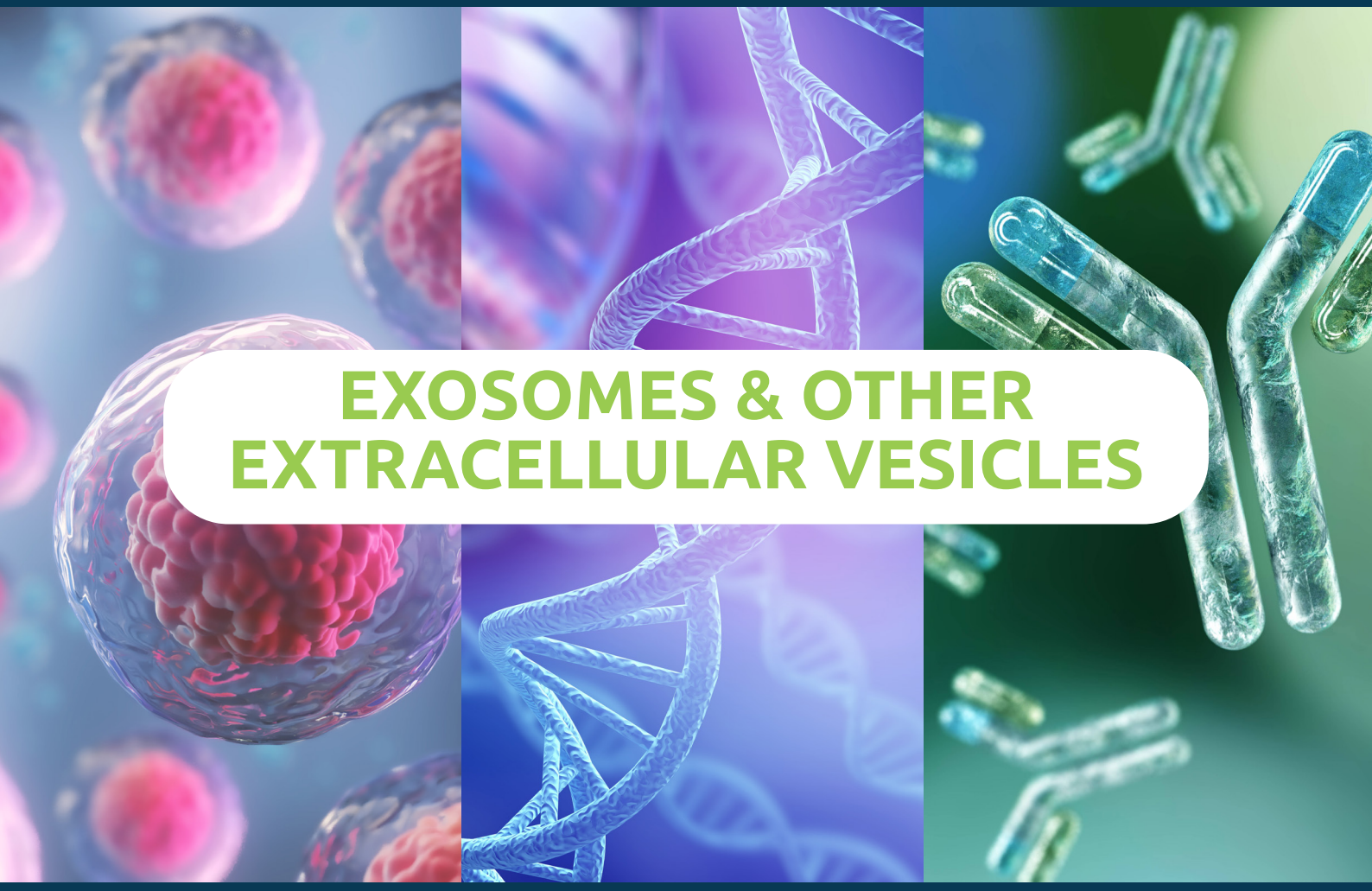
IMMUNOWATCH

EDITION N°9 – JUNE 2024



EXOSOMES & OTHER EXTRACELLULAR VESICLES

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INTRODUCTION

MabDesign's Immunowatch is a one-of-a-kind information monitoring newsletter in the field of biologics. Its aim is to provide members of our association with the most recent and pertinent data gathered or generated through the key expertise of MabDesign and its collaborators in scientific research, business intelligence, market analysis and intellectual property.

Each edition will focus on trending type of biologics. Its general format includes market study research, financial and economic data, invited contributions from scientific teams working in the industry or in academia and a section dedicated to intellectual property. The content of each edition is decided by an editorial composed of two field experts. Decision concerning the theme and conception of each newsletter is done in-house by the permanent members of our editorial team.

Finally, we would like to acknowledge the support of the Ambition Recherche & Développement (ARD) Biomédicaments 2020 Phase II programme, funded by the Centre Val de Loire region during the initial phases of launching this newsletter.

BIOPHARMACEUTICALS
Innovation Dynamics in Health
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VIDEO DROP

Measuring Size and Concentration of Extracellular Vesicules

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In real time (40s)

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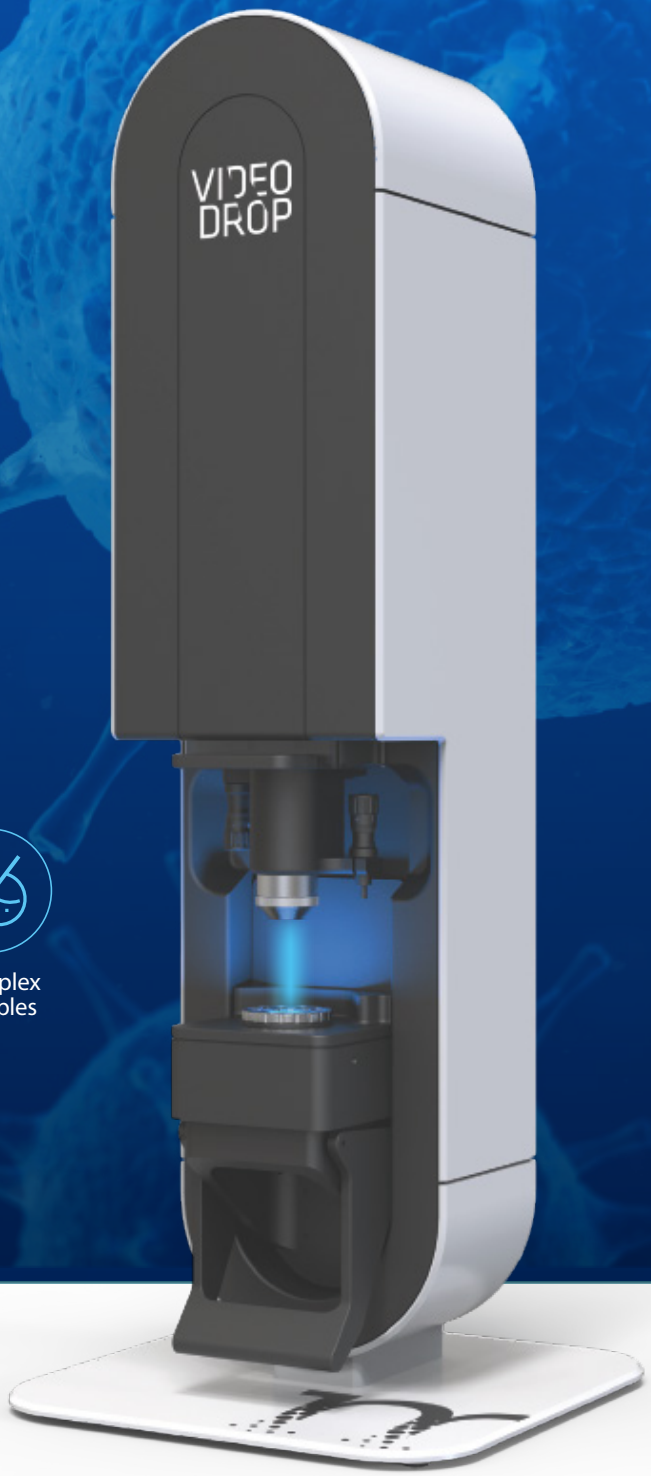
No labeling

Complex samples



Videodrop **simplifies Extracellular Vesicle (EVs) analysis**, saving researchers valuable time & eliminating operational complexity. Videodrop excels at **batch processing** of **heterogeneous EVs** samples, **including small & large vesicles, potential debris, and aggregates**.

