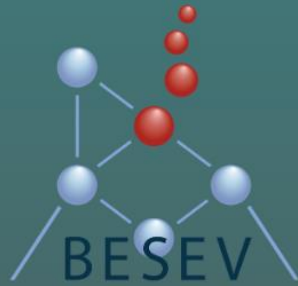
ANNUAL MEETING OF THE BELGIAN SOCIETY OF EXTRACELLULAR VESICLES



Besev 2024

LIEGE, BELGIUM

12 | SEPT 2024

Chateau de Colonster
Allée des Erables 1
4000 Liège

Local organizer
Ingrid Struman
GIGA Institute, ULiège

ACKNOWLEDGEMENT

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WELCOME

Dear participants,

We are happy to welcome you in Liège for this annual meeting organized by the Belgian Society for Extracellular Vesicles.
The objectives are to (i) update and clarify our current understanding of the molecular and cellular mechanisms supporting the formation and activity of these organelles, (ii) offer an opportunity for young biologists, engineers and clinicians to familiarize with the current knowledge and challenges in the field, and (iii) define together future directions to resolve tough questions that remain.

We would be grateful for everyone facilitating vigorous scientific discussion and questioning. We hope this will be a memorable event, providing deeper insight and stimulating creativity for all of us.

Thank you for your active participation.

The organizers,

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Pascale Zimmermann
KU Leuven -CRCM

Program BESEV 2024

12th of September

Annual meeting of the Belgian Society of Extracellular vesicles

8:30	**Registration desk open**	
9:00	**Official welcome**	

Session 1

Chair Pascale Zimmermann

Time	Speaker	Title
9:15	**Keynote speaker** **Romaric Lacroix** *C2VN, Marseille, France*	**A coagulolytic balance of extracellular vesicles: interest for infectious coagulopathies.**
10:00	**Inge Mertens** *VITO, UAntwerpen*	**Molecular characterization of EV-derived biomarkers in neurodegenerative conditions**
10:30	***Coffee break***	

Chair Stephanie Herkenne

Time	Speaker	Title
11:00	**Maureen Cambier** *ULiege* ***Abstract pitch***	Unraveling the significance of exosomal immune checkpoint protein PVR/CD155 in Lung Carcinoma progression
11:05	**Annelore Haems** *VIB, Ugent* ***Abstract pitch***	The role of exosomes in TNF-driven inflammation
11:10	**Lukas HYKA** *KU Leuven* ***Abstract pitch***	A split Nano-Luciferase approach for measuring extracellular vesicle content delivery.
11:15	**Martina Pannetta** *University of Salerno, I* ***Abstract pitch***	Characterization and functional analysis of extracellular vesicles from Lactobacillus rhamnosus GG
11:20	**Chenggong Tu** *VUB-KU Leuven* ***Abstract pitch***	Targeting pro-tumoral stromal exosomes by disrupting the syntenin-CD138/syndecan pathway as a novel therapy in Multiple Myeloma
11.25	**David Van Morckhoven** *ULB* ***Abstract pitch***	microRNA-203a is enriched in stromal-cell derived extracellular vesicles of chronic lymphocytic leukemia patients and associated with tumor burden

11:30	***Sysmex*** ***Sponsor talk***	**Dive into the possibilities of the new NanoSight Pro**
11:40	***Analis*** ***Sponsor talk***	**ELEVATE your Research**
11:50	***Unchained Labs*** ***Sponsor talk***	**Meet Leprechaun – the sEV specific analytical tool**
12:00	***Particle Metrix*** ***Sponsor talk***	**Importance of Combining Scatter Based and Fluorescence Based Nanoparticle Tracking Analysis for EVs Characterization**
12:10	***Lunch :*** **"Sur le pouce" organized by Héliport Brasserie**	

Session 2

Chair Basile Stamatopoulos

13:30	**Keynote speaker** **Jérôme Paggetti** *Department of Cancer Research, Luxembourg Institute of Health. Luxembourg*	**Extracellular vesicles promote an immunosuppressive and tumor-supportive microenvironment in chronic lymphocytic leukemia.**
14:15	**Stephanie Herkenne** *ULiege* ***Short Talk***	Mitochondria: the new regulator of Extracellular vesicle biogenesis
14:30	**Kyra Defourny** *Utrecht University, NL* ***Short Talk***	A comparison of the release and spread of infectious extracellular vesicles during picornavirus infection across different cell models
14:45	**Zhe Liu** *KU Leuven* ***Short Talk***	FO-SPR biosensor enables specific and sensitive detection of extracellular vesicles
15:00	***Coffee break***	
15:30	**Pascale Zimmermann** *KU Leuven - CRCM*	**PDZ proteins in the biology of extracellular vesicles**
16:00	**Closing remarks and awards**	
16:30	**Reception**	

Romaric Lacroix

C2VN, Marseille, France

A coagulolytic balance of extracellular vesicles: interest for infectious coagulopathies

Romaric Lacroix (PharmD, PhD), 43 years old, is professor of hematology and immunology at the faculty of pharmacy of Marseille and medical biologist in the hematology department of the biogenopole at the Timone universitary hospital. His research activity is conducted in the CardioVascular and Nutrition Research Center (C2VN) at Aix-Marseille University where he coordinates a research group on extracellular vesicles (EVs).
He completed a Pharmaceutical and Medical Biology educational and training program at Aix-Marseille University in 2007. After a master degree in hemostasis at Paris university, he completed a PhD program in the Françoise Dignat-George's group in Marseille. He worked on the proteolytic impact of large EVs (microvesicles) in the regulation of hemostasis and described their plasminogenolytic activity. He made a post-doctoral experience in the Furie's lab at Harvard Medical School (Boston). He got an assistant professor position at the faculty of Pharmacy in 2011 followed by a PU-PH position in 2020.
R. Lacroix is a recognize expert for the detection and measurement of EVs and focuses his research program on the role of EV in haemostasis. He coordinated several international EV studies of the standardization subcommittee in vascular biology of the International Society in Thrombosis and Haemostasis. He published around 90 indexed publications focused on EVs.
Contact : romaric.lacroix@univ-amu.fr

Jérôme Paggetti

Department of Cancer Research, Luxembourg Institute of Health. Luxembourg

Extracellular vesicles promote an immunosuppressive and tumor-supportive microenvironment in chronic lymphocytic leukemia

Dr. Jérôme Paggetti is heading the Tumor Stroma Interactions group in the Department of Cancer Research at the Luxembourg Institute of Health since 2017. The group is presently composed of 16 members (including 6 PhD students, 6 Postdocs, 1 technician, 1 scientist). After completing his PhD in biochemistry, molecular and cellular biology at Inserm in France studying epigenetic factors in acute myeloid leukemia, Dr Paggetti joined the LIH in 2010 as postdoctoral fellow. He focused his research on B-cell malignancies, especially chronic lymphocytic leukaemia (CLL), with great interest for the interaction between malignant cells and the tumour microenvironment, particularly the immune system using patient samples and murine pre-clinical models. Indeed, CLL is the prototypical microenvironment-dependent leukemia relying on surrounding immune and stromal cells for activation and proliferation in lymphoid tissues. This complex network is crucial for disease progression and response to therapy. His main goal is to decipher the cellular and molecular mechanisms behind this dependency in order to discover novel therapeutic opportunities for leukaemia patients that could be brought to the clinic. Dr Paggetti has established collaborations with well-known universities and cancer institutes in Europe, published in top journal of the field and received several scientific prizes including the FNR award for outstanding publication in 2016.

