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Zeineb IBN ELFEKIH, ICGM, Montpellier, France

Submission form

Please complete the present submission form and upload it on the registration form by **April 15, 2025.**
Selection will be confirmed on **May 15, 2025.**

Title of the abstract	Functional and structural studies of the AcrAB-TolC efflux pump from Escherichia coli in native vesicles
Authors and affiliations	**Florent Beguin** Laboratoire de Biologie Physico-Chimique des Protéines Membranaires, Université de Paris, UMR 7099 CNRS, F-75005, Paris, France Dhenesh Puvanendran Laboratoire de Biologie Physico-Chimique des Protéines Membranaires, Université de Paris, UMR 7099 CNRS, F-75005, Paris, France
Laboratory	Institut de biologie physico-chimique
Email address main author	beguin@ibpc.fr
Abstract about 200 à 300 words *In English*	As an alternative to vesicles made from synthetic PLs, we aim to develop an approach for incorporating AcrAB-TolC directly into native vesicles to maintain its native surrounding environment. This strategy avoids multiple steps, such as detergent extraction, which could alter protein stability and activity. Ultimately, this method will enable the study of the function and structure of this system directly in its membrane environment, providing an in vivo assessment of its real structure and antibiotic transport activity.
Keywords	Escherichia coli Natives vesicles Efflux pumps

Short talk session

Submission form

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Title of the abstract	Optimization of extracellular vesicles-based therapy in the treatment of ischemic stroke
Authors and affiliations	**Ons BEN HADJ HASSEN[1]**, Sarah RAZAFINDRAKOTO[2], Jérôme TOUTAIN[1], Isis BLANCHARD[1], Viktoriia IVANOVA[1], Charlène RENOULT[1,3], Xavier LAFFRAY[4], Minh-Bao HUYNH[4], Carole BRUNAUD[1], Karim BORDJI[1], Gael LE DOUARON[4], Wilton Albeiro Gomez HENAO[4], Mohand Ouidir OUIDJA[4], Agnès CHOPPIN[3], Denis BARRITAULT[3], Dulce PAPY-GARCIA[4], Amanda SILVA BRUN[2]*, Franck CHIAPPINI[3]*, Omar TOUZANI[1]*, Florence GAZEAU[2]*, Myriam BERNAUDIN[1]* *co-authorship (1) Université de Caen-Normandie, CNRS, Normandie Université, ISTCT UMR6030, GIP CYCERON, F-14000 Caen, France (2) NABI, Université Paris Cité, INSERM U1334, CNRS UMR8175, 75006 Paris. (3) OTR3, 4 rue Française, 75001 Paris, France (4) Glycobiology, cell growth and tissue repair research unit (Gly-CRRET), Université Paris Est Créteil (UPEC), F-94010, Créteil, France
Laboratory	UMR 6030-ISTCT
Email address main author	benhadjhassen@cyceron.fr
Abstract about 200 à 300 words *In English*	Ischemic stroke (IS) remains a major health problem. Extracellular vesicles (EV), derived from mesenchymal stem cells (MSC), have been shown to mitigate brain damage and neurological outcome following IS. Though promising, EV production and their route of delivery remain a major challenge. Here, the effects of EV generated by an innovative turbulence approach were characterized on stroke-induced brain lesion and neurological deficits in the rat. EV were isolated from human adipocyte tissue stromal cells (hASC) using a patented high-yield turbulence bioproduction process. EV were quantified and

	characterized using nanoparticle tracking analysis (NTA), transmission electron microscopy (TEM) and ExoView. Rats were subjected to a transient occlusion (1h) of the middle cerebral artery (tMCAO) and treated with EV at reperfusion time (1010 EV/rat; I.A.) or 24h later (1011 EV/rat; I.V.). MRI were performed at 1 and 14 days after tMCAO to quantify brain lesion volume. Behavioral tests were carried out up to 40 days post-tMCAO. Multiple analyses confirmed EV characteristics. While I.V. EV administration showed limited therapeutic effects. I.A. EV delivery at reperfusion time reduced the lesion volume 24h post-tMCAo compared to the control group ($p< 0.05$). Moreover, 7 days after tMCAO, rats treated with EV immediately after reperfusion tend to improve their neurological score ($p= 0.07$) and showed a significantly better limb placing test performance ($p<0.05$). These rats were less lateralized compared to the control ones in the corner test and performed better in the adhesive test 30 days after tMCAO ($p< 0.01$). In contrast, I.V. EV administration 24h post-tMCAO showed only a significant effect on spatial memory ($p< 0.05$, t-test). These results suggest that MSC-derived EV produced by an original turbulence method are efficacious on stroke-induced brain lesion and neurological deficits and optimizing the administration route and timing enhances the efficacy of EV after stroke
Keywords	Stroke, Ischemia, Extracellular-vesicles, mesenchymal stem cells

Short talk session

Submission form

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Poster :

Title of the abstract	First isolation and characterization of EVs in the hemocytes and digestive cells of the oyster *Crassostrea gigas*
Authors and affiliations	**Clothilde Berthelin[1]**, Lola Lévêque[1], Valentin Mary[1], Ange Midroit[1], Nadège Villain-Naud[1], Nicolas Elie[2], Didier Goux[2], Kristell Kellner[1] 1- Université de Caen Normandie, Laboratoire MERSEA - UR7482 - Marine Ecosystems and oRganisms reSEArch lab, 14000, Caen, France 2- Université de Caen Normandie, EMerode Services Unit, CMAbio3, 14000, Caen, France
Laboratory	Laboratoire MERSEA - UR7482 - Marine Ecosystems and oRganisms reSEArch lab, 14000, Caen, France
Email address main author	clothilde.berthelin@unicaen.fr
Abstract about 200 à 300 words *In English*	Extracellular vesicles (EVs) are now recognized as key mediators in the transport of intercellular biochemical messages. Playing a crucial role in various biological processes, their involvement in invertebrates, such as bivalve mollusks, is beginning to be explored. Oysters are common carriers of noroviruses and are responsible for their transmission. Understanding the implication of oyster Evs in viral accumulation and dissemination is of interest in the context of human viral diseases related to the consumption of bivalve shellfish. This study focuses on the first isolation and characterization of EVs from hemocytes (immune defense cells) and digestive cells of the Pacific oyster, *Crassostrea gigas*. These both types of cells contain noroviruses in contaminated oysters (Le Guyader et al., 2006; Maalouf et al., 2011; Su et al., 2018). Isolation, based on ultracentrifugation, resulted in EVs with size heterogeneity observed across samples. The ultrastructure of the EVs was examined using transmission electron microscopy (TEM). The protein content of the obtained EV fractions was quantified. Two EV markers, CD63 and Alix, showed promising results on oyster cross-sections as well as on isolated cells. These first results on EVs oyster characterization allowed further development based on transcriptomic and proteomic approaches.

Keywords	Oyster EVs, Hemocytes, Digestive cells, Noroviruses

Identification of surface biomarkers on circulating EVs for monitoring hepatic tumor progression by liquid biopsy

C. Berthy[1,2]*, M. Allaire[3], M. Boissan[1,2,3], I. Petit[1]
[1]INSERM, U938, CRSA, Hôpital Saint-Antoine, [2]Sorbonne University, [3]AP-HP, Hôpital Pitié-Salpêtrière, Paris, France.
* Corresponding Author clement.berthy@inserm.fr

Abstract
Tumor extracellular vesicles (EVs) are present in biofluids and serve as a potential source of accessible tumor markers for disease monitoring and precision therapeutics. In this study, we aim to identify surface proteins on circulating EVs that could reflect the tumor progression and/or predict the response to immunotherapy in hepatocellular carcinoma (HCC). HCC is the most common primary liver cancer and is associated with a particularly poor prognosis due to late diagnosis. Since 2021, the first-line systemic treatment for advanced HCC consists of an immunotherapy combination targeting PD-L1 (atezolizumab) and VEGF (bevacizumab), with a response rate of only 27%. Therefore, there is an urgent need to identify biomarkers that can aid in the disease diagnosis and/or predict treatment response in patients receiving immunotherapy. Here, we present the purification and analysis of circulating EVs from plasma samples of HCC patients at different stages, as well as cirrhotic patients and healthy controls. Using beads-based multiplex analysis by cytometry, we identified several markers specific to HCC samples. Additionally, we investigated the presence of the soluble ligands PD-L1 and VEGF, both of which can associate with EVs and are targeted by the immunotherapy and compared their levels to the clinical profiles of the patients.
This work highlights that EV surface profiling may help identify novel biomarkers for HCC detection or longitudinal monitoring using liquid biopsies.

Submission form

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Poster :

Title of the abstract	High fat diet in mice affects the physicochemical properties and the miRNA signature of skeletal muscle release extracellular vesicles which alter macrophage phenotype
Authors and affiliations	Sanjay B. Vasan[1*], Stefano Tacconi[1,2*], Simone Dinarelli[3], Elizabeth Errazuriz-Cerda[4], Christelle Cassin[4], Joao Pais-De-Barros[5], Jennifer Rieusset[1], Sophie Rome[1] **co-authors* 1-Laboratoire CarMeN, UMR INSERM 1060/INRAE-1397, University of Lyon, FRANCE 2-Department of Biology and Biotechnology "C. Darwin", Sapienza University, Rome, ITALY 3-Istituto di Struttura della Materia, CNR, Rome, ITALY 4-Centre d'Imagerie Quantitative Lyon-Est (CIQLE), Université of Lyon, FRANCE 5-Plateforme de Lipidomique, University of Bourgogne, INSERM UMR1231 / LabEx LipSTIC, UFR des Sciences de Santé, Dijon, FRANCE
Laboratory	Laboratoire CarMeN, UMR INSERM 1060/INRAE-1397, University of Lyon, FRANCE
Email address main author	sanjay.b.vasan@gmail.com; srome@univ-lyon1.fr
Abstract about 200 à 300 words *In English*	**Introduction**: Chronic low-grade inflammation is a major etiologic component of the pathogenesis of insulin-resistance (IR) in diabetes and the skeletal muscle (SkM) is the first organ affected. SkM continuously self repairs in response to physical activity, nutrition and inflammation, through the release of cytokines in the regenerative niche. Pro-inflammatory M1 macrophages clear damaged tissue, while anti-inflammatory M2 macrophages support tissue repair. However, the diabetic condition hampers the transition from M1 to M2 altering muscle regeneration and inducing SkM weakness. We hypothesize that SkM-derived EVs modulate macrophage phenotype during muscle regenation, but that IR disrupts this cross-talk. **Methods**: 5-month-old male C57BL/6 mice were fed a high-fat diet (HFD: 60% fat, 20% carbohydrate, including sucrose 7%) or a standard diet (SD: 7,5% fat) for 15 weeks. *Gastrocnemius*-released EVs (HFD-EVs, SD-EVs) were

	purified and characterized by WB, Transmission and Atomic Force Microscopy (TEM, AFM), lipidomics and sequencing of the miRNA population. **Results**: HFD mice were obese and insulin-resistant. The HFD diet affected the EV lipid composition as HFD-EVs were enriched in sphingomyelin and phosphatidylcholine, but had lower level of free fatty acids, *vs* SD-EVs. AFM showed that HFD-EV lipid changes were associated with reduced stiffness and lower sizes, *vs* SD-EVs. SD-EVs induced the M1 marker CD86 in macrophages and their migration. After 30min, HFD-EVs were better internalized into macrophages, *vs* SD-EVs, but induced anergic macrophages. In addition, HFD-EVs altered macrophage migration. The level of 13 miRNAs was increased in HFD-EVs. We predicted their conserved target genes and analysed their Gene Ontology function. The most significant Kegg pathway was "*Cytokine Signaling in immune System*", suggesting a direct role of SkM-EVs on macrophage homeostasis. **Conclusion**: These data show that dietary fat affects the lipid composition of SkM-EVs and consequently their incorporation into recipient macrophages. They also suggest that SkM EVs might participe in the altered SkM regeneration in diabetic subjects.
Keywords	skeletal muscle, metabolism, lipids, miRNAs, inflammation, macrophages, diabetes, insulin-resistance

