*DELIVERABLE 1.1 Report on EVs derived from tumor/osteoblastic cells ready and characterized*
*DESCRIPTION: The deliverable will consist of a report describing the tumor/osteoblastic cells chosen as source of EVs and of the protocols used for EV extraction and characterization and related results*

Authors: Marije Kuipers and Esther Nolte-'t Hoen

To identify molecules on natural EVs that can mediate targeting to bone cells, we selected the PC3 and PC3M cell lines. The PC3 cell line originated from a bone metastasis of a prostate tumor (human). PC3-derived EVs have been reported to interact with bone cells, including osteoclasts (Tamura et al, Int J of Mol Sci, 2020). PC3M is a derivative cell line with higher metastasizing potential.

Following our conventional protocol for EV isolation from cell culture supernatant, we first enriched for EVs by differential (ultra)centrifugation and further purified EVs by density gradient ultracentrifugation. We verified the presence of EVs using high-resolution flow cytometry and western blot analysis. Our data indicate that EVs from PC3 cells have buoyant densities of 1.04- 1.10 g/ml, and contain the characteristic tetraspanin proteins CD9, CD63. We also analyzed the proteomes of purified PC3 EVs and source cells by MS/MS. As much as 3600 unique proteins were detected in EVs and many of these were enriched compared to the source cell proteome. The top EV proteins detected were actin, integrins (including β4, β1, α2, α3) and CD44, heat shock proteins (90, 70, 27), and the tetraspanin proteins CD9, CD81 and CD63. Together, these data indicate that PC3 EVs can be purified and collected in sufficient quantities to allow in-depth proteome analysis.