Molecular characterization of EV-derived biomarkers in neurodegenerative conditions

Y. Hirschberg 1,2, K. Schildermans 1, S. Engelborghs 3,4, I. Mertens 1,2

1 Health Unit, Flemish Institute for Technological Research (VITO), Mol, Belgium

2 Centre for Proteomics (CfP), University of Antwerp, Antwerp, Belgium

3 Department of Neurology and Bru-BRAIN, Universitair Ziekenhuis Brussel and NEUR Research Group, Center for Neurosciences (C4N), Vrije Universiteit Brussel (VUB), Brussels,Belgium

4 Department of Biomedical Sciences, University of Antwerp, Antwerp, Belgium

Presenting author: **Inge Mertens**

Dementia is a leading cause of death worldwide, with increasing prevalence as global life expectancy increases. The most common neurodegenerative disorders are Alzheimer's disease (AD), dementia with Lewy bodies (DLB) and Parkinson's disease dementia (PDD). With this study, we took an in-depth look at the proteome of the (non-purified) cerebrospinal fluid (CSF) and the CSF-derived extracellular vesicles (EVs) of a first cohort including patients with AD (N=64), PD (N=29), PD-MCI (Parkinson's disease with mild cognitive impairment) (N=13), PDD (N=27) and DLB (N=55), and cognitively healthy controls (N=48) analysed by label-free mass spectrometry. This has led to the discovery of differentially expressed proteins that may be helpful for differential diagnosis. We observed a greater number of differentially expressed proteins in CSF-derived EV samples (N = 276) compared to non-purified CSF (N = 169), with minimal overlap between both datasets. This finding suggests that CSF-derived EV samples may be more suitable for the discovery phase of a biomarker study, due to the removal of more abundant proteins, resulting in a narrower dynamic range. As disease-specific markers, we selected a total of 39 biomarker candidates identified in non-purified CSF, and 37 biomarker candidates across the different diseases under investigation in the CSF-derived EV data. The initial step in validating these results involved analyzing larger patient groups, comparing the proteomes of patients with AD (N=220) to cognitively healthy controls (N=221), in non-purified CSF. This approach successfully validated one of the 10 previously selected AD-specific biomarker candidates. Future validation efforts will include different neurodegeneration disorders and CSF-derived EVs. Once the selected proteins are further explored and validated, they can be used to further differentiate between the included dementias and may offer new avenues for research into more disease-specific pharmacological therapeutics.

PDZ proteins in the biology of extracellular vesicles

Pascale ZIMMERMANN

KU Leuven & CRC Marseille

Our understanding of the molecular mechanisms regulating the composition and the selective uptake of extracellular vesicles (EV) by recipient cells is limited. This impairs their rational use in diagnostics and therapeutics. I will illustrate that PDZ proteins, intracellular membrane scaffolds that co-evolved with multicellularity (ca. 150 proteins in humans), are master regulators of EV formation, heterogeneity and (re)internalization.

In particular, I will show that tetraspanins (CD9, CD63 and CD81) directly interact with a limited number of PDZ proteins while syndecans (SDCs) engage 20% of the human PDZ proteome. Using RNAi-mediated depletion, I will illustrate that PDZ proteins support SDC proteolytic cleavage and that SDC C-terminal fragment (CTF) may help predicting small EV number (on the contrary to tetraspanins and syntenin). Moreover, I will document that PDZ proteins differentially fine-tune EV composition in a cell-type dependent manner and control the intracellular and cell-surface distribution of tetraspanins and SDCs. Finally, I will show that PDZ proteins regulate the uptake of CD63-positive EVs, most probably by controlling the levels of heparan sulfate (long unbranched sugar chains carried by SDC) that support 2/3 of the uptake of such EVs by recipient cells.

This work uncovers part of the complex molecular mechanistic behind EV-signaling by (i) highlighting an important unsuspected function for PDZ proteins in EV-mediated cellular exchanges, (ii) complementing our understanding on how PDZ-mediated interactions act on the compartmentalization of cell signaling and (iii) reinforcing the notion that the SDC are major constituents and key regulators of small EV exchanges.

Intercellular Communication: What Syndecan-Syntenin May Teach us About the Molecular Mechanisms That Support Vesicular Exchanges by Rania GHOSSOUB, Guido DAVID and Pascale ZIMMERMANN – Bradshaw Ralph A, Hart Gerald W and Stahl Philip D (eds) Encyclopedia of Cell Biology, Second Edition vol 2, pp 401-407 Oxford: Elsevier (2023)

Castro-Cruz M, Hyka L, Daaboul G, Leblanc R, Meeussen S, Lembo F, Oris A, Van Herck L, Granjeaud S, David G, Zimmermann P. PDZ scaffolds regulate extracellular vesicle production, composition, and uptake. Proc Natl Acad Sci U S A. 2023 Sep 19;120(38):e2310914120.

[1] Mitochondria: the new regulator of Extracellular vesicle biogenesis

Tristan Drouet, Violaine Vandenhooft and Stephanie Herkenne

Laboratory of mitochondria in cell-to-cell communication (MICC), GIGA, University of Liege, Belgium

Presenting author: **Stephanie Herkenne**

Mitochondria, once known primarily as the cell's powerhouse, are now recognized as dynamic organelles orchestrating a delicate balance between fusion and fission. Emerging research has unveiled their role as tethering hubs, connecting with various cellular compartments such as the plasma membrane, endoplasmic reticulum, lysosomes, and peroxisomes. In our groundbreaking study, we have demonstrated for the first time that mitochondria engage in a pivotal interaction with the endosomal compartment. Our findings reveal that mitochondria are not merely passive participants but active controllers of endosomal dynamics. Specifically, we discovered that mitochondria act as transfer stations, delivering RAB5 proteins to early endosomes. This transfer initiates the crucial process of endosomal maturation, guiding early endosomes towards their late-stage counterparts. Moreover, our research uncovers a novel dimension of mitochondrial influence: the regulation of late endosome fate. We show that mitochondria direct these endosomes either towards lysosomal degradation or towards the plasma membrane, facilitating the release of extracellular vesicles (EVs). This discovery underscores mitochondria's role as key regulators in the biogenesis of EVs. In conclusion, our study highlights a previously uncharted mechanism wherein mitochondria not only regulate the formation of extracellular vesicles but also influence their composition and functional properties. This novel insight positions mitochondria at the center of cellular communication and homeostasis, redefining our understanding of their multifaceted roles within the cell.

