PNRR Missione 4, Componente 2, Investimento 1.4 "Potenziamento strutture di ricerca e creazione di "campioni nazionali di R&S" su alcune Key Enabling Technologies"
Iniziativa finanziata dall'Unione europea –- NextGenerationEU.
National Center for Gene Therapy and Drugs based on RNA Technology
Sviluppo di terapia genica e farmaci con tecnologia a RNA
Codice progetto MUR: **CN00000041** – CUP UNINA: **E63C22000940007**

Doctorate of National Interest

RNA THERAPEUTICS AND GENE THERAPY

SELECT ONE OF THE FOLLOWING RESEARCH AREA:

☐ **Mechanisms of Diseases and Drug Target Identification**

☐ **Design and Delivery of New Gene Therapy and RNA-Based Medicines**

X **Validation and Safety In Preclinical and Clinical Studies**

LOCATION OF THE RESEARCH ACTIVITY (INSTITUTION/DEPARTMENT):

UNIVERSITY OF PAVIA – Department of Molecular Medicine

TUTOR: Silvia Giuliana Priori

PROPOSED RESEARCH ACTIVITIES (max 300 words)

This project represents the translational component of the fundamental research conducted during the ERC advanced grant awarded to the Tutor and her team of scientists. In the discovery phase of the ERC-funded project, RNA-interference strategies were developed and successfully tested in animal models of two inherited diseases that cause arrhythmic death: Catecholaminergic Polymorphic Ventricular Tachycardia and Long QT Syndrome Type 8. (Patent number: 11591602 February 2023).
The project now aims to turn "molecules" into "products" to be tested in a "first in human – FIH" study the biological therapies for affected patients.
1.AAV vectors are the most common delivery strategy for gene therapies, but they are still associated with adverse events. We will therefore compare efficacy of AAV-mediated delivery with alternative delivery methods such as Lipid or Calcium phosphate nanoparticles (NaNos).
2.The research will also improve the native silencing molecules adding chemical modification with the goal of reducing the immune response to RNA, Capsids, NaNos, and to increase the stability and the half-life of the RNA therapy.

3. We will test the modified therapies and the efficiency of their delivery with different carriers in genetically modified swine models. Since these animals have a heart similar to the heart of humans in size and electrophysiologic properties. the data collected will be very informative for the transition to a FIH study. Since the genetically modified animals are hosted in our twin laboratory in Madrid, part of the research will be conducted at the CNIC Institute in Spain (https://www.cnic.es/).
4: Phenotyping of swine before and after gene therapy- We will be able to measure the arrhythmic burden in swine models by implanting ECG monitors to compare arrhythmias before and after therapy.
To gather accurate information on the improvement in the electrophysiology of the heart, we will map in vivo the heart's electrical activity using the same electroanatomic mapping systems used in humans in hospitals to determine the presence of arrhythmogenic substrates.
At the end of phenotyping, once information on the efficacy of therapy and efficiency of delivery strategies, the animals will be sacrificed and the following studies will be performed on tissue samples from different organs:

1) Tissue samples from heart, lungs, liver, spleen, brain, and skeletal muscle will be used to perform Real time PCR / Digital PCR to quantify
2) Immune response to AAV-Capsids, Lipid and Calcium phosphate NaNos and to native and modified RNA molecules. Experiments will be performed using a human-serum assay developed in our laboratory that also includes testing of the levels of cytokines and TNF alpha with ELISA in heart, lungs, liver, spleen, brain, and skeletal muscle.
3) Immunostaining, western blot, electron microscopy, and STED super-resolution microscopy will be used to assess whether therapy can correct ultrastructural abnormalities that are part of the phenotype of the diseases under study.

During the third year, we will start writing patents and approaching biotech companies to find partners for clinical translation. We anticipate that the Ph.D. student will be involved and will actively participate in this final part of the research project.