REVOLUTIONARY RNA DELIVERY TECHNOLOGY ENABLES SAFE AND EFFICIENT GENE TRANSFER IN ORGANOIDS, IN VIVO MODELS, IPSCS, AND STEM CELLS

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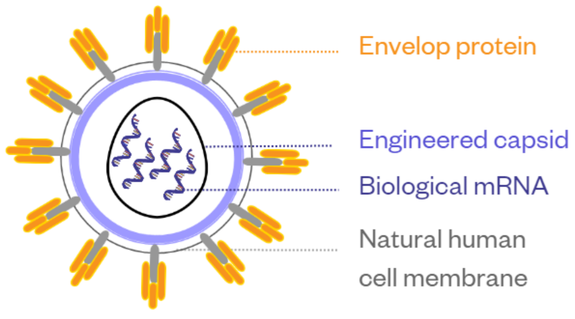
A. FlashRNA® Technology

In **Cell & gene therapy**, RNA delivery technologies are very versatile, and thus can address a large variety of diseases. These approaches target applications in which a **transient expression** is expected.

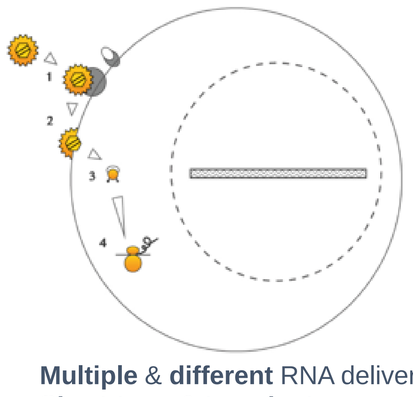
FlashRNA® is a groundbreaking RNA delivery technology, based on a non-integrative bacteriophage-lentivirus chimera, which transfers multiple RNAs safely and efficiently thanks to its unique features. **FlashRNA® particles** can deliver **various types of RNAs** that are protected from degradation by a robust multilayer particle ensuring at the same time their highly efficient delivery into the cytoplasm of all cell types. This innovative approach results in fast and short-term expression, with no risk of cell damage or phenotype alteration.

FlashRNA® Particle



Prel et al. Mol Ther Methods Clin Dev. 2015

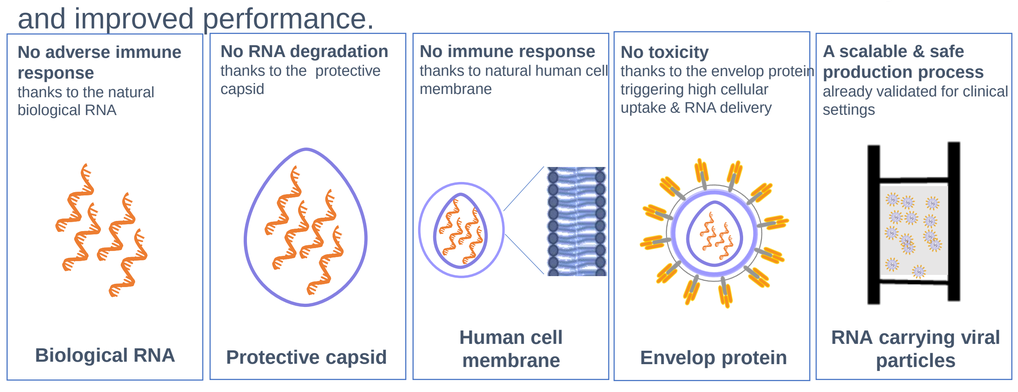
RNA delivery



Multiple & **different** RNA delivery
Short-term & **transient** expression
No insertional mutagenesis
No GMO generated

B. Key Features of FlashRNA®

FlashRNA® is a **safe** and **efficient** biological RNA delivery tool that is helping to advance gene delivery technology. This innovative technology combines the benefits of lentiviral transduction with enhanced safety features and improved performance.