[2] A comparison of the release and spread of infectious extracellular vesicles during picornavirus infection across different cell models

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Kyra Defourny [1,4], Bram Tuinte [1], Xinyi Pei [1], Margarida Viola [2], Charlotte Nurmohamed [1], Renee Maas [2], Joost Sluijter [2], Frank van Kuppeveld [3], Esther Nolte-‘t Hoen [1].

(1) Infection biology Section, Division Infectious Diseases & Immunology, Department of Biomolecular Health Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands. (2) Department of Cardiology, Experimental Cardiology Laboratory, University Medical Center Utrecht, Utrecht, Netherlands. (3) Virology Section, Division Infectious Diseases & Immunology, Department of Biomolecular Health Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands. (4) VIB Center for Inflammation Research, VIB, Ghent, Belgium.

Presenting author: **Kyra Defourny**

Extracellular vesicles (EVs) can contribute to the spread of non-enveloped, positive-sense RNA viruses of the Picornaviridae family via the packaging of infectious virus particles. In doing so, EVs can help viruses evade neutralizing antibodies, and alter their uptake by susceptible cells and cells participating in immunosurveillance. Currently, large efforts are being performed to study the composition, release, and functional properties of EV-enclosed virions. However, few studies have addressed whether viruses induce similar EV release in cells of different tissue origin, and whether the release and functional properties of EV-associated viruses are cell type-dependent. To address this question, we compared the impact of encephalomyocarditis virus (EMCV) and coxsackievirus B3 (CVB3) infection, two causative agents of viral myocarditis, on EV release in various (tumor) cell lines of different tissue origin, as well as human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs). We found that EMCV and CVB3 infections induced an increase in EV release and the production of EV-enclosed viruses in all tested cell types, including cardiomyocytes. Additionally, infection was shown to trigger the release of a similar set of EV markers for both viruses in the different cell lines, indicating that virus-driven EV release is (relatively) conserved across species and cellular contexts. In contrast, we observed that the efficiency by which EV-enclosed virions were able to establish infection compared to naked virions differed between EMCV and CVB3 infection, and demonstrate for EMCV this efficiency is influenced by the cells involved. Together, these data highlight a robust pattern of infection-driven changes in EV release, but emphasize the importance of context when considering the impact of EVs on virus spread

[3] FO-SPR biosensor enables specific and sensitive detection of extracellular vesicles

Zhe Liu (1), Yağmur Yıldızhan (1,2), An Hendrix (3), Jeroen Lammertyn (1), Dragana Spasic (1)

(1)Department of Biosystems–Biosensors Group, KU Leuven, Willem de Croylaan 42, B-3001 Leuven, Belgium.

Presenting author: **Zhe Liu**

Extracellular vesicles (EVs) have attracted great attention as potential biomarkers for cancer diagnostics. However, the majority of the EV detection methods is not yet applicable in clinical settings mainly because they rely on complex EV enrichment methods and/or lack necessary sensitivity/standardization. To overcome this, we established a calibrated fiber optic surface plasmon resonance (FO-SPR) bioassay using well-characterized recombinant EVs (rEVs) as biological reference material. This bioassay was further extrapolated towards other EV model systems from breast cancer cell lines (i.e., MCF7 and SK-BR-3).

In this context, we demonstrated specific EV binding on the FO-SPR probes when using EV-specific capture antibodies (e.g., anti-CD9, anti-CD63 and anti-CD81). By introducing EV-specific detection antibodies (immobilized on the gold nanoparticles) in a sandwich bioassay, we achieved a 10^3 and 10^4 times higher LOD compared to the EV concentration in human blood plasma from healthy or cancer patients, respectively. Moreover, we were able to detect EVs in two different complex matrices, namely: (1) endogenous EVs of HEK293 cells directly in the cell medium without any prior EV purification or enrichment and (2) EVs isolated from two different breast cancer cell lines, spiked in 100-fold diluted blood plasma samples.

While these bioassays have been established using a common EDC/NHS surface chemistry, we also exploited alternative approaches to enhance the reproducibility, sensitivity, and robustness of the FO-SPR EV bioassays, including: (1) NTA for functionalizing FO-SPR probes with his-tagged antibodies/nanobodies, and (2) DSPE for capturing EVs via lipophilic interactions. This flexibility in surface chemistry together with the exceptional sensitivity and specificity of our developed sandwich bioassay even in the complex matrices, offer great prospect for direct EV detection in biological fluids without any prior purification and enrichment steps.

[4] Unraveling the significance of exosomal immune checkpoint protein PVR/CD155 in Lung Carcinoma progression

Cambier M. (1), Remacle C.(1), Beyens J.(1), Herkenne S.(1), Henket M.(2), Gillet H.(2), Polese B.(2), Schyns Joey (2), Sibille A.(2), Louis R.(2), Guiot J.(2), Struman I.(1)

1 Molecular Angiogenesis laboratory, University of Liège - Liège (Belgium)

2 Department of Pneumology, University Hospital of Liège - Liège (Belgium)

Presenting author: **Maureen Cambier**

Introduction: Immunotherapy has revolutionized cancer treatment, but understanding the resistance to this treatment is crucial for improving outcomes. In this study, we investigate the role of extracellular vesicles (EVs) in immune escape through immune checkpoint protein (ICP) expression, focusing on the axis PVR/CD155. We developed a new methodology that identifies several ICPs on the surface of EVs in single-liquid biopsies.

Methodology: We analyzed the expression of several ICPs identified through literature screening and TCGA data analysis. Plasma samples from healthy individuals and cancer patients undergoing immunotherapy were collected. EVs were isolated and characterized using ultracentrifugation. ICPs on EV surfaces were identified via MAGPIX analysis. Knockdowns were generated with siRNA and CRISPR-Cas9 and studied with functional assays.

Results: Analysis of TCGA data revealed high levels of PVR/CD155 in lung cancer. EV-associated PVR/CD155 levels were elevated in patient samples. Furthermore, its expression was even more abundant during immunotherapy in non-responders, suggesting an emergence of resistance. We studied ICP expression in several human and mouse models and discovered an enrichment of PVR in EVs. Knockdown of PVR/CD155 cells presented reduced migration and proliferation rates in human and mouse models.