Extracellular vesicles derived from ovarian cancer cell lines discriminated by biochemical and Fourier transform infrared spectroscopy approaches

Lefkothea Pantazi,[1,2] Valérie Untereiner,[3] Paolo Rosales,[1,4] Romain Rivet,[1] Sandra Audonnet,[5] Isabelle Proult,[1] Laurent Ramont,[1,6] Ganesh D. Sockalingum[7] and **Stéphane Brézillon[1]**

1)Université de Reims Champagne-Ardenne, UMR CNRS 7369, MEDyC, Reims, France. 2) University of Patras, Biochemistry, Biochemical Analysis & Matrix Pathobiology Research Group, Laboratory of Biochemistry, Department of Chemistry, Patras, Greece. 3) Université de Reims Champagne-Ardenne, URCATech, PICT, Reims, France. 4) UNNOBA/CIT NOBA (UNNOBA-UNSADA-CONICET), Laboratorio de Microambiente Tumoral, CIBA, Junín, Argentina. 5) Université de Reims Champagne-Ardenne, URCATech, URCACyt, Reims, France. 6) CHU de Reims, Service Biochimie-Pharmacologie-Toxicologie, Reims, France. 7) Université de Reims Champagne-Ardenne, BIOSPECT, Reims, France.

Ovarian cancer is the most lethal cancer among gynaecological malignancies. Due to the lack of early symptoms and screening tools, patients are diagnosed in advanced stages. Cancer invasion and metastasis through the extracellular matrix (ECM) are enhanced by tumour cell Extracellular Vesicles (EV). The aim of this study was to characterise the EVs derived from two ovarian cancer cell lines (ES2 and SKOV3) using biochemical and vibrational spectroscopic approaches. EVs were prepared by ultracentrifugation and characterised by Nanoparticle Tracking Analysis. Their surface proteins were assessed by MACSPlex EV kit for human exosomes. The presence of MMP14 and integrin subunits was evaluated in EVs and cell protein extracts by Western immunoblotting. Both EVs and cells were measured by Fourier transform infrared spectroscopy (FTIR) and data were analysed by hierarchical cluster analysis (HCA). Spectral differ_ences were observed in the lipids and polysaccharides regions both between the SKOV3 and ES2 cells and their corresponding EVs, which allowed a good delineation by HCA. The differences in the biochemi_cal data were confirmed by similar and specific features exhibited in their respective infrared spectral sig_natures. ES2 EVs exhibited an enrichment in MMP14 in agreement with the aggressiveness of this ovarian cancer metastatic cell line.

Short talk session

Submission form

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Title of the abstract	Versatile Tethering System to Control Cell-specific Targeting of Bioengineered Extracellular Vesicles
Authors and affiliations	**Sheryl Bui**[1], Jeanne Lainé[2], Maud Chevé[1], Stéphane Vassilopoulos[2], Gregory Lavieu[1] [1]Université Paris Cité - INSERM U1334 - UMR 8175 [2] Sorbonne Université, Institute of Myology, INSERM UMRS 974
Laboratory	NABI Lab - Communication and extracellular transport team Université Paris Cité - INSERM U1334 - UMR 8175
Email address main author	sheryl.bui@etu.u-paris.fr
Abstract about 200 à 300 words *In English*	Extracellular Vesicles (EVs) are natural vectors of communication involved in a broad range of physiological functions. Significant efforts have been devoted to use EVs for therapeutic delivery. The main challenges have been controlling and enhancing the key fundamental steps of EV-mediated delivery. We and others have developed solutions to improve cargo/therapeutic loading into EVs and to enhance EV content delivery through the use of viral or non-viral fusogens. Recently, only a few solutions have been proposed to achieve targeting with limited flexibility. Here, we present a versatile system that enables precise EV targeting to specific cell types while allowing quantitative assessment of targeting efficiency using luminescence and fluorescence analysis. In this system, EVs are genetically engineered to express a chimeric adapter protein anchored to their surface via a glycosylphosphatidylinositol (GPI) anchor. This protein includes a fluorescent and/or luminescent domain for visualization/quantification and a streptavidin domain to recruit biotinylated antibodies or ligands specific to cell-surface antigens or receptors. We validated this platform with three different combinations of ligand/target cells, demonstrating up to 40-fold increase in EV uptake. Combined with previously developed modules for enhanced cargo loading and delivery, this adaptable system promises to provide a comprehensive solution for targeted therapeutic delivery using EV-based vectors.
Keywords	extracellular vesicle – drug delivery – targeting – bioengineering – biotin – streptavidin – versatility

THE ENTERIC NITRERGIC PATHWAY, A TARGET OF MICROBIOTA EXTRACELLULAR VESICLES IN AUTISM-RELATED GASTROINTESTINAL DISORDERS

Martial Caillaud[1], Baptiste Ganachaud[1], Irem Buruk, Clémence Lalloué, Mathéus Moreau[1], Justine Marchix[1], Catherine Le Berre-Scoul[1], Michel Neunlist[1], Hélène Boudin[1]

[1]Nantes Université, INSERM, TENS, The Enteric Nervous System in Gut and Brain Diseases, IMAD, Nantes, France.

Objective: Autism spectrum disorders (ASD) are neurodevelopmental disorders defined by deficits in social interactions, repetitive behaviors, and restricted interests. In addition to behavioral disorders arising from neural connectivity abnormalities in the brain, approximately 70% of children with ASDs exhibit gastrointestinal (GI) symptoms (diarrhea, constipation, bloating, abdominal pain). Gastrointestinal disorders significantly affect the quality of life of these patients, and can exacerbate behavioral symptoms. The pathophysiological mechanisms of ASD are still unknown, but dysfunctions of the microbiota-gut-brain axis seem to be particularly involved. Several teams, including ours, have shown that people with ASD show changes in the composition of the gut microbiota. Moreover, our work shows that transplantation of ASD fecal supernatant in mice induces a decrease in intestinal permeability and a remodeling of enteric nervous system (ENS) connectivity, the nervous system which regulates intestinal function. In addition, the transfer of microbiota from ASD patients to mice results in behavioral as well as digestive symptoms, apparently affecting the ENS nitrergic pathway. Thus, one hypothesis is that changes in the gut microbiota of ASD subjects induce digestive disorders via their impact on the connectivity of the ENS nitrergic pathway. Nevertheless, the mediators involved are still largely unknown. Cellular mediators from the gut microbiota include bacterial metabolites, membrane components and extracellular vesicles (EVs). The latter are particularly relevant because of studies indicating that bacterial EVs act as communication vectors between the microbiota and host organs. The hypothesis is that fecal EVs (f-EVs), which contain EVs produced by the microbiota, are mediators between the microbiota and enteric neurons, targeting the intestinal nitrergic pathway and contributing to gastrointestinal disorders in autism.

Methode: We isolated f-EVs from the stools of controls and patients with ASD. The f-EVs were administered to mice by gavage for 2 weeks, and behavioral and digestive tests were performed. The f-EVs were also applied to enteric neuron cultures and their impact on neuronal activity and the nitrergic pathway was assessed.

Results: Firstly, we observed different profiles (size, number, metabolites...) between f-EVs from controls and patients with ASD. In vivo, f-EVs from patients with ASD induced abdominal pain and changes in intestinal motricity associated with changes in the intestinal nitrergic pathway. In vitro, treatment of enteric neurons with f-EV from patients with ASD induced greater activity of this neurons. In addition, we observed an increase in the number of nitrergic neurons and an increase in the expression of neuronal nitric oxide synthase (nNOS), the nitric oxide (NO)-producing enzyme. An increase in NO production was also observed in cultures treated with f-EVs from ASD patients compared with controls. Interestingly, this increase in NO was also observed in the intestines of mice treated with f-EVs from ASD patients.

Conclusion: Our results suggest that the intestinal neuronal nitrergic pathway might be a target of gut microbiota in autism-related gastrointestinal disorders.

