A GAME-CHANGING RNA DELIVERY PLATFORM COMBINING HIGH CELL ENTRY AND MULTIPLE RNA TRANSFER FOR NEXT GENERATION THERAPY : LENTIFLASH®

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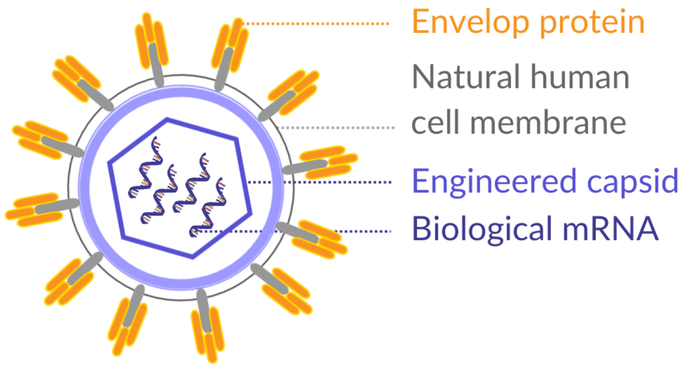
A. Challenges of RNA therapies

Gene therapy approaches show that there is no universal delivery tool for all therapeutic strategies. Compared to DNA delivered-therapies, **RNA therapies** are expected to be more versatile, cover a broad range of applications with minimal regulatory concerns and thus address a large variety of diseases. The technology targets applications in which a **transient expression** is expected.

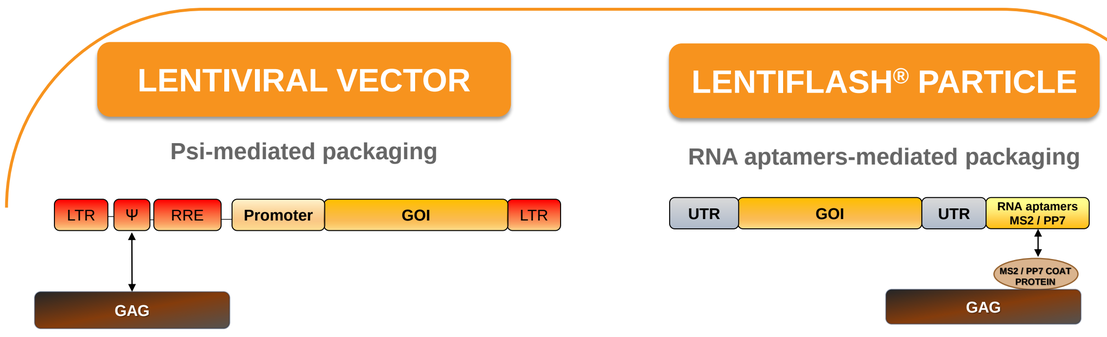
As a game-changing RNA carrier, **LentiFlash®**, a non-integrative bacteriophage-lentivirus chimera, can efficiently and safely deliver **multiple RNA species** that are **rapidly bioavailable,** leading to a **high and short-term expression** of the transferred messenger into the cell cytoplasm.

LENTIFLASH® PARTICLE



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

B. What is LentiFlash® ?



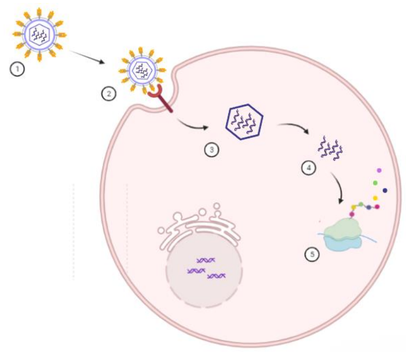
Packaging of two identical RNAs
Lentiviral sequences (LTR, Ψ, RRE)

Packaging of multiple distinct RNAs
No lentiviral sequence

This **biological RNA delivery technology** mediated by a lentiviral particle is an attractive approach as it combines most of the inherited properties of lentiviral vectors (cell entry and tropism) without the potential adverse effects from long-lasting expression or genomic integration.
The lentiviral external structure (membrane, capsid) ensures **RNAs protection.**

Scalable production process already validated for clinical settings

RNA DELIVERY FOR A TRANSIENT EXPRESSION



- **No GMO generated**
  - **No reverse transcription**
  - **No DNA integration, no insertional mutagenesis**
- **Rapid & transient expression**

C. Clinical Context and mouse model

Lymphedema is a lymphatic vascular system disorder characterized by:

- impaired lymphatic return and swelling of the extremities
- accumulation of undrained interstitial fluid/lymph.

It results in fibrosis and adipose tissue deposition in the affected area. It can occur after cancer surgery and lymph node removal. Indeed, 10-15% of women develop arm secondary lymphedema after surviving breast cancer. There is no curative treatment for lymphedema.