Conclusions: Our study introduces a novel method for characterizing circulating ICPs-EVs in lung cancer patients, with implications for patient monitoring and therapeutic target discovery. Further investigation of PVR/CD155+EV in coculture systems with immune cells will deepen understanding of its role in immunotherapy resistance.C

[5] The role of exosomes in TNF-driven inflammation

Annelore Haems [1-2], Jon Huyghe[1-2], Mathieu Bertrand[1-2]

(1) Inflammation Research Center (IRC), Vlaams Instituut voor Biotechnologie (VIB), Zwijnaarde, Belgium;
(2) Ghent University, Ghent, Belgium

Presenting author: **Annelore Haems**

Tumor Necrosis Factor (TNF) is well established as a major driver of inflammation and is consequently a pharmacological target in several inflammatory disorders. Activation of TNFR1 by TNF leads to the successive assembly of two protein complexes. The receptor-bound Complex I forms at the plasma membrane and signals to inflammatory gene activation, while the receptor-dissociated Complex II assembles in the cytosol and possesses cytotoxic potential. TNF-mediated cell death can be beneficial for the host by eliminating infected cells and stripping pathogens of their replication niche. However, uncontrolled/excessive death by TNF can also be highly detrimental and at the basis of a variety of inflammatory pathologies. Consequently, cell death checkpoints are normally in place to prevent TNF cytotoxicity and protect the organism from its potentially detrimental consequences. Recently, an unconventional LC3-independent macro-autophagy pathway was found to prevent apoptosis by eliminating the CASP8-activating Complex II that forms upon TNF sensing. Recently, we have discovered a parallel and non-redundant pathway that additionally prevents TNF-induced apoptosis by detoxifying Complex II. Interestingly, we found that the ESCRT-dependent endosomal micro-autophagy (eMI) pathway contributes to the removal of Complex II from the cytosol by integrating the complex in Intra-Lumenal Vesicles (ILVs) and releasing it extracellularly as part of exosomes. The biological consequences of this phenomenon are currently unknown. We hypothesize that the intercellular transfer of Complex I and/or Complex II via exosomes contributes to the local and systemic organization of the TNF-mediated inflammatory response. We believe that the insights gained by this study will greatly increase our fundamental understanding of the TNF response and potentially reveal new therapeutic approaches for the treatment of TNF-related inflammatory disorders.

[6] A split Nano-Luciferase approach for measuring extracellular vesicle content delivery

Lukas HYKA(1), Sofie MEEUSSEN(1), Clotilde THERY(3), Guido DAVID(1,2), Alain JOLIOT(3) and Pascale ZIMMERMANN(1,2).

(1)Department of Human Genetics, KU Leuven, Leuven, Belgium

(2)Centre de Recherche en Cancérologie de Marseille, France

(3)Institut Curie, PSL University, Paris, France

Presenting author: **Lukas Hyka**

Extracellular vesicles (EV) are organelles mediating cell-to-cell communication, appreciated for their potential therapeutic applications. Yet, whether EV efficiently transfer their content to the cytosol of recipient cells remains a matter of debate.

Here, we established a protocol allowing to measure the administrated EV dose, internalized EV fraction, and cytosolic delivery of internal EV content. We use luminescence-based assays whereby luciferase is split into two nonfunctional parts (LgbiT and HiBit) that can recover enzymatic activity upon complementation. Concentrated conditioned media (CCM) from HEK293 cells expressing HiBit-CD63 or VSVG-HiBit fusions (containing EV with the Luciferase moiety oriented inside EV - verified in protease-protection assays) was added to Panc-1 recipient cells homogenously expressing LgbiT. To determine the total dose of EV, CCMs were incubated with saturating concentrations of recombinant LgbiT in the presence of Triton X-100, added at concentration optimal for permeabilization. To measure the fraction of EV that is internalized and the cytoplasmic delivery of their internal content in recipient cells, we incubated the HEK293 CCM with Panc-1 cells for 4 hours (for saturation of recipient cells with EV). EV-treated Panc-1 cells were then mildly trypsinized to remove non-internalized EV from their surface and split into two fractions to quantify (i) the total internalized EV fraction (in the presence of Triton X-100 plus recombinant LgbiT) and (ii) the cytoplasmic delivery of HiBit fusions (in the absence of treatments).

We found that solely 0.01% of the HiBit-CD63 and 0.1% of the VSVG-HiBit EV administrated accumulate in the cells after 4 hours internalization. At that time, HiBit that has been delivered to the cytoplasm corresponds to approximately 10% of the total internal pool. These data validate an approach to quantify EV content delivery and support the notion that EV can deliver their internal cargo inside recipient cell.

[7] Characterization and functional analysis of extracellular vesicles from Lactobacillus rhamnosus GG

Martina Pannetta (1,2), Daniela Eletto (1), Alessandra Tosco (1), Niké Guilbert (3,4), Amélie Vander Cruyssen (3,4), An Hendrix (3,4), Amalia Porta (1)

(1)Department of Pharmacy, University of Salerno, 84084 Fisciano (SA), Italy;

(2)PhD Program in Drug Discovery and Development, University of Salerno, 84084 Fisciano (SA), Italy;

(3)Laboratory of Experimental Cancer Research, Department of Human Structure and Repair, Ghent University, Ghent, Belgium;

(4)Cancer Research Institute Ghent, Ghent, Belgium.

Presenting author: **Martina Pannetta**

Both Gram-negative and Gram-positive bacteria release extracellular vesicles (bEVs), which are nano-sized, lipid membrane-delimited particles that transport biologically significant cargo from their mother cell. Recently, bEVs have garnered attention as novel mediators of bacterium-bacterium and bacterium-host interactions. Despite this, research on Gram-positive bacteria EVs has been limited due to the challenge posed by their thick cell wall. However, these vesicles exhibit unique structural and functional properties due to the absence of an outer membrane.

Probiotics, known for promoting both intestinal and extraintestinal health benefits, may exert these effects through their bEVs, though the exact mechanisms remain unclear. This study aims to characterize the composition and functions of bEVs derived from the probiotic Gram-positive bacterium Lactobacillus rhamnosus GG (LGG).

Due to the challenges associated with bEV purification, sequential size-based and density-based approaches have been combined to obtain pure LGG EVs: ultrafiltration, density-gradient centrifugation and size-exclusion chromatography (SEC). Next, LGG EVs were characterized in compliance with MISEV 2023 guidelines, using electron microscopy (EM), nanoparticle tracking analysis (NTA), and a TLR2 based reporter assay. LGG bEVs-enriched fractions showed low abundance, nevertheless EM analysis revealed their morphology and confirmed their purity.

Proteomic analysis identified 194 proteins, confirming that LGG EVs carry potential functional molecules. Experiments demonstrated that LGG EVs were successfully internalized by an eukaryotic model and provided a protective effect against LPS-induced intestinal inflammation. However, they did not show antibacterial, antiviral or antifungal properties.

A deeper understanding of the mechanisms behind microorganism-host cell communication (inter-kingdom communication) facilitated by bEVs could lead to significant advancements in therapeutic strategies.

[8] Targeting pro-tumoral stromal exosomes by disrupting the syntenin-CD138/syndecan pathway as a novel therapy in Multiple Myeloma

Chenggong Tu(1,2), Raphael Leblanc(3), Pascale Zimmermann (2,3), Eline Menu(1)

(1)Hematology and Immunology Research Group, Vrije Universiteit Brussel, Brussels, Belgium; (2)Laboratory for Extracellular Vesicle Research, K. U. Leuven, Leuven, Belgium; (3)CRCM, Marseille, France

Presenting author: **Chenggong Tu**

Introduction: Exosomal communication between bone marrow stromal cells (BMSCs) and Multiple Myeloma (MM) cells has been extensively studied as a critical step in the progression and drug resistance of myeloma. However, the specific targeting of pro-tumoral exosomes remains elusive. The PDZ protein syntenin which couples to syndecan 1, has been identified as an exosome marker which can regulate the biogenesis of exosomes. In this study, we aimed to explore whether specific inhibition of syntenin alters exosomal output by BMSCs and whether this could counter BMSC-induced drug resistance in MM.

