

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):

National Research Council (CNR) of Italy

via Ugo La Malfa, 153 - 90146, Palermo, ITALY

TUTOR:

Antonella Bongiovanni

PROPOSED RESEARCH ACTIVITIES (max 300 words):

A next generation platform of nature designed delivery system of RNAs for therapeutic applications

Safe, efficient and specific nano-delivery systems are essential for the current preventive and therapeutic medicine. Extracellular vesicles (EVs) are cell-secreted natural vehicles with a considerable intrinsic complexity. The high biotechnological potential of EVs is boosting their exploitation as carriers of bioactive compounds (e.g., RNAs) towards theranostic applications¹. Beside the interest in stem cell-derived EVs, a considerable novel interest is growing for “green” natural sources-derived EVs. In that, microalgae constitute a rich reservoir of bioactive metabolites and are suitable for growth in industrial-scale and GMP-compliant photobioreactors. We have identified microalgae as a scalable and sustainable bioresource for the

¹ Witwer and Wolfram Extracellular vesicles versus synthetic nanoparticles for drug delivery. Nature Reviews Materials 2022

production of EVs in the context of two European VES4US and BOW H2020-FET projects (Bongiovanni/Manno/Pocsfalvi patent PCT/EP2020/086622). Hence, we defined a new generation of microalgal EVs (e.g., nanoalgosomes) which were demonstrated to be biocompatible and bioactive^{2,3,4,5,6,7,8}.

The main objective of this PhD project is to expand these pioneering results to harness EV natural properties in RNA-based pharmaceutical formulations that relies on scalable and sustainable production of green EVs (i.e., from plants and microalgae) and our tailor-made microalgal-EV bioengineering capabilities. At first, we will scale-up the upstream and downstream manufacturing processes for green EV production, to meet the final products' specifications in terms of quality, process reproducibility and validation. Then, we will setup loading strategies to encapsulate RNAs (including fluorescent-tagged RNAs) to maximize the loading efficiency into EVs. For both purposes, we will take advantages of up-to-date biochemical/biophysical/biological methods. Lastly, RNA-loaded EVs will be tested by: safety and potency assays on 2D/3D cultures of different cell lines; biological validation in in vivo preclinical models using different administration routes (e.g., oral, ocular). The proponent laboratory hold all the multidisciplinary expertise and methodologies needed; the achievement of project outcomes is further ensured by the active collaborations with several international universities and industries.

² Paganini et al. Scalable production and isolation of EVs: Available sources and lessons from current industrial bioprocesses. *Biotechnol. J.*, 2019.

³ Bongiovanni, Pocsfalvi, Touzet, Manno. Extracellular vesicles from microalgae. Patent: PCT/EP2020/086622

⁴ Picciotto et al. Isolation of extracellular vesicles from microalgae. *Biomaterials Science*, 2021

⁵ Adamo et al. Nanoalgosomes: Introducing extracellular vesicles produced by microalgae. *JEV*, 2021

⁶ Picciotto S, et al., Extracellular Vesicles From Microalgae: Uptake Studies in Human Cells and *Caenorhabditis elegans*. *Front Bioeng Biotechnol.* 2022..

⁷ Paterna, et al., Isolation of Extracellular Vesicles From Microalgae: A Renewable and Scalable Bioprocess. *Front Bioeng Biotechnol.* 2022

⁸ Adamo et al., Microalgae as a novel bio-factory for biocompatible and bioactive extracellular vesicles. *bioRxiv* 2023.04.04.535547; 7