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Editorial

Florence Gazeau

Université Paris Diderot

Jeanne Volatron

EverZom

Extracellular vesicles have emerged as new therapeutic modalities for cell and gene therapy relying in their intrinsic therapeutic properties (naïve EVs) and/or their capacity to carry molecules and therefore their potential to host exogeneous new medicine (engineered EVs). In the last decade several companies became leading actors in the EV landscape with new EV-based drug candidates in their pipelines with a strong focus on EVs as drug delivery system. A first hype wave of engineered EV-based product development (2017 – 2020) was initiated by renown players such as Codiak BioSciences (USA, founded in 2015, clinical stage, engineered EVs to treat multiple cancer, fundraising > $100M), Evox Therapeutics (UK, 2016, founded in 2016, preclinical stage, engineered EVs for rare disease raised >$100M). The golden age of this first wave occurred around 2019-2021 with the announcement of some huge deals between these companies and big pharma (2019 - Codiak-Biosciences / Jazz Pharmaceutical - $56M upfront ($1B total), 2020 – Codiak Biosciences / Sarepta Therapeutics - $72.5M upfront, 2020 – Evox Therapeutics / Eli Lilly - $20M upfront ($1.2B), 2020 - Evox Therapeutics / Takeda - $44M upfront ($882M in total). But in 2023 no real evidence show a high-value product causing a notorious disillusion and an interruption in some of these co-development programs. To crown this bust, Codiak Biosciences filed for bankruptcy in march 2023. However, the scope and the number of deals clearly showed that the pharmaceutical industry recognized the promise of EVs but maybe too early and several points needed to be address to accelerate the clinical transfer and diffusion to patients. One of them is the manufacturing challenges technology to ensure bioproduction at scale and with required quality. EV field is the crossroads of biology and nanoscience. It involves complex objects and new characterization methods that are still under development. There is no approved product on the EU market and the regulatory route has yet to be mapped out. Some of EV players are now working hard to solve the EV manufacturing challenge.

Biomanufacturing of new modalities has always been a major challenge, and is now one of the priority of France through the France 2030 plan (€7Mds dedicated to health including €800M for Biotherapy and Bioproduction), with specific call for bioproduction and biotherapy (for which more than 20 French companies have applied in 2024) and Prioritary Equipment Research Program (PEPR) focused on Biotherapy and Bioproduction that have been allocated a total of €80M. Extracellular vesicle bioproduction and engineering are a strategic axis in the France 2030 plan with 3 PEPR projects funded in 2023 (among 12 projects) and also the designation of the first industrial Integrator IVETh dedicated to EVs. These priority projects aim at developing robust and scalable EV bioproduction processes and quality controls, while optimizing the cellular sources or the loading with therapeutic RNAs for next generation vaccines. IVETh integrator develops AI-powered cutting edge multimodal and multiscale methodologies for EV engineering and characterization, offering expertise and training to accelerate the rise in TRL for academics and industrials in the EV field. In addition, a clinical trial initiated by the Professor Philippe Menasche, has been launched in 2023 to assess the safety and efficacy of EV-enriched secretome of cardiovascular progenitor cells (naïve EVs) in patients with a left ventricular dysfunction secondary to non-ischemic dilated cardiomyopathy (NCT05774509). The product is manufactured at the MEARY center, another industrial integrator specializing in cell therapy.

From an industrial perspective, Direct Biologics (USA, 2017, fundraising >$100M) who is currently the only EV company that have received FDA phase III approval, announced the extension of its pipeline of the product Exoflo (naïve EVs) to inflammatory bowel disease, and ExoBiologics (Belgium, 2021) announced €16M fundraising to accelerate its EVs (naïve) clinical developments and launch a phase 1/2 trial in Europe for the prevention of bronchopulmonary dysplasia in premature newborns (orphan disease).

These recent advances, focusing mainly on naive EVs and capitalizing on their intrinsic therapeutic properties, are probably the beginnings towards the second hype EV-wave. The year 2025 could be a landmark year with the potential first approval of an EV based therapeutic product with the completion of Direct Biologics's phase 3.

GLOBAL exosomes & other extracellular vesicles market

Discover the marketed products, pipeline drug candidates, major deals and biopharmaceutical companies

EXTRACELLULAR VESICLES & EXOSOMES

By MabDesign

Previously regarded as mere cellular debris with no biological function, exosomes and, more generally, extracellular vesicles (EVs) are now recognized as vectors of biological material with a prominent role in intercellular communication. The growing body of knowledge on the subject has not only highlighted their involvment in certain pathologies, but also revealed their potential as biomarkers and therapeutic tools.

Definition & biogenesis

Extracellular vesicles (EVs) are vesicles of **various sizes and origins** secreted by eukaryotic cells into the extracellular environment. These vesicles **carry a cargo** of lipids, nucleic acids and proteins, in particular proteins associated with the plasma membrane, the cytosol and those involved in lipid metabolism.

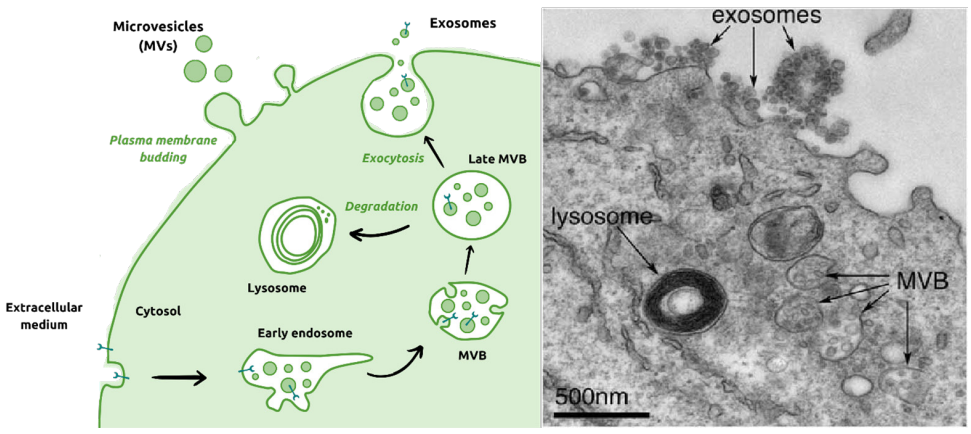
Over the course of time and research, they have been given numerous names, and many subtypes exist, but the most recent nomenclature classifies them into **three main categories**: exosomes, microvesicles (MVs), and apoptotic bodies. These three categories are distinguished by their mode of formation, which also has an impact on their protein profile and size:

- **Exosomes** are 30-150nm in size, and **form via the endosomal pathway**: exosomal vesicles form by budding within the membrane of early endosomes, which then transform into multivesicular bodies (MVBs). Mature MVBs then either follow the lysosomal pathway for degradation, or release their contents by exocytosis at the plasma membrane, with exosomes entering the extracellular space ;

- **Microvesicles** are highly variable in size, ranging from 100 nm to 1 µm, and **form by outward budding and pinching of the plasma membrane**, releasing newly formed MVs directly into the extracellular environment ;

- **Apoptotic bodies** are released by **dying cells** into the extracellular space. Their diameter varies from 50 nm to 5000 nm, the majority being rather large. These bodies are formed by the separation of the plasma membrane from the cytoskeleton as a result of increased hydrostatic pressure following cell contraction.

The rest of this article will focus on extracellular vesicles produced by non-apoptotic cells, i.e. exosomes and EVs. Often, by misuse of language, «extracellular vesicles» refers to all vesicles that are not exosomes (microvesicles and other subtypes).



