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):

Institute of Genetics and Biophysics - CNR

Via Pietro Castellino 111 – 80131 Napoli

TUTOR:

Sandro De Falco

PROPOSED RESEARCH ACTIVITIES (max 300 words):

Gene therapy to inhibit pathological neovascularization in age-related macular degeneration.

Age-related macular degeneration (AMD) is an eye disease that progressively impairs vision. It is categorized into dry and wet AMD; with the former involving the gradual breakdown of macular cells and the latter, also known as neovascular AMD (nAMD), characterized by the occurrence of choroidal neovascularization (CNV). Currently, nAMD is treated with repeated intravitreal injections of anti-*Vascular Endothelial Growth Factor* (VEGF) drugs to hinder angiogenesis, the formation of new blood vessels.

Placental Growth Factor (PlGF), a member of the VEGF family, plays a crucial role in pathological angiogenesis also for its ability to drive inflammation. Its genetic ablation reduces pathological angiogenesis. We have generated a variant of PlGF named PlGF-DE that is unable to bind its specific receptor (VEGFR-1) but is still able to generate heterodimer with VEGF-A. The knock-in of PlGF-DE shows remarkable angiogenesis impairment in pathological conditions compared to both PlGF knock-out and wild type mice (Apicella et al. 2018, *Cell Reports, 23: 3635–3646*). This is due to the inactivation of PlGF-DE/VEGF-A heterodimers in addition to that of PlGF. Furthermore, gene therapy approaches with PlGF-DE variant have already demonstrated effective to inhibit laser CNV model and corneal angiogenesis in vivo (Tarallo et al. 2012, IOVS, 53: 7989-7996).

Since immune cells, such as microglia and macrophages, are crucial in nAMD and express high levels of VEGFR-1, the first objective of this project is to study their infiltration after laser induced CNV in PlGF-DE mice. The second part of the project aims to evaluate the feasibility of a gene therapy approach based on CRISPR-CAS9 gene editing. *Lipid nanoparticles* (L-NPs) will be used as delivery vehicles for the PlGF-DE variant and CRISPR-Cas9 components (L-NPs-PlGF-DE). The efficacy of a single subretinal delivery of L-NPs-PlGF-DE will be evaluated using a laser-induced CNV model in wild type mice.