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The 5th Israeli Society of Extracellular Vesicels Research (ISREV)

Annual Meeting, May 22, 2025

AGENDA

**8:45 - 9:20 ASSEMBLY AND REGISTRATION**

 **9:20 - 9:40 GREETINGS**: Ran Tur-Kaspa, Director, FMRC

 Lena Koren-Feldman, Director,

 Beilinson Hospital

 Karen Avraham, Dean, Gray Faculty of

 Medical & Health Sciences,

 Tel Aviv University

# 9:40 - 11:00 FIRST SESSION

**Chairs:** Tomer Cooks, Ben Gurion University; Neta Regev-Rudzki, Weizmann Institute of Science

9:40 - 10:00 **Shoshana Greenberger**, Sheba Medical Center. *Melanoma extracellular vesicles induce lymphangiogenesis and immunotolerance*

10:00 - 10:20 **Daniella Levy Erez**, FMRC. *Beyond the needle: Urinary EVs as liquid biopsies in kidney transplantation*

10:20 - 10:40 **Yeshayahu Talmon**, the Technion. *The Study of Extracellular Vesicles by Cryogenic-temperature Electron Microscopy*

10:40 - 10:50 **Tal Manko**, Ben Gurion University. *Neuroblastoma extracellular vesicles mediate blood-brain-barrier permeability and carry tight junctions’ proteins cargo*

10:50 - 11:00 **Ewa Kozela**, Weizmann Institute of Science. *Single vesicle approaches for characterization of nucleic cargo in malaria-derived extracellular vesicles.*

**11:00 - 11:30 Coffee Break**

# 11:30 - 13:25 SECOND SESSION

**Chairs:** Orit Uziel, FMRC; Yael Heifetz, Hebrew University of Jerusalem

11:30 - 11:40 **Shlomit Rak Yahalom**, Rhenium. *Overcoming flow cytometry challenges in the research of nanoparticles*

11:40 - 12:00 **Rachel Sarig**, Weizmann Institute of Science. *A clinical proof-of- concept for the reparative outcomes of glatiramer acetate in acute decompensated heart failure patients*

12:00 - 12:20 **Benjamin Dekel**, Sheba Medical Center*. An organoid-based secretome therapy for kidney failure*

12:20 - 12:40 **Moran Yadid**, Bar-Ilan University. *Endothelial extracellular vesicle: innate aid packages?*

12:40 - 12:50 **Igor Paz**, NurExone. *Addressing critical manufacturing challenges for scalable production and reliable characterization in exosome- based therapeutic development*

12:50 - 12:55 **Lital Kalich-Philosoph**, Sheba Medical Center. *Cord blood proteomics reveals mitochondrial and endoplasmic reticulum dysregulation as early autism biomarkers*

12:55 - 13:00 **Irit Rosenhek-Goldian**, Weizmann Institute of Science. *Employing atomic force microscopy to analyze the mechanics of extracellular vesicles and target cell membrane*

13:00 - 13:05 **Sirhan Saeed**, Tel Aviv Sourasky Medical Center. *Extracellular vesicles as therapeutic nanoparticles for pancreatic cancer*

13:05 - 13:15 **Alisa Komsky-Elbaz**, Hebrew University of Jerusalem*. The role of extracellular vesicles and lipid droplets in early implantation: the dynamics of embryo-maternal cross talk*

13:15 - 13:25 **Aladin Samara**, FMRC. *Advancing NK cell-derived extracellular vesicles towards a clinical-grade immunotherapy product for hematological malignancies*

**13:25 - 14:25 LUNCH and Poster Session**

# 14:25- 16:00 THIRD SESSION

**Chairs:** Ofir Bahar, Volcani Center; Moran Yadid, Bar-Ilan University

14:25 - 14:45 **Shiri Soudry**, FMRC. *Small heat shock superfamily of proteins as a retinal defense mechanism against pathogenic amyloid-β*

14:45 - 15:05 **Vadim Krivitsky**, Acytronix GmbH, Switzerland. *Ultrapure extracellular vesicle sub-populations in 10 minutes: Innovative device for precise immuno- purification, liquid biopsy, vesicle loading, labeling, and therapeutics*

15:05 - 15:15 **Hagit Shoyhet**, Technion. *Skeletal muscle-derived extracellular vesicles and their impact on glucose regulation in Type 2 Diabetes*

15:15 - 15:25 **Isabelle Petit**, Sorbonne University, France. *Identification of surface biomarkers on circulating EVs for monitoring hepatic tumor progression by liquid biopsy*

15:25 - 15:35 **Mark Polikovsky**, Weizmann Institute of Science. *Crossing barriers: Communication between kingdoms through extracellular vesicles shared by duckweed and its associated bacteria*

15:35 - 15:40 **Ayelet Lotan**, Technion. *Regenerative potential of mesenchymal stem cell-derived EVs loaded with PTEN siRNA for facial nerve injury*

15:40 - 15:45 **Shiri Navon-Venezia**, Ariel University. *Multidrug-resistant Klebsiella pneumoniae release outer membrane vesicles (OMVs) that facilitate bacterial survival in human serum*

15:45-15:55 **Nora Raz**, Almog. *Bio-fabrication Meets Nanomedicine: Innovations in 3D Bioprinting for Exosome Delivery*

**16:00- 16:10 Concluding Remarks and Student Talk Prize**: Neta Regev-Rudzki, Weizmann Institute of Science; Orit Uziel, FMRC.

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**Melanoma extracellular vesicles Induce Lymphangiogenesis and immunotolerance**

Shoshana Greenberger

Sheba Medical Center

Malignant Melanoma, one of most aggressive human tumors, initiates within the epidermis; during progression, cells invade into the dermis and become metastatic through the lymphatic and blood system. Before melanoma cell invasion into the dermis, an increased density of dermal lymphatic vessels (lymphangiogenesis) is observed, that negatively affects patients' prognosis. In addition, melanoma induces immunotolerance in its microenvironment, leading to primary or secondary failure of current checkpoint inhibition therapy. The mechanism by which MM induce these effects of the tumor microenvironment is not fully understood. To this end, we studied the effect of melanoma extra-cellular vesicles (ECVs) on lymphangiogenesis and immunotolerance.

We show that, while at the primary epidermal stage (in situ), melanoma cells secrete melanosomes, which are uptaken by dermal lymphatic cells, leading to transcriptional and phenotypic pro-lymphangiogenic changes. Mechanistically, melanoma-derived melanosomes traffic mature let-7i to lymphatic endothelial cells, which mediate pro-lymphangiogenic phenotypic changes by the induction of type I IFN signaling. In addition, melanosomes secreted by melanoma cells induce immunotolerance, evidenced by Tumor-induced Lymphocyte (TILs) inactivation, leading to reduced apoptotic fraction and increased viability of melanoma cells. Moreover, our results suggest that carcinoembryonic antigen-related cell adhesion molecule-1 (CEACAM1), a surface protein with immunomodulatory properties, plays a role in the immunomodulatory effects of melanoma derived ECV melanosomes on the lymphatic endothelium.

**Beyond the Needle: Urinary EVs as Liquid Biopsies in Kidney Transplantation**

Levy Erez D1-4, Eisner S2,5, Issa E2-3, Sabah I 2, Levin L6, Chalifa-Caspi V 6, Langman S 2 Landau D 1-2 , Haskin O1-2, Alfandari H1-2, Tur-Kaspa R2,3,7,8, Nesher E 2,5, Zemel R 2-3

1Schneider Children’s Medical Center Israel; 2Tel Aviv University 3Molecular Hepatology & Transplantation Immunology Research Lab. Felsenstein Medical Research Center, Beilinson campus, Rabin Medical Center Israel; 4Children’s Hospital of Philadelphia, PA, USA; 5Department of Organ transplantation, Beilinson campus, Rabin Medical Center Israel; 6Ilse Katz Institute for Nanoscale Science & Technology, Ben-Gurion University of the Negev, Beer-Sheva, Israel; 7Liver Institute, Beilinson campus, Rabin Medical Center, Israel; 8Azrieli faculty of Medicine, Bar-Ilan University, Safed, Israel,

**Introduction:**

Early detection of rejection following kidney transplantation is key to maintaining long-term graft function. Thus, there is a need for a non-invasive diagnostic technique with good early predictive values to determine graft injury and to provide accuracy in titrating immunosuppression.

**Objectives:**

In this study, we aimed to discover novel biomarkers for rejection through analysis of urinary Extracellular vesicle proteome from pediatric transplant recipients to identify markers for early detection of rejection.

**Methods:**

In a single-center pilot study, urine from pediatric kidney transplant recipients(KTR) at SCMCI and healthy controls were collected. EVs were isolated via ultracentrifugation, and proteomic analysis was conducted using Liquid chromatography–mass spectrometry (LC/MS). Patients were categorized into four groups: stable kidney function, acute rejection, chronic rejection and healthy controls. Longitudinal samples were collected from 3 patients experiencing AR. Stable transplant was used as a baseline value and a fold change per protein expression was calculated for all the other groups. Protein level fold change was compared between the various allograft states.

**Results:**

The study included 29 kidney transplant recipients and three healthy controls, with a median age of 14.5 years (IQR 13-19).Proteomic analysis revealed 192 differentially abundant proteins. AR patients displayed a distinct proteomic signature characterized by upregulation of immune-related processes compared to stable patients. Longitudinal data demonstrated dynamic changes in the EV proteome, reflecting transitions between rejection and recovery states.

**Conclusion:**

Urinary EV proteomics offer valuable insights into graft status, highlighting their potential as non-invasive biomarkers for early detection and monitoring of acute rejection in pediatric transplant recipients. Larger studies are required to validate these findings.

**The Study of Extracellular Vesicles by Cryogenic-temperature Electron Microscopy**

Yeshayahu (Ishi) Talmon

Department of Chemical Engineering

Technion – Israel Institute of Technology, Haifa 3200003

Cryogenic-temperature electron microscopy (cryo-EM) is essential for high-resolution directimaging of any liquid or semi-liquid material system. These methodologies allow to image, by cryogenic transmission electron microscopy (cryo-TEM) and cryogenic transmission electron microscopy (cryo-SEM), a wide range of synthetic and natural liquid systems, aqueous and nonaqueous, while preserving their original nanostructure. Cryo-TEM is ideal for imaging lowviscosity liquids with nanoaggregates smaller than about 300 nm, while cryo-SEM is the methodology of choice for the study of viscous liquids containing microparticles, un which nanostructures are to be resolved. Quite often the two methodologies are complementary. Both methodologies are based on ultrafast cooling of the liquid (thermal fixation), kept at controlled conditions prior to cooling, the transfer of the thermally-fixed specimen under controlled conditions into the microscope, and its imaging, while it is kept at cryogenic temperatures.

Over the past ten years we have studied a wide range of EV systems by cryo-EM. In addition of the characterization of the EV nanostructure, we followed their formation processes from human cells and from a bacterium. We have also shown how cryogenic storage affects their nanostructure.

In my presentation I will describe briefly the basics of cryo-TEM and cryo-SEM, will demonstrate how they are applied as complementary methodologies, and give a number of examples.