Biogenesis of exosomes & EVs- *Left : adapted from Kowal et al., 2014 ; right – cancer cell secreting exosomes, Edgar et al., 2016. MVB : multi-vesicular bodies*

Diverse and context-dependent biological functions

For a long time, exosomes and MVs were regarded as a means for cells to eliminate excess and/or non-functional cellular components. Since then, numerous research studies have highlighted their role in a wide range of biological processes: **cell maintenance** (recycling of cell surface proteins and signaling molecules), **cell-cell communication**, angiogenesis, antigen presentation, coagulation, cell homeostasis, inflammation and more.

It is thanks to their ability to transport and transfer a wide variety of active cargoes and deliver them to another cell, whether nearby or far away, that extracellular **vesicles are capable of modifying the functions of the receiving cell**, and thus impacting numerous biological processes. Exosomes have been **isolated from a wide range of body fluids**, including blood, saliva, plasma, urine, sperm and cerebrospinal fluid. The cell «receiving» the exosomes will interact with them by endocytosis, membrane fusion or ligand/receptor interaction, resulting either in internalization of their contents, or in the triggering of signaling cascades, for example.

This impact on biological processes is at play in healthy cells, but **also in the context of numerous pathologies** such as cancer, neurodegenerative diseases, infections or autoimmune diseases.

Medical applications of exosomes & EVs

The potential of EVs and exosomes as biomarkers

Exosomes and EVs are produced by cells in **both healthy and pathological conditions**. They contain proteins, lipids, metabolites and nucleic acids that reflect the physiological state of their cell of origin, making them a **source of biomarkers** for clinical diagnosis. In the literature, several biomarkers typical of pathologies have been isolated from exosomes: for Parkinson's disease for example, alpha synuclein has been isolated from plasma exosomes. What's more, EVs are present in many bodily fluids: they can therefore be harvested **non-invasively by liquid biopsy**.

In oncology, diagnosis via exosomal markers could enable **early detection of genetic or phenotypic alterations**, thanks in particular to the nucleic acids contained in the vesicles. This diagnostic approach therefore has a number of advantages over the conventional method of tissue biopsy, which is invasive and biased by tumor heterogeneity. In addition to early diagnosis, exosomes are also of interest for the development of **personalized treatments, prognosis and overall treatment follow-up**.

The potential of EVs and exosomes as therapeutic tools

Two approaches are mainly used in products currently under development:

- The use of EVs as **vectors for targeted drug delivery** ;
- The use of purified cell-derived EVs **as a therapy itself** ;

The potential of EVs and exosomes as therapeutic tools

In this approach, vesicles can be **loaded with a variety of therapeutic cargoes** depending on treatment needs. Exosomes can be **modified or functionalized** to specifically target certain cells or tissues in the body, enhancing treatment efficacy and precision. As «**natural carriers**», exosomes offer considerable advantages: they have low immunogenicity and toxicity, protect their cargo from degradation, and are able to cross the cytoplasmic membrane and the blood-brain barrier. This approach could therefore help reduce undesirable side effects, improve treatment efficacy and reach specific tissues.

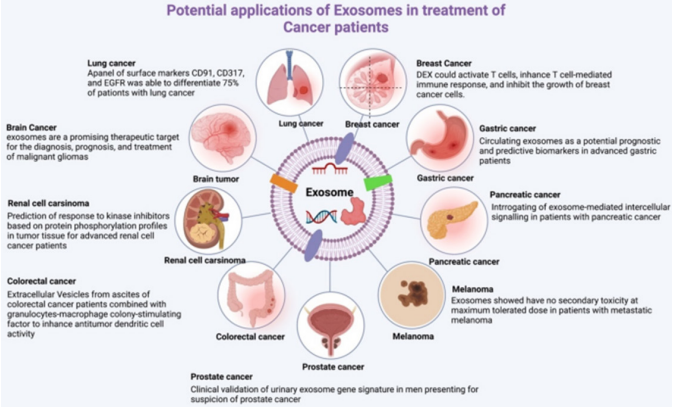
Cell-derived EVs as therapy

With this strategy, EVs, often exosomes, are **collected from the culture medium of specific cells**: often

stem cells (mesenchymal, neural, etc.) but also cancer cells, platelets, immune cells, etc.

Cell-derived exosomes can be used as **therapeutic products**, without the addition of specific cargo. Indeed, exosomes **«inherit» therapeutic effects** similar to those of their parent cells: exosomes derived from mesenchymal stem cells, for example, have great potential in regenerative medicine. This **«cell-free» alternative to stem cell therapies** could offer several advantages, such as lower immunogenicity and toxicity, easier access, better preservation, and the absence of tumorigenic potential.

Both exosomal therapies and the use of exosomes as drug-delivery vectors are **widely investigated approaches, notably in oncology**, with numerous projects in development for the treatment of cancers.



Source : Hussen, B.M et al., 2022

Challenges

Despite the density of research into extracellular vesicles and exosomes, and the rapid growth of the sector, there are still a number of technical and other challenges to overcome.

Purification and characterization

Isolation and purification techniques are still **suboptimal and poorly standardized**. The isolation of exosomes is difficult because some components of biological fluids, such as lipoproteins or other EVs, **have sizes that overlap with those of exosomes** (30-150 nm).

On top of these difficulties is the complexity of characterization: in-depth analysis of EV characteristics is often **hampered by the heterogeneity of isolates**, resulting in a mixed size distribution, and difficulties in profiling vesicle contents.

From these issues flow questions regarding **exosome bioproduction**, in terms of reproducibility, safety and cost. Guidelines and standards need to be defined, in compliance with biopharmaceutical regulations, to enable exosomes to be used in the clinic.

Targeted delivery, dosing and pharmacokinetics

A major challenge for the future development of EVs is to **control their delivery to target sites** and **maintain their efficacy**. To achieve this goal, several issues need to be addressed: the frequency of treatment required to maintain their effect (dosage, half-life of exosomes in tissues, etc.) and the side-effects of treatment, particularly in the long term. A better understanding of the biology of VEs and exosomes, and in particular of cargo encapsulation mechanisms, is needed to optimize these steps.

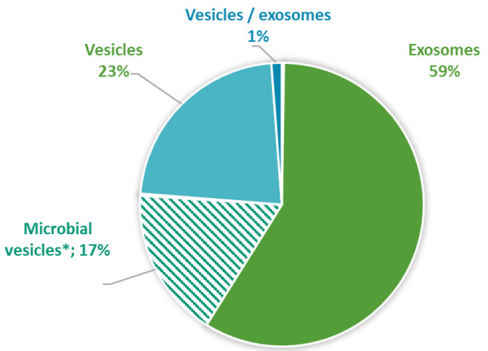
Market analysis of exosomes and extracellular vesicles

Pipeline analysis

As development of the exosome and extracellular vesicle field is **fairly recent**, there are currently no products on the market. Nevertheless, given the promising prospects as a diagnostic tool, therapeutic tool or technological platform, enthusiasm for these approaches is high, resulting in a **rich global pipeline** with 420 unique products in development.

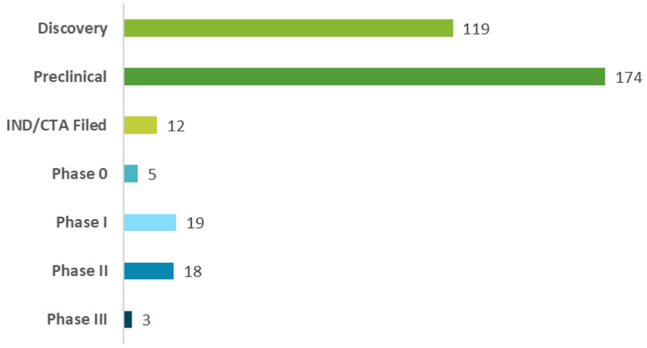
The majority of products in the pipeline are **exosomes (59%)**, or **extracellular vesicles (23%)**. Extracellular vesicles refer to all vesicles of eukaryotic origin that are not exosomes. Microbial vesicles, such as bacterial outer-membrane vesicles (OMVs) or viral-like vesicles (VLVs), also account for a significant proportion of the pipeline (17%). These projects **have been removed from the rest of the analysis** to focus on the exosome and intracellular vesicle pipeline, i.e. **347 unique products**.