Short talk session

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Poster :

Title of the abstract	*ExoSPY – An innovative tool for early diagnosis of pancreatic cancer using engineered exosomes*
Authors and affiliations	**Chloé CHAMBARD***, Selma KOCIER*, Lina VERSCHUERE*, Yacine EL BICHARA*, Justine PELLIER**, Héloïse PLOYON***, Guillaume BANON***, Matthieu VIEZZI***, Thibault SOULA*** * «Master 1 Sciences et Management des Biotechnologies» _UGA ** «Licence 3 Biotechnologies pour la Santé» _UGA *** Biomedical Engineering _INP Phelma
Laboratory	UGA iGEM Team, 1 Place Commandant Nal, Bâtiment Jean Roget, 38700 La Tronche
Email address main author	uga.igem.2025@gmail.com
Abstract about 200 à 300 words *In English*	Pancreatic cancer remains one of the deadliest cancer due to its late diagnosis and unfavourable prognosis. In fact, the 5-year survival rate is 10 percent thus there remains a need for an innovative diagnostic tool. We are a multidisciplinary team composed of nine students at the University Grenoble Alpes taking part in the iGEM competition. iGEM is an international competition of synthetic biology including teams of students working on a wide range of subjects going from software to arts. We decided to focus on oncology, particularly on the early diagnosis of pancreatic cancer. Up to now, numerous studies have shown the existence of the interaction between the *Clostridium perfringens* enterotoxin (CPE) and Claudine-4 (CLDN4), a tight junction protein, which is not accessible in normal cells, but becomes attainable in cancerous tissues. Our project consists in the production of modified exosomes

	expressing on their surface the CPE ligand to target pancreatic cancer cells in the human body. After modelling the ligand-receptor interaction, the plasmids of interest will be designed. They code for the specific CPE ligand fused to a membrane anchor and a GFP-type fluorescent protein, which will be then transfected into HEK293 cells. The exosomes produced by the recombinant cells will be extracted by ultracentrifugation and analyzed (measurement of size distribution by Nanoparticle Tracking Analysis), observation by electron microscopy and verification of ligand presence by fluorescence). We will determine their ability to interact and bind to the receptor on healthy and cancerous pancreatic cell models, grown in vitro in monolayers and then in organoids to approximate physiological conditions. Once these steps have been validated, the exosomes will be loaded with a paramagnetic molecule so that, once injected into the patient, they can be tracked in the body by MRI and their biodistribution and affinity for the pancreas can be verified.
Keywords	Pancreatic cancer, early diagnosis, engineered exosomes

Short talk session

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Title of the abstract	Identification of key regulators of extracellular vesicle production in *Cryptococcus neoformans*
Authors and affiliations	Alicia C. Piffer[1], Jean-Yves Copee[1], Frédérique Moyrand[1], Cosmin Saveanu[1],Thierry Fontaine[2] and Guilhem Janbon[1] [1] Institut Pasteur, Université Paris Cité, Unité Biologie des ARN des Pathogènes Fongiques, Paris, France. [2] Institut Pasteur, Université Paris Cité, Unité Biologie et Pathogénicité Fongiques, Paris, France.
Laboratory	Unité Biologie des ARN des Pathogènes Fongiques, Institut Pasteur, Paris, France
Email address main author	alicia.corbellini-piffer@pasteur.fr
Abstract about 200 à 300 words *In English*	*Cryptococcus neoformans* is a human pathogen classified by WHO as belonging to the critical group of the fungal priority pathogen list. As observed in all fungi species studied to date, *C. neoformans* secretes extracellular vesicles (EVs), transporting several types of molecules, including lipids, polysaccharides, pigments, proteins and RNAs to the extracellular environment. Although their functions are still not fully understood, studies suggest that they are associated with fungal pathogenicity. Despite their importance, the pathways involved in their biogenesis are still unclear. To address this, we took advantage of the relationship between EV biosynthesis and azole susceptibility and screened a library of knockout mutants for their susceptibility to fluconazole aiming to identify genes regulating EV production. One of the first fluconazole-resistant and low EV-producer mutant identified was a strain knockout for HAD1, a putative target of calcineurin in *Cryptococcus*. Using pharmacological inhibitors and genetic approaches, we confirmed that the calcineurin pathway positively regulates EV biogenesis in this yeast. Supporting this, we have shown that the chelation of calcium in the culture medium also alters EV production. In contrast, the transcription factor Crz1, which is one of the most studied targets of the calcineurin pathway in fungi, negatively regulates EV biosynthesis, suggesting a dual role of this pathway in coordinating EV production. By biochemical and genetic approaches, we characterize Had1 as a glycerol-phosphatase, converting glycerol-3-phosphate in glycerol. The

	mechanisms by which glycerol metabolism influences EV generation in *C. neoformans* are still unknown; nevertheless, several strategies, including lipid assays, are being considered to address this question.
Keywords	Fungal EVs, EV biogenesis, glycerol metabolism, calcineurin pathway

Short talk session

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Selection will be confirmed on **May 15, 2025.**

Title of the abstract	Extracellular Vesicles from Canine Hemangiosarcoma Cell Lines as Potential Anti-Cancer Drug Delivery Systems
Authors and affiliations	**Dadi Y[1].**, Filipe J.[2], Zamboni C[4]., Roccabianca P[1]., Stefanello D[1]., Dell'Aere S[1]., DeMatos L[1]., Di Monte M[1]., Ceciliani F[1]., Crippa E[3]., Villa A[3]., Ciana P[3]., Lecchi C[1]. 1 Department of Veterinary Medicine and Animal Sciences, University of Milan, Lodi, Italy. 2 Department of Veterinary Science, University of Pisa, Pisa, Italy. 3 Department of Health Sciences, University of Milan, Milano, Italy. 4 Clinaxel, Clinglobal group Company, Lille Cedex, France
Laboratory	General pathology of the veterinary medicine department in the university of Milan
Email address main author	Yasmine.dadi@unimi.it
Abstract about 200 à 300 words *In English*	Canine splenic hemangiosarcoma (HSA) is an aggressive endothelial cell cancer of blood vessels, mainly affecting geriatric dogs. Splenectomy and adjuvant doxorubicin (DOX) alone or with doxorubicin-based-protocol are the most common treatment, but prognosis remains poor due to metastatic dissemination to organs (lungs, heart, liver, kidney, and brain). Extracellular vesicles (EVs), naturally secreted by almost all cells, can encapsulate different molecules, offering a promising tool for drug delivery due to their immuno-compatibility and targeting properties. This study investigates the potential of EVs derived from canine HSA patients as a DOX vehicle, to target neoplastic cells and reduce its side effects. Primary cell lines from spontaneous splenic HSA samples collected from owned dogs were established and phenotyped using CD31 and Factor VIII-RA antibodies. EVs were isolated from the cell medium by ultracentrifugation and size exclusion

	chromatography and were loaded with DOX through enhanced diffusion. The incorporation of DOX was assessed by NTA and FACS analysis. HSA primary cell lines were challenged with different concentrations of free DOX, EV-loaded DOX, and empty EVs for 4 hours, and cell viability and apoptosis were evaluated. Cultured spindle cells expressed Factor VIII-RA, confirming endothelial origin and demonstrating HSA culture establishment. DOX was efficiently loaded into EVs. After 4 hours of coincubation, a time-dependent increase of apoptosis and decreased viability were observed, indicating that the DOX-free or loaded-in EVs had a cytotoxic effect on the tumoral cells. In conclusion, this pilot study highlights the potential of EVs as an innovative drug delivery system for HSA treatment.
Keywords	Tumor, hemangiosarcoma, Drug delivery, chemotherapy.

Short talk session

Submission form

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Selection will be confirmed on **May 15, 2025.**

Title of the abstract	**Regulation of the extracellular vesicle production in *Cryptococcus neoformans ?***
Authors and affiliations	**Cassandre Deculty[1,2], Patricia Flamant[2], Frédérique Moyrand[2], Pierre-Henri Commere[3], Sophie Novault[3], Rayen Elj[4], Mariette Matondo[4], Gérard Pehau-Arnaudet[5], Adeline Mallet[5], Guilhem Janbon[2] and Isabelle Mouyna[2]** [1] Unité de Biologie des ARN des Pathogènes Fongiques, Institut Pasteur, Université Paris Cité, Paris, FRANCE [2] Unité de Biologie des ARN des Pathogènes Fongiques, Institut Pasteur, 75015 Paris, FRANCE [3] Plateforme de Cytométrie (UTechS), Institut Pasteur, 75015 Paris, FRANCE [4] Plateforme de Protéomique, Unité de Spectrométrie de Masse pour la Biologie (MsBio), CNRS UMR 2000, Institut Pasteur, 75015 Paris, FRANCE [5] Plateforme de Bio imagerie Ultrastructurale (UBI), Institut Pasteur, 75015 Paris, France
Laboratory	Unité de Biologie des ARN des Pathogènes Fongiques, Institut Pasteur, 75015 Paris
Email address main author	cassandre.deculty@pasteur.fr
Abstract about 200 à 300 words *In English*	Fungal pathogens infect each year over 6,55 million people leading to 3,75 million deaths [1]. *Cryptococcus neoformans* is among the three major human fungal pathogens recently identified by the WHO in the critical group of the fungal priority pathogens list, cryptococcal meningitis affecting 194 000 people, with 147 000 deaths

	(75,8%). Extracellular vesicles (EVs) are lipidic nanosized particles that deliver complex molecular cargo between cells and organisms. Since their recent discovery in *C. neoformans*, EVs have been identified in many other pathogenic fungi and have been shown to be involved in many biological processes like intercellular communication, antifungal resistance or biofilm formation. Recently in our laboratory, we described the structural and compositional aspects of *C. neoformans* EVs and we showed a coregulation between EV production and antifungal drug sensitivity [2,3]. However, to date, the biosynthesis pathways regulating EV production remain poorly understood. We investigated the dynamics and genetics of *C. neoformans* EV biogenesis by screening a library of kinase mutant strains for EV production. We showed that the three MAP kinase mutants ***mpk1Δ***, ***mkk2Δ*** and ***bck1Δ***, involved in the cell wall integrity (CWI) pathway, produce a higher amount of EVs. Moreover, EV associated to ***mpk1Δ*** mutant showed an increase in size as demonstrated by Cryo-electron microscopy analysis, as well as a different protein composition and structure compared to the wild strain suggesting different type of EV populations. These mutants are also impaired in cell wall organization as revealed by their sensitivity to cell wall disrupters and by an increase of cell wall permeability. These results suggest that the cell wall composition is a crucial parameter regulating EV production in *C. neoformans*. [1] Denning DW. Lancet Infect Dis. 2024. [2] Rizzo J et al. J Extracell Vesicles. 2021. [3] Rizzo J et al. mBio. 2023.
Keywords	Cell wall integrity, MAP kinases, EV secretion, EV structure, *Cryptococcus neoformans*

Metabolic reprogramming and drug resistance in chronic lymphocytic leukemia (CLL): Role of tumor microenvironment exosomes

Kenza Dubois[1], Karim Maloum[2], Damien Roos-Weil[1,3], Elise Chapiro[1,2], Florence Nguyen-Khac[1,2], Santos A. Susin[1], Delphine Garnier[1]

[1] Sorbonne Université, Université Paris Cité, Inserm, Centre de Recherche des Cordeliers, Cell Death and Drug Resistance in Lymphoproliferative Disorders Team, Paris, France

[2] Service d'Hématologie Biologique, Hôpital Pitié-Salpêtrière, APHP, Sorbonne Université, Paris

[3] Service d'Hématologie Clinique, Hôpital Pitié-Salpêtrière, APHP, Sorbonne Université, Paris

Abstract:

Chronic lymphocytic leukemia (CLL) is the most common adult leukemia in Western countries. It is characterized by an accumulation of mature $CD5^+$ $CD19^+$ B lymphocytes in the peripheral blood, bone marrow, and secondary lymphoid organs. In Europe, clinical prognosis is based on the Binet classification, with stage A corresponding to an indolent form of the disease, while stages B and C are more aggressive and require treatment. The clinical course of CLL is highly variable due to significant molecular heterogeneity, such as *IGHV* mutational status (immunoglobulin heavy chain variable region genes) and cytogenetic abnormalities. Despite therapeutic advances, particularly with targeted therapies such as BTK inhibitors (e.g., ibrutinib) and BCL-2 antagonists (e.g., venetoclax), CLL remains incurable, with frequent relapses and the emergence of resistance. Beyond intrinsic genetic alterations, tumor cell activity is strongly influenced by the tumor microenvironment (TME), which consists of normal cells interacting with the tumor. This microenvironment plays a crucial role in leukemic cell survival, immune evasion, metabolic reprogramming, and resistance to treatment. Among the key mediators of this intercellular communication are exosomes, small extracellular vesicles carrying various molecules (nucleic acids, proteins, lipids). Their involvement in numerous tumor-related processes makes of them some very promising therapeutic targets, including in CLL treatment. In this context, my PhD project investigates the role of TME-derived exosomes as an essential support for CLL cells, particularly as regulators of their metabolic reprogramming, thereby contributing to drug resistance.