Methods: Syntenin knockout bone marrow stromal cells (BMSCs) were generated by CRISPR/Cas9 technology. Their exosomes were isolated by ultracentrifugation and protein markers were identified by western blot. The effects of syntenin knockout in BMSCs on myeloma cells were determined by cell viability, apoptosis assay and western blot analysis. A small chemical compound targeting the PDZ2 domain of syntenin, termed SyntOFF was used to block syntenin loading into exosomes and its therapeutic effect in combination with bortezomib was evaluated in vitro and in the preclinical 5T33MM mouse model.

Results: Syntenin (SDCBP) expression correlates to poor survival in MM patients and is enriched in bone marrow stromal cells. Knockout of syntenin in BMSC alters the exosomal output and abolishes BMSCs-induced bortezomib resistance of MM cells via regulation of STAT3, MAPK, and AKT-mTOR pathways. SyntOFF decreases syntenin sorting into exosomes and enhances the therapeutic effect in vitro and in vivo.

Conclusion: Here we show that syntenin controls the secretion of pro-tumoral exosomes in BMSCs. Blocking syntenin therefore disrupts the communication between BMSC and MM cells and serves as a possible novel target in multiple myeloma.

[9] microRNA-203a is enriched in stromal-cell derived extracellular vesicles of chronic lymphocytic leukemia patients and associated with tumor burden

David Van Morckhoven, Nathan Dubois, Dominique Bron, Laurence Lagneaux, Basile Stamatopoulos

Laboratory of Clinical Cell Therapy, Jules Bordet Institute, Université Libre de Bruxelles (ULB), Brussels, Belgium

Presenting author: David Van Morckhoven

Background: Crosstalk between the leukemic B cells and the microenvironment plays a fundamental role in the progression of chronic lymphocytic leukemia (CLL). In this context, the extracellular vesicles (EVs) of bone marrow mesenchymal stromal cells (BM-MSCs) were shown to promote survival, migration and chemoresistance of the malignant B cells.

Methods: To further investigate their content, EVs isolated from conditioned media of BM-MSCs of (1) healthy donors and CLL patients, of (2) control/inflammatory conditions and of (3) patient/healthy serums were obtained by serial ultracentrifugation. Subsequently, conditioned medium EVs were characterized by electronic microscopy, direct flow cytometry, nanotrack analysis and microRNA screening by real-time PCR (qPCR).

Results: MicroRNA screening revealed that miR-203a-3p was significantly enriched in CLL and under inflammation BM-MSC EVs compared to controls (n=4, +616-fold, P <0.05 and +74 fold, P<0.05 respectively). Quantification by qPCR on independent samples confirmed these findings in EVs for both CLL (n=7 P=0.0068) and EVs produced under inflammatory conditions (n=8 pairs, P=0.0391). Interestingly, miR-203a-3p is not enriched in the cells themselves compared to controls. The level of miR-203a-3p was 13x higher in patient serums (n=190) compared to healthy controls (n=18) (P<0.0001) and associated with CLL prognostic factors such beta-2microglobulin (P=0.0002), lymphocyte doubling time (P=0.0389), solubleCD23 (P=0.0230), all associated with tumor burden. PBMC of CLL patients were treated with PKH67 labelled EVs to highlight the cells internalizing the EVs: we observed preferential internalization of BM-MSC EVs by monocytes.

Conclusions: miR-203a-3p is enriched in CLL BM-MSC EVs compared to healthy donors and in MSC-EVs produced under inflammatory conditions. miR-203a serum level is statistically higher in CLL patients and is associated with tumor burden. Monocytes preferentially internalize BM-MSC EVs.

[10] EV-based therapy to decrease neuroinflammation in the scope of multiple sclerosis

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Multiple sclerosis is the most common chronic inflammatory disease of the central nervous system (CNS) affecting young adults. It is characterized by multifocal inflammatory demyelinating lesions and axonal loss, leading to various symptoms such as sensory and motor deficits. Anamdamide (AEA) is an endogenous ligand of cannabinoid receptors. This bioactive lipid displays immunomodulatory proprieties by decreasing chronic inflammation in several diseases, including multiple sclerosis. But the short half-life of AEA in vivo resulting from its rapid catabolism and poor solubility requires its incorporation in a drug delivery system (DDS). In recent years, many studies focused on extracellular vesicles (EV) as promising biological DDS have emerged. EV are lipid-bilayers vesicles secreted by almost all cell types and play a role in cell-to-cell communication. EV have a better ability to cross the BBB compared to the others nanocarriers and thus are appropriate for drug delivery for brain treatment. Therefore, we have hypothesized that encapsulation of AEA in EV would enhance its delivery in the CNS, in addition to improving its stability.

We aim to compare the encapsulation of AEA into EV by using different loading methods described in the literature: co-incubation, permeabilization by saponin and sonication. Our results showed that the saponin method was the most efficient one. However, we worked with saponin concentrations higher than the critical micellar concentration and we thus expect the formation of saponin micelles. After experimentally confirming the presence of saponin micelles, whether the highest AEA loading with the saponin method was really the result of its encapsulation in EV or if it was actually be encapsulated in saponin micelles was investigated. We showed that AEA was preferentially loaded in micelles than in EV.

[11] Insights Into The Role Of Endothelial Extracellular Vesicles In Pre-Metastatic Niche Formation And Metastasis In Breast Cancer

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Breast cancer (BC) is one of the most common cancers worldwide. While it can be treated, metastatic BC remains incurable. Previously, our research group demonstrated that endothelial cell-derived extracellular vesicles (EVs) enriched in miR-142-5p, miR-183-5p, and miR-222-3p participate in the polarization of macrophages towards an M2-like phenotype, thus promoting tumour growth in a BC mouse model (Njock et al., 2022, JEV). However, the impact of these microRNAs on metastasis remains unknown. A crucial step during metastasis is the formation of a pre-metastatic niche (PMN), which is notably initiated by EVs.

With this project, we aim to unravel the impact of these microRNAs on PMN formation and metastasis in vitro and in vivo.

In this study, we isolated EVs from mouse endothelial cell lines, characterized them using Western blotting (WB) and Nanosight Tracking Analysis (NTA). Next, we evaluated their incorporation into macrophages and fibroblasts, which are two major cell types in PMN formation, using confocal microscopy. Afterwards, we electroporated the EVs with the three microRNAs and added them to macrophages and fibroblasts. To determine the effects of these EVs on cell differentiation, we analysed the expression by qPCR of several genes known to be involved in PMN formation.

Our data showed that endothelial EVs were successfully incorporated into the recipient cells. Our qPCR results demonstrated that microRNA-enriched EVs upregulate the expression of pro-tumorigenic genes Csf3, Cxcl1, Col3a1, Il-1β, and Ccl3. Currently, we are further investigating the role of microRNA-enriched endothelial EVs in PMN formation in a 4T1-BC mouse model.