Pipeline per type of technology

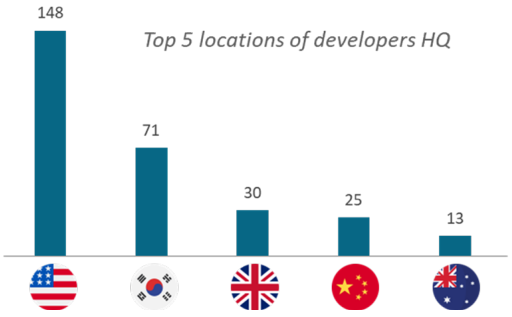


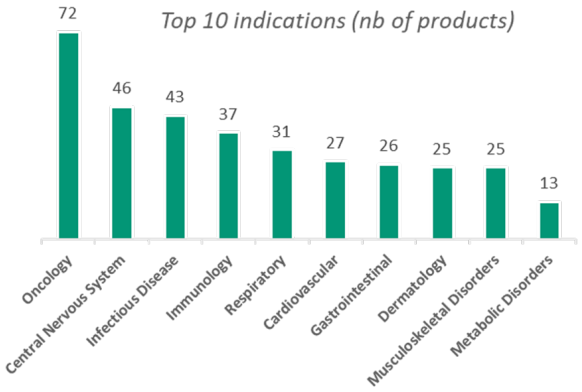
*excluded from further analysis

Pipeline per phase



The projects in the pipeline are mainly in the early stages (discovery or preclinical), with **only 11% in the clinical phase**. As the pipeline is quite rich, this still corresponds to **40 projects** involving EVs or exosomes currently in clinical trials. The five countries with the most products in development are the USA, South Korea, the UK, China and Australia. The **USA and South Korea** are far ahead, together accounting for **219 unique products in development, or 63% of the pipeline**.





In terms of **therapeutic areas**, applications are quite varied, as illustrated by the top 10 by number of products in development. Note that the top three targeted indications are oncology, neurology, and infectious diseases.

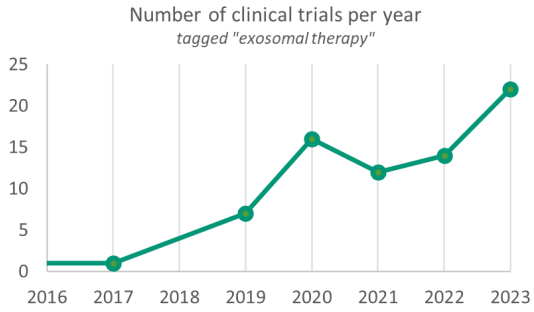
Among the most advanced projects is **ExoFlo™, developed by Direct Biologics**. This is a product containing growth factors and extracellular vesicles, notably exosomes, isolated from mesenchymal stem cells. The candidate is currently in **Phase III clinical trials**, notably for the treatment of moderate to severe acute respiratory distress syndrome, post-acute sequelae of COVID-19, transplant rejection and refractory Crohn's disease. **Diasome pharmaceuticals** has also begun a **Phase III clinical trial** for its **hepatocyte-targeted vesicle technology (HDV platform)**, aimed at the targeted delivery of insulin in the treatment of type 2 diabetes.

Main players & Trends

The exosome market is still very new and is **growing year on year**. This upward trend is illustrated in particular by the **number of clinical trials** underway bearing the tag «exosomal therapy», which has been on the rise since 2017.

The enthusiasm surrounding exosomes and EVs is also reflected in the market by the **plurality of players**, as well as by **major deals**. For example, **Evox Therapeutics** signed deals in 2020, first with Takeda for a partnership focused on rare diseases worth over $800 million, then a partnership worth over $1 billion with Eli Lilly, for the treatment of neurological diseases. Both deals concern the development of therapies using Evox Therapeutics' exosome-based technology platform.

There have also been a number of rounds of financing for exosome biotechs. Most recently, **EXO Biologics** raised $16 million to further develop therapies based on extracellular vesicles derived from mesenchymal stem cells.



Key exosome players

Conclusion

Exosomes represent an **innovative and promising modality** in the field of regenerative medicine and diagnostics. Their ability to act as **natural therapeutic vectors**, their potential for **early disease diagnosis** and their role in regulating biological processes offer vast opportunities for medical applications. Ongoing research efforts have brought projects to the clinical stage, and continue to lead to technical and medical innovations that have the potential to **improve the treatment and management** of numerous pathologies.

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scientific articles

Read the different inputs from the scientific community on various aspects of exosomes & other extracellular vesicules

The Simple Way to Characterize Extracellular Vesicles with Simple Western Assays

Extracellular Vesicle Research: Promises and Challenges

Extracellular vesicles (EVs) and endosome-derived exosomes play an important role in many pathological conditions, such as cancer, infectious diseases, and neurodegenerative diseases.[1] Much attention is focused on EV detection in liquid biopsy methods and engineering EVs and lipid nanoparticles (LNPs) for drug delivery. However, isolation and characterization of EVs can be challenging as EVs are found in complex samples and body fluids with contaminants that must be removed during isolation. This problem is compounded by their relatively low abundance, heterogeneity, and size variance.

Due to these challenges in isolation, EV samples are often limited, which can prohibit molecular profiling by traditional methods like Western blot. Furthermore, there are few exclusive biomarkers to identify bona fide exosomes from other EVs and cellular debris, and validated antibodies for biomarkers can be hard to find. For EV research to advance, standardized methods for isolation and analysis of extracellular vesicles are needed, and emerging innovative technologies need to be integrated.

A Powerful Analytical Solution for EV Protein Biomarker Analysis

Simple Western™ is a capillary immunoassay platform that revolutionizes protein analysis for EV research by providing specific biomarker detection with pg-level sensitivity using only 3 µL of sample. Unlike traditional western blot, Simple Western seamlessly combines protein size separation and immunodetection in a 3-hour hands-free run for highly reproducible and fully quantitative immunoassay readouts. With Simple Western instruments like Jess™, researchers have flexible multi-channel multiplex detection for simultaneous analysis of multiple protein targets and total protein detection. As supported by the rapidly growing publication record in the field of EVs,2-91 Simple Western is emerging as the gold-standard method for EV protein analysis.

Streamlining the EV Isolation and Characterization Workflow

Simple Western is an open platform, and any conventional antibody may be used for detection. Close to 5,000 antibodies have been validated for Simple Western so far, including nine targets provided by "Minimal Information for Studies of Extracellular Vesicles" (MISEV) guidelines (Fig. 1).1 MISEV recommends characterizing EV preparations for transmembrane-, cytosolic- and contaminating non-EV proteins. Compliance with MISEV can mean a considerable effort to the individual laboratories due to a lack of easy and robust analytical protocols.

Here, we present a simple method for the isolation of EVs and automated protein separation and immunodetection of MISEV-recommended proteins using Simple Western. We developed a new workflow for EV protein characterization using intact EVs eluted from the exoEasy™ kit (QIAGEN) as input for automated capillary western analysis on the Simple Western Jess instrument. We utilized this workflow for the detection of 9 prominent targets in categories 1-3 provided by the MISEV guidelines (Fig. 1).

Optimized Protein Assays

	MISEV category	Target	Catalogue #
EV	1a - Transmembrane	CD63	Ab68418[&]
EV	1b - Transmembrane	CD9	13403S *
EV	2a - Cytosolic	Alix	NBP1-49701
EV	2a - Cytosolic	Annexin V	MAB3991-SP
EV	2a - Cytosolic	Flotillin-1	Ab133497[&]
EV	2b - Cytosolic	ß-Actin	MAB8929
Non-EV	3a - Free Proteins	Albumin	MAB1455-SP
Non-EV	3a - HDL Particles	ApoA1	AF3664-SP
Non-EV	3a - HDL & (V)LDL	ApoE	NBP2-67565

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* Cell Signaling
& Abcam

Figure 1. Antibodies used in Simple Western assays for the detection of prominent MISEV targets in EV samples.

Results of the EV Isolation and Characterization Workflow

A single isolation yields 0.25 mL of EV eluate, which can be used for > 60 individual Simple Western assays due to the low input requirements of our method (4 µL) or for total protein detection by the BCA assay, protein modification reactions like deglycosylation, or frozen future analysis. Using Simple Western's capillary electrophoresis-based protein separation and chemiluminescence-immunodetection, we generated quantitative expression data with MW characterization for all 9 protein EV biomarkers (Fig. 2-3).