My research demonstrates that in aggressive forms of CLL (Binet B stage), TME-derived exosomes enhance the glycolytic pathway and counteracts the effects of glucose uptake inhibitors in tumor cells. Activation of the B-cell receptor (BCR) pathway is crucial for CLL cell survival and is coupled with metabolic reprogramming toward glucose metabolism. By inhibiting this pathway, ibrutinib disrupts the metabolism of CLL cells, leading to their apoptosis. However, my findings suggest that TME-derived exosomes counteract this effect by promoting glucose utilization, thereby contributing to the development of ibrutinib resistance, particularly in patients with aggressive CLL.

My results reveal that TME-derived exosomes promote resistance to targeted therapies, particularly ibrutinib, by stimulating glucose metabolism in aggressive forms of CLL, unlike CLL cells from patients with indolent disease. This highlights the therapeutic potential of targeting metabolic reprogramming induced by these exosomes to optimize existing treatments. Blocking tumor communication via exosomes, either by inhibiting their secretion or preventing the transfer of their contents to tumor cells, could pave the way for innovative therapeutic strategies. In this way, this approach may help overcome resistance to current therapies and more effectively eradicate leukemic cells in patients with aggressive CLL.

Keywords:

Exosomes, Chronic Lymphocytic Leukemia, Metabolism, Drug resistance

Rosalie MOREAU[1], Mikaël CROYAL[2], Carla CRIBELIER[1], Laurent MATHIOT[3], Jean-Sébastien FRENEL[3], Gwennan ANDRE-GREGOIRE[1,3], Julie GAVARD[1,3] , Laëtitia GUEVEL[1]

[1] Team SOAP, Centre de Recherche en Cancérologie et Immunologie Intégrée Nantes-Angers (CRCI[2]NA), Inserm, CNRS, Nantes Université, Nantes, France

[2] CRNH-Ouest Mass Spectrometry Core Facility, Nantes, France

[3] Institut de Cancérologie de l'Ouest (ICO), Saint Herblain, France

Abstract:

Characterization of plasma extracellular vesicles from triple-negative breast cancer patients: towards a non-invasive predictive biomarker of treatment efficacy.

Triple-negative breast cancer (TNBC) has a poor prognosis in both early and advanced stages. A key prognostic factor in early TNBC is the achievement of a pathological complete response (pCR) after chemotherapy. Currently, the combination of chemotherapy and pembrolizumab (a PDL1 inhibitor) significantly increases the percentage of pCR, establishing this treatment as a standard.

The concept of liquid biopsies has emerged in oncology as a prominsing tool of guiding treatment. In this context, **E**xtracellular **V**esicles (EVs) are attracting growing interest. We have recently developed a simplified protocol for the analysis of EVs from plasma liquid biopsies, enabling a large number of samples to be processed from a minimal volume of plasma. The procedure consists of three steps: 1- EVs are enriched by semi-automated size exclusion chromatography (SEC); 2- The concentration of EVs, called vesiclemia, is determined by nanoparticles quantification via Interferometric Light Microscopy (ILM); and 3- The EV lipid composition (and more specifically the sphingolipid signature) is analyzed by mass spectrometry.

The aim is to use plasma EVs as a biomarker applicable to clinical routine for patients with metastatic TNBC. This approach has been validated with a longitudinal characterization of plasma EVs from patients treated for RH^+ metastatic breast cancer under iCDK4/6 treatment in the EPICURE cohort (Richard et al., 2024). The combination of vesiclemia and EV sphingolipid profile can predict early response to treatment, paving the way for their use in personalized medicine. In this new project, we carried out a preliminary study on 10 TNBC patients, treated with chemotherapy alone or in combination with pembrolizumab. The aim is to identify whether treatment influences the EV lipid cargo, and whether variations in vesiclemia and sphingolipids signature during treatment could predict early relapse in TNBC patients.

Overall, we anticipate that our results will increase the understanding of the correlation between tumor progression, relapse with respective plasmatic EV production and lipid content. Consequently, we believe to optimize management by facilitating early follow-up, reducing ineffective treatments and side effects.

Poster session

Submission form

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Poster :

Title of the abstract	Visualizing proteopathic Tau seeds within Alzheimer's disease brain-derived extracellular vesicles by single molecule localization microscopy
Authors and affiliations	Dalya Gulseren[1] (CNRS UMR9199, CEA, Université Paris-Saclay, Fontenay-aux-Roses) Christian G. Specht[2] (INSERM U1195, Université Paris-Saclay, Le Kremlin-Bicêtre) Mehdi Kabani[1] (CNRS UMR9199, CEA, Université Paris-Saclay, Fontenay-aux-Roses)
Laboratory	[1]Laboratoire des Maladies Neurodégénératives, UMR9199, CNRS, CEA MIRCen, Université Paris-Saclay, Fontenay-aux-Roses [2]Maladies et Hormones du Système Nerveux, U1195, INSERM, Université Paris-Saclay, Le Kremlin-Bicêtre
Email address main author	dalyaglsrn@gmail.com (presenting author) christian.specht@inserm.fr (senior author) mehdi.kabani@cnrs.fr (senior author)
Abstract about 200 à 300 words *In English*	Misfolding and aggregation of Tau into highly ordered amyloid filaments is characteristic of Alzheimer's disease (AD) and other tauopathies. Tau lesions progressively invade the brain by a prion-like mechanism of self-replication and spreading of diffusible Tau seeds. Extracellular vesicles (EVs) are major vehicles for Tau dissemination, but the ultrastructural and molecular properties of Tau-containing EVs are scarcely known. Here, we aimed at characterizing Tau-containing EVs isolated from AD brains at single-vesicle resolution using single molecule localization microscopy (SMLM). Due to its high spatial precision and outstanding sensitivity, SMLM is ideally suited to provide both detailed ultrastructural information as well as quantitative molecular information about the association of Tau with EVs. Post-mortem human brain tissue samples (parietal and frontal cortex) from AD (Braak V/VI) and non-demented age-matched control subjects were obtained from the NeuroCEB brain

	bank. After gentle enzymatic dissociation of the tissues, EVs were isolated by ultrafiltration and size-exclusion chromatography, and characterized by immunoblotting and negative staining transmission electron microscopy (TEM). The presence of pathological Tau within EVs was confirmed by immunogold EM. For SMLM, EVs were selectively captured on multichannel coverslips and labelled with specific antibodies against EV marker proteins and their pathogenic cargo. With this approach, we were able to visualize pathological Tau seeds within brain-derived EVs from AD patients. The simultaneous detection of thousands of immobilized EVs per recording generates comprehensive datasets for categorizing and quantifying Tau-containing EV subpopulations. We will apply this methodology for a more systematic analysis of the ultrastructure and molecular composition of EVs in neurodegenerative diseases.
Keywords	Brain-derived EVs, Tau, Alzheimer's disease, SMLM

Extracellular vesicles from adipose tissue-derived mesenchymal stromal cells are functionally different in patients with systemic sclerosis

Dalia Ibarissen, Claire Bony-Garayt, Elise Dumas, Christian Jorgensen, Alexandre Maria, Danièle Noël

Abstract: Systemic sclerosis (SSc) is a rare autoimmune disease characterized by three pathological processes: vasculopathy, fibrosis and immune dysregulation. The more severe forms of diffuse SSc involve fibrosis of the skin and internal organs, leading to a poor prognosis, particularly in the case of pulmonary involvement. In the absence of curative treatment, we have previously investigated the therapeutic interest of adipose tissue-derived mesenchymal stromal cells (ASCs) and demonstrated that ASCs and their derived extracellular vesicles (EVs) can slow disease progression by reducing fibrosis and inflammation in the HOCl-induced SSc mouse model. In patients with diffuse SSc, the adipose tissue is scarce and dysfunctional suggesting that ASCs might be altered. In the present study, we aim to determine whether EVs released from healthy and SSc ASCs are functionally similar.

Methods: ASCs were isolated from healthy donors and diffuse SSc patients and EVs were isolated by differential ultracentrifugation. EVs were characterised structurally by cryo-TEM, by size and concentration using NTA and by phenotype using nanocytometry. The antifibrotic and immunosuppressive properties of ASCs and EVs were evaluated in vitro using TGFβ1-induced myofibroblastogenesis assay and mixed lymphocyte reaction (MLR). The therapeutic function of ASCs was assessed in the HOCl-induced SSc model. Proteomic and miRNome analyses of EVs were performed by LC-HRMS and small RNAseq, respectively.

Results: Both healthy and SSc ASCs exerted an anti-fibrotic effect by reducing the expression of pro-fibrotic markers such as αSMA, COL1A1 and COL3A1 and increasing remodelling markers such as MMP1 and MMP3. Notably, SSc ASCs induced a pro-apoptotic effect on stimulated fibroblasts, a feature not observed with healthy ASCs. In MLR assays, healthy ASCs showed a dose-dependent inhibition of PBMC proliferation, reflecting a strong immunomodulatory activity, but SSc ASCs exert a significantly reduced inhibitory capacity. EVs from SSc ASCs tended to be smaller in size (215 ± 11 nm) compared to healthy ASCs (231 ± 6 nm) and to be released in slightly higher amounts. The total protein content and phenotype were comparable. Functionally, similar trends as parental cells were observed with EVs derived from SSc ASCs displaying anti-fibrotic activity and lower immunosuppressive function. The proteomic analysis revealed 27 differentially expressed proteins, of which 13 were upregulated in EVs from SSc patients.

Conclusion: EVs isolated from SSc ASCs have been characterised phenotypically and functionally. Further studies are needed to determine how the different protein cargo of SSc EVs may affect their functions.

