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**

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

LOCATION OF THE RESEARCH ACTIVITY (INSTITUTION/DEPARTMENT):

University of Padova

Department of Pharmaceutical and Pharmacological Sciences

TUTOR: Paolo Caliceti

PROPOSED RESEARCH ACTIVITIES (max 300 words):

The project will be focused on investigating the engineering of nanoformulations with enhanced, RNA loading, fusogenic/endosomal escape performances and targeting abilities. These activities will be carried out in the UNIPD unit of Spoke 8. A set of fusogenic agents will be preliminarily selected according to literature. Chemical protocols will be set up to obtain the functional modules if not available from the market; the modules will be designed with fit-for-purpose approach and rationally combined to enhance the performances of the nanovehicles for nucleic acid delivery (typically nanoparticles, lipoplexes and polyplexes). The materials will be fully characterized by spectrometric and chromatographic analyses. Libraries of formulations containing the RNA loading enhancer and fusogenic compounds corresponding to reference compositions will be prepared

and tested to evaluate the biopharmaceutical features. The intracellular delivery and endosomal escape will be investigated in order to select the most promising intracellular penetration enhancers and fusogenic materials and formulations. The study will be carried out by using fluorescents probes such as fluorescent labelled nucleic acid and cytofluorimetric and confocal analyses. Endosome colocalization and escape studies will be performed to quantify the trafficking of the RNA payload. Studies will be performed with siRNA and mRNA models that inhibit or express GFP or luciferase as models that will provide the evidence of intracellular delivery and fusogenic efficiency of the new formulations.

In the second part of the work, targeting agents will be selected according to the input deriving from vertical spokes. The targeting agents will be properly functionalized to decorate the nucleic acid delivery systems. Libraries of formulations containing different amounts of targeting agents will be prepared and will be tested with cell models to evaluate the targeting efficiency. Different targeting agents will be considered to overcome dedicated biological barriers.

The study will allow to optimize the nucleic acid delivery composition.