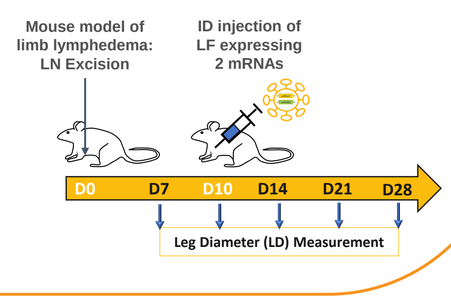
Theralymph clinical program's main objective is to establish a multiple gene therapy for secondary lymphedema, based on the transient expression of two factors allowing the restoration of a normal lymphatic function. LentiFlash® will be used to deliver the two therapeutic RNAs by intra-dermal injections.

THERALYMPH

HORIZON 2020

LYMPHEDEMA MOUSE MODEL

Secondary lymphedema is induced in mice by mastectomy of the 2nd mammary gland, associated with brachial and axially lymphadenectomy. LentiFlash® (LF) is intra-dermally injected in limb at day 10.



D. Pre-clinical results

After lymph nodes resection, mice exhibit a reproducible reduction of lymphatic drainage associated with dermal backflow (fig. 1) and increase of leg diameter (fig. 2) up to 4 weeks post-surgery.

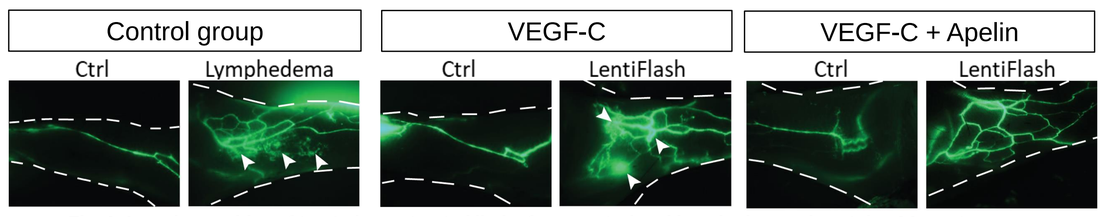


Fig. 1: Lymphographies of treated or untreated limbs from control and lymphedema mice at day 14 post-surgery

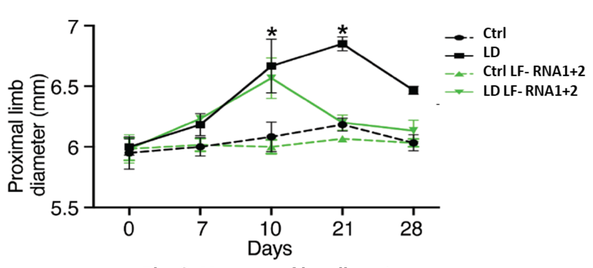


Fig. 2: Measure of leg diameter

Lymphedema resolution after therapeutic RNAs delivery by LentiFlash®

- Lymphatic vessels restoration and suppression of dermal backflow (fig. 1)
- Decrease of limb diameter upon LF injection at day 10, reaching the normal control value at day 21 (fig. 2)

LentiFlash® induces a strong protein expression increase

At day 14 after injection of LF expressing Apelin+VEGF-C or Apelin alone, or an integrative Lentivector (iLV) expressing both genes, we observed a 2-fold increase of Apelin expression level, showing that LF is able to provide equivalent protein expression as iLV *in vivo*, even when it delivers 2 different mRNAs (fig. 3).

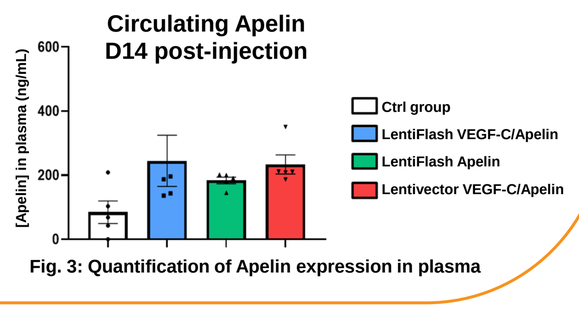


Fig. 3: Quantification of Apelin expression in plasma

E. Conclusion

The LentiFlash® properties, associated with our own **lentiviral production platform** compliant with the **cGMPs**, offer additional safety considerations making it a versatile and safe mean for human therapy.

A first-in-human phase I/IIa clinical trial on patients who developed lymphedema after breast cancer using RNA delivery by LentiFlash® will be performed in 2024 at the Toulouse University Hospital, France.

Furthermore, LentiFlash®, as an RNA delivery tool, can be used for a broad range of applications, such as **gene editing** (Mianné et al. 2022) and **vaccination/immunotherapy applications** for both infectiology and oncology purposes.

- LentiFlash® packages **biological RNAs of human origin**, without any lentiviral sequence.
- LentiFlash® can deliver **multiple RNAs.**
- LentiFlash® does **not** lead to **adverse immune responses.**
- Transduction by LentiFlash® does **not** result in **GMO generation.**
- It combines the efficient delivery of lentiviral vectors with the safety of RNA delivery since it enables **highly efficient transfer** and **transient expression:**
  - ✓ LentiFlash® particles display a very large tropism thanks to **VSV-G pseudotyping.**
  - ✓ **All cell types** can be efficiently transduced by LentiFlash® without altering cell viability nor phenotype.

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