**A Clinical Proof-of-Concept for the Reparative Outcomes of Glatiramer Acetate in Acute Decompensated Heart Failure Patients**

Rachel Sarig

The Weizmann Institute of Science

We recently showed that glatiramer acetate (GA), a well-known drug prescribed for the treatment of multiple sclerosis, has considerable therapeutical responses in rodent models of acute and chronic myocardial ischemia, as evidenced by improvements in cardiac function and reduction in infarct size. We showed that GA positively affects key pathways required for cardiac repair, including cardiomyocyte protection, inducing a pro-reparative immune milieu, restricting fibroblast activation and enhancing angiogenesis (Aviel *et al*., *Repurposing of Glatiramer Acetate to Treat Cardiac Ischemia in Rodent Models*, *Nature CVR*, 2024). Overall, these findings highlight GA’s potential as a promising candidate for the treatment of cardiovascular disease.

Building on these preclinical studies, and as GA is a well-established drug with an excellent safety profile, we conducted a small proof-of-concept, randomized-controlled clinical trial to assess its effects on heart failure (HF) patients. Strikingly, a short-term (14 days) add-on administration of GA to patients hospitalized due to acute decompensated HF resulted in marked reduction in the cytokine surge associated with acute exacerbations, as well as in the prognostic marker NT-proBNP, relative to control patients. Mechanistically, GA induces a distinct proteomic signature in the plasma of patients and potentially exerts its effects through pro-reparative extracellular vesicles, as demonstrated in a murine model of acute myocardial infarction (MI) model. These results underscore the potential of repurposing GA for treating HF patients resulted from complicating MI.

**An organoid based secretome therapy for kidney failure**

Benjamin Dekel, Sheba Medical Center

No abstract available

**Endothelial Extracellular Vesicle: Innate Aid Packages?**

Moran Yadid

The Azrieli Faculty of Medicine, Bar-Ilan University

The Galilee Medical Center

Regenerative medicine offers different approaches for treating damaged organs. In my research I use tissue engineering approaches to establish advanced in-vitro models to explore physiological repair mechanisms and acellular approaches for organ regeneration as routes to induce self-repair.

I will present the development of in vitro assays to model and assess the function of the human myocardium and vasculature. These assays are used for studying extracellular vesicles (EVs) mediated interactions between endothelial cells and cardiomyocytes under various pathophysiological conditions. I will demonstrate how a “human heart on chip” platform is utilized to assess the regenerative function of EVs in a model of cardiac injury, and the use of proteome data to shed light on the possible mechanisms of action. The combination of the functional data derived from the in vitro model, and the deep proteome analyses suggest that endothelial EVs target multiple pathophysiological routes related to cellular stress, as well as metabolic routs, and thereby induce an innate protective mechanism, which may be abolished in pathological conditions related to endothelial dysfunction.

**Small heat shock superfamily of proteins as a retinal defense mechanism against pathogenic Amyloid-β**

Shiri Zayit-Soudry1,2,3

1Department of Ophthalmology, Rabin Medical Center, Israel; 2Felsenstein Medical Research Center, Tel Aviv University, Israel; 3Faculty of Medical and Health Sciences, Tel Aviv University, Israel

Age-related macular degeneration (AMD) is a leading cause of vision loss, yet its underlying mechanisms remain incompletely understood, critically affecting the development of effective therapies. Amyloid β (Aβ), best known for its role in Alzheimer’s disease, is increasingly recognized as a key contributor to retinal degeneration in AMD. Given the complexity of Aβ-related pathology in AMD—impacting multiple cell types and stress pathways—treatment strategies that can broadly modulate the retinotoxic environment are needed.

We explored the potential of adipose tissue-derived mesenchymal stem cell exosomes (AT-MSC-exosomes) to counteract Aβ-induced retinal damage. Using both in vivo and in vitro models, we show that pretreatment with AT-MSC-exosomes preserves retinal function and cell viability in the presence of toxic Aβ42 assemblies. In wild-type rats, intravitreal administration of MSC-exosomes prevented Aβ-induced retinal dysfunction as measured by electroretinography. In parallel, the exosomes protected retinal pigment epithelial (RPE)-like cells in culture from Aβ42-induced toxicity. Using fluorescent labelling, we also observed that AT-MSC exosomes are actively recruited to stressed retinal cells in vivo and in vitro, suggesting targeted homing to sites of amyloid-induced damage. Proteomic and immunohistochemical analyses revealed that exosome treatment dampens molecular stress responses typically triggered by Aβ, including the induction of heat-shock proteins and astrocyte-associated α-crystallin expression.

Together, these findings position AT-MSC exosomes as a promising candidate for cell-free intervention in Aβ-associated retinal degeneration. They offer not only a potential therapeutic tool for AMD but also a platform for broader investigation into amyloid-related retinal pathology and exosome-based retinal neuroprotection.

**Ultrapure Extracellular Vesicle Sub-Populations in 10 Minutes: Innovative Device for Precise Immuno-Purification, Liquid Biopsy, Vesicle Loading, Labeling, and Therapeutics**

Vadim Krivitsky

 Acytronix GmbH, Switzerland.

Extracellular vesicles (EVs) are potent biomarkers and therapeutic carriers, but isolating them rapidly with high purity remains challenging. The Portable Microstructured Electrochemical Device (PMED) transforms this process by rapidly isolating ultrapure EV sub-populations from complex biofluids, including serum, plasma, urine, and cell cultures, in less than 10 minutes. Outperforming traditional methods in yield and efficiency, PMED employs antibody-coated microstructured electrodes for precise immuno-purification, unlocking transformative applications in diagnostics and therapeutics. PMED’s versatility spans multiple domains: (1) **Liquid Biopsy**, capturing tissue-derived EVs for non-invasive disease profiling and precise multiomic analysis; (2) **Therapeutics,** generating drug-enriched EVs from patient tissues for targeted chemotherapy with reduced toxicity, and hybrid EV-polymer particles for safe, efficient gene delivery; (3) **Labeling and Loading**, modifying EVs with therapeutic agents, removing unreacted material to lower immunogenicity; (4) **Environmental Monitoring**, isolating EVs from biofluids to assess pollutant exposure; and (5) **Regenerative Medicine**, enhancing cell proliferation through specific EV subpopulations from stem cell treatment. The results highlight tight EV size distribution, intact protein markers (e.g., CD9, TSG101), and superior performance in regenerative applications. This innovative platform integrates microfluidics and bioelectronics to deliver rapid, scalable solutions for personalized medicine, enabling early detection of diseases like cancer, optimizing treatments with minimal side effects, and extending to environmental health monitoring. PMED’s portability and unmatched purity position it as a game-changer in EV research and clinical translation.

**Overcoming Flow Cytometry Challenges in the Research of Nanoparticles**

Shlomit Rak Yahalom

Rhenium

Flow Cytometry (FC) is a robust and widespread method to assess various cellular characteristics. It is known in its statistical strength to analyze multiparameter characteristics of populations. In recent years studies involving extracellular vesicles (EVs) are rapidly growing in number and depth and FC, as a powerful statistical method, is often examined for this purpose. However, as FC has been classically designed for particles in the size range of microns, its use for nanoparticle characterization is challenging. In this talk we will review some physics boundaries, technical and instrument related limitations in using FC for EVs analysis, and highlight tolls to mitigate them, from sample preparation through instrument settings and up to technological innovations. These suggested guidelines together with the utilization of technological innovations, would allow the use of FC in nanoparticle studies with increased reliability, accuracy, and reproducibility.

**Addressing Critical Manufecturing Challenges for Scalable Production and Reliable Characterization in Exosome- Based Therapeutic Development**

Igor Paz1,Nirit Drori Carmi1, Shy Rubin1, Alina Freiman1, Kineret Taler1, Isabella Solomon1, Neta Kutner1, Sharon Baumgarten-Soueid1, Tali Kizhner1, Ina Sarel1, Lior Shaltiel1, Shulamit Levenberg1,2

1NurExone Biologic, [Haifa, Israel]; 2Faculty of Biomedical engineering, [Technion Israel institute of Technology, Haifa, Israel]

Objective:

NurExone has developed ExoPTEN, an exosome-based therapeutic approach for acute spinal cord injury (SCI), using exosomes produced from bone marrow-derived mesenchymal stem cells (BM-MSCs) to deliver a proprietary siRNA targeting PTEN. The current research aimed to scale up production while ensuring consistency and robust manufacturing processes for clinical application.

Methods:

Exosome production was scaled from 2D culture to 3D bioreactor systems with solid scaffolds to optimize BM-MSC culturing. Screening was conducted to identify the optimal combination of BM-MSC donor and expansion media to maximize exosome yields. An analytical tool was developed to monitor cell growth on the scaffolds, enabling precise tracking of cell numbers. Exosome purification was carried out using tangential flow filtration (TFF) and size-exclusion chromatography (SEC). Robustness was evaluated by analysing exosome size, yields, and surface markers across batches. The therapeutic effects were assessed in two SCI rat models via intranasal and intrathecal administration.

Results:

The defined combination of BM-MSC donor and media resulted in high exosome yields and low population doubling time (PDT) even at high passages, indicating potential for improvement in process cost efficiency. Testing confirmed consistent exosome yield, size, and surface marker expression across batches from the same donor. In preclinical SCI models, ExoPTEN significantly enhanced motor, sensory, and structural recovery.

Conclusions:

This study establishes a scalable, reproducible platform for ExoPTEN production, overcoming challenges in donor variability and production consistency, supporting its clinical application for SCI and nerve regeneration.

**Bio-fabrication Meets Nanomedicine: Innovations in**

**3D Bioprinting for Exosome Delivery**

Nora Raz

Almog

 The convergence of biofabrication and nanomedicine is unlocking new frontiers in therapeutic delivery. Advances in 3D bioprinting for exosome-based applications enable controlled, localized release of extracellular vesicles for regenerative medicine, cancer therapy, and beyond. These bioprinted systems demonstrate versatility across clinical and resource-limited settings, offering scalable, point-of-need solutions. By merging nanotherapeutics with customizable biofabrication, this approach highlights a promising strategy for next-generation, personalized treatment platforms across a range of biomedical applications.

**Neuroblastoma Extracellular Vesicles mediate Blood-Brain-Barrier permeability and carry tight junctions’ proteins cargo.**

Tal Manko1, Ishai Luz1, Meshi Zorsky2, Gad Vatine2, Tomer Cooks1

1The Shraga Segal Dept. of Microbiology,Immunology & Genetics, Faculty of Health Sciences; 2The Department of Physiology and Cell Biology, Ben-Gurion University, Beer Sheva, Israel

Introduction

Extracellular Vesicles (EVs) play an important role in cell-to-cell communication particularly when released by cancer cells, allowing them to interact with their tumor microenvironment. Neuroblastoma (NB) is a cancerous solid tumor commonly arising in pediatric children across the sympathetic nervous system, which causes multisite neuronal damage to children and might lead to even death. NB is considered an enigma with spontaneous remission, relapses, and primary tumor site, despite extensive research of NB there are still many gaps in our knowledge regarding its tumorigenesis, metastasis and pathology.

Results

Our results indicate that NB EVs and educated NSC34 cells significantly impact bEND3 integrity. Mass spectrometry analysis of EVs from N2A EVs unraveled several proteins that affect tight junctions’ assembly, suggesting a first reported evidence of BBB manipulations via NB cancer cells. Higher levels of tight junctions’ proteins and were found in the late-stage NB EVs (NS20), including key proteins such as Occludin, claudin and ZO-2, matching with our TEER measurements showing higher degree of destabilization of the BBB.

