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**

SPOKE 5: Inflammatory and Infection disease

Inhalable RNA-loaded nanoplatforms for local management of lung fibrosis and chronic inflammation

Nowadays, there is increasing evidence of the involvement of extracellular vesicles (EVs) in the pathogenesis of lung diseases including lung cancer, chronic obstructive pulmonary disease and pulmonary fibrosis [1]. EVs seems to play a pivotal role as mediators of intercellular communication and as co-operators in the development of lung diseases. In the framework of the research activity envisaged by WP3 of Spoke 5 of the National Center for Gene Therapy and Drugs based on RNA Technology (*Inflammatory and infectious disease*), EV-coupled biomarkers (miRNA, proteins, etc.) are being identified as potential targets for therapy of pulmonary fibrosis and related chronic inflammation [2]. This opens the path to novel specific RNA-based therapies for local management of this severe lung disease. Nonetheless, the unsuccessful history of inhaled RNAs points out the urgent need of safe and effective delivery systems to transfer them from lab to bedside. To fill this gap, lipid/polymer nanoparticles offer the unprecedented opportunity to improve RNA availability in the lung [3-5]. With this idea in mind, this PhD project aims to produce and to validate in lab inhalable RNA-loaded lipid/polymer nanoparticles (iNPs). A panel of iNPs loaded with reporter gene RNAs will be produced to assess, at the early stage of nanoplatform development: i) the correct operating conditions to produce RNA-loaded nanoparticles in lab and, expectantly, in an industrial setting; ii) the target properties of iNPs to move them in preclinical models provided for in Task 5.3.2. To this purpose, as an alternative to conventional multistep production techniques (e.g., emulsion-solvent diffusion), nanoplatforms will be produced by scalable microfluidic techniques and, if needed, further processed in form of long-term stable dry powders by either freeze-drying or spray-drying. In view of their application for local therapy of pulmonary fibrosis, iNPs will be characterized for size, surface, RNA entrapment efficiency, release kinetics, aerodynamic behaviour and interactions with in vitro models of lung extracellular and cell barriers. Inhalable RNA formulations validated in lab will be loaded with the identified RNA target sequence and moved to in vivo preclinical studies. Inhalable RNA prototype formulations will be expectantly produced in GMP-like conditions and progress to GLP preclinical studies provided for in Spoke 9.

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2. d'Alessandro M et al., *Int J Mol Sci*, 24(4):4071, 2023.
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4. Comegna et al., *Sci. Rep*., 11:6393, 2021.
5. Conte et al., *ACS Appl. Mater. Interfaces* 14, 7565−7578, 2022