P-505

Ligand-Functionalized LNPs for Active Targeting of RNA Therapeutics

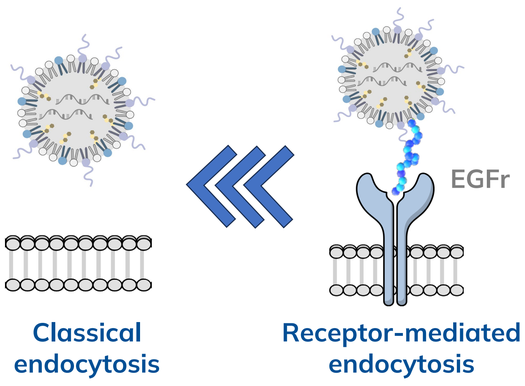
Laura de Miguel, Andrei Mihai Stoian[1], Diego de Miguel[1], Esther Pérez-Herrán[1], Diego Casabona[1], Javier Giménez-Warren[1] and Juan Martínez[1]

[1] *Certest Pharma, Certest Biotec S.L., San Mateo de Gállego, Zaragoza, Spain*

ldemiguel@certest.es

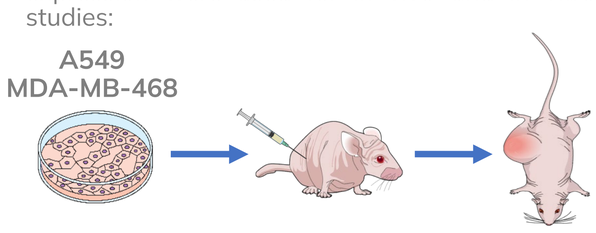
Introduction

Active targeting is crucial to direct RNA therapeutics to specific extrahepatic cells. We present a modular platform for developing ligand-directed Lipid Nanoparticles (LNPs) and oligonucleotide conjugates to achieve cell-type-specific delivery.



Epidermal Growth Factor receptor (EGFr)

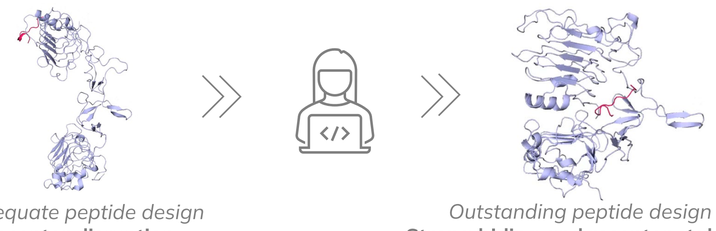
EGFr is overexpressed in multiple solid tumors and represents an attractive therapeutic target. Two cell lines with high constitutive EGFr expression were used for *in vivo* and *in vitro* studies:



Materials and Methods

Ligand Design

We employed molecular dynamics simulations to guide the rational design of peptide ligands, enabling the identification of stable conformations and key interaction hotspots with the target receptor.



Inadequate peptide design
-Receptor disruption-

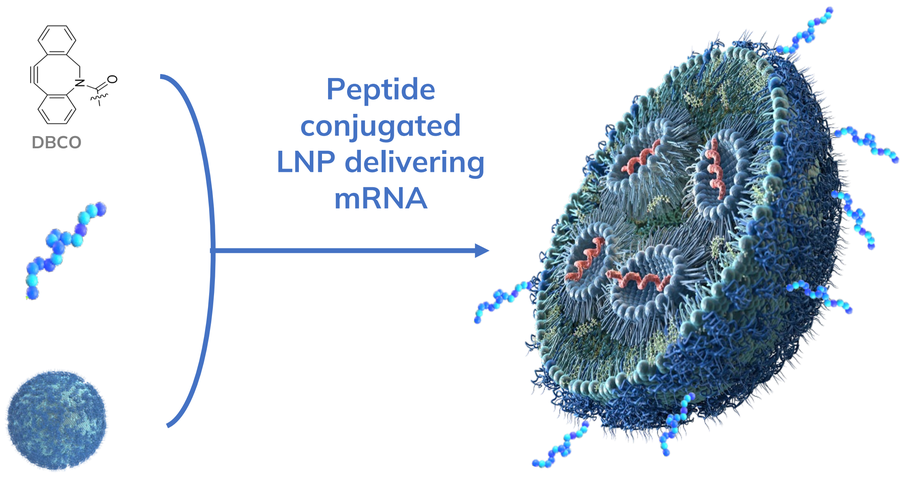
Outstanding peptide design
-Strong biding and receptor stability-

