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 Diseasesand Drug Target Identifixcation**

X **Design and Delivery ofNew Gene Therapy and RNA-Based Medicines**

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

LOCATION OF THE RESEARCH ACTIVITY (INSTITUTION/DEPARTMENT):

Università della Campania "Luigi Vanvitelli" – Dipartimento della Donna, del Bambino e di Chirurgia Generale e Specialistica.

TUTOR:

Professoressa Francesca Rossi

PROPOSED RESEARCH ACTIVITIES (max 300 words):

Tumor microenvironment cells (mesenchymal stromal/stem cells-MSC, fibroblasts, osteoblasts, osteocytes, osteoclasts, immune and vascular cells) play a key role in the onset and progression of osteosarcoma (OS), by releasing growth factors, cytokines, chemokines, and extracellular vesicles.
The aim of this project will be to use extracellular microvesicles to deliver, *in-vivo* and *in-vitro*, synthetic tumor suppressor miRNAs (mimic RNAs) or inhibitors of endogenous oncogenic miRNAs (anti-miRNAs) in the tumor microenvironment as a new therapeutic approach for OS.
miRNoma obtained from primary cultures of tumor microenvironment cells isolated from OS patients and healthy subjects will be analyzed by smallRNAseq in order to identify differentially

expressed miRNAs and their potential involvement in the tumorigenesis process as oncogenes (oncomiRs) or tumor suppressors. Different bioinformatics tools will be used to predict the target transcripts of these miRNAs.

Identified and validated miRNAs/anti-miRNAs will be transfected into microvesicles isolated from healthy subjects' MSCs.

The antitumor effects of these microvesicles will be evaluated on three-dimensional cell cultures (obtained by spheroids or scaffolds) and on a mouse xenograft model. In three-dimensional cultures (constituted by OS cell lines and primary cells of the tumor microenvironment) different pathways involved in cell cycle, apoptosis, senescence and invasiveness will be analyzed. In the mouse model (obtained through xenotransplantation of three-dimensional cultures in a nude mouse) tumor volume and distant metastases will be evaluated using different imaging techniques (high-resolution ultrasound, in situ hybridization, confocal and multiphoton microscopy, LCM microdissection).

This project proposes a new and promising therapeutic approach in OS, improving its prognosis and limiting the short- and long-term side effects associated with current therapeutic protocols.