Summary/Conclusion

This study presents characterization of NB EVs and shows their capacity to promote disassembly of tight junctions, leading to destabilization of the BBB. Our results also suggest that EVs from NB source can contain proteins involved in tight junctions biology, leading to a new frontier of EVs biomarkers and potentially, full intact tight junctions formations that can interact with target sites.

**Single vesicle approaches for characterization of nucleic cargo in malaria-derived extracellular vesicles**

Ewa Kozela, Neta Regev-Rudzki

Departments of a Chemical Research Support, Life Sciences Core Facilities and Biomolecular Sciencesc, Weizmann Institute of Science, Rehovot, Israel

 Extracellular vesicles (EVs) are membrane-bound nano-organelles released by living cells across various kingdoms, including human parasites. EVs facilitate cell-to-cell communication by transferring bioactive molecules, thereby influencing cell function and fate. Combining cellular, biophysical and omics approaches, we have shown that malaria parasite (Plasmodium falciparum, Pf) while residing inside the human red blood cells (RBCs) utilizes EVs to regulate its growth and interaction with host immune cells. Here, we employed two advanced single vesicle technics, spectral flow cytometry and super resolution microscopy (dSTORM), to enable Pf EV (sub)phenotyping. We used these approaches to detect EVs carrying nucleic cargo and isolated from Pf infected human RBCs. We performed double EV tagging which combines co-staining of membrane lipids and RNA or DNA molecules. Using spectral flow cytometry we observed that Pf EVs are enriched in RNA cargo as compared to EVs isolated from non infected cultures. We also identified Pf EV subpopulations carrying DNA cargo. dSTORM nanoscopy showed that the detected nucleic signal is indeed encapsulated within parasitic EVs. Our results indicate that single vesicle technologies assist in the detection, quantitation and monitoring of nucleic acid cargo in EVs, advancing our understanding of EV-mediated host-pathogen cross talk.

**The role of extracellular vesicles and lipid droplets in early implantation: the dynamics of embryo-maternal cross talk**

Alisa Komsky-Elbaz1, Rita Shuhmaher1, Javier Arturo Sanchez-Lopez1, Yoav Soen2, Yael Heifetz1

1Department of Entomology, The Hebrew University of Jerusalem; 2Department of Biomolecular Sciences, Weizmann Institute of Science

Intercellular communication is essential for healthy embryo development, yet the role and dynamics of extracellular space in the maternal-embryonic dialogue remains unclear. Furthermore, little is known of maternal and embryonic metabolic states during early endometrial preparation and once the embryo enters the uterine cavity. We hypothesized that embryo-maternal bidirectional simultaneous communication begins upon the embryo’s arrival at the endometrium and is mediated by extracellular vesicles (EVs). To investigate this, we established an in-vitro co-culture model using human endometrial cells and embryonic trophoblast spheroids. Utilizing EV-specific tools, we dynamically tracked EV secretion, uptake, and functional integration between the embryo and the endometrium. Our results reveal that hormonal stimulation alters endometrial secretory output, leading to the production of distinct EV populations. Stimulated endometrium-derived EVs differed from non-stimulated EVs in size, secretion dynamics and uptake efficiency. Metabolomic analysis demonstrated that stimulated-EVs selectively package energy-related metabolites, indicating a potential mechanism for enhancing endometrial receptivity. Additionally, lipid droplets (LDs), influenced by both endometrial- and embryo-derived EVs, were actively secreted and taken up by embryonic cells, highlighting their role in early implantation. Importantly, EVs were not only exchanged between the embryo and endometrium but were also rapidly internalized and functionally integrated, influencing key cellular processes and enhancing spheroid attachment. Our findings suggest that EVs, extracellular metabolites, and LDs mobilized between the endometrium and embryo, coordinate to promote embryo attachment and implantation. This study advances our understanding of embryo-maternal EV-mediated communication and provides a valuable model for investigating EV-mediated simultaneous intercellular bidirectional crosstalk in other biological contexts.

**Advancing NK cell-derived extracellular vesicles towards a clinical-grade immunotherapy product for**

**hematological malignancies**

Aladin Samara1,2, Ayala Gover-Proaktor1,2, Lamis-Qassim1,2,3, Ido Rosen1,3,4, Shirly Partouche1,2, Saar Shapira1,2, Ido Lubin1, Pia Raanani2,3, Galit Granot1,2,3

1Felsenstein Medical Research Center, Rabin Medical Center, Petah Tikva; 2Institute of Hematology, Davidoff Cancer Center, Rabin Medical Center, Petah Tikva; 3Faculty of Medical & Health Sciences, Tel Aviv University, Tel Aviv; 4Sagol Center for Regenerative Medicine, Tel Aviv University, Israel

 Cell-based immunotherapies have transformed hematological malignancy treatment but face challenges such as severe toxicities, complex manufacturing, and immune evasion. Extracellular vesicles (EVs) have emerged as promising alternatives, offering advantages like low immunogenicity, targeting capability, and lack of proliferation. We previously demonstrated that NK-derived EVs (NKEVs) exert anti-leukemic effects in-vitro, ex-vivo, and in-vivo, highlighting their potential as a feasible immunotherapy. However, clinical translation is hindered by low-yield isolation methods that compromise purity and scalability. To facilitate clinical applications of NKEVs it is vital to develop standardized, scalable, GMP- compliant isolation methods for efficient, reproducible EV isolation in sufficient quantities. To date, most studies evaluating EV therapeutic efficacy have relied on small-scale, low-quality production methods, such as ultracentrifugation (UC). Tangential flow filtration (TFF) has emerged as a promising alternative enabling rapid efficient separation and concentration of EVs from large volumes. We developed a GMP-compatible, scalable TFF combined with size exclusion chromatography (TFF-SEC) protocol for NKEV isolation. TFF-SEC demonstrated superior performance over UC, yielding 24-fold more NKEVs than UC (2.9×10 9 vs. 3.5×10 8 particles/ml of NK conditioned media) with enhanced purity (5.89×10¹⁰ vs. 5.2×10¹⁰ particles/μg protein). NTA showed a more homogeneous size distribution (median peak: 135.7nm vs. 152.8nm). Functional assays confirmed that TFF-SEC isolated NKEVs exceed cytotoxicity and retain uptake efficiency

comparable to UC-isolated EVs. Our optimized TFF-SEC method enables the high-yield production of clinical-grade NKEVs with improved purity and functionality, addressing key barriers to clinical translation. This scalable approach facilitates the development of NKEVs as a cell-free off-the-shelf immunotherapy for hematological malignancies.

**Obesity-Mediated Molecular Alterations in Adipocyte Extracellular Vesicles Drive Gastric Cancer Progression**

Hagit Shoyhet1,2, Yahel Cohen3,4 Yifat Herman Bachinsky2 ,Dina Safina2

Eran Horenstein3,4, Shulamit Levenberg2

1 The Norman Seiden Multidisciplinary Graduate program in Nanotechnology & Nanoscience, Technion; 2Department of Biomedical Engineering, Technion; 3 Department of Molecular Biology, Weizmann Institute of Science; 4Department of Molecular Neuroscience, Weizmann Institute of Science

 Type 2 diabetes (T2D) is wide-spread chronic disorder marked by insulin resistance and disrupted cellular communication, leading to inflammation and metabolic dysregulation. Conventional treatments for T2D focus on symptoms rather than underlying intercellular signaling deficits. Extracellular vesicles (EVs), which transport crucial molecular cargo, could revolutionize therapeutic strategies. Tissue engineering of skeletal muscle has emerged as a promising approach to harness EVs for T2D intervention.

 We previously demonstrated that engineered skeletal muscle overexpressing GLUT4 (G4OE-EMC) significantly improves diabetic indicators in Diet-Induced Obesity (DIO) mice, enhancing blood glucose levels, insulin sensitivity, and cytokine profiles. In this study, we isolated EVs from GLUT4-overexpressing 3D muscle constructs and characterized them using morphological and functional assays. When applied to wild-type myotubes, G4OE-EMC derived EVs increased glucose uptake; in vivo studies in diabetic mice, showed improved glucose tolerance and response. Proteomic and microRNA analyses revealed that these EVs modulate key metabolic pathways—namely IGF-1 and insulin signaling—partly through downregulation of specific MiRs, including MiR-122, MiR-486, and MiR-16.

 These findings suggest that EVs derived from engineered skeletal muscle can transmit metabolic benefits to recipient tissues, helping explain the systemic improvements observed after implanting even a small construct. By targeting the root causes of T2D-related dysfunction, this tissue engineering strategy could offer a powerful platform for developing EV-based therapies that go beyond conventional interventions.

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

Clément Berthy1,2, Manon Allaire3, Mathieu Boissan1,2,3 and Isabelle Petit1\*.

1INSERM, UMR938, Saint-Antoine Hospital, 2Sorbonne University, 3Pitié-Salpétrière Hospital, Paris, France.

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Background:

 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.

Methods:

 EVs were isolated from plasma samples of HCC patients at different stages, as well as cirrhotic patients and healthy controls. Beads-based multiplex analysis by cytometry was used to measure the expression of various known surface proteins. We also investigated by ELISA the presence of the soluble ligands PD-L1 and VEGF, both of which can associate with EVs and are targeted by the immunotherapy. The expression of the identified markers was then compared to the clinical profiles of the patients.

Results:

 The analysis identified several EV markers specific to HCC samples compared to healthy ones as well as markers that correlate with clinical parameters or immunotherapy outcomes.

Conclusion:

 Our preliminary results reveal several markers with prognostic or predictive potential for HCC patients. This work highlights that EV surface profiling may help identify novel biomarkers for cancer detection, prognosis or longitudinal monitoring using liquid biopsies.

**Crossing Barriers: Communication between Kingdoms through Extracellular Vesicles Shared by Duckweed and Its Associated Bacteria**

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 Plant extracellular vesicles (EVs) are membrane-bound nanostructures known to mediate pathogen resistance. However, until now, plant EVs have been exclusively studied in terrestrial species. Here, we report the first isolation of EVs from the aquatic plant duckweed (Lemna japonica). We developed a novel isolation protocol combining multiple filtration steps, ultrafiltration concentration, and differential ultracentrifugation to purify EVs from duckweed exudates. The vesicles were comprehensively characterized using multiple visualization techniques including Nanoparticle tracking analysis (NTA), Atomic Force Microscopy (AFM), Transmission Electron Microscopy (TEM), and Cryo-Electron Microscopy (Cryo-EM). These analyses provided detailed insights into EV morphology and size distribution. Molecular characterization through lipidomics, semi-polar metabolomics, and proteomics revealed enrichment of defense-related proteins, membrane transporters, and enzymes involved in secondary metabolism within the isolated EVs. To investigate functional aspects, we established a natural bacterial synthetic community from the duckweed's native habitat. Notably, duckweed EVs were extensively incorporated into bacterial cells and significantly changed their growth patterns. These findings suggest that duckweed EVs function as critical mediators in establishing and maintaining the plant's natural microbiome. Our study provides the first comprehensive characterization of EVs from duckweed and highlights their potential role in cross-kingdom communication within freshwater ecosystems, offering new insights into plant-microbe interactions in aquatic environments.