Our current findings reveal a previously unrecognized role of microRNA-enriched endothelial EVs on macrophage and fibroblast differentiation in vitro. These preliminary findings reveal a potential role of endothelial EVs in PMN formation, offering avenues for novel approaches in BC treatment

[12] Role of the exosomal protein Syntenin in autophagy

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Autophagy is a process allowing cell survival upon stress and the clearance of dysfunctional proteins and organelles. During autophagy, specialized organelles are formed from the endoplasmic reticulum and mature to form autophagosome that ultimately fuse with lysosomes. Recent evidence suggests that autophagy and the production of exosomes may be interdependent. Yet, molecular mechanisms supporting autophagy-exosome crosstalk remain obscure. Previous studies indicate that syntenin, a protein supporting exosome production, may influence the levels of the autophagosomal protein LC3B. Here we studythe role of syntenin in autophagy and document that it controls the formation and morphology of autophagosomes and lysosomes. We also illustrate that syntenin controls the cellular levels and the exosomal secretion of proteins involved in different steps of autophagy. Our data indicate that syntenin acts as a down-regulator of autophagosome biogenesis and as a promoter of autophagosome-lysosome fusion. The present study thus identifies a dual role for syntenin in autophagy and one piece of the molecular framework that governs the interplay between exosomes and autophagy.

[13] Study of the role of microRNAs-encapsulated in extracellular vesicles in osteosarcoma

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The tumor microenvironment is crucial in tumor progression, significantly influenced by secreted factors like extracellular vesicles (EVs). In osteosarcoma (OS), EVs play a role in promoting tumor growth and invasion. Additionally, EVs derived from bone marrow mesenchymal stem cells (BMSCs) regulate cell proliferation, migration, and survival, contributing to OS drug resistance. Conversely, EVs from adipose-derived stem cells (ASCs) have been shown to reduce OS cell proliferation.

Our study aims to elucidate the impact of ASC-EVs on OS growth and develop a technology that enhances their therapeutic potential by modifying their surface and cargo.

To explore the therapeutic potential of miRNAs encapsulated in isolated EVs, we conducted functional assays on OS cells transfected with five specific miRNAs*. Our findings suggest these miRNAs may act as tumor suppressors by inhibiting OS cell migration and proliferation. Additionally, co-culture experiments were performed to assess the effects on microenvironmental components by indirectly co-culturing miRNA-transfected OS cells with fibroblasts and endothelial cells. The results indicate that this co-culture significantly influences the migratory behavior of fibroblasts and endothelial cells in an angiogenic environment. Future studies will further investigate the therapeutic potential of miRNA-encapsulated EVs on OS cells. We are also exploring the efficacy of ASC-EVs with surface modifications achieved through peptide* grafting, showing promising effects on cell viability.

Our findings highlight the potential of cultivating ASCs in a three-dimensional scaffold-free extracellular matrix to enrich miRNAs within EVs, enhancing their anti-tumor activity. Future research will focus on assessing the clinical viability of using these EVs in treating osteosarcoma, paving the way for innovative therapeutic strategies against this challenging bone malignancy. *Names are withheld due to patent and publication considerations.

[14] Gaining insight in central nervous system derived extracellular vesicles utilizing vitreous humor Development of new therapies against multiple sclerosis based on bioactive lipid-loaded extracellular vesicles

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Within the central nervous system (CNS), extracellular vesicles (EVs) have sparked interest for their potential use as drug delivery tools, biomarkers, and therapeutic targets for neurodegenerative diseases due to their evolving role in intercellular communication. Currently, CNS EVs are isolated from tissue, cerebrospinal fluid or plasma, albeit all having drawbacks. Here, we propose using vitreous humor derived-EVs to investigate CNS EVs. The retina, being an integral part of the CNS, shares a similar cellular composition and (patho)physiological processes with the brain. Only separated by the inner limiting membrane, the vitreous humor is suggested to be a liquid biopsy of the retina, and is known to be rich in EVs. Up till now, using vitreous-derived EVs to study neurodegenerative processes has remained unexplored. In this study, mouse vitreous-derived EVs were isolated via size exclusion chromatography, and characterized using nanoparticle tracking analysis, electron microscopy and western blot for EV and cell-type markers. Briefly, EV concentrations isolated from 30µL vitreous comprised on average 10E10 particles/mL, with diverse morphologies and sizes ranging between 50-350nm. Western blot analyses confirmed that isolated EVs display neuronal and glial markers. In a second step, retinal neurodegeneration and -inflammation was induced via an optic nerve crush or lipopolysaccharide injection, which impacted the EV release/uptake ratio without altering EV size. Moreover, depending on the model, neuronal and/or glial markers were upregulated. An ongoing proteomic analysis will assess the EV cargo in these experimental conditions. In conclusion, this study demonstrates the potential of the vitreous humor as a liquid biopsy to obtain and study retinal EVs. The presence of neuronal and glial EVs suggests that this biofluid provides unique opportunities to study CNS-derived EVs, including their involvement in neuroinflammatory and -degenerative processes.

[15] The display of an anti-CS1 nanobody by small extracellular vesicles does not improve disease targeting in multiple myeloma

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Introduction: Multiple myeloma (MM) is an incurable plasma cell cancer primarily found in the bone marrow. Due to the inevitable development of refractory disease, new therapeutic options are essential. Small extracellular vesicles (sEVs) show promise as therapeutic delivery vehicles because of their high biocompatibility and ability to cross biological barriers. This study aims to engineer HEK293-derived sEVs to display a nanobody (Nb) targeting CS1, a recognized surface marker of MM cells, to enhance sEV specificity to MM-associated organs and cells.
Methods: HEK293 cells were stably transfected with Nb-SDC1CTF fusion proteins, linked to the CD4 juxtamembranal domain to prevent membrane cleavage. Western blot and confocal microscopy confirmed correct sEV sorting and membrane topology. Control sEVs without Nb and with an irrelevant Nb were included. Binding of anti-CS1 Nb-displaying sEVs to CS1 was assessed by incubating sEVs with soluble CS1 and analyzing size-exclusion chromatography fractions for co-elution by western blot. DiR-labeled sEVs were injected intravenously in 5T33MM mice, and after 24 hours, organs were imaged. MM cell specificity was determined by measuring DiR fluorescence via flow cytometry.
Results: Cleavage-resistant constructs were expressed correctly and enriched in sEVs. Both human and murine anti-CS1 Nb-displaying sEVs bound soluble CS1 forms. However, no enhanced sEV specificity towards MM cells was observed in mononuclear cells from spleen, spine, and legs. Interestingly, in myeloma-bearing mice, anti-CS1 Nb increased sEV accumulation in the liver and lungs.
Conclusion: Anti-CS1 Nbs were successfully displayed on the sEV surface. While these EVs bind soluble CS1 efficiently, they do not improve MM targeting in vivo. Further work will explore suborgan distribution in the lungs and expand the flow cytometric panel to include immune cell populations.

