Finanziato dall'Unione europea NextGenerationEU
Ministero dell'Università e della Ricerca
Italiadomani PIANO NAZIONALE DI RIPRESA E RESILIENZA
Università degli Studi di Napoli Federico II

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

TITLE OF THE RESEARCH PROJECT
Developing and testing methods to assess RNA delivery, endosomal escape and siRNA downregulation efficiency in 3D models

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):

Department Biomedical Sciences

TUTOR:
Tito Calì

PROPOSED RESEARCH ACTIVITIES (max 300 words):
The project will be focused on developing and testing methods to assess RNA delivery, endosomal escape and siRNA downregulation efficiency in 3D models. These activities will be carried out under the supervision of Prof. Tito Calì in the UNIPD unit of Spoke 9 coordinated by Prof. Marisa Brini. The designed split GFP delivery reporters (SPLIDS) will be used to establish stable cell lines and organoids to test the efficiency of the delivery methods (e.g., aptamers, lipid nanoparticles, polyplexes). Selected mRNA or siRNA will be delivered with different methods along with the non-fluorescent β11 strand of the SPLIDS either in the form of peptide or mRNA. Different constructs with in-tandem β11 strands will also be designed and tested in order to evaluate the degree of delivery with a tunable fluorescent reporter. Libraries of cell lines and organoids containing the loaded RNA will be assessed by confocal microscopy techniques. The SPLIDS reporter will be modified to assess delivery and endosomal escape by targeting

GFP1-10 fragments to the lysosomal compartment. Endosome colocalization and escape studies will be performed to quantify the trafficking of the RNA payload. In a second part of the work, inhibitory β11 peptides will be designed and tested in 2D cell lines and 3D organoids obtained by stable transfected hIPSC clones expressing the GFP1-10 portion . The aim of these tests is to correlate the GFP1-10 intensity to the siRNA induced downregulation of a target of interest (tagged with an inhibitory peptide). These studies will be performed initially in vitro with purified GFP1-10 protein and WT and Mutant β11 peptides as well as in cell lines overexpressing the constructs encoding the two split GFP fragments and then in organoids. The final goal will be to apply SPLICS reporters to set up an automatized high throughput screening platform to validate different delivery technologies in terms of amount of RNA therapeutics that can reach a precise cell population in 3D organization, in terms of segregation in the intracellular compartments, i.e. endosomes or lysosomes where they can be prone to degradation, and in terms of efficiency to set up their threshold level as well as to test factors/manipulations that may influence their bioavailability, i.e. the stability of the molecules and the lack of passive diffusion.