**Cord Blood Proteomics Reveals Mitochondrial and Endoplasmic Reticulum Dysregulation as Early Autism Biomarkers**

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 Autism spectrum disorder (ASD) is associated with changes in the brain microstructure, particularly affecting neuronal connectivity. While genetic etiologies account for ~30% of ASD cases, the majority likely result from environmental influences during prenatal brain development. Extracellular vesicles (EVs) are critical mediators of cellular communication and are associated with the pathological conditions affecting brain development. This study utilizes Mass-spectrometry‐based proteomic analysis of EVs derived from umbilical cord blood plasma (UCB-PL) to identify potential biomarkers and pathways associated with ASD. Samples were collected from 30 children diagnosed with ASD, representing three severity levels, and 30 neurotypical (NT) individuals. The analysis identified 565 proteins with significantly different expression levels, most of which were higher in the ASD group. Interestingly, protein expression variance was markedly lower in the ASD group, suggesting a shared biological signature despite ASD’s heterogeneous nature. Differentially expressed proteins primarily formed clusters associated with mitochondria and endoplasmic reticulum (ER), with an additional cluster comprising a mixture of immune system and cytoskeleton proteins. Further analysis found 11 of the most distinguishing proteins with near-perfect predictive value in Receiver operating characteristic (ROC) analysis, along with 13 brain-specific proteins mainly associated with synaptogenesis. These findings suggest that, despite diverse etiologies, ASD may converge on a common final pathway involving mitochondrial and ER dysfunction, resulting in abnormal synaptogenesis. This study demonstrated the potential for early diagnosis of ASD susceptibility at birth and provides a foundation for further investigation of the underlying mechanism.

**Employing Atomic Force Microscopy to Analyze the Mechanics of Extracellular Vesicles and Target Cell Membrane**

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 Atomic force microscopy (AFM) serves as a powerful technique for high-resolution imaging and nanomechanical characterization of extracellular vesicles (EVs), attached to a surface under wet conditions in liquid environment, particularly in the study of host-pathogen interactions.

Comparing the mechanical properties across different EV populations, particularly those with varying sizes, could be challenging due to variations in tip-sample contact area and influence of the underlying substrate. To overcome these limitations, we employed AFM puncture tests on supported lipid bilayers formed from two distinct malaria-derived EV subpopulations: small diameter (10-70 nm) and large diameter (30-500 nm). Combined with data and machine learning analyses, this approach revealed significant differences in biophysical properties between the subpopulations, effectively avoiding size-related artifacts. Moreover, we have successfully expanded our AFM methodology to investigate the biophysical components of the immune host cells that govern the internalization of malaria-derived EVs. By comparing giant plasma membrane vesicles (GPMVs) derived from host T cells or from monocytes, we found that the internalization of malaria-EVs depends on the biophysical properties of the host cell membrane rather than solely on EV-cell membrane interactions. This integrated AFM-based approach offers a unique biophysical framework for understanding the impact of membrane stiffness on EV uptake pathways.

1 Abou Karam P. et al. (2022) EMBO Reports 23:e54755.

2. Alfandari D, et al. (2025) ACS nano, TBD.

**Extracellular Vesicles as Therapeutic Nanoparticles for**

**Pancreatic Cancer**

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Background:

Pancreatic cancer (PC) is a leading cause of cancer-related mortality, often presenting as a systemic metastatic disease with poor prognosis and limited therapeutic options. Novel therapies are urgently needed to improve patient survival. Extracellular vesicles (EVs) are spherical nanovesicles that have gained attention as therapeutic agents due to their ability to deliver therapeutic cargos to tumor sites. MiR-141, a known tumor suppressor, is downregulated in PC and plays a key role in epithelial to mesenchymal transition (EMT). This study explores the therapeutic potential of EVs enriched with miR-141 (EV-miR-141) using an innovative 3D spheroid model.

Methods:

EVs enriched with miR-141 were generated from human embryonic kidney (HEK293) cells engineered to stably express miR-141 via lentiviral shMIMIC-miR-141. Mir-141 levels in both engineered cells and their EVs were quantified by qRT-PCR. The effects of EV-miR-141 on the proliferation, migration, and invasion of PC cells were assessed through various in vitro assays. EMT-related gene expression in treated PC cells was analyzed using qRT-PCR. Additionally, 3D spheroids derived from human PC cells were generated and treated with EV-miR-141. Tumor cell viability and spheroids size were monitored over time using live-cell imaging.

Results:

HEK293 cells stably expressing miR-141 showed a 150-fold increase in miR-141 levels. EVs isolated from these cells demonstrated a 300-fold enrichment of miR-141. Treatment of human PANC1 cells with EV-miR-141 significantly reduced proliferation, migration and invasion compared to control-EVs. In addition, EV-miR-141 reduced the expression levels of the EMT related genes ZEB1, Snail and Vimentin, while increasing the expression level of the epithelial marker E-cadherin. Finally, we engineered a 3D PC spheroid model of PANC1 cells. The EV-miR-141 reduced effectively the spheroid size and growth and induced tumor cells apoptosis.

Conclusions:

Our findings demonstrate that EVs enriched with the tumor-suppressor miR-141 effectively suppress PC cell growth and EMT, highlighting their potential as a targeted therapy for PC.

**Regenerative potential of mesenchymal stem cell-derived EVs loaded with PTEN siRNA for facial nerve injury**

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Facial nerve injuries are a significant cause of peripheral nerve damage, affecting millions worldwide and impairing both physical and psychological well-being. Current treatments, such as corticosteroids and nerve grafting, often fail to ensure full recovery, highlighting the need for innovative regenerative strategies. Mesenchymal stem cell (MSC)-derived extracellular vesicles (EVs) have shown potential in nerve repair by supporting Schwann cells, promoting angiogenesis, and reducing inflammation. Downregulating phosphatase and tensin homolog (PTEN) has been linked to improved nerve regeneration, and MSC-derived EVs provide an effective vehicle for targeted siRNA against PTEN (siPTEN) delivery.

Human bm-MSC-derived EVs were isolated by differential centrifugation and loaded with glucose-conjugated siPTEN to enhance nerve regeneration. To determine the optimal administration route, we conducted an in vivo biodistribution study in a rat model of facial nerve injury. Labeled EVs were delivered via intravenous, intranasal, or intralesional (IL) routes, with distribution tracked using IVIS imaging.

IL administration resulted in the most targeted delivery, with significantly higher DiR signal in the injured nerve. The EVs + siPTEN group showed the greatest improvements in facial symmetry and eyelid closure, outperforming all other groups. Notably, symmetry improved within one day post-injury, suggesting a neuroprotective effect. By day 28, CMAP analysis confirmed the highest functional recovery. Additionally, gene expression analysis revealed the greatest upregulation of nerve growth factors and Schwann cell markers, further supporting the regenerative potential of this treatment.

These findings support bm-MSC-derived EVs loaded with siPTEN as a promising, minimally invasive therapy for facial nerve injuries, warranting further preclinical studies.

**Multidrug-resistant Klebsiella pneumoniae release outer membrane vesicles (OMVs) that facilitate bacterial survival in human serum**

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 Background and aims: Klebsiella pneumoniae (Kp) is a major bacterial pathogen causing life-threatening bloodstream infections (BSIs) that necessitate bacterial survival in human serum. Like other Gram-negative pathogens, Kp release OMVs that serve as cargo nanostructures with various host effects. However, their role in survival in human serum has not been investigated. We hypothesize that Kp OMVs facilitate bacterial survival in human serum. To investigate this, we isolated OMVs from serum-resistant and serum-sensitive strains and investigated their cross-effects on serum survival.

Methods: OMVs were recovered from three Kp isolates - a serum-resistant isolate, KpB199 and from two serum-sensitive strains - KpB10 and KpATCC13383, using Tangential Flow Filtration apparatus. OMVs were characterized using nanoparticle tracking analysis (NanoSight) and visualized using TEM and fluorescence microscopy. Proteomics was performed by LC/MSMS and analyzed using DIA-NN1.9.2. OMVs functionality was evaluated and compared by assessing their effects on the survival of each Kp strain in pooled human serum compared to survival without exogenous OMVs.

Results: We successfully isolated OMVs from the three Kp isolates exhibiting typical OMVs morphology sized 100-250nm. OMVs consisted of proteins, DNA and RNA. OMVs derived from the serum-resistant strain (KpB199) enhanced significantly (15 and 40-fold) the serum survival of the two serum-sensitive Kp strains - KpB10 and KpATCC13383, respectively (p<0.001). In contrast, OMVs derived from the two serum-sensitive strains did not affect the serum survival of either Kp strain, suggesting the unique properties of the serum-resistant-derived OMVs. Comparative proteomic analysis of the three purified OMVs revealed enrichment of unique proteins in KpB199 OMVs.

Conclusion: We demonstrate a new role for Kp OMVs in bacterial survival in human serum. Structure-function studies are under investigation. These findings highlight the important role of OMVs as a strategy for Kp-host pathogenesis during BSIs and may contribute to potential future diagnosis and targeted therapy against MDR Kp.

**Scalable and reproducible EV isolation from patient samples using optimized SEC and automation**

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Background/Introduction: Extracellular vesicles (EVs), released by all cell types into biofluids, hold great promise as a new class of biomarkers. However, the lack of scalable, high-yield, and high-purity isolation methods has limited their clinical utility.

Methods: We developed Single Molecule Array (Simoa) digital ELISA assays for EV markers (CD9, CD63, CD81) and non-EV contaminants (Albumin, ApoB-100).¹ These tools enabled precise evaluation of EV isolation by size exclusion chromatography (SEC). We optimized SEC by tuning resin type, column volume, and fraction collection, and developed high-throughput platforms, including semi-automated and fully automated formats.1,3 We also introduced Dual-Mode and Tri-Mode Chromatography to selectively deplete ApoB and Albumin, enhancing sample quality for downstream proteomics.¹ To quantify a specific internal EV cargo, we coupled the optimized SEC with a protease protection assay and applied it to measure total and phosphorylated α-synuclein in plasma EVs.2

Results: We developed a semi-automated platform capable of processing eight SEC columns in parallel and used it to measure α-synuclein species in plasma.1,2 We observed modestly elevated levels in Parkinson’s disease patients and found that phosphorylated α-synuclein was enriched inside EVs.² To further increase throughput, we adapted SEC to a 24-well plate format and engineered custom hardware for integration with liquid handling systems.³ This enabled fully automated EV isolation with high reproducibility across clinical samples.

Conclusion: We present new tools that overcome key barriers in EV research by enabling scalable, reproducible, and high-purity EV isolation from plasma and other biofluids. These advances facilitate the use of EVs in biomarker discovery, enable accurate measurement of disease-relevant EV cargo, and support the development of EV-based diagnostics for neurodegenerative diseases and beyond.

References:

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**Bio-fabrication Meets Nanomedicine: Innovations in 3D Bioprinting for Exosome Delivery**

Tali Kizhner

NurExon

 The convergence of biofabrication and nanomedicine is unlocking new frontiers in therapeutic delivery. Advances in 3D bioprinting for exosome-based applications enable controlled, localized release of extracellular vesicles for regenerative medicine, cancer therapy, and beyond. These bioprinted systems demonstrate versatility across clinical and resource-limited settings, offering scalable, point-of-need solutions. By merging nanotherapeutics with customizable biofabrication, this approach highlights a promising strategy for next-generation, personalized treatment platforms across a range of biomedical applications.