[16] MAFB Expression in LPS-Induced Exosomes: Revealing the Connection to sepsis-triggered Hepatic Injury

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Sepsis poses a significant global health threat, necessitating extensive exploration of indicators tied to its pathological mechanisms and multi-organ dysfunction. While murine studies have shed light on sepsis, the intricate cellular and molecular landscape in human sepsis remains enigmatic. Exploring the influence of activated monocyte-derived exosomes in sepsis sheds light on a promising pathway for understanding the intricate cellular and molecular mechanisms involved in this condition in humans. In sepsis, exosome-borne mRNA and miRNA orchestrate immune response gene expression in recipient cells. Yet, the specifics of exosome-mediated cell-to-cell communication, especially how mRNA cargoes modulate gene expression in recipient cells, remain poorly understood. This study focuses on the role of activated monocyte-derived exosomes in sepsis, specifically investigating how exosomal mRNA cargoes, particularly MAFB, influence gene expression in liver cells. THP-1 cells were treated with LPS to induce changes in exosomal RNA profiles. Exosomes were isolated and characterized using microscopy and mass spectrometry. The most abundant exosomal mRNAs were subjected to GO analysis for functional annotation analysis and KEGG database analysis to identify the involved enriched pathways. PCR (Polymerase Chain Reaction), RNA sequencing, and Western blotting were involved to analyze changes in gene expression, protein levels, and signaling pathways within the liver cells(HepG2) after exposure to MAFB.

This study pinpoints exosomal MAFB as a potential key regulator linked to liver cell damage during sepsis, along with associated genes (miR155HG, H3F3A, and possibly JARD2) forming a crucial molecular pathway contributing to liver cell injury, Together, these elements indicate a vital molecular pathway that plays a significant role in the emergence of liver cell injury during sepsis.
Keywords: sepsis; exosome; MAFB; LPS-induced THP cells; sepsis-triggered hepatic injury

[17] Bacterial extracellular vesicle analysis: challenges and opportunities

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INTRODUCTION: Bacterial extracellular vesicles (BEV) enable intercellular communication between bacteria and their natural habitat, creating a unique ecosystem that affects physiology and disease. Consequently, the study of BEV has rapidly gained attention with recent research revealing considerable promise for diagnostic and therapeutic applications. Although diverse technologies are available, the composition of their source, their heterogeneity in biophysical and biochemical features, and their multi-faceted cargo composition challenges their analysis. We conducted a systematic review to map the current practices in BEV research.
METHODS: We analyzed 845 publications released between 2015 and 2021, reporting 3338 BEV-related experiments. For each experiment a checklist of 233 parameters related to source, preparation protocol and characterization method was completed. The extracted data are publicly available on the EV-TRACK platform (http://evtrack.org).
RESULTS: Across these 845 BEV-related studies, heterogeneous nomenclature is applied to denote particles released from bacterial cells. The term “bacterial extracellular vesicle” is reported in less than 20% of studies, although its implementation increases over time. The 845 studies analyzed, report 51 unique methods and 934 unique protocols to prepare BEV from their source. Differential (ultra-)centrifugation is the most implemented method (97%), followed by filtration (90%). Only 24.5% of experiments perform both particle and protein characterization. Strikingly, one fourth of experiments report particle nor protein characterization.
CONCLUSION: In conclusion, mapping current BEV research practices, identifying knowledge gaps, and offering recommendations will refine guidelines, raise awareness, and promote informed dialogue among researchers. As interest in BEV research grows, ensuring transparency and reproducibility will be essential for advancing biological insights and developing BEV-based applications.

[18] Mitochondria regulate endosomal maturation in VEGF-pathway

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The role of endosomal compartment in cell physiology is now more and more studied, and a huge variety of regulation mechanisms depending on context, pathways,… has been recognized. In our lab, we dove into the study of the Vascular-Endothelial Growth Factor (VEGF) regulation at the level of early endosomes. Indeed, VEGF binds its receptor VEGFR2, which dimerizes, is activated by trans-phosphorylation and is internalized by endocytosis. The activated receptor thus reaches early endosomes, where effectors are recruited on several phosphorylated residues located on cytosolic domain of the receptor and then mediate diverse intracellular pathways.

Very interestingly, we noticed that mitochondria regulate negatively VEGF pathway by controlling endosomal maturation. Indeed, VEGF induces the production of mitochondrial-derived vesicles (MDVs) that enter in contact with early endosomes. Preventing the production or trafficking of MDVs inhibits endosomal maturation. This leads to an hyperactivated state of VEGFR2 and its effectors. These results show for the first time a role of MDVs in signaling and place mitochondria as central regulator in the endosomal compartment.

We will find out the biochemical mechanisms behind MDV-mediated regulation of endosomal maturation.

[19] Placental Exosomes as Novel Identified Carriers of Black Carbon to Fetal Organs

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Background: Black carbon (BC), a key component of particulate matter from combustion, poses significant health risks, especially during fetal development. BC accumulates in syncytiotrophoblasts, the primary placental cells responsible for nutrient exchange and exosome production. This study, part of the ENVIRONAGE birth cohort, explores the role of placental exosomes in transporting BC at the maternal-fetal interface.
Methods: Placental tissue and cord blood samples were collected from 10 mother-child pairs at East-Limburg Hospital (Genk, Belgium). Placental exosomes were isolated using precipitation, size exclusion chromatography (SEC), and PLAP-coupled magnetic beads. BC levels were measured in placental tissue and exosomes using white light generation under femtosecond pulsed illumination. Z-stack confocal microscopy, with Ceramide staining, confirmed BC particles inside the exosomes.
Results: BC was detected in placental tissue and exosomes in all samples, confirmed by z-stack confocal imaging. The advanced isolation techniques and imaging provided strong evidence of BC within placental exosomes. A Spearman correlation between the log10-transformed BC levels in placental tissue and exosomes yielded a coefficient of 0.20, with a non-significant p-value of 0.58, indicating preliminary but supportive findings.

Conclusion: Despite the small sample size, this study provides strong preliminary evidence that placental exosomes can carry BC to fetal organs. The rigorous isolation methods and confirmation of BC internalization suggest a novel pathway for BC's impact on fetal development. Further research is needed to understand the implications of maternal-fetal transmission of air pollutants. This study enhances our understanding of how air pollution affects fetal health, highlighting the importance of further investigation into this critical public health issue.