LNP-conjugation technology

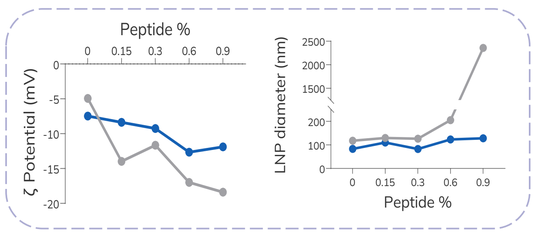
Chemical anchor
- **Click Chemistry**
  - Maleimide
  - Amide bond
  - Disulfide bond

Cargo
- **Peptide**
  - mAbs
  - Fab
  - ScFV
  - Nanobodies

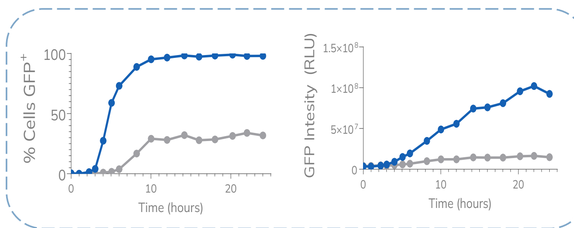
LNP activation
- **Post-insertion**
  - Pre-conjugation
  - Post-conjugation



LNP physical properties preservation



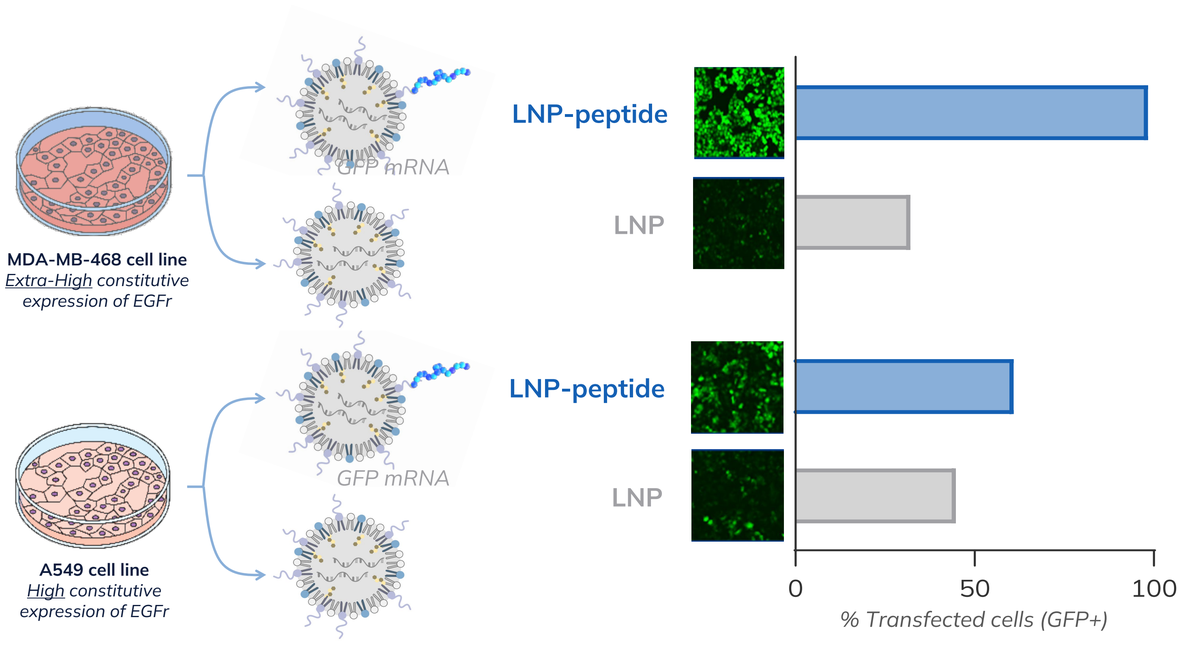
LNP functionality preservation



● *Established methods* ● *Certest conjugated LNPs*

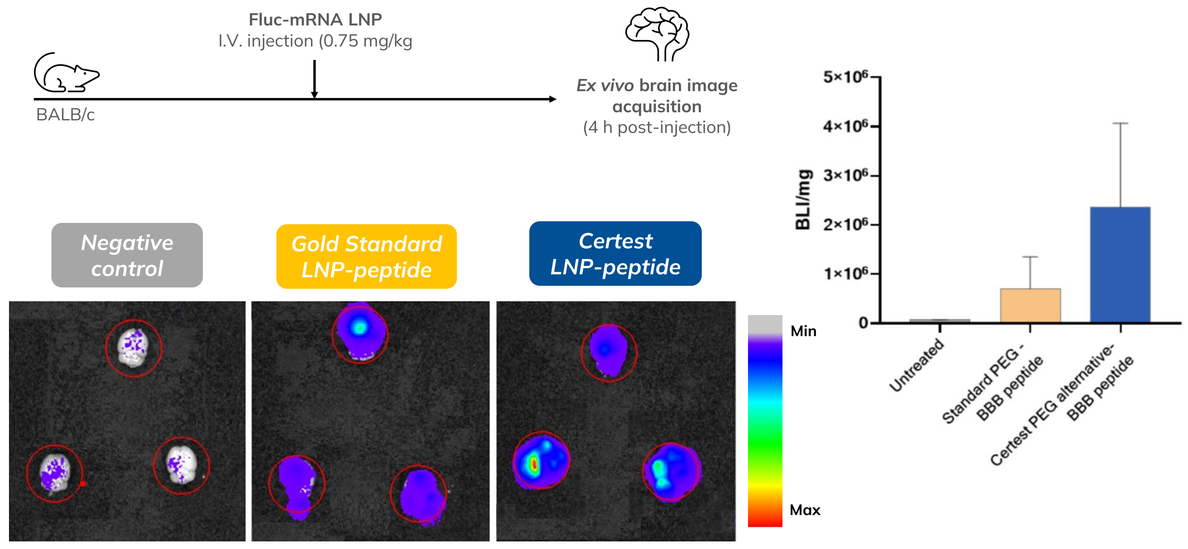
Peptide-Conjugated LNP Targeting

Peptide-conjugated LNP targeting EGFr improves internalization *in vitro*



In vitro assays using high EGFr-expressing cell lines (MDA-MB-468 and A549) demonstrated enhanced GFP mRNA delivery, with a significantly higher transfection efficiency compared to unconjugated LNPs.

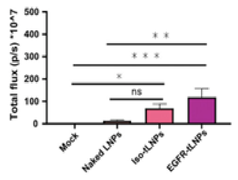
Organ-specific targeting



Peptide conjugation of our LNP formulation resulted in a 4-fold increase in bioluminescence signal in the mouse brain compared with the non-conjugated control, demonstrating substantially improved delivery efficiency and enhanced accumulation across the blood–brain barrier in vivo.

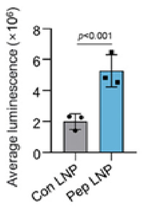
Tumor-specific targeting

State of the Art



8-fold increase

Masarwy R, Peer D, et al. Adv Sci (2025). Targeted CRISPR/Cas9 Lipid Nanoparticles Elicits Therapeutic Genome Editing in Head and Neck Cancer.

