COLLEGE ED BIOLOGIE DOCTORAL SANTE PAYS DE LA LOIRE

Year 2023 /2024

THESIS TOPIC

Subject N° (to be completed by the ED):	**FUNDING:** ☐ **Requested** ☒ **Acquired**	**Funding origin: CHU (intern)**
Thesis title: **mRNA-loaded lipid nanocapsules: an innovative approach to treat pseudoxanthoma elasticum (PXE)**		3 keywords: Genetic diseases Messenger RNA Nanomedicines
Unit / team: **MINT INSERM 1066 CNRS 6021**		
Supervisor's name: **Dr. Elise Lepeltier**		Phone number: +33244688535 Email address: elise.lepeltier@univ-angers.fr

Socio-economic and scientific context (approximately 10 lines):

Pseudoxanthoma elasticum (PXE) is an autosomal recessive disease affecting approximately 1/40 000 people. PXE presents with skin changes in teenage years. Yellowish papules emerge on the neck and flexural areas. With time, papules coalesce and skin finally becomes wrinkled. Dermal elastic fibers are fragmented and calcified. The most serious manifestations of PXE appear later in life. The vision progressively decreases until central blindness in relation to the calcification of the Bruch's membrane. In addition, patients have cardiovascular manifestations due to the dystrophic mineralization in the media of arterial vessels. The main causes of PXE are mutations in the ABCC6 gene, translated in a protein belonging to the superfamily of ATP-binding cassette (ABC) membrane transporters. The liver is the primary organ expressing ABCC6. Indeed, ABCC6 at the basolateral surface of hepatocytes is responsible for cellular efflux of ATP from hepatocytes to the blood. Briefly, ATP is converted by ectonucleotidases into AMP and inorganic pyrophosphate (PPi) that inhibit the calcification process. Thus, ABCC6 sequence changes determine a low level of plasma PPi, subsequent hydroxyapatite crystal deposition, leading to mineralization of elastic fibers in the skin, retina, and blood vessel walls.

Working hypothesis and aims (approximately 8 lines):

Messenger RNA (mRNA) is an emerging class of therapeutic agents with a broad potential to prevent and treat a variety of diseases. mRNA approach is safe with no risk of integration or mutation into the genome. In addition, mRNA does not require to reach the nucleus (as opposed to DNA) because the translation machinery resides in the cytosol. Nevertheless, there are some challenges related to mRNA features. First, its instability due to the rapid degradation by RNAses and second its poor cell membrane diffusion and limited cellular uptake due to their hydrophilic nature and negative charge. Those challenges can be overcome by using a nanocarrier able of improve the intracellular delivery of intact mRNA. The recent success of the mRNA vaccine loaded lipid nanoparticle produced by Moderna and Pfizer/BioNTech to protect against COVID-19 pandemic, demonstrated the huge potential of mRNA loaded nanomedicines for revolutionizing medical research. In MINT laboratory, lipid nanocapsules (LNC) have been patented in 2016. LNC are obtained by phase inversion process and composed only of regulatory FDA approved excipients. They are able to encapsulate, protect and deliver siRNA, miRNA and plasmid DNA. Compared to other lipid systems such as liposomes, they demonstrated a great colloidal stability and a thin control of their diameter. Interestingly, after an intravenous injection LNC are known to accumulate into the liver, the targeted organ of this project. In this project, we propose to optimize LNC to efficiently deliver ABCC6 mRNA as supplemental protein therapy.

Grandes étapes de la thèse (env. 12 lines) :

WP1. Production of the humanized ABCC6 mRNA

This part will be done in Prof. Pichon's laboratory, possessing all technological arsenal and state of the art equipment to produce and purify any mRNA of interest. The production will be produced first by classical *in vitro* transcription as proof of concept. The coding sequences and flanking sequences (3' and 5') will be optimized to improve expression of mRNA. Library of UTRs will be screened to identify hepatocytes specific 5 'or 3' UTRs to create different master cloning vectors ensuring a better expression in cellulo. *In vitro* translation assay will be performed to check the expression of the correct ABCC6 mRNA (31 exons) sequence (Western blot).