[20] Role of Extracellular Vesicles in Parkinsonian disorders

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Parkinson's disease (PD) is the fastest-growing neurodegenerative disease, of which both α-synuclein (αSyn) aggregation and propagation are known to be a major event in its pathophysiology. PD diagnosis is based on clinical motor symptoms, which only arise after irreversible neuronal damage has already occurred. Of note, the neuropathological changes in the brain can precede symptoms by decades, highlighting a still unmet need for new non-invasive diagnostic and prognostic PD biomarkers. In this context, mounting evidence points to extracellular vesicles (EVs) as a source of biomarkers, being mediators of intercellular communication and carriers for disease-associated proteins including αSyn. More specifically, neuronal-derived EVs (nEVs) separated from peripheral biofluids can provide a snapshot of ongoing pathological changes in the brain, thereby constituting a promising diagnostic tool of PD. In our work, EVs were isolated from human plasma samples via a combination of SEC and density gradient centrifugation, whereafter EV concentration, size, morphology and tetraspanin distribution were characterized with NTA, TEM and ExoView. To enrich for nEVs, immunoprecipitation with the neuronal marker L1CAM is explored. To validate whether αSyn levels in (n)EVs are representative for αSyn brain pathology, we established a preclinical mouse model based on the intrastriatal injection of αSyn PFFs in wildtype mice. Preliminary results indicate successful EV isolation from plasma. Furthermore, we confirmed the presence of several pathological hallmarks including the deposition of αSyn aggregates, neuroinflammation and loss of dopaminergic neurons in our PD mouse model, supporting its feasibility to validate whether plasma (n)EVs can represent a liquid brain biopsy. This study will demonstrate if αSyn (n)EV levels hold promise as diagnostic biomarker for PD and if αSyn (n)EV levels correlate with severity of PD pathology in both preclinical mouse models and human samples.

[21] Extracellular vesicle-microRNAs: key players in lipopolysaccharide-induced inflammation in the eye

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Parkinson's disease (PD) is the second most prevalent neurodegenerative disease worldwide, currently lacking adequate diagnosis and treatment. The disease is characterized by the loss of dopaminergic neurons in the substantia nigra, and the accumulation and aggregation of alpha-synuclein into cytoplasmic inclusion bodies called Lewy bodies. Another major hallmark of PD is excessive and/or sustained neuroinflammation, though the molecular and cellular players underpinning this event remain incompletely understood. Recently, a role emerged for extracellular vesicles (EVs) in the neuroinflammatory process, being important mediators of intercellular communication. These nanosized membrane-enclosed vesicles have been shown to transport cytokines, misfolded proteins such as alpha-synuclein, as well as microRNAs (miRNAs) over short and long distances. Of note, increasing evidence points to the dysregulation of several miRNAs being a major driver in PD pathogenesis and, more specifically, in neuroinflammation. However, the exact role of EV-miRNAs in neuroinflammation in PD in vivo remains unclear. In this project, we are filling this knowledge gap applying an unbiased multi-target systematic approach. Considering the high prevalence of visual dysfunction/retinal abnormalities in PD and the unique advantages of the eye as a research model, we use the eye as a window to the brain. Neuroinflammation and other PD characteristics are induced in vivo in the mouse eye via a systemic injection of lipopolysaccharide (LPS). Exploiting this novel eye neuroinflammation model, the microglial EV-miRNA profile is determined and the role of these EV-miRNAs in neuroinflammation is studied. Ultimately, this project will lead to a better understanding of the function of EV-miRNAs in neuroinflammation and reveal novel therapeutic targets and promising biomarkers for the diagnosis of PD and other related disorders.

[22] Smart bioprocess technology platform enabling scalable and sustainable process development of upstream, adherent cell-based extracellular vesicle manufacturing

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Extracellular vesicle (EV)-based therapies offer new possibilities for patients, but their clinical development is hindered by the lack of technologies to scale lab methods into manufacturing processes that produce large quantities of high-quality EVs. Enhancing the availability, accessibility, and affordability (3A) of EVs is crucial.

Antleron has co-developed a bespoke, scalable, adherent cell-based EV manufacturing process with convEyXO for the latter's proprietary stem cells, using a smart bioprocess platform that integrates process modeling and 3D-printing. This approach links wet lab solutions to digital twins and in silico experiments, enabling faster and smarter bioprocess development in small-scale bioreactors (mL) with results predictive of larger production scales (L). This innovative platform addresses process development and scalability issues more efficiently and cost-effectively.

The EV manufacturing process achieved up to 3E5 EVs/cell (mean of 2.08E5 across tested scales using microfluidic resistive pulse sensing) in an animal component-free medium. This represents a 20x higher yield compared to similar cells in culture flasks (0.1E5 EVs/cell), demonstrating the process' efficiency. EXO-Harvest, an affiliate of convEyXO, has developed a dedicated upstream bioreactor platform for EV production that can accommodate the designed process and any custom 3D-fixed bed. Ongoing developments aim to improve scalability (up to L scale) and reproducibility across scales using DoE and predictive computational modeling.

In conclusion, Antleron's smart bioprocess platform reduces experimental load and accelerates time-to-market, lowering development costs. The combined wet lab and in silico results show that the platform provides efficient and affordable EV production, essential for ensuring the 3As of future EV-based therapies for patients.

[23] Biomarker potential and pathological role of bacterial derived extracellular vesicles in Parkinson's disease

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There is growing evidence for an important role of the gastrointestinal tract, and more specifically the residing gut microbiota, in the etiology and progression of Parkinson's disease (PD). Our research group has recently shown that a single faecal microbiota transplantation (FMT) induces long-lasting beneficial effects on motor symptoms in early-stage PD patients, underscoring the therapeutic potential of gut microbiome modulation. Notably, changes in microbiota composition may be reflected in extracellular vesicles (EVs) released by these bacteria (bacterial EVs or bEVs), making bEVs particularly interesting from a biomarker perspective. Additionally, we and others have shown that bEVs can exacerbate pathology in mouse models of neurodegenerative diseases including Alzheimer's. However, whether bEVs affect PD pathology remains unclear.
In this study, we aimed to investigate the effects of bEVs derived from faeces of a PD patient versus a healthy control on PD pathology. Thereto, we used a PD injection model in which murine recombinant alpha-synuclein (α-syn) pre-formed fibrils (PFFs) are injected in the striatum of wildtype mice. Eight months after PFF injection, we administered the bEVs through multiple oral gavages over a three-week period. We then assessed the impact of the bEVs on PD pathology by evaluating motor function, α-syn pathology, microglial activation and dopaminergic neuron loss. However, we found no significant effect of bEVs on PD pathology in neither the control nor the PD bEV conditions.
In conclusion, our data indicate that bEVs derived from faeces of a PD patient did not influence pathology in a late-stage PD mouse model. Further research is needed to validate these results across different disease stages and using bEVs from additional samples. Moreover, we will explore the biomarker potential of (b)EVs for PD and their utility in monitoring treatment response in our FMT trial.

[24] Mitochondria in tumor cell-to-cell communication

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Mitochondria are known to be highly dynamic organelles capable of fusion, fragmentation, and numerous inter-organelle contacts. In our laboratory, we view mitochondria as platforms that regulate signaling pathways and control cell-to-cell communication. Preliminary data indicate that mitochondria influence the secretion and composition of extracellular vesicles, which play a crucial role in cancer cell communication with the tumor microenvironment. Our research has shown that by controlling extracellular secretion, mitochondria are also involved in the interaction between cancer cells and their microenvironment. This highlights mitochondria's significant role in modulating cellular communication in cancer.