**The potential use of CAR EV’s as a therapeutic strategy for solid tumors**

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Introduction

 Solid tumors are a leading cause of cancer-related mortality in developed countries, primarily due to late-stage diagnoses resulting in poor prognosis. Chimeric Antigen Receptor (CAR) T-cells, engineered to recognize tumor-associated antigens, have shown success in hematological cancers. However, their effectiveness is limited in solid tumors due to challenges in infiltrating the tumor niche, possibly influenced by the immunosuppressive tumor microenvironment. Immunotherapy based extracellular vesicles (EVs) may overcome some of these limitations. EVs, nano-metric membraned vesicles originating from various cells, express antigens/proteins and genetic molecules reflects their parental cells. EVs interact with target cells and transfer diverse cargo, to recipient cells. CAR EVs represent a novel approach combine the benefits of both EVs and CAR T cells. CAR EVs has the recognition of the CAR to the cancer cell, while the content has all that is needed to kill a cancer cell. This study aimed to explore the *in-vitro* and *in-vivo* mechanisms of action of CAR EVs against solid tumors, and their potential to facilitating solid tumor infiltration while minimizing side effects and toxicity.

Materials and methods

 High-expression EGFR and CD276 cancer cell lines were carefully selected using Flow cytometry analysis. Four CAR retro constructs (anti-CD276.1/2 and anti-EGFR.1/2) were transduced into T cells, and their efficiency was assessed using cytotoxic assays.

After stimulating cells with antigen coated beads (CD276 or EGFR), CAR EVs were isolated using the ultracentrifuge method. The size and protein content of the EVs were characterized, and their impact on cancer cells was subsequently evaluated.

Results

Higher transduction were found in anti-CD276.1 and anti-EGFR.1. Cytotoxic abilities of these CAR-T cells were tested on three lung cancer cell lines, anti-CD276.1CAR and anti-EGFR.1CAR exhibit superior performance across different effector-to-target ratios and higher IFNγ secretion. Additionally, anti-CD276.1 and anti-EGFR.1 CAR EVs exhibit high cytotoxicity against lung cancer cells. These findings suggest the potential of these CAR-T cells and EVs as effective therapies for specific lung cancer subtypes.

Conclusion

Next, we will assess the CAR EV’s potential *in vivo*, using a mouse model. We are confident that CAR EVs have the potential to revolutionize solid tumor therapy by enhanced and efficient delivery to the tumor niche, while maintaining a comparable killing effect to traditional CAR-T cells.

**EVs from PDAC cells harboring mutant p53 can drive tumor microenvironment remodeling and fibroblast activation**

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Background: Pancreatic ductal adenocarcinoma (PDAC) has a poor survival rate due to late-stage diagnosis. Over 75% of advanced cases carry mutations in TP53, which encodes the p53 protein. Tumors expressing p53 gain-of-function (GOF) mutations induce pro-invasive alterations to the extracellular matrix (ECM) secreted by cancer-associated fibroblasts (CAFs), which make up over 70% of the tumor mass. We hypothesized that EVs from mutant p53 cells activate CAFs, altering their fiber secretion to form a desmoplastic ECM. Uncovering the role of these EVs may reveal a mechanism by which mutant p53 cancers drive tumor progression and resistance to therapies.

Methods: Human and mouse PDAC cells (WT, KO, or mutant p53) were cultured, and EVs were isolated and characterized. The EVs were then incubated with primary fibroblasts in 3D cultures. Fibroblast activation and ECM changes were analyzed using immunofluorescence staining for fibronectin, α-SMA, and palladin. Additionally, labeled EVs were injected into the pancreas of mice, with subsequent histological and morphological analysis of pancreatic tissue.

Results: Fibroblasts treated with mutant p53-derived EVs showed significantly higher expression of α-SMA and palladin, indicating activation and ECM remodeling. These EV-treated fibroblasts also displayed altered fibronectin fiber orientation and density. In vivo, we injected EVs that reached the pancreatic tissue, and pancreases treated with mutant p53 EVs exhibited observable changes in tissue morphology and a significant increase in weight, supporting the in vitro findings of TME modulation.

Conclusions: Mutant p53 is a driver mutation in advanced PDAC cases. EVs from PDAC cells carrying a mutation in TP53 might play a crucial role in reshaping the TME by modifying ECM structure and activating fibroblasts. Targeting exosome-mediated signaling pathways could be a promising approach to disrupt PDAC progression and improve therapeutic outcomes.

**The Paracrine Cross-Talk between Human Adipocyte-Like Cells and a Human Skin Equivalent Model During Burn Injury Regeneration**

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Severe burn injuries, which affect an estimated 11 million people worldwide and cause almost 200,000 deaths annually, are associated with long hospitalization periods and pose a substantial financial burden. Until recently, studies of cutaneous burns and wound healing focused mainly on the upper layers of the skin – epidermis and dermis. However, recent dogmas have suggested that hypodermal adipocytes may have an important role in wound healing by providing energy as lipids and promoting cellular proliferation and differentiation. The release of lipids from adipocytes occurs in two pathways: breakdown of intracellular neutral lipids by lipolysis and shuttling of intact neutral lipids in extracellular vesicles (EVs). To date, no human-relevant models have been used to investigate the effect of a cutaneous burn injury on adipocyte lipid metabolism or the impact of adipocyte paracrine signalling on dermal-epidermal wound healing. Therefore, we focus on the paracrine communication between human models of adipocytes and skin in a burn injury setting.

In this work, we established a platform for the automated induction of contactless thermal injuries in in vitro samples. Moreover, we developed an in vitro human skin equivalent (HSE) model composed of a dermal and epidermal compartment, and a human adipocyte-like cell model. To study the effect of a burn on the paracrine cross-talk between HSE and adipocyte-like cells, we analyze gene expression levels by RNAseq, measure the levels of secreted lipids and polar metabolites, measure cytokine levels, examine tissue and cellular morphology and analyze the quantity and size of released EVs.

**Host Immune Cell Membrane Deformability Governs the Uptake Route of Malaria-Derived Extracellular Vesicles**

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The malaria parasite, Plasmodium falciparum, secretes extracellular vesicles (EVs) to facilitate its growth and to communicate with the external microenvironment, primarily targeting the host’s immune cells. How parasitic EVs enter specific immune cell types within the highly heterogeneous pool of immune cells remains largely unknown. Using a combination of imaging flow cytometry and advanced fluorescence analysis, we demonstrated that the route of uptake of parasite-derived EVs differs markedly between host T cells and monocytes. T cells, which are components of the adaptive immune system, internalize parasite-derived EVs mainly through an interaction with the plasma membrane, whereas monocytes, which function in the innate immune system, take up these EVs via endocytosis. The membranal/endocytic balance of EV internalization is driven mostly by the amount of endocytic incorporation. Integrating atomic force microscopy with fluorescence data analysis revealed that internalization depends on the biophysical properties of the cell membrane rather than solely on molecular interactions. In support of this, altering the cholesterol content in the cell membrane tilted the balance in favor of one uptake route over another. Our results provide mechanistic insights into how P. falciparum-derived EVs enter into diverse host cells. This study highlights the sophisticated cell-communication tactics used by the malaria parasite.

**Extracellular vesicle-based delivery platform for the targeted administration of therapeutic siRNA by inhalation**

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Extracellular vesicles (EVs) are naturally secreted nanoparticles that, due to their supreme biocompatibility and natural role in encapsulating RNA fragments, have substantial potential as therapeutic RNA delivery vehicles. Small interfering RNA (siRNA)-mediated gene silencing has emerged as a promising therapeutic modality. However, effective targeting and delivery of siRNA remain significant challenges. We developed an EV-based platform for the targeted delivery of siRNAs via nebulization, enabling hyper-localized administration to the lungs while minimizing off-target effects.

o increase specificity, we genetically engineered the EVs to target cells expressing the ACE2 receptor, such as alveolar type 2 cells in the lung. This was achieved by displaying a high-affinity mutant of the SARS-CoV-2 receptor-binding domain on the EV surface (EVs-RBD62). We optimized a protocol for the encapsulation of siRNA targeting GFP (siGFP) into EVs-RBD62 and demonstrated efficient in vitro knockdown of GFP in cells after EV nebulization, as well as ACE2-dependant specificity. We also identified stability buffers that protect EVs during nebulization, ensuring their functional integrity. Subsequently, we loaded EVs-RBD62 with siRNA targeting JAK1 (siJAK1), an important pro-inflammatory mediator implicated in various autoimmune and inflammatory pulmonary conditions. We confirmed significant in vitro JAK1 knockdown in ACE2-overexpressing cells treated with nebulized, EVs-RBD62 containing siJAK1. We present early-stage in vivo results demonstrating notable JAK1 knockdown in mice treated with nebulized EVs-RBD62 loaded with siJAK1. The modular nature of this delivery platform allows for easy substitution of both the siRNA cargo and the membrane-bound EV targeting protein, allowing for a huge variety of therapeutic applications.

**Unlocking the mysteries of fibromyalgia syndrome (FMS): Exploring extracellular vesicles (EVs) as new promising biomarkers**

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 Fibromyalgia syndrome (FMS) is a chronic pain condition affecting 3%-6% of the population, with a higher prevalence in women. It is characterized by widespread musculoskeletal pain, fatigue, and cognitive impairment. The underlying mechanism of FMS remains elusive, and no definitive laboratory test exists for FMS diagnosis, underscoring the urgent need for reliable biomarkers.

 Extracellular vesicles (EVs) are nano-sized, lipid bilayer enclosed particles, released by various cell types. They play a crucial role in intercellular communications and are involved in processes such as inflammation, stress response and immune signaling. Due to their presence in biological fluids, their ability to cross the blood-brain barrier (BBB), and their bio-functional cargo (RNA, DNA, and proteins), EVs hold significant potential as diagnostic/prognostic biomarkers and as a natural carrier for drugs.

 In the current study, we characterized circulating EVs in FM patients and compared their protein profile with that of healthy controls. Plasma-derived EVs were isolated using iodixanol density gradient combined with size exclusion chromatography and analyzed using nanoparticle tracking analysis, transmission electron microscopy and western blot analysis.

 Preliminary proteomic analysis of plasma-derived EVs from FM patients revealed distinct protein expression patterns with several proteins uniquely present in FMS patients as compared to healthy controls. These proteins were associated with key biological pathways, including immune regulation (e.g. Complement component), mitochondrial function (e.g., ATP5), neuronal activity (e.g. Cofilin-1), and oxidative stress (e.g. Superoxide dismutase).

**Anti Her2 Chimeric Antigen Receptor (CAR)-T Extracellular Vesicles (EVs), an innovative and safe cancer therapy**

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Introduction: Chimeric antigen receptor (CAR)-T cells are genetically engineered T cells targeting tumor-associated antigens. Extracellular vesicles (EVs) derived from CAR-T cells (CAR-T EVs) preserve CAR-T function and may overcome limitations of CAR-T cell therapy. This study investigates the potential of CAR-T EVs as an effective immunotherapy for solid tumors.

Methods: Anti-Her2 CAR-T cells were produced and stimulated using SKOV-HER2+ cells or recombinant Her2-coated beads. EVs were isolated from the cell media at multiple time points and compared with EVs from un-transduced (UT) cells. EVs were characterized for size, concentration, and protein content using nanoparticle tracking analysis, protein arrays, and western blot (WB). The cytotoxic effects of EVs were assessed on breast cancer cells (SKBR-Her2+), ovarian cells (SKOV-Her2+), glioblastoma cells (GBL, U251-Her2+), and primary healthy cell cultures. Cytotoxic effects were measured using methylene blue killing assay and caspase 3/7 activity assay (Incucyte live imaging).

