Efficient and safe delivery of multiple mRNA using non-integrative bacteriophage-chimeric retrovirus-like particles for in vivo application

Régis Gayon[1], Emeline Benuzzi[2], Florine Samain[1], Audrey Turban[1], Alexandra Iché[1], Nicolas Martin[1], Christine Duthoit[1], Barbara Garmy-Susini[2] and Pascale Bouillé[1]

[1]Flash Therapeutics – 3 Avenue Hubert Curien, 31100 Toulouse, France; [2]Institute of Metabolic and Cardiovascular diseases of Toulouse (I2MC), INSERM UMR 1297, CHU Rangueil, 1 av Jean Poulhès, 31432 Toulouse Cedex 4, FRANCE

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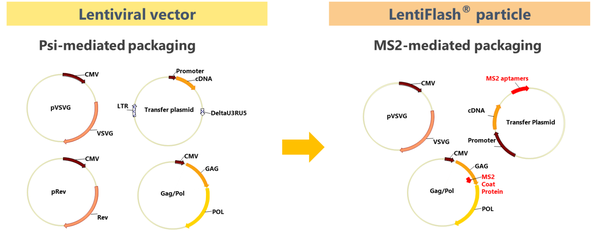
Introduction

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 transiently expressed into the cell cytoplasm directly available to be translated into protein.



iLV transfer plasmid contains lentiviral sequences (LTR, Ψ, RRE)

LentiFlash® transfer plasmid contains no lentiviral sequence

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

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. From a therapeutic perspective, great advantage of such system is its ability to carry different RNA species.

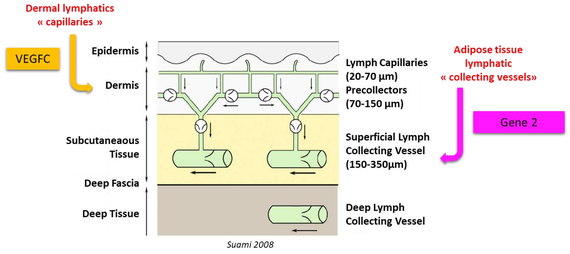
Here we show LentiFlash® delivering two different mRNAs after intradermal injection to treat a murine lymphedema model.

Clinical context

Lymphedema is a disorder of the lymphatic vascular system 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 lymphedema after surviving breast cancer. However there is no curative treatment for lymphedema.



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We are developing a **regenerative gene therapy** with non-integrative LentiFlash® vectors expressing two different mRNAs (VEGFC + Gene2) to restore the lymphatic function in the lymphedematous arm.

Theralymph is funded by the European Union's Horizon 2020 Research & Innovation program, with as a main objective establishing a multiple gene therapy for lymphedema. We are focused on women who developed secondary lymphedema after breast cancer surgery.

Results in murine lymphedema model treated by LentiFlash® (LF)

Mouse model of lymphedema was performed after the 4th mammary gland mastectomy associated with brachial and axially lymph node dissection. Mice exhibit a reproducible reduction of lymphatic drainage associated with dermal backflow (Fig1A) and increase leg diameter (Fig 2A) 2 weeks post-surgery.

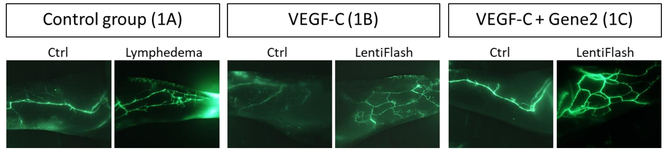

Fig.1: Lymphographies of treated or untreated limbs from control and lymphedema murine model.

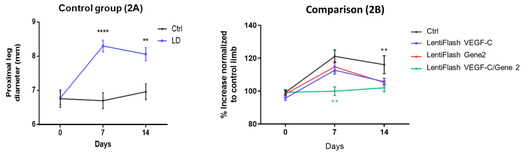

Fig.2: Measure of leg diameter.

After LF-VEGF-C intradermal injection, we observed an increase of lymphatic vessel density (Fig 1B) that is not sufficient for restoring the flow lymphatic to decrease lymphedema (Fig 2B). LF-VEGF-C+Gene2 allowed formation of large draining lymphatics with regular shape (Fig 1C) allowing to restore lymphangiogenesis, flow lymphatic, leading to a complete reduction of the limb swelling (Fig 2B).

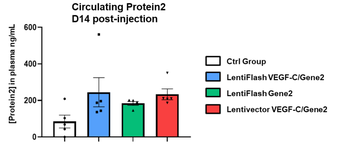

Fig.3: Quantification of protein2 expression in plasma.

2 weeks after injection of LentiFlash® (LF) expressing either Gene2 alone or Gene2 + VEGF-C or integrative Lentivector (iLV) expressing 2 candidate genes, we observed :

- a 2-fold increase of protein2 expression level for LF or iLV than control group
- the same expression level of protein2 whatever the delivery system used, showing that LentiFLash® is able to express a protein as much as an iLV in vivo.
- Even if LentiFlash® delivers 2 mRNAs, the protein expression level is as high as when LentiFlash® delivers only one mRNA.

Conclusion

The RNA Technology LentiFlash® :

- ✓ Combines the efficient delivery of lentiviral vectors with the safety of RNA delivery since it enables highly efficient transfer and transient expression
- ✓ Deliver multiple RNAs
- ✓ Maintain original cell phenotype and cell viability (safe RNA delivery)

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

A Phase I/IIa gene therapy clinical trial on patients who developed lymphedema after breast cancer using RNA delivery (LentiFlash®) will be performed on 2024 at the Toulouse University Hospital, France.

LentiFLash®, as a RNA delivery tool, can be used for a broad range of applications, such as **gene editing** (Mianne et al. 2022, and poster W-85 ASGCT 2022) , **vaccination/immunotherapy applications** for both infectiology and oncology purposes.