To demonstrate the functionality of the new workflow, we initially analyzed a titration of human plasma for the presence of EV biomarker, Flotillin-1 (Fig. 2). All six EV-positive protein markers from MISEV categories 1- 2 were successfully detected in EV eluates and their signals scaled with plasma input (Fig. 2-3). As expected, co-isolated IgG (Fig. 2) and other non-EV plasma constituents from MISEV category 3 were not detected (Fig. 3).

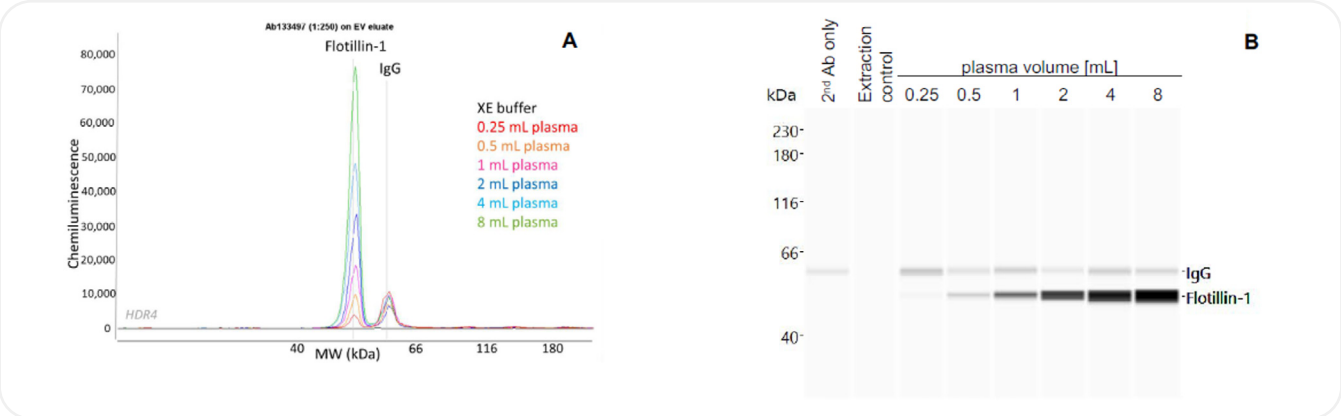


Figure 2. Simple Western immunodetected Flotillin-1 signal in 1:10 diluted human plasma derived EV eluate shown as (A) electropherogram graph view and (B) virtual lane view at expected MW of 50 kDa. Flotillin-1 signal increases with input volume while background signal from plasma-contained antibodies (IgG) stays at the same level and can be excluded via 2nd-antibody-only-control.

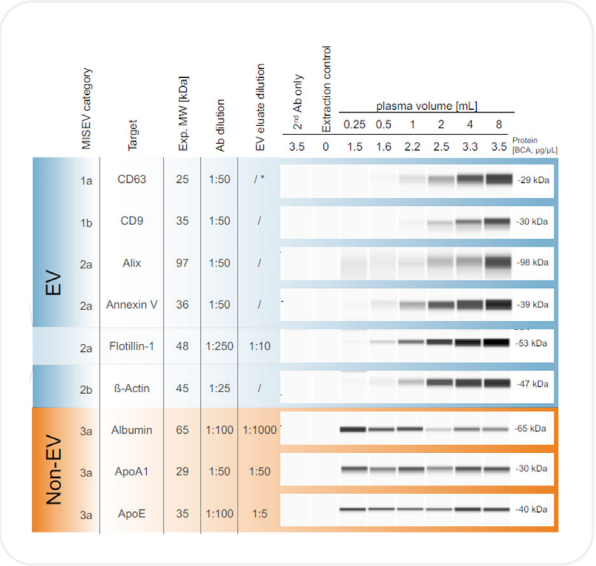


Figure 3. Virtual lane overview of all immunodetected proteins in plasma-derived EV eluate ordered by MISEV classification and specified settings. For CD63 protein (marked with *) a deglycosylation reaction (PNGaseF, NEB) was essential to detect a band at the expected MW. Increasing human plasma input lead to a dose-response in protein assay signal for immunodetected EV proteins (MISEV category 1-2), but not for contaminants (MISEV category 3).

EV protein signals correlated with the total protein amount measured by the BCA assay (Fig. 4), demonstrating a linear range of detection and quantitative nature of the new workflow using as little as 0.25 mL human plasma.

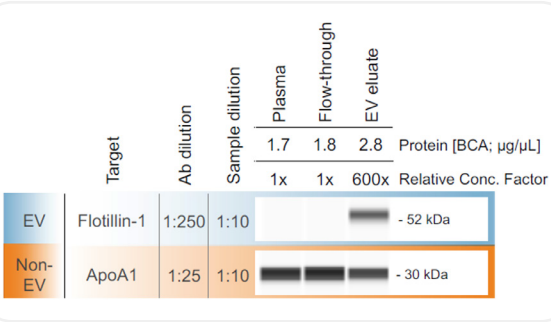


Figure 5. Comparison of Plasma Fractions. EV eluate from 3 mL plasma compared to the extraction's input (neat plasma; 1:50 diluted) and the unbound fraction found in the column flow-through (diluted 1:25 to a final 1:50). EV marker Flotillin-1 is not detectable in neat plasma but in EV eluate, while HDL-marker ApoA1 is reduced in EV eluate compared to plasma and flow-through fraction.

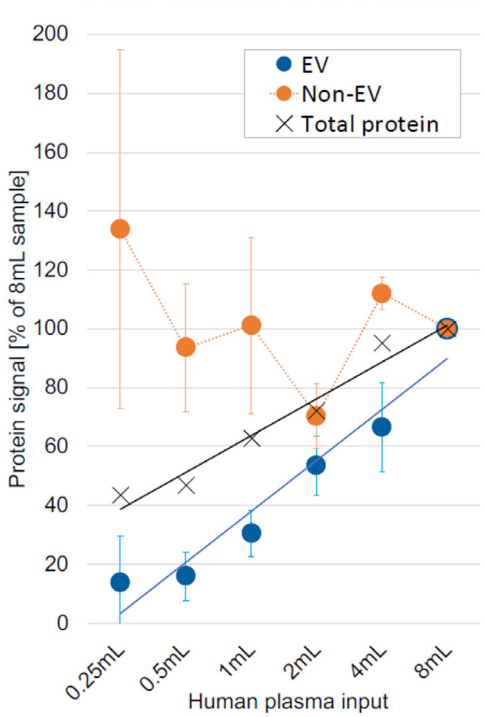


Figure 4. Relation of total protein quantity to immunodetected proteins. Averages and standard deviations of calculated peak areas from six EV proteins (blue) and three Non-EV proteins (orange) compared to BCA measurement displayed as percentage of maximum input sample. Increasing human plasma input lead to an increase of EV proteins similar to the increase in total protein but not co-eluted non-EV proteins.

To get a better estimation on the amount of contaminating protein, we analyzed 3 different fractions from the same EV isolation (Fig. 5). EV proteins were not detectable in neat plasma but only when enriched by isolation (e.g. Flotillin-1). Non-EV proteins (e.g. ApoA1) are depleted >600-fold in the EV-eluate.

Save Your Precious EV Sample with Sensitive Protein Biomarker Assays

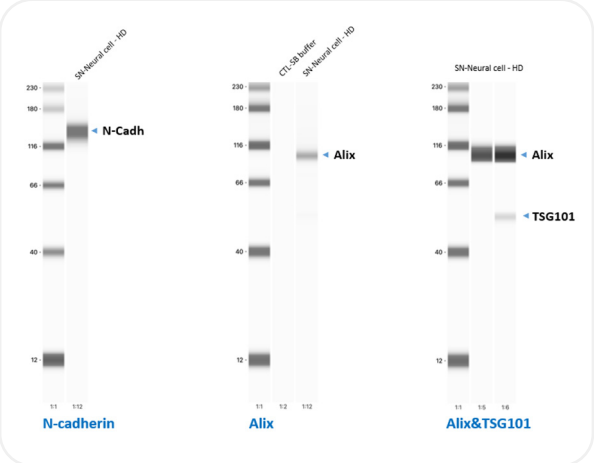
Here, we provide a simple workflow for general EV protein characterization that is robust and quantitative, using just a few microliters of sample. Biomarker signals of 9 EV targets recommended by MISEV were detected with Simple Western which correlated with sample load and total protein concentration. Contrastingly, signals from contaminating proteins did not scale with the sample load and were efficiently reduced. These optimized protein assays are compliant with MISEV guidelines for EV protein characterization and are compatible with exosome isolation using the ExoEasy isolation kit (QIAGEN).

