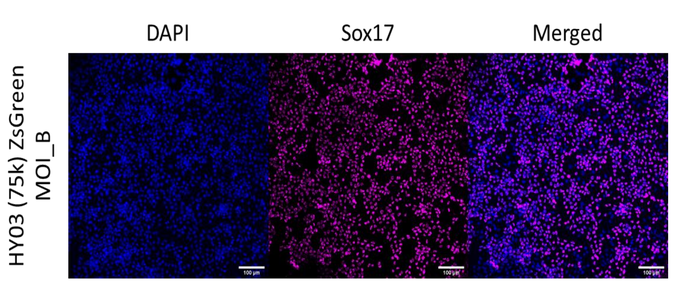
C. Safe and efficient gene editing on human iPSCs

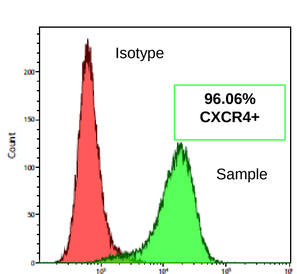
hiPSCs are efficiently transduced with LentiFlash® particles delivering CRISPR system (**sgRNA + Cas9 in a single particle**), they are **efficiently edited**, with **no cytotoxicity** and **no adverse effect on differentiation potential.**











Transduced hiPSCs retain a pluripotent morphology, express pluripotency markers (NANOG, OCT3/4, SOX2, SSEA4) and can be differentiated into definitive endoderm that expressed CXCR4, FOXA2 and SOX17.

D. Conclusion

Here, we show the potential of LentiFlash® particles for **gene therapy clinical applications:**

LentiFlash® particles can deliver CRISPR-Cas9 components for an efficient gene editing while :

- ✓ Preserving **cell viability**,
- ✓ Maintaining **cell phenotype**
- ✓ Preserving stem cell **differentiation capacity**,
- ✓ Ensuring low or **no genotoxicity**.

These properties, and the ability to easily produce LentiFlash® particles using existing **GMP production platform**, provide a robust and reliable method for safe and efficient therapy in Humans, with **additional safety considerations** compared to other therapeutic approaches.
LentiFLash® RNA delivery tool can be used for a broad range of applications, such as **regenerative medicine**, or **vaccination/immunotherapy** for both infectiology and oncology purposes.

FLASH THERAPEUTICS Centre d'Innovation Langlade, 3 Avenue Hubert Curien, 31100, Toulouse, France **Tel : +33 5 61 28 70 75**

LEARN MORE