Results: CAR-T EVs contained a mixture of small and large EVs. EVs from bead-stimulated CAR-T cells consisted solely of CAR-T EVs, while EVs from SKOV-stimulated cells contained both cancer cell EVs (~60%) and CAR-T EVs (~40%). EVs from bead-stimulated CAR-T cells had higher levels of cytokines (e.g., GM-CSF, I-309, IL-13, IL-17, RANTES) and induced significant killing rate (60-85%) of Her2+ cancer cells compared to UT EVs, similar to EVs from SKOV-stimulated cells, but did not affect the viability of healthy cells.

Conclusion: CAR-T EVs produced by Her2-coated beads may offer a novel, pure, and potent immunotherapy approach, demonstrating effective cytotoxicity without affecting healthy cells.

**Extracellular vesicles (EVs) as biomarker for cGVHD, and the therapeutic potential of placental cell-derived EVs**

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Introduction: Graft-versus-host disease (GVHD) is a serious complication following allogeneic hematopoietic cell transplant (HCT). Chronic GVHD (cGVHD) is a multi-organ, immune-mediated, life-threatening disorder affecting organs such as the lungs and skin. Placental extracellular vesicles (EVs) may be used for cGVHD treatment. This study explored the potential use of EVs as biomarkers of cGVHD state and the anti-inflammatory and anti-fibrotic effects of placental EVs on cytokine-induced dermal and lung cells.

Methods: EVs were obtained from 22 healthy controls (HC-EVs) and 16 cGVHD patients and characterized using nanoparticle tracking analysis, western blot, flow cytometry, and RT-PCR. The effects of patient-derived EVs versus HC-EVs on normal human dermal fibroblasts (NHDF) were studied. The anti-inflammatory and anti-fibrotic effects of placental EVs on cytokine-induced NHDF, human epidermal keratinocytes (HACAT), lung fibroblasts, and normal epithelial airway cells (AECs) were explored.

Results: cGVHD-EVs displayed elevated αSMA protein levels and fibrosis-related miRNA-221 expression compared to HC-EVs. Exposure of NHDF cells to patient-derived EVs increased TGFβ and SMAD7 expressions, indicating their pathological role. Placental EVs attenuated cytokine stimulation (TNFα/INFγ 0.01-0.1 ng/µL) effects on HACAT cells and normalized cell proliferation. Treatment of NHDF cells with a combination of TGFβ and placental EVs reduced the stimulatory effects of TGFβ on αSMA production by over 40% (p = 0.0286). Placental EVs mitigated cytokine-induced anti-proliferative effects in AECs and decreased IL-6 gene and protein levels in TGFβ-stimulated lung fibroblasts.

Conclusion: cGVHD-EVs can serve as biomarkers for disease states. Placental EVs may be used to regulate dermal inflammation and fibrosis.

**Obesity-Mediated Molecular Alterations in Adipocyte Extracellular Vesicles Drive Gastric Cancer Progression**

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Background: Peritoneal metastases of gastric cancer (GC), particularly in the omentum, indicate advanced disease and poor prognosis. The omentum, composed primarily of adipocytes, plays a key role in metabolic, inflammatory, and cancer-related processes, which are further enhanced in obesity, creating a favorable pre-metastatic niche. Extracellular vesicles (EVs) facilitate cell-to-cell communication in the tumor microenvironment by transferring bioactive cargo. This study examines the interaction between GC cells and EVs from omental adipocytes of lean and obese GC patients (Ad-EVs). Materials and Methods: Ad-EVs were isolated from the omentum of lean and obese GC patients and characterized by standard criteria. They were co-cultured with a GC cells for functional assays and bulk RNA-seq, while their microRNA cargo was profiled separately. A murine peritoneal metastasis model was used, involving peritoneal injections of GC-luciferase cells with lean or obese Ad-EVs. Results: Both lean and obese Ad-EVs enhanced GC cell proliferation, with obese EVs significantly increasing migration and invasion. We identified 30 differentially expressed microRNAs in lean vs. obese Ad-EVs. In immunocompromised mice, obese Ad-EVs increased metastatic rates compared to lean Ad-EVs. GC cells stimulated with Ad-EVs exhibited significant transcriptomic changes linked to key biological pathways. Conclusions: This study reveals that omental adipocyte-derived EVs from obese vs. lean GC patients may promote GC progression. Our findings highlight a novel mechanism linking obesity and GC aggressiveness via EV cargo, suggesting potential RNA-based therapeutic targets.

**Elucidating the involvement of Acidovorax temperans-derived outer membrane vesicles in lung cancer progression**

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Introduction

Dysbiosis is common in solid tumors, but its direct contribution to tumor development is unclear. Our previous work identified the Gram-negative Acidovorax temperans as enriched in tumors of smokers and TP53 mutation patients, where it accelerated tumor growth through pro-inflammatory cell infiltration in the lungs. This study investigates the role of outer membrane vesicles (OMVs) shed by A. temperans in driving inflammatory dynamics and tumorigenesis.

Methods

A. temperans OMVs were isolated using serial centrifugations, filtration, and an OptiPrep density gradient. Characterization was performed using NTA, TEM, and proteomics. A549 lung cancer cells and PMA-differentiated THP-1 macrophages were used as in vitro models. OMV uptake was assessed by confocal microscopy. qRT-PCR and transcriptomics were used to evaluate downstream effects of OMV treatment. For in vivo studies, OMVs were administered intranasally to C57BL/6 mice, and their delivery to the lungs was confirmed by ex vivo imaging. Flow cytometry identified OMV uptake by immune cells. CyTOF profiled immune dynamics in OMV-treated lungs. RNA-Seq of OMVs characterized their sRNA cargo.

Results

A temperans OMVs were efficiently internalized by A549 and THP-1 cells. Transcriptomic analysis of OMV-treated cells revealed dysregulation of genes involved in immune signaling and tumor-associated pathways. In vivo, OMVs reached the lungs, increased cytokine secretion, and were preferentially taken up by CD11b⁺ myeloid cells. CyTOF analysis of OMVs-treated lungs revealed that OMVs increased infiltration of dendritic cells, macrophages, and T helper 17 cells. OMV RNA-seq identified a unique sRNA signature including various fragmented tRNAs that are selectively loaded in OMVs and are predicted to target host mRNAs.

Summary/Conclusion

Interactions between OMVs shed by A. temperans and lung cancer microenvironment lead to accelerated, inflammation-based tumorigenesis.

**Dialogue on the fly: Drosophila as a model for tissue communication in the female reproductive tract**

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 Successful reproduction involves a series of timed events, which require precise communication between the female reproductive tissues, as well as between them and the male gametes and seminal fluid. Among the agents of such communication, extracellular vesicles play a major role. We use Drosophila melanogaster as a model organism to better understand EV-mediated processes in inter-tissue and inter-sex communication within the female reproductive tract. Drosophila shares many traits with the mammalian models, such as ovulation, sperm storage and guidance, and final maturation of the egg and the sperm. Similar to mammals, these processes are dependent on the successful transmission and reception of a multitude of signals between the female tissues, the sperm, and the seminal fluid that accompanies it. Unlike mammals, these processes are readily manipulated and visualized using the powerful Drosophila genetic tools.

 Here we show that EVs secreted by the reproductive endocrine glands of Drosophila, specifically the spermathecae and accessory glands, are taken up by all tissues of the female reproductive tract, and the involvement of clathrin-mediated endocytosis in this process. Additionally, we show how mating influences both the secretion of these EVs and their uptake. Finally, we demonstrate the significance of the secretions from both glands for female fertility.

**NPCE EVs Adding to the Oxidative Stress Burden of the Trabecular Meshwork: Lipidomic Insights and Phospholipase Activity**

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 The non-pigmented ciliary epithelium (NPCE) plays a crucial role in aqueous humor (AH) homeostasis, secreting extracellular vesicles (EVs) that contribute to intercellular communication. In primary open-angle glaucoma (POAG), oxidative stress disrupts AH dynamics and trabecular meshwork (TM) function, yet the role of NPCE-derived EVs in this process remains unclear. Here, we investigate the impact of oxidative stress on NPCE-EV lipid composition, phospholipase activity, and their potential contribution to TM oxidative burden.

Human NPCE cells were subjected to acute (15 mM AAPH, 90 min) and chronic (1.5 mM AAPH, 24 h) oxidative stress conditions. Lipids from NPCE-EVs were extracted using the Bligh and Dyer method and analyzed via MRM-based untargeted lipidomics. Phospholipase presence and activity were assessed. Statistical analysis was performed using MetaboAnalyst 6.0, applying unpaired t-tests (p < 0.05) and volcano plots (1.2-fold change, p < 0.05).

Oxidative stress induced significant alterations in NPCE-EV lipid profiles, notably increasing cholesteryl esters, diacylglycerols, sphingolipids, and oxidized phospholipids. Phospholipase activity was detected, suggesting a role in lipid remodeling within NPCE-EVs. These modified EVs may be internalized by TM cells, exacerbating oxidative stress and potentially impairing AH outflow.

Future studies should explore the downstream effects of oxidized NPCE-EVs and phospholipase-driven lipid remodeling on TM function. Understanding NPCE-EV lipid dynamics and enzymatic activity may offer novel therapeutic targets for glaucoma management.

**Investigating Viral Membrane Fusion with a Controlled Giant Plasma Membrane Vesicles Based Platform**

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Membrane fusion is fundamental to biological process such as membrane trafficking and viral infection. However, studying fusion mechanisms in live cells is challenging due to their complex composition and topography. To overcome this challenge, we established a controlled cell-free system using giant plasma membrane vesicles (GPMVs) to investigate the receptor- binding and fusion process of SARS-CoV-1 Spike protein with the human ACE2 receptor in a physiologically relevant yet simplified environment. We generated two distinct populations of GPMVs, one expressing ACE2-GFP and the other expressing Spike and cytoplasmic mCherry. Using live confocal microscopy, we visualized and monitored the docking kinetics of Spike GPMVs onto ACE2 GPMVs in the presence of calcium, confirming receptor accumulation at the docking interface. To induce fusion, we introduced Trypsin, a protease known to activate Spike, and used ImageStream technology to quantify fusion efficiency, providing a high-throughput assay for studying membrane fusion. This GPMV based platform offers a versatile tool for dissecting fusion mechanisms beyond viral interactions, eliminating the complexities associated with live cell studies while preserving the native characteristics of the plasma membrane. By enabling precise control over experimental conditions, this system provides new opportunities for studying fusion processes with applications in drug delivery, therapeutic design, and fundamental membrane biology research.