Isolation of mitochondria-containing extracellular vesicles from PAH-exposed endothelial cells using density gradient

Camille Chauvin[1], Joan Guillouzouic[1], Valentine Brouard[1], Agnès Burel[2], Florian Barathon[1], Odile Sergent[1], Dominique Lagadic-Gossmann[1], Lydie Sparfel-Berlivet[1] and Éric Le Ferrec[1]

1- Univ Rennes, Inserm, EHESP, Irset (Institut de recherche en santé environnement et travail) - UMR_S 1085, 35000, Rennes, France
2- MRic Cell Imaging Platform, BIOSIT, University of Rennes, 2 avenue du Pr Léon Bernard - CS 34317, 35043, Rennes, France

Polycyclic aromatic hydrocarbons (PAHs) are persistent environmental pollutants from incomplete organic combustion, widely found in air, water, and soil. Due to their carcinogenic and genotoxic properties, chronic PAH exposure poses significant health risks. Investigating PAH mixtures, which mirror real-life exposure, is crucial to understanding their complex cellular effects.

Recent studies suggest that PAHs disrupt mitochondrial function, inducing oxidative stress, energy metabolism impairment, and apoptotic pathways. Mitochondria are thus emerging as key targets of PAH toxicity. Endothelial cells, which form the interface between blood and tissues, are particularly exposed to circulating PAHs. Our team previously showed that individual PAHs, including benzo[a]pyrene (B[a]P), benz[a]anthracene, and dibenzo[a,h]anthracene, enhance extracellular vesicle (EV) release by human microvascular endothelial cells (HMEC-1).

Here, we investigated the effects of three PAH exposure scenarios: PAH18, a complex mixture of 18 PAHs representing environmental contamination; PAH4, a subset of four priority PAHs identified by the European Commission's Scientific Committee; and B[a]P alone. HMEC-1 cells were exposed to 100 nM or 1000 nM of these mixtures or B[a]P for 24 hours. Total EV production and mitochondria-containing EVs (Mito-EVs) were analyzed using MitoTracker Green labeling, nanoparticle tracking analysis and iodixanol density gradient ultracentrifugation.

Our results show a mixture-dependent increase in both total EV and Mito-EV release. Gradient fractionation allowed the isolation of a distinct EV subpopulation enriched in mitochondrial content.

We are now characterizing vesicular, mitochondrial, and endothelial protein markers in each fraction and assessing the effects of PAH mixtures on these markers to further elucidate PAH-induced endothelial toxicity.

This study highlights the relevance of gradient-based EV isolation in toxicology and positions Mito-EVs as potential biomarkers of PAH-induced cellular effects.

Role of extracellular vesicles in the metastatic tropism of triple negative breast cancer

Sarah Mehenni1, Tala Hammadé1, Blandine Chazarin1, Tristan Cardon1, Soulaimane Aboulouard1, Isabelle Fournier1, Michel Salzet1, Franck Rodet1* and Christophe Lefebvre1*

1Univ. Lille, Inserm, CHU Lille, U1192 - Protéomique Réponse Inflammatoire Spectrométrie de Masse - PRISM, F-59000 Lille, France

Background:

Triple-negative breast cancer (TNBC) is considered as the most aggressive and metastatic subtype. It exhibits metastases in preferential target organs: bones, lungs, liver, and brain. However, chemoresistance is leading to an alarming increase in brain tropism which represents a major cause of death due to the challenging of their treatment. The spread of tumor cells is a non-random process because it requires the preparation of a pre-metastatic niche (PMN). The underlying molecular mechanisms involved in the conditioning of this PMN and the messengers delivered in return to recruit tumor cells, are not well described. Recent studies suggest the importance of extracellular vesicles (EVs) in this molecular communication. EVs (exosomes and microvesicles) are important messengers by carrying biological signals at by providing effectors or regulators as proteins, lipids and nucleic acids. This project aims to understand the role of EVs derived from primary TNBC cells in the development of PMN in brain.

Material and methods:

In this project, EVs from a parental cell line of human TNBC were compared to those of its derivative metastatic cell lines collected in brain, lung and bone marrow. A large-scale proteomic approach was dedicated to identify EV surfaceomes and contents since they respectively play a crucial role for the recognition and modulation of recipient cells in the future PMN. In this context, EVs were used to challenge astrocytes in order to evaluate the impact on subsequent protein signatures and secretory activity.

Results:

The first proteomic results obtained on the different EV subpopulations revealed differential and common signatures. Moreover, EVs from different metastatic origins have different effects on the proteome and secretome of astrocytes. Interestingly, various key proteins such as CCL22, CXCL5 VEGF and IGFBP2 were more secreted by astrocytes treated with EVs derived from brain metastatic cell line. These molecules are known to recruit immune cells and promote brain metastasis and may therefore contribute to the preparation of brain PMN.

Conclusion:

Our results reveal a preferential effect of EVs derived from brain metastatic TNBC cell line on astrocytes. This supports the idea of a specific tropism of these EVs towards nervous cells. To test this hypothesis, the identification of EV surfaceomes are currently under progress.

Keywords: Brain metastasis, Extracellular vesicles, Glia

Short talk session

Submission form

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Selection will be confirmed on **May 15, 2025.**

Poster :

Title of the abstract	Investigating the crosstalk between Tau and the autophagy-lysosomal pathway
Authors and affiliations	**Valentine Thomas**[1,2], Christel Brou[1], Chiara Zurzolo[1] [1]Membrane Traffic and Pathogenesis Unit, Department of Cell Biology and Infection, CNRS 18 UMR 3691, Institut Pasteur, Université Paris Cité, Paris, France. [2]Sorbonne Université, ED394 - Physiologie, Physiopathologie et Thérapeutique, Paris, France.
Laboratory	Institut Pasteur, Membrane Traffic and Pathogenesis Unit, PI : Chiara Zurzolo
Email address main author	valentine.thomas@pasteur.fr
Abstract about 200 à 300 words *In English*	Neurodegenerative diseases like Alzheimer's and Parkinson's have been associated with the pathological aggregation of misfolded proteins[1,2], respectively Tau and α-synuclein, which seed and spread from cell-to-cell in a prion-like manner. Previous data suggests that aggregate-containing cells are dysfunctional in the autophagy-lysosomal pathway, leading to aggregates persistence and neurodegeneration[3,4]. We and others have shown that exogenous α-synuclein fibrils accumulate in lysosomes in human and mouse neuronal cells, resulting in their swelling and decreased activity[5]. However, in the case of Tau, we previously reported that endogenously formed repeated-domain (RD)-Tau aggregates were recognized as autophagic cargos but failed to be delivered to lysosomes[6]. Yet, whether aggregates are not degraded because they are

	intrinsically resistant to degradation or because they impair the autophagy-lysosomal pathway remains an open question. My study aims at better characterizing the fate of full-length (FL)-Tau aggregates and at understanding whether these cause autophagy-lysosomal disruption. In human SH-SY5Y neuronal cells, we stably overexpressed soluble or aggregated P301S-FL-Tau-mClover3, an aggregation-prone mutant that is associated with early-onset frontotemporal dementia[7], based on a Tet-off system. Using immunofluorescence and live imaging, I show that in these cells lysosomes are affected in their morphology (bigger), positioning (more peripheral), motility (faster). However, unlike in the presence of α-synuclein aggregates, they are still labeled with Lysotracker, suggesting that their acidification is not impaired. Additionally, I could confirm by super-resolution that endogenous FL-Tau aggregates can overlap onto lysosomes but don't accumulate inside, which differs compared to α-synuclein aggregates. However, I detect in these cells less autolysosomes (co-labeled with LC3-LAMP1) and an increase in the nuclear translocation of TFEB, a transcription factor regulating autophagy and lysosome biogenesis, showing that the autophagy machinery is active. Using western blot, I observe that aggregates of Tau are more stable than soluble proteins. I am currently investigating whether or not these are targeted for autophagy-lysosomal degradation. Future work will help understanding the mechanisms governing Tau aggregate formation, maintenance, and downstream effects on lysosomes, notably via lysosome purification and immunoprecipitation, in comparison to α-synuclein.
Keywords	Neurodegeneration, Tau aggregate, autophagy-lysosomal dysfunction

Submission form

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Selection will be confirmed on **May 15, 2025.**

Title of the abstract	Lipid metabolism and extracellular vesicle production in *Cryptococcus neoformans*
Authors and affiliations	Keita Kayama, Thierry Fontaine, and Guilhem Janbon Institut Pasteur, Paris, France
Laboratory	RNA Biology of Fungal Pathogens
Email address main author	keita.kayama@pasteur.fr,
Abstract about 200 à 300 words *In English*	**Extracellular vesicles (EVs)** produced by the human fungal pathogen *Cryptococcus neoformans* have been extensively studied. In our laboratory, we have analyzed the RNA and protein cargo of EVs produced by this fungus. However, the lipid components of EVs and the role of lipid metabolism in EV biogenesis remain largely unexplored. We recently performed a genome-wide genetic screen to identify 260 mutant strains with altered EV production. However, many of these genes have unknown functions making their involvement in lipid metabolism difficult to predict. To further investigate the role of these genes in EV production regulation, we are assessing the sensitivity of the 260 mutants to various lipid metabolism inhibitors. We hypothesize that mutants affecting the same metabolic pathway will have similar drug sensitivity profiles, allowing us to cluster the genes accordingly. Our preliminary results suggest that several lipid metabolic pathways, including the sphingolipid biosynthesis pathway, are important for EV production in *Cryptococcus.*
Keywords	EV, lipid, fungi, pathogens

Non canonical function of ATG9A: EV secretion induction for plasma membrane repair

Maribel Lara Corona[1], Tom Vennin[1], Etienne Morel[1], Cédric Delevoye[1], Aurore Claude-Taupin[1]

[1]Université Paris Cité, INSERM U1151, CNRS U8253, Institut Necker-Enfants Malades, Paris, France.

Proximal tubule epithelial cells in the kidney undergo shear stress, which activates autophagy via the AMPK signaling pathway. This process relies on ATG9A, a transmembrane scramblase essential for the expansion of the phagophore, the precursor to the autophagosome. Autophagy is crucial for kidney function since it supports energy-consuming processes essential for maintaining homeostasis such as glucose reabsorption and gluconeogenesis. However, excessive shear stress—as observed in patients with Chronic Kidney Disease (CKD)—can damage the plasma membrane and impair autophagy induction. In our lab, murine Kidney Epithelial Cells (KECs) cultured under controlled flow conditions mimicking physiological and pathological shear stress reveal that high shear stress promotes the recruitment of ESCRT proteins by autophagy proteins like ATG9A, leading to increased ectosome secretion. Notably, KECs KO for ATG9 show no change in extracellular vesicle (EV) release under either flow condition, suggesting that ATG9A plays a non-canonical role in coordinating membrane repair via EV secretion. Understanding the link between ATG9A and secretory pathways may open new avenues for therapeutic strategies against CKD.