Additional Resources:

- Download the Exosome Poster
- Learn More About EV Characterization with Simple Western
- Learn More About Jess

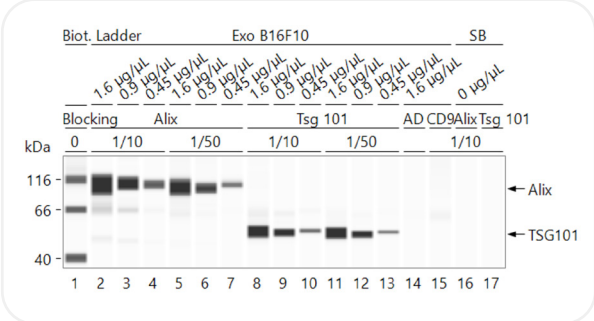
In the Hands of Your Peers: French Researchers Use Simple Western for EV Analysis

1. Collaboration with Dr Aurélie de Thonel - UMR7216 « Epigénétique et Destin cellulaire » - Paris, France



Expression of exosomes markers in extracellular vesicles derived from neural cells. This figure shows Simple Western analysis of EVs markers on exosomes isolated from neural cells by ultracentrifugation.

2. Collaboration with Dr Jessica GOBBO – HSP-Pathies / CGFL / Inserm 1231 – Dijon, France



Exosomes isolated by ultracentrifugation from B16F10 cell cultures (mouse melanoma) tested at 1.6, 0.9 and 0.45 µg/µl. The figure shows Simple Western analysis of proteins detected with different dilutions of Anti-Alix and Anti-TSG101.

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ADVANCING NON-INVASIVE DIAGNOSTICS: UNLOCKING THE POTENTIAL OF EXOSOMES WITH INNOVATIVE SOLUTIONS

By Promega

Exosomes are small extracellular vesicles that play a crucial role in cell-to-cell communication, carrying proteins, lipids, and nucleic acids between cells. They have been implicated in a variety of biological processes and are increasingly recognized for their potential as biomarkers for disease diagnosis and prognosis, particularly in the field of liquid biopsy along with circulating tumor cells and cell free circulating nucleic acid. This emerging area of research holds promise for non-invasive cancer diagnostics, allowing for early detection and monitoring of disease progression through the analysis of exosomes in bodily fluids.

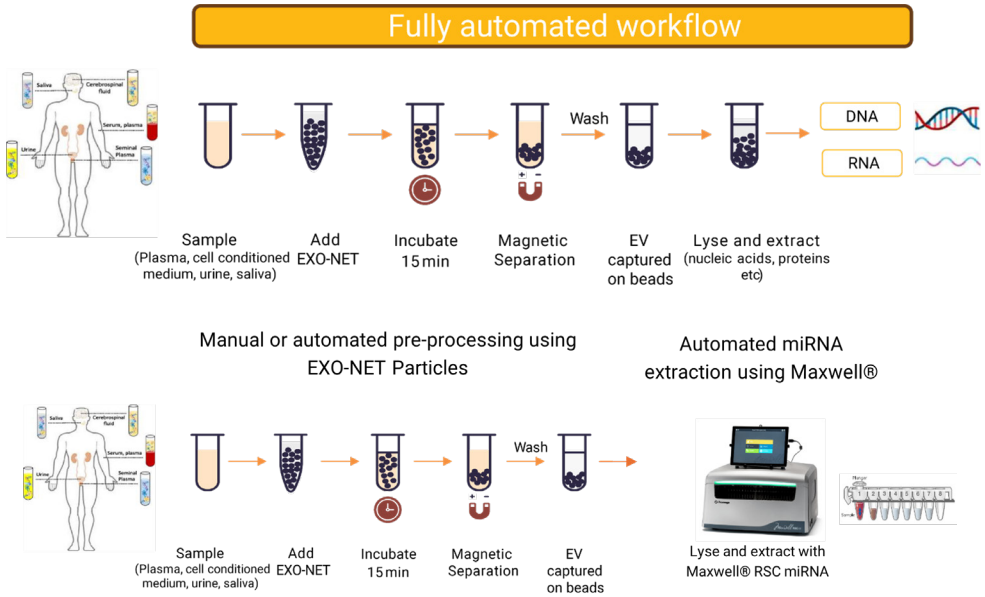
Interview with Dr Gregory Rice, Chief Scientific Officer at Inoviq on EXO-NET:

1. What are the key benefits of using EXO-NET for exosome research and diagnostics?

The key benefits of using EXO-NET for isolating extracellular vesicles (including exosomes) are:

1. increased enrichment of exosomes,
2. a simple, rapid and scalable process,
3. cost effective,
4. compatibility with routine clinical pathology laboratory equipment and workflows.

Key design criteria in developing EXO-NET included the targeted capture of relevant exosome populations, reduced co-isolation of non-exosome vesicles and particles, and speed and ease of use. For exosome diagnostics the key requirements were: appropriate diagnostic performance; compatibility with existing pathology laboratory equipment and analytical platforms, speed, and timeliness. For an exosome-based test, this necessitates cost-effective, high-throughput exosome isolation (e.g., up to 4-500 samples per day) and high-specificity analytics. With the exception of EXO-NET, few currently available exosome isolation methods meet these criteria. Of promise are 96-well plate systems and magnetic particle movers (including Promega's Maxwell® instrument and 96-well particle moving platforms). Promega has successfully implemented EXO-NET on both of these platforms, resulting in semi- and fully-automated, high-throughput exosome isolation from biofluids, followed by downstream RNA or DNA extraction. Such developments hold exciting potential for advancing EV-based testing in a clinical setting.



Exosome workflow

2. Could you discuss any specific case studies or research findings that highlight the effectiveness of EXO-NET in clinical applications?

1. Proof-of-Concept Studies. The EXO-NET-miRNA high throughput system has been effectively utilized to identify differentially expressed miRNAs in exosomes isolated from plasma samples (0.5 ml) obtained from women with breast and ovarian cancer. Using EXO-NET, Promega's Maxwell® HT miRNA Plasma and Serum kit on a 96-well plate particle mover, up to 100 differentially expressed miRNA were identified by RNASeq. On submission of identified miRNA to Net Analysis, 1500-4000 gene interactions were reported, including extracellular vesicle- and cancer-associated proteins and signaling pathways (see Promega's online webinar).

In addition, miRNA prepared using the EXO-NET-miRNA high throughput system was screened for a panel of exosomal miRNA using RT qPCR. All 16 targets were identified, 3 were constitutively expressed and one was significantly increased with cancer stage.

These studies establish the utility of the EXO-NET-miRNA high throughput platform for exosomal miRNA analysis and its utility for clinical biomarker discovery.

2. Identifying biomarkers of periodontitis. In a recent publication [1], the performance of EXO-NET was compared to that of size exclusion chromatography (SEC) for the enrichment of small extracellular vesicles (sEV) from saliva, the identification of biomarkers of periodontitis, and co-isolation of bacteria-derived contaminants. EXO-NET sEVs contained more EV-specific protein and substantially higher expression of EV surface markers (CD9, CD81, CD63), but less pathogenic DNA (bacterial) was detected compared to that in SEC-EVs. Additionally, EXO-NET EVs from periodontitis patients contained higher amounts of IL-6 and IL-8, and decreased IL-10, compared to those from non-periodontitis patients. The authors concluded that "immunoaffinity capture (EXO-NET) is a dependable method for salivary EVs enrichment, resulting in a higher yield of host EVs with reduced bacterial DNA detection compared to SEC."