**Out-of-the-Box EV-labeling: Cytolight as a Promising Alternative to Conventional Dyes**

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Efficient labeling of extracellular vesicles (EVs) is necessary for studying their dynamics both in-vitro and in-vivo. Yet, current EV-labeling protocols pose serious limitations. Some dyes undergo aggregation, leading to inaccurate quantifications. Additionally, membrane labeling may alter EV surface properties, affecting interactions, uptake and targeting capabilities. Fluorescence transfer between labeled EVs and surrounding membranes is another concern. Additionally, some dyes are prone to diffusion and photobleaching, limiting long-term imaging and others are restricted to in-vitro applications. In this study, we assessed the efficacy of Cytolight Rapid Red, traditionally used for live-cell imaging, as a label for EVs and compared its performance to commonly used EV-dyes such as ExoBrite, CFSE and PKH-26. Using NTA and single-EV flow cytometry, we found that Cytolight-labeled EVs exhibited a significantly higher percentage of fluorescent particles compared to CFSE- and ExoBrite-labeled EVs, demonstrating superior staining efficiency. Moreover, Cytolight significantly outperformed other dyes in terms of signal intensity and retention. Notably, unlike PKH, known for self-aggregating, Cytolight showed no such tendency, ensuring more reliable vesicular visualization. Cellular-uptake studies using microscopy and flow cytometry further confirmed Cytolight's efficacy, revealing a significantly higher signal-to-background ratio than other dyes. Importantly, Cytolight enabled successful in-vivo EV biodistribution analysis via ex-vivo fluorescence imaging. Our findings establish Cytolight as a reliable, aggregation-free and highly effective approach for labeling EVs with minimal residual dye, offering distinct advantages over conventional dyes like ExoBrite, CFSE and PKH in terms of labeling efficiency, stability, and analytical reliability.

**Microbiota transfer following liver surgery involves microbial extracellular vesicle migration that affect liver immunity**

Shmuel Jaffe Cohen et al.,

TLMC

Background: Short-term perioperative administration of probiotics was shown to alleviate postoperative complications and promote liver recovery among patients undergoing resection for liver malignancy. The mechanisms by which probiotic bacteria effectively influence the gut microbiome composition during the perioperative time are controversial. Here, we aim to elucidate the short-term direct biological effect of probiotic microbiota–derived vesicles on host liver cells during the perioperative period.

Methods: Probiotic-derived vesicles (pbMVs) were administered postoperatively. pbMVs were isolated and characterized from probiotics, mainly from the bacteria genus Lactobacillus, Bifidobacterium, and Lactococcus. Mice underwent bile duct ligation, sham laparotomy (SHAM), or 70% partial hepatectomy (70%PH). pbMVs were tracked in vivo, and intrahepatic cellular and molecular aspects were analyzed by flow cytometry and qRT-PCR techniques. Liver sinusoidal endothelial cells (LSECs) analysis for Vascular Cell Adhesion Molecule-1(VCAM-1) expression following pbMV stimulation of cultured liver non-parenchymal cells which had been activated by LPS.

Results: The administered pbMV rapidly translocated to the liver after surgery. pbMV administrations following surgeries enhanced neutrophil clearance; there was a dramatic decline in the liver neutrophil-to-lymphocyte ratio Ly6G+/CD3+ and an increase in IL6 levels. pbMVs reduced intrahepatic VCAM1 and ICAM2 expression compared with control following SHAM and decrease in IL10 levels following 70%PH. The administration of pbMV improved liver regeneration 72 hours following surgical liver resection with a significant decrease in IL17 expression. pbMVs modulated VCAM-1 on liver sinusoidal endothelial cells in liver cell culture.

Conclusions: Our study findings provide mechanistic insights into the liver-gut axis following surgery and illustrate how probiotic vesicles can reduce adhesion molecule expression and affect immune cell invasion and liver immunity, resulting in improved liver recovery following hepatic surgery.

**Targeted Migration of Milk-Derived Exosomes to Brain Inflammation Sites: Implications for Neurological Therapeutics**

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Milk-derived exosomes have emerged as potential therapeutic vehicles for brain injuries due to their ability to cross biological barriers. This study investigates the migration of milk exosomes to inflammation sites in the brain. Using a mouse model, we induced localized inflammation in the striatum by injecting either lipopolysaccharide (LPS) or endothelin-1. Milk exosomes were administered intranasally or orally (per os) to assess their biodistribution. Our findings demonstrate that milk-derived exosomes successfully migrate to the lesion site in the brain, regardless of the administration route. This targeted accumulation of exosomes at the site of inflammation suggests their potential as natural nanocarriers for delivering therapeutic agents to damaged brain areas. The ability of milk exosomes to reach inflamed brain regions after non-invasive administration highlights their promise as a novel approach for treating various neurological disorders and brain injuries.

**Studying the Role of Outer Membrane Vesicles from Plant**

**Pathogenic Bacteria in Host Plant infection**

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 Recent studies have advanced our understanding of the role of extracellular

vesicles (EVs) play in inter-organismal interactions. EVs are nanometric

structures surrounded by a lipid bilayer, which encapsulate, preserve, and

deliver their cargo to target sites. EVs play a key role in pathology by

stimulating the host immune system and enhancing pathogen virulence.

However, their interactions with plants remain largely unexplored.

Preliminary work in our laboratory demonstrated that EVs derived from the

pathogenic bacterium Xanthomonas campestris pv. campestris (Xcc) induced

a broad immune response in the plant host Arabidopsis thaliana. To study the

roles of EVs further, we sought to characterize the molecular cargo of Xcc-

derived EVs to identify factors that may influence plant-pathogen interactions.

We performed proteomic, DNA, mRNA, and sRNA analyses of EVs, revealing

the presence of sRNA sequences that complement genes in Arabidopsis and

tomato. This led us to hypothesize that Xcc delivers sRNA molecules into

plant cells via EVs to regulate host gene expression and facilitate infection.

**The contribution of Bone Marrow-Derived EVs to Venetoclax Resistance in Acute Myeloid Leukemia**

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 Acute Myeloid Leukemia (AML) is a complex and aggressive hematological

malignancy characterized by differentiation blockage and proliferation of clonal hematopoietic stem or progenitor cells leading to bone marrow (BM) failure. The introduction of targeted agents to treat AML, such as venetoclax, a selective B-cell lymphoma 2 (BCL-2) inhibitor, has revolutionized treatment protocols. Venetoclax promotes apoptosis of leukemic cells, leading to improved clinical outcomes. However, the emergence of resistance to venetoclax remains a critical barrier. Recently, the pivotal role of the tumor microenvironment in mediating drug resistance was addressed. Specifically, extracellular vesicles (EVs, exosomes), nano-sized vesicles facilitate communication within the tumor microenvironment. They carry a cargo of proteins, lipids, RNA, and other molecules that can modulate cellular responses, including survival and drug resistance. Our study aims to investigate the role of AML-derived EVs in mediating venetoclax resistance by inducing stromal cells to secrete cytokines. To this end, we exposed 4 AML cell lines to venetoclax and showed their differential response to the drug. We isolated EVs from these cells and showed the kinetics of their uptake by SH-5 stromal cells. In addition, we created cells that are resistant to the drug and show the effect of their exosomes in conferring resistance to the sensitive cells. Finally, the effects of the tested AML exosomes on the secretion of IL-6 and IL-8 by SH-5 cells are shown.

 Ultimately, this study will deepen our understanding about the tumor microenvironment's impact on treatment resistance, paving the way for more effective therapeutic strategies in AML.

**The role of alternative splicing factors in exosomes isolated from hematological and solid malignancies**

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 Hematological malignancies comprise a heterogeneous group, varying in their diagnostic methods. Overall, there is a luck of sensitive and not invasive methods for the diagnosis of this group of diseases. Similarly, there are no easily detectable tumor biomarkers for the clinical evaluation of brain tumors (GBM, meningiomas and low glioma). Existing MRI based methods suffer from a limited ability to differentiate between therapy effects and the progression of the tumor. Alternative splice (AS) factors regulating alternative splicing process are suggested to serve as biological markers in solid and hematological cancers. Based on these studies and on our preliminary results we aimed to assess the validity of three AS factors within exosomes as predictive markers for both types of malignancies. To this end, we have isolated exosomes from the sera of patients with both malignancies and found that the three alternative splice factors: HNRNPA2B1, PTBP1 and SRSF6 are differentially expressed in their secreted exosomes compared to healthy individuals. To decipher exosomal AS factors putative roles in the communication between cancer and microenvironmental cells we exposed endothelial cells to K562 and U87 exosomes and found that they were taken up by endothelial cells in a time and dose dependent manner. Currently we study the putative role of these AS factors by following changes in phenotype and in the levels of their targets in the recipient cells. The results of our study may advance future development of liquid biopsies based method to evaluate disease diagnosis and progression.

**The role of fibroblast exosomes in the tumor microenvironment of mycosis fungoides in promoting immune suppression**

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Introduction: Cancer-associated fibroblasts (CAFs) are a major component of the tumor microenvironment (TME) stroma. Exosomes are nanosized extracellular vesicles secreted by all cells to facilitate intercellular communication. We previously demonstrated the presence of CAFs in the TME of mycosis fungoides (MF). In this study, we investigated the effects of exosomes derived from MF fibroblasts (MF-Fs) versus normal fibroblasts (N-Fs) on immune cells.

Material and Methods: Primary fibroblasts were established from MF patient biopsies and normal skin. Exosomes were isolated from the supernatant of MF-F and N-F cultures via ultracentrifugation and characterized using electron microscopy, Nanosight, and flow cytometry (FACS). The internalization of fibroblast-derived exosomes into normal peripheral blood mononuclear cells (PBMCs) and monocytes (from healthy donors) was confirmed through microscopy of labeled exosomes. Immune cell characterization was performed using mass cytometry (CyTOF) with a panel of 41 antibodies. M1 and M2 macrophage polarization was analyzed by FACS and qRT-PCR for protein markers and cytokine expression. PD-L1 expression was assessed by FACS. Specific microRNA expression was analyzed via qRT-PCR. Primary monocytes, CD4+ T cells, and CD8+ T cells were isolated by negative selection, and T cell viability was evaluated using trypan blue staining.

Results: CyTOF analysis of nPBMCs revealed that N-F exosomes increased Th1 and CD8+ cytotoxic T cells, whereas MF-F exosomes led to a reduction in Th1 cells, M1 macrophages, and Th17 cells and promoting M2 macrophage polarization. The polarization of monocytes into M2 macrophages was further confirmed by the upregulation of M2 cytokines following incubation of primary monocytes with MF-F exosomes compared to N-F exosomes. Additionally, both MF-F and N-F exosomes increased PD-L1 expression in M1 and M2 macrophages, with a more pronounced effect observed in MF-F exosomes. Monocytes polarized into M2 macrophages with high PD-L1 expression by MF-F exosomes, compared to those polarized by N-F exosomes, exhibited a stronger suppressive effect, reducing the viability of both cytotoxic and helper T cells. To investigate the molecular mechanisms underlying exosome-mediated PD-L1 upregulation and M2 polarization, we analyzed microRNA expression in MF-F versus N-F exosomes. We found that miR-23a-3p, a known positive regulator of PD-L1 and M2 polarization, was significantly upregulated in MF-F exosomes compared to N-F exosomes.

Our findings suggest a novel mechanism by which MF fibroblast-derived exosomes contribute to an immunosuppressive microenvironment. Specifically, these exosomes promote M2-like macrophage polarization with high PD-L1 expression, leading to the suppression of T cell responses. This highlights a potential new avenue for improving immunotherapy in MF by

targeting fibroblast-derived exosome signaling.