Submission form

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Selection will be confirmed on **May 15, 2025.**

Title of the abstract	Bacterial versus host extracellular vesicles: critical elements of host-microbiota interplay
Authors and affiliations	Béatrice Schaack[1,2], Maya Katby[2], Corinne Mercier[2], **David Laurin[3]** **U**niversité **G**renoble **A**lpes [1]CEA, CNRS, Institut de Biologie Structurale [2]CNRS UMR 5525, Laboratoire TIMC TrEE [3]INSERM U1209, CNRS UMR 5309, Ets Français du Sang, Institut pour l'Avancée des Biosciences
Laboratory	IAB & TIMC
Email address main author	david.laurin@efs.sante.fr
Abstract about 200 à 300 words *In English*	Extracellular vesicles (EVs) are key players in inter- and intra-kingdom communications, particularly within animal holobionts - including humans. We addressed the question of exchanges between the intestinal microbiota and the host via EVs. First, we analyzed the presence of bacterial EVs (BEVs) in healthy donors, in the absence of barrier disruption (on therapeutic blood products), in search of markers enriched in enterobacterial EVs. Next, we produced and used fluorescent Escherichia coli EVs to monitor their fusion with blood mononuclear cells. Finally, we followed their diffusion from the mouse intestine into the circulating blood to the organs. Our study shows that BEVs are critical and highly active elements of cellular communication between the host and the microbiota.
Keywords	Bacterial EVs, Blood EVs, Microbiota, Biodistribution

Submission form

Please complete the present submission form and upload it on the registration form by **April 15, 2025.**
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Title of the abstract	Optimization and preclinical validation of chemistry, manufacturing and control (CMC) framework for bioengineered extracellular vesicles in phase I oncology clinical trial
Authors and affiliations	Xiangliang (XL) LIN, Esco Aster Pte Ltd
Laboratory	Esco Aster Pte Ltd
Email address main author	xl.lin@escoaster.com
Abstract about 200 à 300 words *In English*	Introduction: Extracellular vesicles (EVs) exhibit structural and molecular heterogenicity due to their diverse origin. Their production conditions may influence the characteristics of EVs. A CMC framework is required to support consistent and scalable manufacturing, complement with analytical characterization of EVs, that meet regulatory quality standards for clinical applications. Methods: The stable cell clone of engineered cells was expanded into a 2L packed-bed tide motion bioreactor to produce EVs under GMP conditions. Critical process parameters were optimized to maintain cell growth. EVs were purified using a sequential process of tangential flow filtration (TFF) and size exclusion chromatography (SEC). The EVs were formulated in Hartmann's solution with trehalose as the drug product. The product was aseptically filled into vials and stored at -20 °C. Preclinical studies were conducted to evaluate the ability of the EVs to reach the target cells. Results: More than 95% of the cells were adhered to the packed bed in the bioreactor, with an average viability of approximately 80% at the end of the culture. The deviation of total number of EVs was within 15% among sub-optimal culture conditions. The TFF- SEC purification process achieved an average yield of 70% with minimal aggregation. Flow cytometry analysis confirmed EVs had lipid bilayers when labelled with membrane-labeling dye and 60-70% of EVs expressed the surface marker X. Preclinical studies demonstrated that the bioengineered EVs were rapidly accumulated in the target cells, in contrast to naïve EVs. Summary: The CMC framework establishes a foundation to ensure a consistent production of high-quality EVs. This

	supports reproducibility in research and development, enabling biotech companies to achieve reliable outcomes. Funding: This project is sponsored by ShineOn Biomedical Co. Ltd.
Keywords	Bioengineered EVs, Quality design, Manufacturing

Short talk session

Submission form

Please complete the present submission form and upload it on the registration form by **April 15, 2025.**
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Title of the abstract	*EVs as circulating biomarker of in radio-induced cardiotoxicity in breast cancer (BC)*
Authors and affiliations	1. **Celine Loinard** (ASNR, Fontenay aux Roses) 2. MOHAMED AMINE BENADJAOUD ((ASNR, Fontenay aux Roses)) 3. HAWA BACHILY (ASNR, Fontenay aux Roses)) 4. SOPHIE JACOB (ASNR, Fontenay aux Roses)) 5. ROMARIC LACROIX (Aix-Marseille University, C2VN, INSERM 1263, INRAE 1260, Marseille, France) 6. RADIA TAMARAT (ASNR, Fontenay aux Roses))
Laboratory	ASNR
Email address main author	Celine.loinard@asnr.fr
Abstract about 200 à 300 words *In English*	Breast cancer radiotherapy (RT) can lead to irradiation of the heart, leading to an increased risk for a variety of heart diseases arising many years after radiotherapy. The release of EVs important biological functions, including inflammation, angiogenesis and influences the development of cardiotoxicity. The present study tested the hypothesis that EV generation in blood after RT of BC women could predict radio induced cardiotoxicity. EVs isolated from plasma of 200 women treated for BC before RT, end of RT, 6 and 24 months after the end of RT in which were characterized as large vesicles expressing monocyte, platelets and endothelial markers by cytometry and small EVs

	by Nanosight. Interestingly, large EVs generated after RT, but not small EV, increased significatively after the end of the RT: large EV derived from Platelets and Endothlial cells significantly increased at the end of the RT, 6 and 24 months after the end of the RT by 30% and 100% respectively compared to before RT ($P< 0.05$). Large EV derived from Monocyte increased significantly by 40 % 24 months after the end of the RT compared to before the RT ($P< 0.05$). Large EV isolated from patients with cardiac dysfunction (gls < 15%) at 6 months after RT showed an increase in the wound healing process by 5%,8% and 10% respectively in 2 Gy, 5 Gy and 10 Gy irradiated HUVEC compared to EVs isolated from patients with normal GLS. VEGF, eNOS and ICAM protein level was significantly increase by 1.8-, 2- and 2- fold change in large EVs isolated from patients with cardiac dysfunction (GLS < 15%) at 6 months after RT compared to large EVs isolated before RT. Finally, we demonstrated that Tissue Factor is significantly correlated to GLS < 15% of patient at 6 months after the RT. These results demonstrate that RT increases the generation of large EVs derived from platelets, endothelial and monocytes cells in breast cancer. Large EVs could represent an early circulating biomarker of cardiotoxicity crucial for developing effective strategies for primary and secondary prevention, which are needed to optimize the CV and cancer outcomes of BC survivors.
Keywords	Biomakers, radiotherapy, breast cancer

Comparison of Extracellular Vesicle characterization methods – focus on super resolution microscopy

Marie Maumus, Zoé Servant, Dalia Ibarissen, Danièle Noël.

IRMB, University of Montpellier, INSERM, Montpellier, France.

***Introduction*.** Accurate quantification of extracellular vesicles (EVs) is essential for both basic research and clinical applications but remains challenging due to their small size, low refractive index, and heterogeneity. Several techniques have been developed to assess EV size and concentration, each with distinct advantages. Commonly used methods include Nanoparticle Tracking Analysis (NTA) and Interferometric Light Microscopy (ILM), which track particle movement to estimate size and number. Other approaches such as nano-Flow Cytometry (nFC), offer complementary information to investigate single-EV heterogeneity, while bulk protein quantification methods provide indirect estimations. Super-resolution microscopy offers high sensitivity and spatial resolution, revealing phenotypic and structural variability at the individual vesicle level.

Choosing the appropriate technique depends on the sample type, required sensitivity, and whether bulk or single-particle resolution is needed. A combined use of multiple methods is often recommended to achieve a comprehensive and reliable EV quantification. In this work, we characterized EV derived from Mesenchymal Stromal Cells (MSC) different technics to fully understand EV characteristics.

Materials and Methods. EV from MSC-conditioned media were isolated by differential ultracentrifugation and EV pellet were resuspended in PBS, aliquoted and stored at -80°C until use. EV size and concentration were quantified in parallel by NTA using the Zetaview from Particle Metrix, by ILM using the Videodrop from Myriade or by nFC using the Nanoanalyzer from NanoFCM. EV and contaminant markers (Alix, TSG101, Flotilin1, Lamin-A/C and ApoA1) were analyzed by simple western (Jess from Biotechne). Tetraspanin profiling were analyzed by nFC as well as by super resolution microscopy (Nanoimager from ONI).

***Results*.** MSC-derived EV concentration and size were measured using NTA and ILM. NTA yielded 2-fold higher EV concentrations than ILM and reported smaller median diameters. EV concentration measured by but showed comparable concentrations measured by NTA but the median diameters are 72 nm by nFC and 172 nm by NTA. Simple Western provided sensitive, quantitative EV marker analysis compared to classical western blot. To characterize EV with super-resolution microscopy, two capture methods—tetraspanin-trio (CD9/CD63/CD81) and phosphatidylserine (PS)—were compared.

PS captured more EVs, but both methods showed similar size and marker profiles. Super-resolution imaging allowed single-EV tetraspanin profiling and antigen quantification. Dual staining with membrane dye (Pan-EV staining) and the 3 tetraspanin antibodies (Tet-trio) revealed that ~50% of EVs express at least one tetraspanin. This double staining showed tetraspanin gathered in a core and the diameter were larger than the diameter measured on EV labelled with only the 3 tetraspanins.

***Conclusion*.** Our results highlight that no single technique provides a comprehensive EV profile, emphasizing the need for multi-modal approaches to fully understand EV characteristics. Our results highlight also the importance to know the strengths and limitations of each technics, to keep the same technics along the study and to report which technic is use to characterize EV in publications. Finally, we show the high potential of super resolution microscopy in EV field to study surface marker co-localization and cargo distribution.

