either from actively circulating viruses – including VSV-G, rabies, influenza, and mokola viruses – or from human endogenous retroviruses (HERVs) Env proteins – such as syncytin-1.

Summary/Conclusion: EVs produced in the absence of viral Env machinery are poorly fusogenic and are unlikely to be efficient mediators of cell-to-cell communication via the delivery of EV contents to the cytoplasm. In contrast, viral Env proteins significantly enhance EV fusogenicity, suggesting that EV fusion and communication may occur and play a significant role during viral infections. Furthermore, cells expressing the HERV Env syncytin-1 – including many human cancers – also give rise to fusogenic EVs that may contribute to tumour establishment, growth, and metastasis. These findings suggest that blocking syncytin-mediated EV fusion may be an effective strategy to block EV communication in human cancers.

OS24.03

Preferential accumulation of copper-free click chemistry-modified exosomes to own pancreatic xenograft *in vivo*

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Introduction: Pancreatic cancer (PC) is one of the deadliest malignancy with few effective approaches available for early diagnosis or therapy. Exosomes (Exo) as one type of extracellular vesicles are currently being investigated as potential theragnostic tools in cancer. However, it is not yet well-understood how Exo are taken up by PC cells. This work aims to study the Exo dosimetry and preferential Exo-cell affinity in PC cells *in vitro* and *in vivo* for exploitation of Exo-based delivery of therapeutics.

Methods: Exo are isolated by sucrose cushion ultracentrifugation and characterized for exosomal marker expression, number, purity and shape. Exo were fluorescently labelled by copper-free click chemistry to enable uptake quantification in cells using the Design of Experiments (DoE) approach. Cellular uptake of Exo was investigated using flow cytometry and confocal microscopy. Factors studied are donor Exo source, dose, receipt cell type, and incubation time. Responses identified are Exo "Taken up numbers" and "Percentage uptake" per cell. Candidate PC Exo uptake was then assessed *in vivo* and compared between PC and melanoma xenograft models in NSG mice following intravenous administration.

Results: Cellular uptake of Exo was time- and dose-dependent profiles. PC derived PANC-1 Exo showed significantly higher and not saturable uptake in PANC-1 cells compared to B16-F10 Exo (cancer-derived) and HEK-293 Exo (non-cancer derived) which showed lower and saturable uptake profile at 24 h. *In vivo* biodistribution studies of PANC-1 Exo in subcutaneous PC xenograft further confirmed that PANC-1 Exo favoured accumulation in PC tumours over melanoma (B16-F10) tumours.

Summary/Conclusion: A simple and highly efficient surface modification approach via click chemistry was developed enabling both *in vitro* and *in vivo* tracking of Exo. DoE modelling predicted PC cells' preference to PC-derived Exo which was confirmed also *in vivo*. This Exo dosimetry study could facilitate a rationalized approach in Exo-based therapeutics for treatment of cancer in pre-clinical studies.

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OS24.04

Specific transfer of hollow gold nanoparticles within exosomes is determined by the exosome origin

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Introduction: Exosomes are considered key elements for communication between cells but very little is known about the mechanisms and selectivity of the transference processes involving exosomes released from different cells.

Methods: In this study we have investigated the transfer of hollow gold nanoparticles (HGNs) between different cells when these HGNs were loaded within exosomes secreted by human placental mesenchymal stem cells (MSCs). These HGNs were successfully incorporated in the MSCs exosome biogenesis pathway and released as HGNs-loaded exosomes, by using time-lapse microscopy and atomic emission spectroscopy

Results: Those studies allowed us to demonstrate the selective transfer of the secreted exosomes only to the cell type of origin when studying different cell types

including cancer, metastatic, stem or immunological cells.

Summary/Conclusion: In this study we demonstrate the selectivity of *in vitro* exosomal transfer between certain cell types and how this phenomenon can be exploited to develop new specific vectors for advanced therapies. We show how this preferential uptake can be leveraged to selectively induce cell death by light-induced hyperthermia only in cells of the same type as those producing the corresponding loaded exosomes. We describe how the exosomes are preferentially transferred to some cell types but not to others, thus providing a better understanding to design selective therapies for different diseases.

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OS24.05

A high-throughput screen for functional extracellular vesicles

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Introduction: Prions are infectious protein aggregates that self-propagate and infect naïve cells by direct cell contact or via secreted vesicles. Several lines of evidence argue that also protein aggregates associated with common neurodegenerative diseases can intercellularly propagate their aggregated states in a prion-like manner. Thus, targeting extracellular vesicles (EVs) has potential clinical implications for neurodegenerative diseases. We have developed a mouse neuroblastoma cell-based assay to identify compounds that modulate exosome uptake and subsequent protein aggregate formation in recipient cells. In this novel cell-based assay, we take advantage of the non-toxic Saccharomyces cerevisiae prion domain Sup35NM that forms self-templating protein aggregates in mammalian cells capable of spreading through cell cultures. The addition of fibrils produced from bacterially expressed Sup35NM to cells expressing soluble NM efficiently induces appearance of NM aggregates which are faithfully inherited by daughter cells. Importantly, EVs released from donor cells containing NM aggregates are infectious and induce the aggregation of soluble NM-GFP in recipient cells after 12 h incubation time. We here introduce a high throughput assay to screen for functional EVs that trigger NM reporter protein aggregation in target cells.

Methods: We have developed a quantitative high-throughput screen assay to identify modulators (inhibitors and activators) on exosome uptake. The read-out of this functional EV assay is the percentage of recipient cells with induced NM-GFP aggregates.

Results: A total of 4135 small molecules were screened from three well-defined compound libraries (LOPAC, TOCRIS and SELLECKCHEM). Thirty-three inhibitors and 35 activators were found to decrease or increase the EV-mediated aggregate induction in recipient cells, respectively. Lead compounds identified in this screen affect active and selective EV uptake in recipient cells.

Summary/Conclusion: We successively developed a cell-based assay for functional extracellular vesicles and performed high-throughput screening to identify the mechanisms of active extracellular vesicle uptake. I will present some interesting findings out of the screen.