Innovative Nanoparticles for *ex vivo* and *in vivo* Genome Editing

Héloïse Pilet[1], Julie Gérard[1], Hyewon Jang[2], Hyongbum Henry Kim[2], Nicolas Grandchamp[1]

1: GEG Tech, Paris, France
2: Yonsei University College of Medicine, Seoul, Republic of Korea

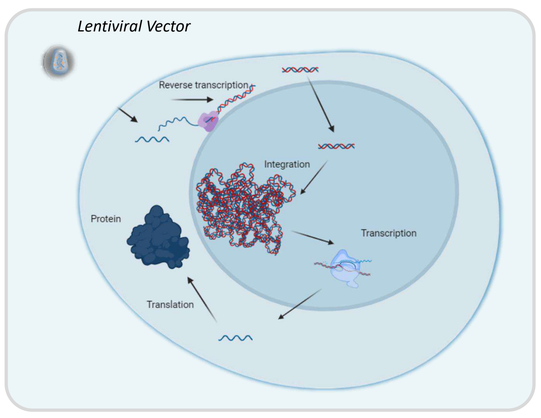
Abstract

The field of genome editing for gene therapy has experienced an unprecedented boost these last years through the discovery and design of new genome editors. However, to deliver these new systems *ex vivo* to primary cells, or *in vivo* directly into patients, there are several interlink challenges to address: suitable biosafety, efficacy and stability. Existing vector systems do not combine these characteristics. **Synthetic vectors** such as Lipid NanoParticles (LNPs) have generally a **good level of biosafety**, but their use are often **limited by their efficacy and stability**. Conversely, the **vectors derived from viruses** have a **good efficacy and stability**, but usually a **low level of biosafety** due to their DNA forms. Furthermore, **AAV** which is mostly used for in gene therapy is **sorely limited by its cargo capacity**, affecting its efficacy to deliver genome editors which are large.

GEG Tech has designed **new generation of nanoparticles combining the advantages of LNPs and viral vectors**. These new generations are constituted by a viral packaging enabling a good efficacy and stability, containing non-viral RNA and/or protein conferring a high level of biosafety. We have demonstrated that these new nanoparticles are very efficient to entry in primary cells and are able to induce genome editing *in vitro* and *in vivo* in the retina of **rd12 mice** to **restore rpe65 expression** thanks to a **prime editing** system efficiently deliver by our nanoparticles.

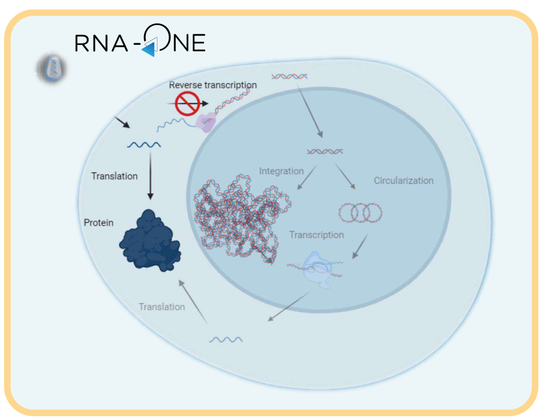
Design of a new Nanoparticle: RNA-ONE

RNA-ONE is derived from the lentivirus HIV-1 which contains a specific mutation into the reverse transcriptase coding sequence



Standard Lentiviral Vector cycle

After entry in the cell, RNA vector genome is reverse transcribed in DNA which is integrated into cell's genome.