Submission form

Please complete the present submission form and upload it on the registration form by **April 15, 2025.**
Selection will be confirmed on **May 15, 2025.**

Title of the abstract	Nutritional regulation of extracellular vesicles - Implication of dietary polyphenols
Authors and affiliations	Laurent-Emmanuel MONFOULET Université Clermont Auvergne, INRAE, Human Nutrition Unit, Clermont-Ferrand, France
Laboratory	Human Nutrition Unit (UMR 1019, UCA-INRAE)
Email address main author	Laurent-emmanuel.monfoulet@inrae.fr
Abstract about 200 à 300 words *In English*	Extracellular vesicles (EVs) are gaining significant attention in physiology, medical diagnostics, and therapeutics due to their role in inter-tissue communication, their plasma level variations linked to pathophysiological conditions, and their ability to deliver various molecules. Recent human studies show that dietary habits impact fasting plasma EV levels long-term. Daily meal intake and its nutritional quality lead to transient changes in plasma EV levels in the postprandial state, with EVs characterized by surface markers from platelets, leukocytes, and endothelial cells. However, the mechanisms driving EV secretion after food intake and their biological functions remain unclear. Dietary micro-constituents, especially polyphenols from plant-based foods, play a key role in maintaining cellular functions and explain some health benefits of plant-rich diets. Few studies have examined EV biology in response to polyphenol consumption. Studies show that cocoa or red wine polyphenol supplementation improves endothelial function and reduces fasting endothelial EVs in patients with coronary heart disease and in rodent models of hypertension. A recent study found that hydroxytyrosol, a simple polyphenol, modulates plasma EV levels and their microRNA content. We hypothesize that EVs mediate the effects of diet on health. Our research aims to characterize EVs in response to food intake, especially polyphenols. In a cellular model, we demonstrated that circulating polyphenol metabolites, like hesperidin, can correct alterations in EV secretion and miRNA content induced by postprandial inflammatory stimulation. Our ongoing work suggests that polyphenols modulate the secretion, content, and function of EVs, contributing to vascular homeostasis.
Keywords	Nutrition, Polyphenols, EVs

Short talk session

Submission form

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Title of the abstract	Development of Microfluidic and Electrokinetic approaches for Advanced Detection, Characterization, and Sorting of Extracellular Vesicles
Authors and affiliations	Sameh Obeid[1], Delaram Zohouri[1], Charlotte Neel[1,2], Thanh Duc Mai[1], Zuzana Krupova[3], Joseph Chamieh[4], Hervé Cottet[4], Sakina Bensalem[2], Bruno Le-Piouffle[2] and Myriam Taverna[1] [1]Institut Galien Paris Saclay, UMR 8612 CNRS, Université Paris-Saclay, Orsay [2]University Paris-Saclay, ENS Paris-Saclay, Gif-sur-Yvette 91190, France [3]Excilone, Elancourt, France [4]IBMM, Université de Montpellier, CNRS, ENSCM, Montpellier, France
Laboratory	Institut Galien Paris-Saclay (IGPS), UMR 8612 CNRS, Université Paris-Saclay, Orsay
Email address main author	sameh.obeid@universite-paris-saclay.fr
Abstract about 200 à 300 words *In English*	Extracellular vesicles (EVs) have emerged as promising biomarkers for disease diagnostics and as nanoscale platforms for drug delivery. However, their biochemical heterogeneity, nanometric size and presence within complex biological matrices present substantial obstacles to their reliable isolation and characterization. Conventional techniques, such as nanoparticle tracking analysis, dynamic light scattering, transmission electron microscopy and tunable resistive pulse sensing, often lack the ability to simultaneously assess multiple physicochemical properties of intact EVs. Moreover, they offer limited resolution of EV subpopulations and often struggle to differentiate EVs from co-isolated contaminants.

	To address these limitations, we are developing innovative and complementary analytical strategies based on microfluidic, electrokinetic and electrical approaches. Specifically, we have developed several capillary electrophoresis (CE) methods for the detection, preconcentration and electrophoretic profiling of EV subpopulations[1]. In parallel, we have introduced capillary Taylor Dispersion Analysis (TDA) as a novel, calibration-free approach for simultaneously assessing EV hydrodynamic size and sample purity[2]. Our recent work demonstrates, for the first time, the feasibility and analytical potential of in-line TDA–CE coupling, enabling concurrent assessment of EV size, purity and electrophoretic mobility. Additionally, we have recently explored the development of a dielectrophoresis (DEP)-based microfluidic platform for the manipulation and electrical characterization of EVs. These approaches provide access to unprecedented features of EVs, such as electrokinetic profiles and dielectric properties, offering a complementary dimension to standard biochemical and size-based analyses. We now aim to integrate these methods into a single microfluidic platform for the selective sorting and multiparametric characterization of clinically relevant EV subpopulations, thus advancing their translation in diagnostics and precision therapeutics. <u>References:</u> *[1]Zohouri et al., J. Chrom A, 2024. 10.1016/j.chroma.2024.465116* *[2]Obeid et al., J. Chrom A, 2023. 10.1016/j.chroma.2023.464189*
Keywords	Extracellular Vesicles, Microfluidic, Capillary electrophoresis, Taylor Dispersion Analysis, Dielectrophoresis

Poster

Submission form

Envoyé par:

Iona Pâques (Ms) Heales (Healthy Life Extension Society), Brussels Belgium iona.paques@gmail.com

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Poster :

Title of the abstract	Feasibility of intravenous injections of pig plasma extracellular particles into rats — and the next step
Authors and affiliations	Authors: Nicolás Cherñavsky[1] Stepheny C. de Campos Zani[2] Marcelo A. Mori[2] Nina T. Zanvettor[1] Affiliated Institutions: 1 Rejuvenation Science Institute, Campinas, São Paulo, Brazil, 2 Department of Biochemistry and Tissue Biology, University of Campinas, Campinas, São Paulo, Brazil.
Laboratory	Laboratory of Aging Biology, Department of Biochemistry and Tissue Biology, University of Campinas
Email address main author	ninatzanvettor@gmail.com
Abstract about 200 à 300 words *In English*	Extracellular particles (EPs), especially small extracellular vesicles (EVs), extracted from young animals are increasingly being studied in animal models as agents for regeneration and rejuvenation, with studies using EPs from one species injected into another showing no immune reaction. In this study, we aimed to investigate if the injection of Pig Plasma Extracellular Particles (PPEPs) into rats would produce an acute immune or toxic reaction. To do so, blood from a young pig was collected, PPEPs were isolated by size exclusion chromatography and injected into young male Sprague-Dawley rats, while the control group received a sterile saline injection. After 9 days, the animals

	were euthanized and their organs were histologically analyzed for signs of cellular damage or immune infiltration. The treated rats showed no signs of acute immunological reaction, behaving normally immediately after the injections and during the 9 days since the first injection. Throughout the trial period, the animals continued gaining weight normally and the histological analysis of their liver, kidney and spleen showed no signs of acute toxicity. Our findings underscore that PPEPs from young animals do not cause an acute immune or toxic response when injected intravenously into young male Sprague-Dawley rats. Our next experiment, scheduled to be performed in June 2026, aims to test if the PPEPs are able to rejuvenate old rats and/or keep young rats young. Thus, 10 rats aged 25 months and 10 rats aged 7 months old will be injected with PPEPs and several health markers will be measured to assess possible rejuvenation and/or youth maintenance, including epigenetic age, grip strength, memory and blood markers levels.
Keywords	Pig Plasma Extracellular Particles (PPEPs), small extracellular vesicles, blood, Sprague-Dawley rats, injection, histology, immune reaction, rejuvenation

Short talk session

Submission form

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Poster :

Title of the abstract	Uranyl acetate substitution for TEM imaging of extracellular vesicles
Authors and affiliations	Christine Péchoux[1], Martine Letheule[2], **Mélissa Reyre[3,†]**, Marthe Vilotte[1], Zuzana Krupova[3] [1] Université Paris-Saclay, INRAE, AgroParisTech, GABI, 78350, Jouy-en-Josas, France [2] Université Paris-Saclay, INRAE, AgroParisTech, BREED, 78350, Jouy-en-Josas, France [3] Excilone, Molecular biology R&D Department, 78990, Elancourt, France [†] Presenting author
Laboratory	Excilone/GABI INRAE
Email address main author	melissa.reyre@excilone.com
Abstract about 200 à 300 words *In English*	Uranyl acetate (UA) is commonly used to contrast biological samples in blocks or thin sections. It is also applied as a contrasting agent for negative staining of biological macromolecules such as proteins, viruses or extracellular vesicles. Negative staining is a fast and simple technique for many biological applications. However, as the use and purchase of radioactive products such as UA is now prohibited, we have tested an alternative procedure for negative staining using different contrast agents. **Materials and method:** Negative staining is a simple technique in which the sample is first adsorbed onto a substrate, then a stain drop is applied, blotted, and dried to form a thin electron-dense layer embedding the particles. However, sample behavior can vary significantly depending on staining conditions. This has led to the development of diverse substrate preparation methods, staining reagents, as well as washing and blotting techniques. Four different contrast agents (samarium, gadolinium, ammonium molybdate and phosphotungstate acid) were initially tested and compared to uranyl acetate for staining of extracellular vesicles isolated from bovine milk and pig plasma by size-exclusion chromatography. Then, optimization was carried out on ammonium molybdate refining key parameters such as concentration, staining time and/or the use of methylcellulose. **Results:** Although all contrast agents showed more-or-less

	satisfactory results, the protocol using 1% ammonium molybdate at pH 8 for 10 seconds has provided the highest-quality images for the visualization of EVs. As a next step, we aim to validate this protocol on viral proteins, extending its potential applications.
Keywords	Extracellular vesicles, transmission electron microscopy, negative staining, contrast agent

Short talk session

Submission form

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Title of the abstract	Coated for capture: extracellular vesicles determining biological particle uptake in skin
Authors and affiliations	Salavessa Laura[1,2], Benito-Martinez Silvia[1], Macé Anne-Sophie[5], Picas Laura[3], Van Niel Guillaume[4], Raposo Graca[1,5], Delevoye Cédric[1,2,5] [1]Institut Curie, PSL Research University, CNRS, UMR144, Structure and Membrane Compartments, 75005 Paris, France. [2]Institut Necker Enfants Malades, Université Paris Cité, INSERM UMR-S1151, CNRS UMR-S8253, Cellular & Membrane Dynamics in Stress Response, Paris, France. [3]Institut de Recherche en Infectiologie de Montpellier, UMR 9004 CNRS / UM, Biologie Quantitative du Trafic Membranaire et Pathogénèse, 34293 Montpellier, France. [4]CRCI2NA, UMR-S1307, Team 7bis EVICAT, 44 007 Nantes, France. [5]Institut Curie, PSL Research University, CNRS, UMR144, Cell and Tissue Imaging Facility, 75005 Paris, France.
Laboratory	Cellular & Membrane Dynamics in Stress Response (MEMBRAMICS)
Email address main author	laura.salavessa@inserm.fr
Abstract about 200 à 300 words *In English*	The exchange of biological materials between cells is essential for tissue homeostasis. Extracellular vesicles (EVs) are active players in this process, yet their physiological functions remain difficult to define due to the ambiguous identity of EV-producing and -recipient cells. Here, we investigate a biological system in which both donor and recipient cells are well characterized: the transfer of melanin particles from melanocytes to keratinocytes in human skin—a key step in pigmentation and genome photoprotection. Melanin pigments produced by melanocytes are

	secreted and internalized by neighboring keratinocytes, which store them to protect nuclear DNA from UV damage. The mechanisms underlying pigment recognition and uptake by keratinocytes remain poorly understood. We developed a model of human keratinocytes exposed to extracellular melanin particles, recapitulating *in vitro* their *in vivo* pigmentation and photoprotective functions. We found that secreted melanin particles are decorated with small lipid vesicles with morphological features of EVs, and that keratinocytes use filopodia protrusions to capture them prior to uptake. Using a melanin-producing cell line KO for CD63, a tetraspanin required for intraluminal vesicle biogenesis, we obtained melanin particles devoid of surface EVs, enabling the study of their molecular identity and function in melanin capture and uptake by keratinocytes. At the molecular level, proteomic analysis revealed that CD63-KO melanin particles lack molecules known to bind LRP1, a receptor enriched at melanin entry sites. At the functional level, CD63-KO melanin particles showed delayed internalization in keratinocytes, suggesting that their melanin-associated EVs are drivers of melanin uptake. Our results show that EVs on the surface of melanin particles confer a specific molecular signature essential for their recognition, interaction, and uptake by keratinocytes. We propose a physiological model in which EVs are pre-loaded onto secreted biological particles to mediate selective engagement with recipient cells. This mechanism underlies skin pigmentation and photoprotection, and provides broader insights into the role of EVs in intercellular communication and biological particle exchange required for tissue function.
Keywords	Extracellular vesicles; Cell-to-cell communication; Bioparticle exchange; Skin pigmentation.

