

## Isolation and characterization of tumor-derived Extracellular Vesicles

### Introduction

Isolation and downstream analysis of Circulating Tumor Cells (CTCs) from blood of patients with metastatic cancer can serve as a non-invasive liquid biopsy giving insights on the mutational status and the possible treatments that a patient can undergo. However, CTCs are very rare events with their frequency being around 0-2 CTCs/mL of blood in a background of millions of white blood cells and trillions of red blood cells. Tumor-derived extracellular vesicles (tdEVs), on the other hand, are membrane-encapsulated particles derived from tumor cells with a size range between 50 nm and 5  $\mu$ m. tdEVs serve as cell messengers transporting cellular information (miRNAs, mRNAs and proteins) to recipient cells of even distant sites through circulation. Moreover, they can reprogram the recipient cells in order to promote metastasis and cancer progression. Their higher frequencies in the blood of the patient and the fact that they are secreted by numerous cancer cells suggests that their cargo could be more indicative of the mutational status of the patients and their treatment-decision making.

### Ongoing research

We are aiming to tdEV isolation using different techniques, either affinity- or size-/density- based) as well as tdEV characterization in terms of their morphology (using electron microscopy) and their downstream analysis in regards to the expression of their surface markers (using flow cytometry and fluorescence imaging) and their cargo analysis (mRNAs and miRNAs).

### Projects:

#### *Downstream RNA analysis of tdEVs*

*The gene expression of a tumor can be traced down by the isolation of its secreted tdEVs and their downstream RNA cargo analysis. Digital droplet PCR will be applied and standardized in MCBP using first RNA isolated from prostate cancer cell lines (PC3 and LNCaP) to detect cancer specific RNA (mRNA of CK and EpCAM) and RNA isolated from white blood cells (mRNA of CD45 and CD16). The next step will be the isolation of RNA from tdEVs produced by the aforementioned cancer cell lines and EVs from plasma of healthy volunteers to define the minimum amount of tdEVs required for their mRNA to be detected. Finally and most importantly, RNA isolated from tdEVs of prostate cancer patients will be analyzed and evaluated for the presence of cancer specific mRNA. Ideally, RNA sequencing of the isolated EVs from the plasma of prostate cancer patients will be performed to have an overview of all the cancer-specific RNAs present.*