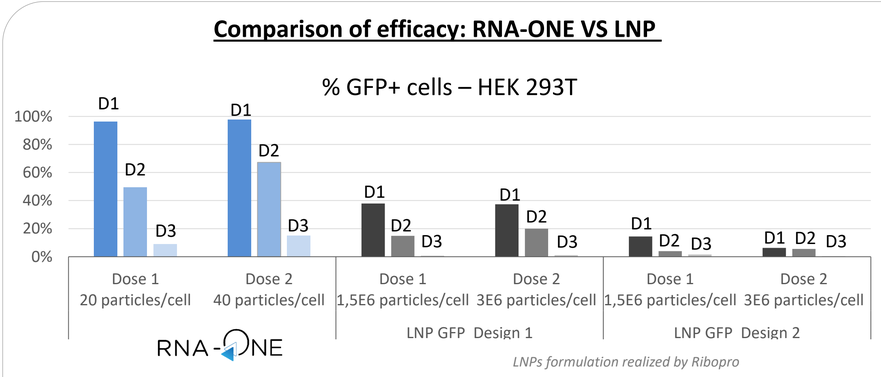


RNA-ONE vector cycle

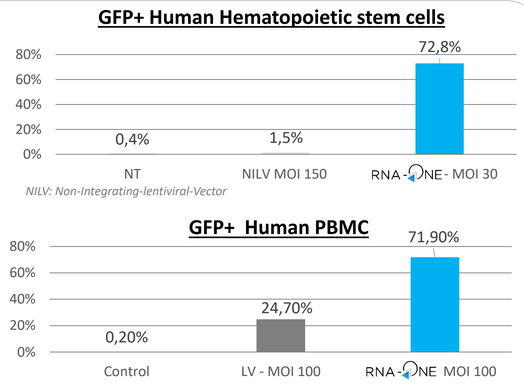
After entry in the cell, RNA vector genome is directly translated into protein without any DNA intermediate.

RNA-ONE is derived from lentiviruses. The reverse transcriptase has been specifically mutated and almost all viral sequences deleted. Consequently, this new nanoparticle enables to deliver mRNA with the lentiviral packaging, keeping the advantages in term of efficacy, stability and pseudotyping of lentiviruses.

RNA-ONE *in vitro* studies – Comparison of efficacy with LNPs & Lentiviral Vectors

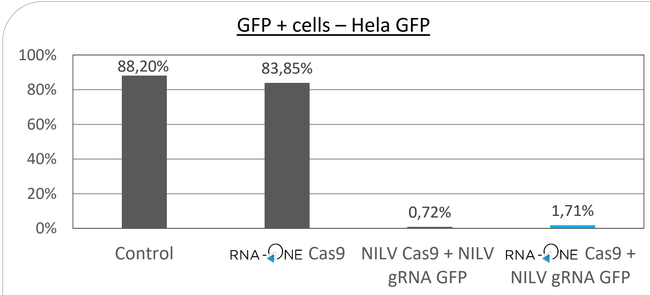


RNA-ONE efficacy is more than **two times higher than LNPs.**

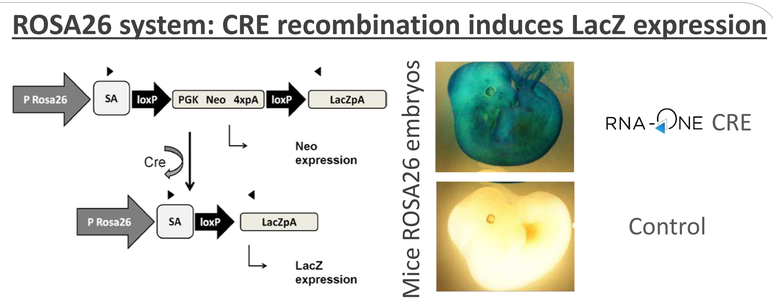


RNA-ONE efficacy is **higher than lentiviral vector efficacy** on human primary cells

RNA-ONE to induce genome edition *in vitro* & in transgenesis



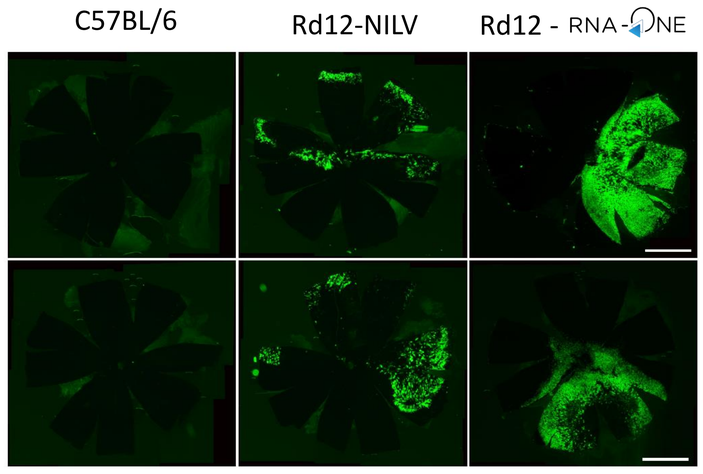
RNA-ONE Cas9 KO efficacy is comparable to NILV Cas9.