**The crosstalk between senescent and stem cells: the impact of MSC-derived EVs**

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 Cellular senescence (CS) is a phenomenon aimed at preventing the damaged cells from cancer transformation. However, senescent cells per se could promote pro-inflammatory and pro-cancerogenic microenvironment. At the same time, CS was suggested to be an important or even obligatory component of cell reprogramming. A growing body of evidence indicates that there is a crosstalk between senescent and stem cells, and this crosstalk could, to a great

extent, be attributed to EVs. To further evaluate the role of EVs in regulating the pools of stem and senescent cells, we examined the impact of MSC-derived EVs on CS and the abundancy of stem cells in primary cultures of human pulmonary fibroblasts (HPFs). Stem cells in HPF cultures (so called Side Population; SP) were evaluated using Hoechst staining followed by FACS analysis. CS was evaluated based on the rate of cell growth, cell morphology, and molecular markers. We found that the effect of MSC-derived EVs depends

on the stage of CS. EVs concomitantly increased the number of senescent cells and SP stem cells in pre-senescent HPF cultures but decreased the number of senescent cells with a simultaneous increase in a pool of stem cells in senescent HPF cultures. The rate of an in vitro wound healing, which commonly attenuates in the course of CS, was significantly higher in the EV-treated senescent HPF cultures compared to untreated ones. Our findings further strengthen the existence of regulatory loops between senescent and stem cells, which could be modified by MSC-derived EVs.

**Endothelial Dysfunction Alters Extracellular Vesicles’ Character and Secretion Profile**

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Introduction:

 Endothelial cells (ECs) line the lumen of blood vessels and play a central role in regulating vascular and tissue homeostasis. They are known to release endothelial extracellular vesicles (EEVs), which transport bioactive cargo to recipient cells. EEVs have been shown to promote cytoprotection against ischemia-reperfusion injury (IRI) while supporting cardiomyocyte viability and function. In metabolic diseases such as diabetes and obesity, ECs undergo endothelial dysfunction (ED), contributing to cardiovascular complications. We hypothesize that ED associated with metabolic disease is linked to changes in the properties of their associated EEVs, thereby altering the cytoprotective effects of EEVs and their homeostatic function.

Methods:

 Human umbilical vein endothelial cells (HUVECs) were cultured under normal, high glucose, high lipid, or combined conditions to mimic diabetes-like environments. EEVs were isolated using differential ultracentrifugation and size exclusion chromatography. HUVEC morphology and function were assessed to confirm ED. EEVs were analyzed for particle number, biomechanical properties, surface markers, and cargo content.

Results:

 HUVECs cultured in hyperglycemic and/or hyperlipidemic conditions exhibited morphological changes, impaired barrier function, and impaired angiogenic capacity —consistent with ED. EEVs from these cells showed altered secretion profiles, surface characteristics, biomechanical properties, and cargo composition, indicating a disease-modulated phenotype.

Conclusions:

 Our findings demonstrate that diabetes-like conditions induce ED in HUVECs and significantly alter the properties of their secreted EEVs. These changes may undermine EEV-mediated cytoprotection and contribute to the increased cardiovascular risk associated with metabolic diseases.

**ExoPTEN: A Revolutionary Exosome-Based Therapy for Spinal Cord Injury and Nerve Regeneration**

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 NurExone is a platform company pioneering exosome-based therapies to address nerve damage and support nerve regeneration across multiple clinical indications. Our first product, ExoPTEN, targets acute spinal cord injury (SCI), utilizing exosomes derived from bone marrow mesenchymal stem cells (MSCs) to deliver a proprietary siRNA targeting PTEN, an inhibitory regulator in the mTOR pathway. Exosomes, as natural carriers, provide a highly effective and targeted delivery system for therapeutic molecules, while their ability to cross biological barriers makes them ideal for regenerative therapies.

Methods: Our exosome platform has been tested in two well-established preclinical rat models of SCI: full spinal cord transection and spinal cord compression. The siRNA-loaded exosomes were administered via intranasal and intrathecal routes. Efficacy was measured through MRI, BBB motor scoring, von Frey sensory tests, and immunohistochemistry. Homing ability was confirmed through fluorescent labeling, up to 7 days post-injury.

Results: ExoPTEN demonstrated significant efficacy in both tested preclinical rat models. Improvements were observed in motor, sensory, and structural functions, as confirmed by MRI and histological analyses. ExoPTEN exhibited strong and effective homing capacity to the injury site, up to 7 days post-injury.

Conclusion: These results, combined with orphan drug designation for acute SCI and strong FDA support from the pre-IND submission, highlight the robustness and versatility of our exosome technology. This manufacturing platform allows for the loading and delivery of diverse therapeutic payloads, extending its application to other clinical indications marked by nerve damage, such as glaucoma. NurExone is advancing this platform to serve unmet medical needs in regenerative medicine, particularly in conditions requiring nerve regeneration.

**Extracellular vesicles derived from polarized anti-inflammatory MSCs increase hippocampal neurogenesis and ameliorate cognitive aging in mice**

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 Age-related decline in cognitive functions is associated with reduced hippocampal neurogenesis and changes in the systemic inflammatory milieu. Mesenchymal Stem Cells (MSC) obtain key therapeutic properties originating from their rich secretome. In addition, MSC are known for their immunomodulatory properties. Accordingly, MSC are a leading candidate for cell therapy and to alleviate inflammatory diseases and aging frailty via systemic delivery. We have recently developed a protocol for polarizing MSC towards an anti-inflammatory phenotype (pMSC). When administered systemically, pMSC reduced systemic inflammation, increased hippocampal neurogenesis, and ameliorated cognitive deficits in old mice. In the present study, we report that extracellular vesicles (EVs) obtained from pMSC were superior to EVs obtained from naive MSC in promoting hippocampal neurogenesis and cognitive functions in aging mice. In 9-month-old ICR mice, intranasal delivery of EVs derived from pMSC increased proliferating (Ki67+) neuroprogenitors in the subgranular zone of the dentate gyrus and increased spatial learning in the Morris water maze and Y-maze tests. In 18-month-old C57/bl6 mice, intranasal delivery of EVs derived from pMSC significantly increased the number of newly formed neurons (DCX+) in the granular cell layer of the dentate gyrus and improved spatial memory in the Novel object location test. Interestingly, these changes were accompanied by increased plasma levels of Th17 cytokines such as IL-21 and IL-17, well known regulators of neurogenesis and cognitive behavior. Proteomics and metabolomics analysis of EV content revealed slight changes between EVs derived from pMSC and naive MSC, supporting their function in promoting cell migration and immune modulation. In conclusion, EVs can effectively carry the therapeutic properties of their parental pMSC and promote cognitive health during aging.

**Empowering Extracellular Vesicle Research: Advanced Support and Innovation at the Weizmann Life Sciences Core Facilities**

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 The EV Expertise Virtual Unit at the Life Sciences Core Facilities of the Weizmann Institute of Science offers a comprehensive, multidisciplinary platform for advanced extracellular vesicle (EV) research, supporting projects from the early stage of the research to translational applications. Our mission is to empower academic and industry collaborators with cutting-edge, reproducible, and scalable solutions across EV characterization, functional studies, cargo analysis and therapeutic development.

 We provide integrated, end-to-end workflows encompassing EV production, isolation, and in-deep characterization, leveraging advanced biophysical analyses (NTA, DLS, zeta potential), high-resolution imaging (cryo-TEM, super-resolution microscopy, IncuCyte), and small-particle flow cytometry. These capabilities are further enhanced by multi-omics profiling, including next-generation sequencing (NGS), high-resolution proteomics, and targeted metabolomics.

 Our unit collaborates closely with other state-of-the-art Core Facilities at Weizmann such as electron microscopy, image stream flow cytometry, and omics units to deliver fully customized and multidimensional EV analysis pipelines. This synergistic approach enables us to support diverse research goals, from fundamental mechanistic studies in basic science to preclinical pipelines in drug development, diagnostics, and delivery systems.

**Cancer cells release ubiquitinated PCNA via extracellular vesicles**

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Introduction: Proliferating Cell Nuclear Antigen (PCNA) is a nuclear protein that enhances DNA polymerase processivity during DNA replication. It primarily resides in the nucleus, interacting with proteins involved in DNA replication and repair.  We show that PCNA is also present in extracellular vesicles (EVs) from cancer cells. The presence of PCNA in EVs is therefore investigated to delineate whether PCNA carried by EVs and taken up by tumor microenvironment cells might further contribute to tumorigenesis.

Methods: EVs from cancer cell lines including A549 (lung carcinoma), JimT1 (breast carcinoma), PANC1 (pancreatic carcinoma) and Jurkat (T cell leukemia) were isolated via ultracentrifugation following MISEV guidelines. After isolation, EVs were characterized by size and concentration using nanoparticle tracking analysis (NTA). We tested for the presence of exosomal PCNA using a unique antibody and western blotting. Subsequently, we examined the uptake of these EVs by the A549 , Jurkat, and NK cells.

Results: We found that PCNA is present on the surface of EVs derived from lung, breast, pancreatic, and T cancer cells. We discovered that PCNA is in a ubiquitinated form in Lysine 164. A549 and Jurkat cells exhibited uptake of these EVs compared to NK cells, as observed in a time-lapse examination for a 24-hour period. NK cells were co-cultured with PCNA-containing EVs, showing no significant changes in activity markers (e.g., CD107a) by flow cytometry.

Summary: The presence of ubiquitinated PCNA on EVs derived from cancer cells suggests a potential mechanism for immune modulation or as a marker for DNA damage. Further investigation is needed to fully unravel the role of exosomal ubPCNA in the context of cancer, immune modulation and DNA damage response.

**Enhancing Natural Killer (NK) cells -Derived Extracellular Vesicles (EVs) for Targeted Cancer Immunotherapy via Chimeric Antigen Receptor (CAR) Engineering**

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Introduction: EVs derived from immune cells hold promise for cancer immunotherapy. This study investigates the potential of enhancing the anti-tumor capabilities of natural killer (NK) cell-derived EVs by engineering their parental NK cells with chimeric antigen receptors (CARs) targeting HER2+ cancers. This strategy aims to combine the inherent cytotoxicity of NK cells with the specificity of CARs to enhance the targeted delivery of anti-cancer agents via EVs.

Methods: NK-92 cell line was modified to express two generations of CARs: a first-generation (Gen-0) with an anti-HER2 scFv linked to mouse CD3ζ and a second-generation (Gen-2) with an anti-HER2 scFv linked to human CD28 and CD3ζ. These constructs were chosen to investigate the impact of co-stimulatory domains on CAR-NK cell activity and EV production. EVs were isolated from these CAR-NK cells using differential centrifugation and ultracentrifugation techniques. EVs were then characterized using nanoparticle tracking analysis (NTA). The anti-tumor activity of CAR-NK-EVs was evaluated in vitro against the HER2+ (JIMT1) breast cancer cell line using flow cytometry-based cytotoxicity assays.

Results: CAR-NK cells expressing Gen-0 and Gen-2 anti-HER2 CARs were generated, as confirmed by flow cytometry analysis. The EVs exhibited typical characteristics of exosomes, with most EVs falling within the size range of  100-200 nm. NTA analysis revealed a slight increase in size heterogeneity in CAR-NK-EVs, particularly in Gen-2, which may reflect alterations in EV biogenesis or cargo sorting.  Cytotoxicity assays showed that CAR-NK cells and CAR-NK-EVs enhanced activity against HER2+ cancer cells, with Gen-2 exhibiting the highest efficacy, followed by Gen-0, compared to untransduced NK cells and their EVs.