Submission form

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Selection will be confirmed on **May 15, 2025.**

Poster :

Title of the abstract	Microfluidic stimulation of the production of extracellular vesicles by circulating cancer cells in the context of metastasis.
Authors and affiliations	Arthur Salles[1,3], Emile Gasser[1,2], Claire Wilhelm[2], Jean-Yves Pierga[4], Catherine Villard[1] [1] Laboratoire Interdisciplinaire des Énergies de Demain, CNRS & UPCité, UMR 8236, Paris (France) [2] Physique des Cellules et Cancer, Univ. PSL, CNRS & Institut Curie, UMR 168, Paris (France) [3] Fluigent SA, Kremlin-Bicêtre 94270, (France) [4] Département d'oncologie médicale, UPCité & Institut Curie, Paris (France)
Laboratory	Université Paris Cité - LIED (UMR CNRS 8236)
Email address main author	arthur.salles@etu.u-paris.fr
Abstract about 200 à 300 words *In English*	The cancer metastasis cascade includes a dissemination step in the bloodstream. We have developed a unique microfluidic device reproducing the geometry of a capillary bed to investigate how cancer cells respond to harsh bloodstream conditions, i.e. shear stress and repetitive deformation, with the ultimate aim to identify new therapeutic targets that reduce cancer cell survival in circulation. We first studied the survival of breast cancer circulating cells derived from primary (MCF-7) or metastatic (MDA-MB-231) cancers. We report that the more malignant the cell type, the longer it survives in the circulation, both in our device and in a control condition (tubing) where circulating cells are only affected by shear stress. We next observed that biomechanical stresses like those encountered by circulating tumor cells stimulate the production of extracellular vesicles (EVs), providing a possible mechanism for the presence of cancer-derived vesicles in the circulation. Both MDA-MB-231 and MCF-7 cells respond to shear stress and repeated deformations by releasing more EVs as compared to shear stress only. However, the production level as compared to non-recirculated floating cells is much higher in MDA-MB-231 cells, with e.g. a

	34-fold increase after 20 minutes of recirculation in the microfluidic device where a 4-fold increase only is obtained for MCF-7 cells in the same condition. Interestingly, a circulation-induced broadening of the size distribution of EVs is only observed for MCF-7 cells. In the future, we aim to investigate the proteins present at the surface of these EVs to find potential correlations with the metastatic potential of these cell lines. 253/300 words
Keywords	Cancer, Metastasis, Microfluidic, EVs Production

Differential Expression of Extracellular Vesicle miRNAs in Severe Pediatric Malaria Patients from Benin

Samuel Odarkwei Blankson[1], Lionel Forestier[2], Ioanna Deni[3], Zaneta Kidiavai[4], Joshuah Adjah[4], Kelly Aubertin[5], Charlotte Izabelle[6], Bruno Saubamea[6], Sarah Razafindrakoto[5], Magali Blaud[7], Rafiou Adamou[8], Azizath Moussilou[8], Jules Maroufou Alao[9], Annick Amoussou[10], Caroline Padonou[11], Nicaise Ndam[1], Laars Hviid[12], Gordon Awandare[13], Yaw Aniweh[13], **Rachida Tahar[1*]**

1. Université de Paris Cité, MERIT, IRD, F-75006 Paris, France
2. Université de Limoges, Inserm Laboratory U1094, F-87042, Limoges, France
3. Biology of Plasmodium transmission, INSERM U1016, CNRS, UMR 8104, Université Paris Cité, Institut Cochin, Paris, France
4. Freie Universitat Berlin, Institute of Immunology, Center for infection Medicine, Berlin, Germany
5. Université Paris Cité, UMR 7057, IVETh, F-75006 Paris, France
6. Université Paris Cité, US25 INSERM, UMR 3612 CNRS, PICMO, F-75006 Paris, France
7. Université Paris Cité, CiTCoM – UMR CNRS 8038, F-75006 Paris, France
8. Institut de Recherche Clinique du Benin (IRCB), Calavi, Benin
9. Département de Pédiatrie, Hôpital Mère-Enfant la Lagune (CHUMEL), Cotonou, Benin
10. Service de Pédiatrie, Centre Hospitalo-Universitaire, Suruléré (CHU-Suruléré), Cotonou
11. Centre Hospitalier Universitaire de l'Oueme, Porto-Novo, Benin
12. Center for Medical Parasitology, Department of Immunology and Microbiology, University of Copenhagen, Denmark
13. West African Centre for Cell Biology of Infectious Pathogens (WACCBIP), University of Ghana, Accra, Ghana

Abstract

Background: Severe malaria is a complicated form of infection caused by *Plasmodium falciparum*, the most virulent *Plasmodium* species. These complications include several clinical manifestations, with cerebral malaria (CM) being the deadliest. In Sub-Saharan Africa, CM affects particularly children and causes 10-25% of the mortality. CM leads also to neurocognitive impairment due to its direct and indirect effects on the brain. Studies have shown that extracellular vesicles (EVs) produced during murine or human malaria infection carry biomolecules, such as miRNAs, which play a role in malaria pathogenesis. However, there has yet to be an in-depth study on the contribution of EVs derived miRNAs to the pathogenesis of human CM and their potential as biomarkers for assessment of the disease severity.

Methods: A comprehensive analysis of the miRNA content in EVs from 15 plasma samples across three clinical groups of malaria-infected individuals—cerebral malaria (CM), severe

non-cerebral malaria (SNCM), and uncomplicated malaria (UM)—was conducted. To assess the differentially expressed miRNAs in the plasma-derived EVs samples, qRT-PCR was performed using miRNA-array cards containing pre-formulated specific probes and primers for 188 human miRNAs. Additionally, bioinformatics was employed to search for target genes of the significant miRNAs, gene ontology, and their regulatory pathways.

Results: Six differentially expressed miRNAs were identified: hsa-let-7b-5p, hsa-miR-152-3p, and hsa-miR-29c-3p were expressed in both CM and SNCM groups only. The other three, hsa-miR-342-3p, hsa-miR-484, and hsa-miR-376a-3p were over expressed only in CM compared to SNCM and UM.

Conclusion: The six miRNAs identified might be implicated in pathogenesis of CM and SNCM. hsa-miR-342-3p, hsa-miR-484, and hsa-miR-376a-3p, which were specifically upregulated in CM patients, offer promising biomarkers for the prognosis of CM.

Key words: *Plasmodium falciparum***,** *extracellular vesicles, miRNA, cerebral malaria, severe malaria, uncomplicated malaria*

Short talk session

Submission form

Please complete the present submission form and upload it on the registration form by **April 15, 2025.**
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Title of the abstract	**Tailoring the nebulization parameters for Enhanced Pulmonary Delivery of Extracellular Vesicles**
Authors and affiliations	▪ **Zeineb IBN ELFEKIH**, Institut Charles Gerhardt Montpellier UMR 5253 - Montpellier (France) ▪ **Morgane DAURAT**, Institut Charles Gerhardt Montpellier UMR 5253 - Montpellier (France) ▪ **Marie Maumus, IRMB -** Institute for Regenerative Medicine & Biotherapy- Montpellier (France) ▪ **Jade BERTHELOT**, Institut Charles Gerhardt Montpellier UMR 5253 - Montpellier (France) ▪ **Anne AUBERT-POUESSEL**, Institut Charles Gerhardt Montpellier UMR 5253 - Montpellier (France) ▪ **Marie MORILLE**, Institut Charles Gerhardt Montpellier UMR 5253 - Montpellier (France)- Institut Universitaire de France
Laboratory	Institut Charles Gerhardt Montpellier UMR 5253 - Montpellier (France)
Email address main author	zeineb.ibn-elfekih@umontpellier.fr
Abstract about 200 à 300 words *In English*	**Introduction:** EVs derived from mesenchymal stromal cell (MSC) have been described to exhibit anti-inflammatory and regenerative properties which make them excellent candidates in the context of lung inflammation. Compared to systemic administration, pulmonary route is a non-invasive, patient friendly way to deliver drugs directly and rapidly to the pulmonary cells. However, EVs delivery through the pulmonary route is a challenge to be addressed. **M&M:** EVs derived from MSC and isolated using Ultracentrifugation (dUC-EVs) were nebulized using an ePari mesh nebulizer. Different buffer compositions, EVs concentration and volumes were tested to identify the best conditions to preserve EVs. The nebulized EVs were characterized in terms of size, particle concentration and surface antigens to check for eventual modification. EVs were then isolated using alternative strategies: tangential flow filtration (TFF-EVs) and Exodus (Exo-EVs) and nebulized to check for particle's recovery differences as a function of EVs suspension isolation method. **Results:** The dUC-EVs nebulization was performed in different EVs Buffer compositions (DPBS 1X, Trehalose 25mM, polysorbate 80). Interestingly, the use of polysorbate

	80 increased the particles' recovery reaching 53 % of dUC-EVs recovery vs 32% (DPBS 1X). Our results also indicate that post nebulization, the particles recovery increased (from 45% to 58%) when a higher EVs concentration (10^9 vs 10^{10} particles) is initially nebulized. The same observation was obtained with the initial volume used (0.5,1, 2, 3mL). Differences in the particles' recovery were also observed when dUC-EVs, TFF-EVs and Exo-EVs were nebulized with the highest particles' recovery being 100% obtained with TFF-EVs, followed by 78% with Exo-EVs compared to 52% with dUC-EVs. **Conclusion:** We have identified the critical parameters allowing optimal recovery after EVs nebulization, regarding particles concentration and EVs buffer composition. We will now explore the biological function of the different nebulized EVs (dUC-EVs, TFF-EVs and Exo-EVs) using these optimal conditions of nebulization.
Keywords	Pulmonary delivery, mesh nebulization, mesenchymal stromal cell