RNA-ONE is able to induce *in vivo* genome edition.

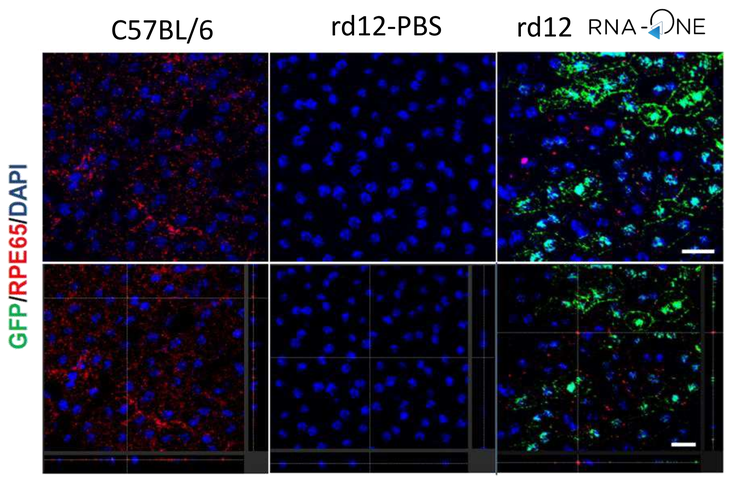
RNA-ONE to induce genome edition *in vivo* for retina gene therapy

GFP vector injections in mouse retina



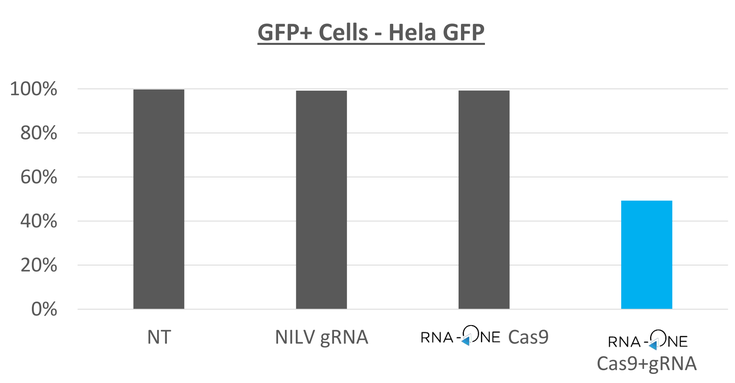
GFP is broadly expressed in the retina of rd12 mouse model injected with RNA-ONE GFP, better than in the retina injected with NILV GFP.

Prime Editing system injected in mouse retina



RNA-ONE enables to express a Prime Editor to restore *rpe65* expression in rd12 mouse model. gRNA is bring with a Non-Integrating Lentiviral Vector.

RNA-ONE next generation for genome editing: All-in-One Nanoparticles



RNA-ONE has been designed to vectorize Cas9 and a gRNA to have the two CRISPR elements in the same nanoparticle and improve its use for *in vivo* applications.

RNA-ONE nanoparticles derived from the HIV-1 enable the delivery of mRNA with higher efficacy than LNPs to transduced/transfected cell lines, and than standard lentiviral vectors to transduced human primary cells such as PBMC or hematopoietic stem cells. Furthermore RNA-ONE enables to induce the expression of genome editors such as Cre, Cas9 or Prime Editor *in vitro*, in transgenesis or *in vivo*. The level of genome editor expression enables to restore the expression of *rpe65* in rd12 mouse model. Finally, the last version of RNA-ONE has been validated to vectorize a genome editor with its gRNA, constituting a free DNA system. This last version of RNA-ONE combines the strong efficacy of viral vectors with a high level of biosafety, making it an excellent candidate for *in vivo* gene therapies.