UNIQUE PROPERTIES

- ✓ **High transduction efficiency**: Effectively delivers RNA to any target cells
- ✓ **Transient expression**: Ensures short-lived activity, reducing adverse effects
- ✓ **Large payload capacity**: Accommodates multiple and/or long RNA sequences
- ✓ **RNA protection**: Multi-layered particle structure prevents RNA degradation
- ✓ **Minimal toxicity**: Preserves cell viability and phenotype

ENHANCED SAFETY PROFILE

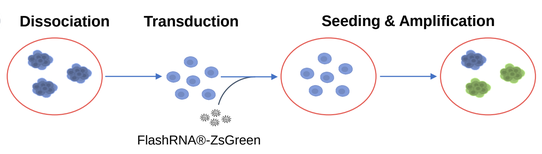
- ✓ **Absence of viral sequences**: Prevents expression of viral genes
- ✓ **Reduced immunogenicity**: Biological RNA origin minimizes immune responses
- ✓ **No integration**: Eliminates risks associated with genomic integration
- ✓ **cGMP-compliant production**: Utilises existing manufacturing platform

C. FlashRNA® for RNA transfer *in vitro* and *in vivo* in Organoids, Immune & Stem cells

HUMAN ORGANOIDS

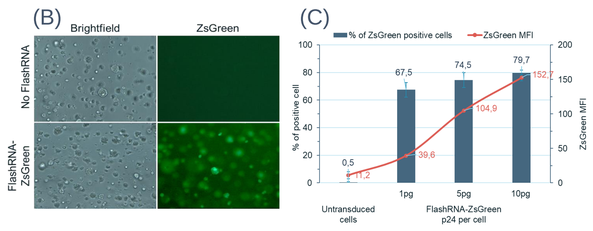
Collaboration with Urosphere, a preclinical research specialist in urogenital field. This partnership enables us to transduce cancerous bladder organoids with FlashRNA® particles, delivering ZsGreen RNA.

(A)



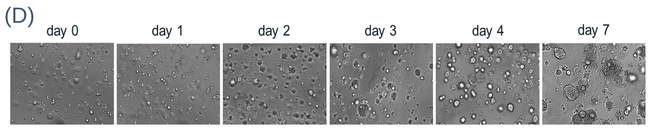
(A) Experimental conditions using FlashRNA® for transferring ZsGreen RNA in human bladder organoids. Organoids are dissociated, transduced, and seeded into Matrigel supplemented with growth factors inducing organoid generation.

(B) (C)



(B) Representative brightfield images of human organoids 48 hours after treatment with or without FlashRNA® carrying ZsGreen RNA. (C) Flow cytometry analysis of ZsGreen expressing organoids treated with a dose range of FlashRNA®. The percentage of ZsGreen cells and mean fluorescence (MFI) are presented (means ± SD, n = 3).

(D)

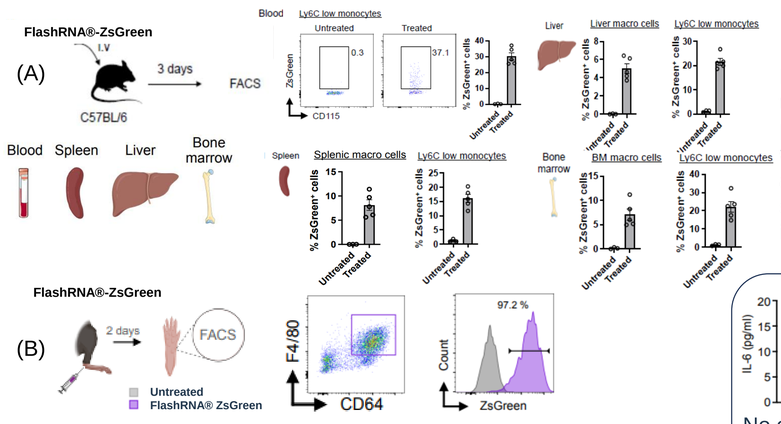


(D) Representative brightfield images of the evolution of FlashRNA® treated-organoids over time

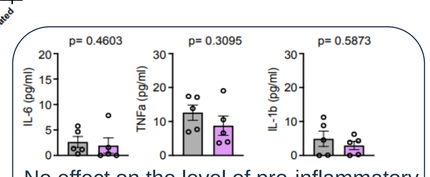
FlashRNA® has been shown to have no adverse effects on organoid formation, making it a safe and effective gene delivery tool for transient protein expression and engineering of organoids.

MOUSE MACROPHAGES

Data courtesy of G.Courties



Mouse macrophages are efficiently transduced *in vivo* with FlashRNA® particles delivering ZsGreen RNA, with no cytotoxicity and no adverse effect, after either systemic or peri-articular injections of C57Bl/6 mice.