3. How does EXO-NET contribute to the accuracy and reliability of exosome-derived liquid biopsies?

One of the major challenges in exosome research and the development of accurate and reliable exosome diagnostics relates to the purity and specificity of the exosome isolation. If non-exosome vesicles and particles are co-isolated with exosomes, they confound robust identification of exosome biomarkers and their validation for diagnostic applications.

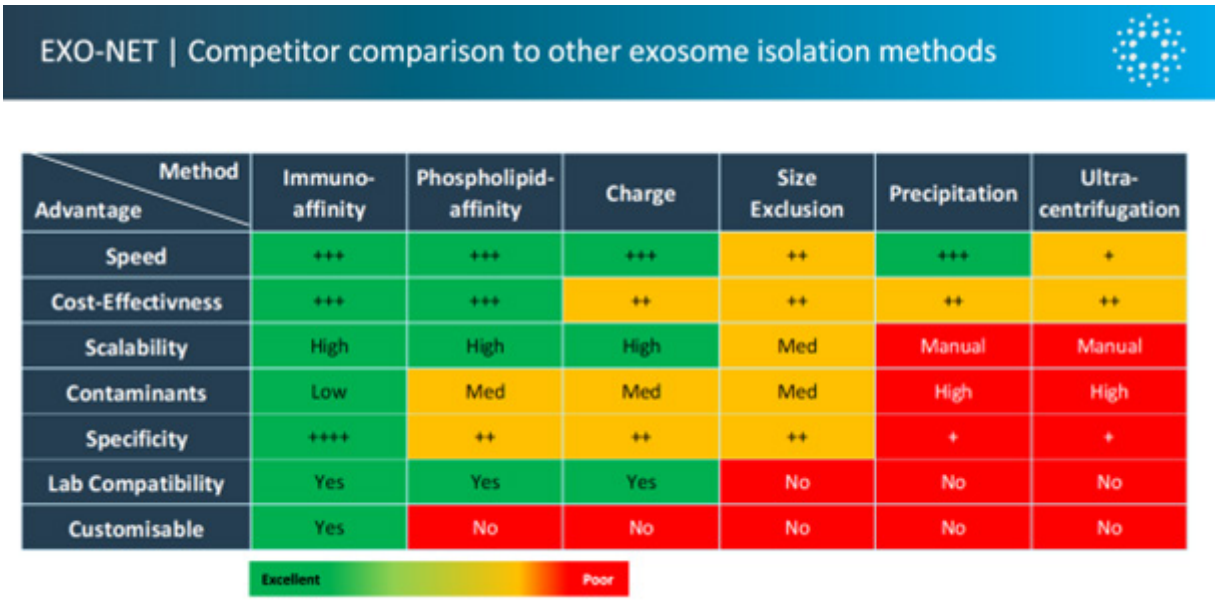
It is widely recognized in the field that isolating particulate fractions from biofluids using methods based on hydrodynamic size, density or charge leads to heterogeneous preparations. [2-6] These preparations often contain a mixture of co-isolating membrane-bound and non-membranous particles. The co-isolation of extracellular particles sharing common physical features complicates the robust characterization of exosomal cargo and meaningful assessment of their specific biological activities. Indeed, it is axiomatic that the isolation of a cell-specific subpopulation of exosomes cannot be achieved using methods that rely on characteristics that are common to all extracellular vesical and/or particles. One promising approach involves epitope-specific ligand capture, where exosomes are isolated based on epitopes expressed on their surface. [3]

EXO-NET® has been developed as a pan-exosome capture tool for On-Bead-Analysis (e.g. FTIR, FACs, ELISA) or ON-Bead-Lysis and downstream analysis (e.g., RNA, protein and lipid analysis). EXO-NET is a magnetic bead-based immunoaffinity matrix that targets 10 protein epitopes that are expressed on the surface of exosomes. It does not rely on common physicochemical properties (i.e., particle size, density and charge) to enrich exosomes. Reducing input sample contamination by non-exosome vesicles and particles coupled with Promega's miRNA isolation technologies enables meaningful and robust analysis

of exosome-associated biomarkers.

4. Can you explain the unique features of EXO-NET and how it differentiates from other exosome isolation techniques?

A comparison with other isolation methods is presented below.



Advantage \ Method	Immuno-affinity	Phospholipid-affinity	Charge	Size Exclusion	Precipitation	Ultra-centrifugation
Speed	+++	+++	+++	++	+++	+
Cost-Effectivness	+++	+++	++	++	++	++
Scalability	High	High	High	Med	Manual	Manual
Contaminants	Low	Med	Med	Med	High	High
Specificity	++++	++	++	++	+	+
Lab Compatibility	Yes	Yes	Yes	No	No	No
Customisable	Yes	No	No	No	No	No

Modified from Salomon et al., 2022 [3]

5. Looking forward, what are the potential applications of EXO-NET in personalized medicine and targeted therapies?

The field of exosomal signaling has the potential to transform our understanding of how cells communicate with each other and how communication pathways are disrupted or altered with pathology. Characterizing such changes affords the opportunity to develop more informative diagnostics and efficacious therapeutic interventions.

1. Exosome Diagnostics. To develop accurate and reliable exosome-based diagnostics requires identification of exosome disease-associated biomarkers and rapid, reproducible and scalable sample processing. Over the next 2-years, targeted immnoaffinity capture of extracellular vesicle EVs (including, exosomes) will enable more precise characterization of subpopulations of EVs. For example, modifying the capture ligands on EXO-NET to target brain-derived EVs enables the enrichment of EVs released from the brain into blood and is providing unique insights into disease-related changes in their associated RNA (messenger, long noncoding, micro and circular) and protein biomarkers. The isolation and analytical technologies being developed by Promega and INOVIQ will enhance our understanding of exosome signaling and facilitate the development of diagnostic applications.

2. Exosome Therapeutics. Effective exosome therapeutics require delivering a therapeutic payload to specific cell populations. Over the next three to five years, exosomes will be developed by genetically engineering the expression of cell-targeting ligands on their surface (tropism) and either utilizing their innate bioactivity or overexpression or loading of bioactive agents to deliver therapeutic benefit. Unlike cell-based therapies, this approach offers advantages including increased tissue accessibility and efficacy, low immunogenicity, and more effective manufacturing and storage.

Interview with Douglas Horejsh, Associate Director, R&D at Promega Corporation on EXO-NET and miRNA Extraction:

1. What are the challenges in miRNA analysis from exosomes, and how does your technology overcome them?

The biggest challenge in purifying miRNA from exosomes is the need for organic solutions such as acidified phenol. Other challenges include difficulty in purifying such small miRNA (about 17-24 ribonucleotides long), complexity of the sample matrix, co-purification of contaminants, and loss of miRNA yield and integrity.

Promega's Maxwell® miRNA chemistry for purification uses a proprietary technology that ensures efficient and selective binding of miRNAs from a complex mixture, reducing the co-purification of unwanted material. Instead of using acidified phenol to remove DNA, the chemistry incorporates a DNase step within the automated workflow. Yield, quality, and integrity of the miRNA purified using this system are compatible with RT-qPCR and sequencing.

2. How does Promega's technology integrate with EXO-NET to enhance exosome research and diagnostics? Can you describe the process and advantages of extracting miRNA from exosomes using Promega's systems?

Using EXO-NET and Maxwell® miRNA purification together enables a highly automated product using two different paramagnetic particle chemistries. The EXO-NET particles can be automated which is a huge advantage in moving towards higher-throughput uses, eliminating the need for ultracentrifugation or cold precipitation methods which are manual and time-consuming. Likewise, the Maxwell® miRNA purification technologies are automated using particles, making the process more amenable to higher throughput and limiting many manual steps needed in organic extraction or the use of spin columns.

Once the exosomes have been purified using EXO-NET, the samples are pre-processed to lyse the exosomes and digest the proteins. This lysate is then added to the Maxwell® cartridge or sample plate with no other hands-on time needed.

3. Could you provide insights into any groundbreaking research or findings facilitated by your technology in the context of exosome analysis? How do you see the role of exosome research evolving in the next few years, and what is Promega's vision in this rapidly advancing field?