WP2. Formulation and characterization of ABCC6 mRNA loaded LNC

The formulation will be performed by phase inversion process. Briefly, the ABCC6 mRNA will be first complexed with a mixture of phospholipids (1:1 in mole DOTAP/DOPE) into lipoplexes, then be added during the phase inversion zone of the LNC formulation process. A purification step by size exclusion chromatography will be performed to keep only the mRNA loaded LNC. The diameter and polydispersity index of the suspension will be obtained by diffusion light scattering, the zeta potential by electrophoretic mobility. Then the mRNA encapsulation efficacy and drug loading will be quantified by fluorescence spectroscopy, using a classical Qubit kit.

WP3. *In vitro* biological evaluations

The enzymatic protection of the mRNA by LNC will be assessed by electrophoresis gel. The cytotoxicity of the mRNA LNC will be studied on HepG2 hepatocytes, by classical MTT and LDH assays. The efficient cell internalization will be verified by flow cytometry and confocal imaging with a lipophilic fluorescent dye encapsulated inside the LNC to follow directly the nanoparticle and a Green Fluorescent Protein mRNA to prove easily the correct mRNA translation. Finally, after treatment by ABCC6 mRNA loaded LNC of 1h, 4h, 8h, 24h and 48h, the different proteins will be extracted from the HepG2 hepatocytes and analyzed by Western Blot to underline an overexpression of the ABCC6 protein (1503 amino acids, 165 kDa). As a comparison, the ovarian cell line SKOV-3 will be studied: this cell line does not express the ABCC6 protein and the mRNA translation could be easily demonstrated.

WP4. *In vivo* proof of concept on PXE murine model

We already have used Abcc6-/- mice from our facility. All animal experiments will be approved by the institutional ethical review committee. One or several intravenous injections will be performed in the tail, after a sterilization step of the suspension, through a 0.2 mm filter. Then, the plasma will be collected after 1h, 4h, 8h, 24h and 48h and the PPi will be quantified by our partner Dr. Duranton. A

biodistribution study by luminescence/fluorescence imaging on living animal be performed with a mRNA expressing the luciferase and a lipophilic NIR dye to follow the LNC.

Scientific and technical skills required by the candidate (2 lines):
The candidate should have a good background in nanomedicines, have experience already in nanoparticle formulation and characterization. Some skills in cell culture and Western Blot would be an advantage

3 publications from the team related to the topic (last 5 years):

- Le Moal B., **Lepeltier E.***, Rouleau D., Levisage C., Benoit JP, Passirani C., Guicheux J., Fusellier M., Clouet J., Lipid nanocapsules for intracellular delivery of microRNA: a first step towards intervertebral disc degeneration therapy, *Int. J. Pharm.*, **2022,** 624, 121941**, IF 6.5**

- Resnier P., **Lepeltier E.*,** Emina A.L., Galopin N., Bejaud J., David S., Ballet C., Benvegnu T., Pecorari F., Chourpa I., Benoit JP., Passirani C., Model Affitin and PEG modifications onto siRNA lipid nanocapsules: cell uptake and *in vivo* biodistribution improvements, *RSC Advances,* **2019***,* 47*,* 27264-27278 **IF 4.0**

- Kauffenstein G., Chappard D., Leftheriotis ., Martin L., ABCC6 deficiency and bone loss: A double benefit of etidronate for patient presenting with pseudoxanthoma elasticum?, *Experimental Dermatology*, **2022**, 31(10), 1635-1637 **IF 4.5**

National and international collaborations:

- **Prof. Chantal Pichon, INSERM UMS55 Orléans: specialist in mRNA production and formulation**
- **Prof. Dominique Bonneau, CHU Angers: genetician**
- **Dr. Christophe Duranton, Université Côte d'Azur: specialist in PPi quantification**