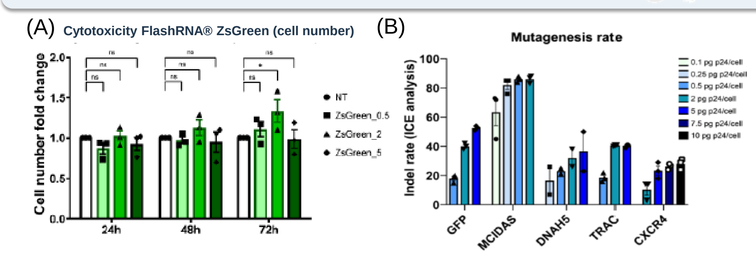


No effect on the level of pro-inflammatory cytokines following injections.
*quantities below detection threshold

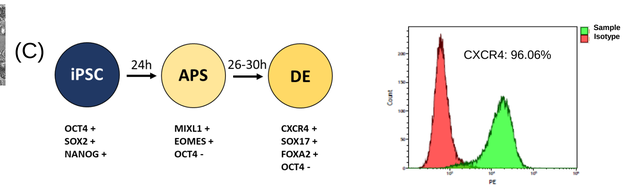
FlashRNA® ZsGreen is injected intravenously (A) or peri-articularly (B) to C57Bl6 mice and delivery to immune cells monitored by flow cytometry

HUMAN iPSCs

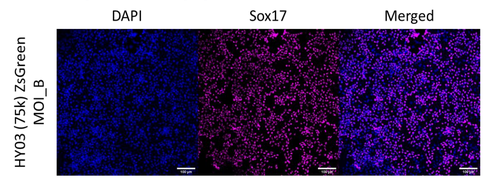
(A) Cytotoxicity FlashRNA® ZsGreen (cell number) (B)



(C)



hiPSCs are efficiently transduced with FlashRNA® particles with no cytotoxicity (A). Efficient genome edition can be achieved with FlashRNA® particles delivering CRISPR system (B), no adverse effect is observed on differentiation potential (C).



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.

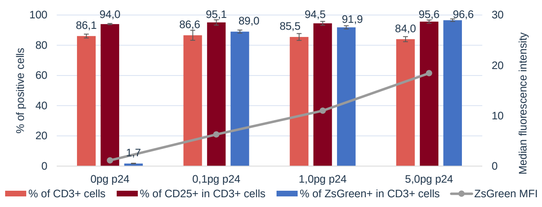
Mianné et al. BMC Biol. 2022

mPB-HSPCs

Cryopreserved human mPB-HSPCs are efficiently transduced by low dose of FlashRNA® particles delivering ZsGreen RNA, without altering the CD34 phenotype.

HUMAN T CELLS

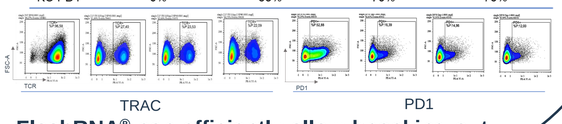
Gene expression using FlashRNA® for coding RNA



Human T cells were isolated from cryopreserved PBMC, activated by CD3 & CD28 co-stimulation for 3 days and cultivated with IL-2. Cells were transduced with FlashRNA® particles 24 hours later.
More than 90% of activated T cells express ZsGreen 48h after transduction. The viability and the phenotype of the transduced cells is unchanged.

Gene Editing using FlashRNA® for CRISPR-Cas9

FlashRNA dose	No dose	Dose 1	Dose 2	Dose 3
KO TRAC	0%	66%	71%	72%
KO PD1	0%	63%	70%	75%



FlashRNA® can efficiently allow knocking-out of different genes in human activated T cells

D. Conclusions

GENE THERAPY · GENE EDITING · CELL-BASED IMMUNOTHERAPY · VACCINATION · REGENERATIVE MEDECINE

THERAPEUTIC VERSATILITY

FlashRNA® is suitable for a wide range of RNA-based therapies: **gene editing** with efficient delivery of CRISPR/Cas9 components; **regenerative medicine** for cell reprogramming and stem cells engineering; prophylactic and therapeutic **vaccination**; **immunotherapy**.

FlashRNA® represents a significant advancement for **RNA delivery,** offering a **robust**, **safe**, and **efficient** method for **various therapeutic applications**. Its **unique properties** and **cGMP-compliant production** process position it as a **versatile tool** for the future of **gene therapy** and **personalized medicine.**

CLINICAL TRIAL MILESTONE

A **first-in-human Phase I/IIa** clinical trial using FlashRNA® for RNA delivery is scheduled for 2025 at Toulouse University Hospital, France. This trial will focus on treating **lymphedema** in patients following breast cancer surgery.

> Introducing an innovative new cell & gene delivery technology: our vector is engineered from the robust envelope & capsid of lentivirus, combined with the RNA packaging system of a bacteriophage. This innovative solution is set to revolutionize the field of gene therapy, vaccine development, and beyond.

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