GENE EDITING ON PRIMARY AND STEM CELLS USING LENTIFLASH® RNA DELIVERY APPROACH: KEY SAFETY CONSIDERATIONS FOR GENE THERAPY CLINICAL APPLICATIONS

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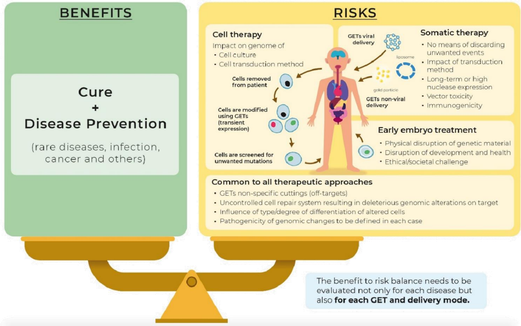
flash THERAPEUTICS

Poster #W-85
Abstract #959

Booth #252

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

Box 1: **Some Currently Recognized Risks of Using Nucleases for GET for Clinic**

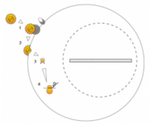
- GETs fit the definition of "targeted mutagens" as agents that increase frequency / extent of a mutation at a given locus.
- With an experimental design that favors GET transient expression, the frequency of Off-Target mutation is likely to be lower than the natural occurrence of genetic drift for any given generation.
- One of GET-associated safety risks is on-target mutations that are due to unwanted DSB repair mechanisms.
- The use of GETs as therapeutic agents is associated with three main risk factors: (1) DNA cutting and the resulting repair events, (2) risks associated with the delivery means, and (3) the biological context in which GETs are employed (organ systems or cell types).
- Each GET is different and displays its own efficiency and target specificity.
- GETs need to be assessed in the cellular context(s) for which they are intended in therapy, as the mechanism by which targeted cells repair specific DSBs may differ among cell types, cell-cycle phase, metabolic status, and level of differentiation.

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. RNA delivery mediated by a lentiviral particle is an attractive approach as it combines cell entry and tropism properties of lentiviral vectors without the potential adverse effects from long-lasting expression or genomic integration.

LentiFlash® Technology



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
- Rapid bioavailability of RNAs
- High and rapid transient expression
- Transduce non-dividing and dividing cells
- Non-integrative technology
- No insertional mutagenesis / No GMO generated

Applications : gene editing, regenerative medicine, vaccination, immunotherapy

E. 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 :

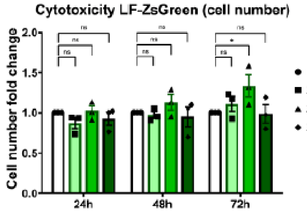
- ✓ Preserving **cell viability**,
- ✓ Maintaining **cell phenotype**
- ✓ Preserving stem cell **differentiation capacity**,
- ✓ Ensuring low or **no genotoxicity**.

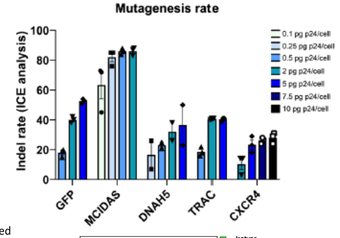
These properties, and the ability to easily produce LentiFlash® particles using existing GMP production platform, provide a robust and reliable method for safe and efficient therapy in Humans, with **additional safety considerations** compared to other therapeutic approaches.
LentiFlash® RNA delivery tool can be used for a broad range of applications, such as **regenerative medicine** (See poster W-12 ASGCT 2022), or **vaccination/immunotherapy** for both infectiology and oncology purposes.

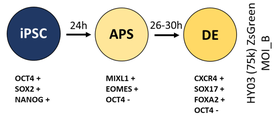
C. Safe and efficient Gene editing on human iPSCs

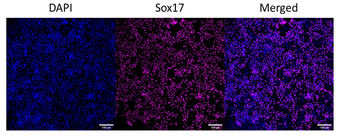
Mianné J, et al. BMC Biol. 2022

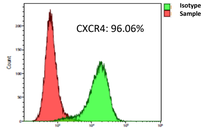
hiPSCs are efficiently transduced with LentiFlash® particles delivering CRISPR system (**sgRNA + Cas9 in a single particle**), they are **efficiently edited**, with **no cytotoxicity** and **no adverse effect on differentiation potential.**









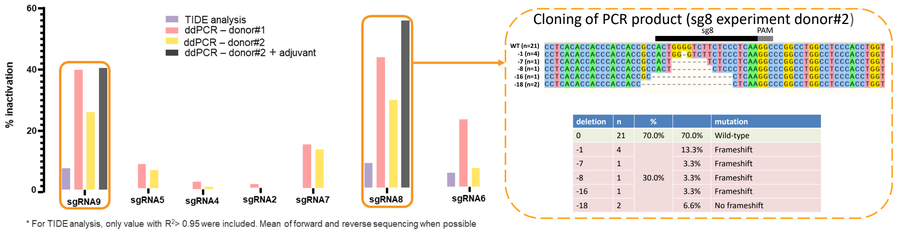


Transduced hiPSCs retain a pluripotent morphology, express pluripotency markers (NANOG, OCT3/4, SOX2, SSEA4) and can be differentiated into definitive endoderm that expressed CXCR4, FOXA2 and SOX17.

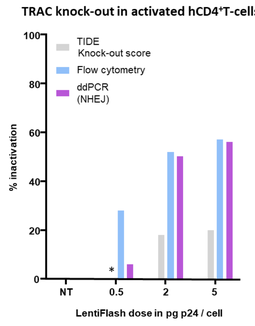
D. Which technology to reliably assess editing efficiency?

We compared 3 methods to evaluate mutations obtained by CRISPR/Cas9 genome editing with LentiFlash® on human CD4+ T cells, 7 days post transduction :

- **TIDE method**: 500-1500 bp are PCR amplified from gDNA isolated from the cell pool treated with Cas9 and 7 different sgRNAs targeting our GOI. PCR products were sequenced using Sanger sequencing and TIDE software is used to evaluate the percent and type of edited cells
- **ddPCR method**: different probes were designed to evaluate the level of NHEJ and small deletions induced by double strand break reparation (see Lindner, L. *et al.* 2021 *Methods*. for details)
- **Cytometry**: For TCR alpha (TRAC) editing a specific TCR immunostaining was made for FACS analysis.



* For TIDE analysis, only value with R^2> 0.95 were included. Mean of forward and reverse sequencing when possible



* no indel detection by TIDE analysis at 0.5 pg p24 / cell

Different technics allow to detect mutations at the target site but the TIDE method underestimate the efficiency of genome editing whereas our design using multiple **ddPCR** probes allows a **better quantification of mutated cells,** which is correlated to the level of protein expression.

These data are part of the **IRIS project** funded by the French National Research Agency (ANR) Investments for the Future program (PIA) under grant agreement No. ANR-18-RHUS-0003