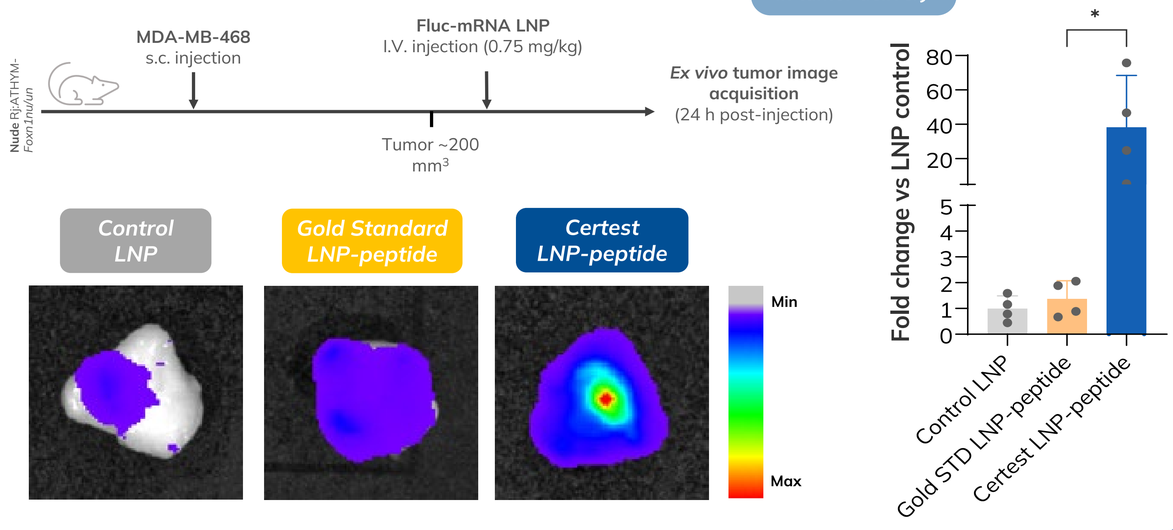


3-fold increase

Kim Y, et al. (2024), Adv Sci. Design of PD-L1-Targeted Lipid Nanoparticles to Turn on PTEN for Efficient Cancer Therapy.

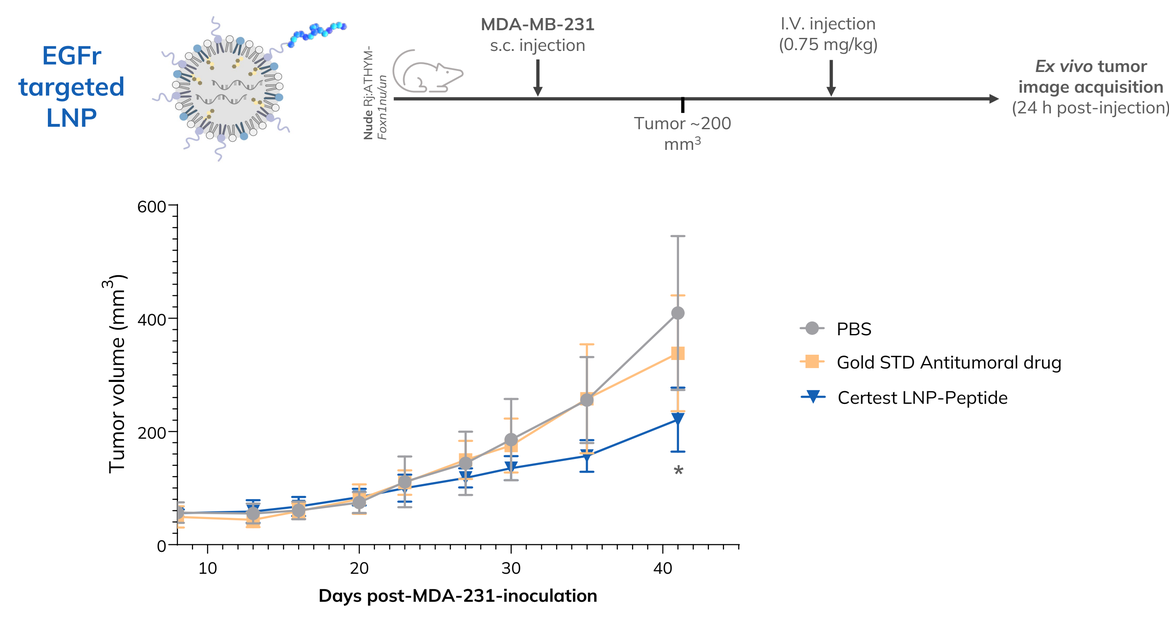
Certest targeted LNPs

38-fold increase in tumor delivery



In vivo, systemic administration of peptide-conjugated LNPs in a subcutaneous MDA-MB-468 tumor model resulted in a **38-fold increase in tumor accumulation**, as visualized by luminescence imaging. This targeting strategy markedly outperforms conventional LNP formulations.

Efficacy Assays



In vivo efficacy assays of antitumoral cargo show significant delay in tumor growth in the Certest LNP-peptide group compared with the PBS control group and improved performance over the antitumoral drug treated group after day 30 post-inoculation.

Conclusion

We present a versatile and modular platform for the targeted delivery of RNA therapeutics based on ligand-functionalized LNPs. Using peptide ligands conjugated via click chemistry, our system enables selective delivery of both mRNA and siRNA to EGFr-expressing cells in vitro and in vivo. The approach preserves LNP functionality and can be adapted to different RNA cargos and targeting ligands, paving the way for precision medicine applications across diverse therapeutic targets.