Promega and INOVIQ performed a trial looking at miRNA expression patterns in Brest and Ovarian cancer. Utilizing the EXO-NET® Pan Exosome reagent and Promega Maxwell® HT miRNA plasma and serum kit coupled with miRNA seq and dPCR, we were able to identify several miRNAs that were either up regulated or down regulated. The combination of EXO-NET and Maxwell HT extraction speeds and simplifies the sensitive purification of EVs and miRNA.

As the field of exosome research evolves, it's becoming increasingly clear that exosomes play a significant role in various biological processes and diseases, such as cancer, neurodegeneration, inflammation, and infection. Promega's vision is to lead in this rapidly advancing field by providing innovative solutions that address current challenges in exosome research, such as the need for efficient, scalable, and reliable isolation techniques, as well as advanced analytical tools for better characterization of exosomes.

Promega continues to stay at the forefront of innovation to simplify nucleic acid purification processes from complex matrices. A better understanding of miRNA from exosomes holds promise for transforming disease diagnosis, elucidating pathogenesis, refining treatment response monitoring, and even pioneering new therapeutic strategies. In our commitment to staying ahead in this dynamic field, Promega actively collaborates with partners and thought leaders, such as INOVIQ to share collective expertise and drive innovation.

In conclusion, the collaboration between Promega Corporation and INOVIQ marks a significant leap forward in exosome research and diagnostics. By combining EXO-NET's unique isolation capabilities with Promega's state-of-the-art extraction and analysis technologies, this partnership delivers unparalleled ease of use, automation, and processing speed. This approach not only sets a new standard for specificity and efficiency but also distinguishes their methodologies from existing alternatives. Such advancements are poised to transform non-invasive disease diagnostics and personalized medicine, making sophisticated analyses more accessible and actionable than ever before.

About Promega Corporation

Promega Corporation is a leader in providing innovative solutions and technical support to the life sciences industry. The company's portfolio of over 4,000 products supports a range of life science work across areas such as cell biology; DNA, RNA and protein analysis; drug development; human identification and molecular diagnostics. These tools and technologies have grown in their application over the last 45 years and are used today by scientists and technicians in labs for academic and government research, forensics, pharmaceuticals, clinical diagnostics and agricultural and environmental testing. Promega is headquartered in Madison, WI, USA with branches in 16 countries and over 50 global distributors. Learn more at www.promega.com.

About INOVIQ LTD

INOVIQ Ltd (ASX:IIQ) (INOVIQ) is developing and commercializing next-generation exosome solutions and precision diagnostics to improve the diagnosis and treatment of cancer and other diseases. The company has commercialized the EXO-NET pan-exosome capture tool for research purposes and the hTERT test as an adjunct to urine cytology testing for bladder cancer. Our cancer diagnostic pipeline includes blood tests in development for earlier detection and monitoring of ovarian, breast and other cancers. For more information on INOVIQ, visit www.inoviq.com

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INTERLABORATORY STUDY PROJECT TO STANDARDIZE EXTRACELLULAR VESICLE METROLOGY

By consortium for EV study

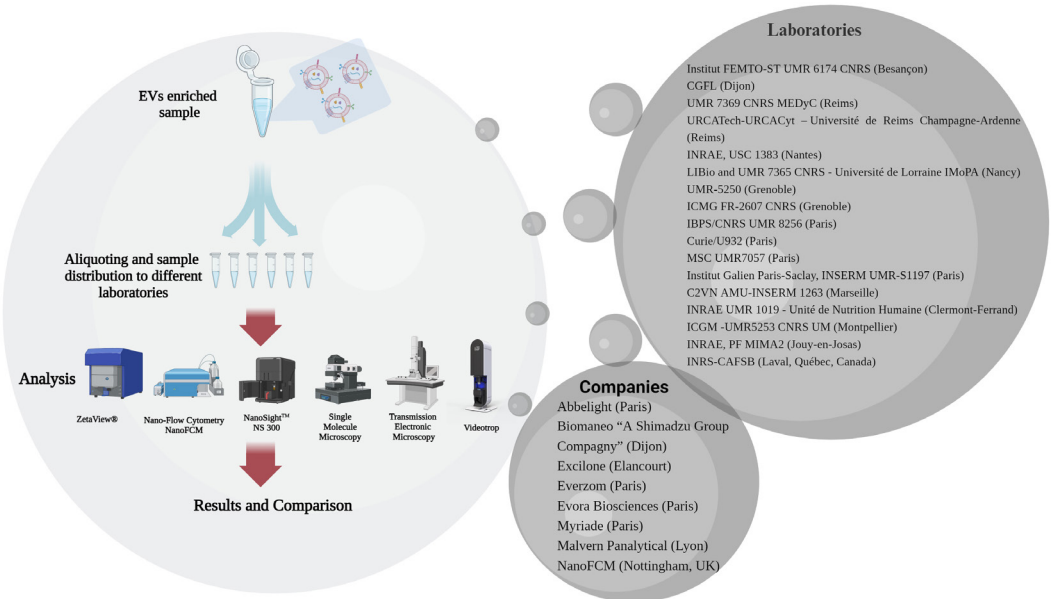

Figure 1 : *Demarche (on the left) and members (on the right) of the interlaboratory EV study devoted to standardizing methods for metrological analysis of EVs.*

The biotherapy development has enabled the growth of so-called personalized medicine, providing proven therapeutic solutions for a wide range of indications (oncology, immunology, infectious diseases, rare diseases, etc.). These solutions often represent a decisive opportunity for patients, as these biomedicines currently account for 50% of clinical trials in progress. At the same time, these new therapies act as an economic challenge, particularly in terms of the sustainability of the healthcare system and the adaptation of care organization.

This market represented 24.3% of the global drug one in 2019, or around $240 billion. It is expected to grow at an average annual rate of between 8% and 9%, reaching €320 billion by 2025.

Extracellular vesicles (EVs) are nano- to micro-sized particles, delimited by a lipid bilayer membrane, ubiquitously released from cells in the extracellular space under normal and pathological conditions. They contain proteins, lipids and nucleic acids (such as miRNA, mRNA, and DNA) that may be transferred from parent to nearby or distant recipient cells, mediating intercellular communication.

The aim of this interlaboratory study is to compare the metrological and concentration measurement results in an extracellular vesicle isolate, obtained by several analytical methods, using the same sample distributed to fifteen laboratories in France and in Canada, in partnership with several companies. Here, a reference analytical method or combination at international level is proposed to meet the need to characterize accurately EV samples in a bioproduction context for biotherapy.

In France, as part of the "Biothérapie Horizon 2030" program, 4 emerging scientific programs have been identified, including one to support an emerging industrial sector focused on EV therapeutics. The medical use of EVs will gradually become a reality, given the number of clinical trials underway worldwide exploiting the potential of EVs for tissue repair, inflammation resolution (particularly in "COVID-19" patients), vaccination, drug delivery or anticancer therapy, among others. Indeed, to date, one EV-based product has already received marketing authorization: Bexsero® (GSK Vaccines), a validated vaccine approach based on the use of Neisseria meningitidis outer membrane vesicles (OMVs).

Many of the biological effects of cell therapy rely on the cell secretome and, in particular, on the biomolecules contained in EVs, which are now being investigated as potential therapeutic agents to recapitulate a substantial part of the parental cell benefits. Preclinical studies have demonstrated the therapeutic effects of EVs enriched secretomes from a variety of cell sources to treat osteoarticular, cardiac, renal, hepatic, cerebral and cutaneous injuries. Non-immunogenic, in addition to these cell-free regenerative approaches, EVs can be engineered in a pre-production or post-production stage to carry natural or chemical molecules that enhance their specific targeting or therapeutic properties. In addition, EVs can encapsulate therapeutic agents to protect them from degradation and potentiate their effects while minimizing their toxicity.

The proposed interlaboratory study is devoted to standardizing methods for metrological analysis of EVs, corresponding to a real need in research and clinical applications today. The use and validation of reference metrological analysis methods will be a guarantee of quality for all R&D and production projects using EVs as therapeutic fundamentals. Moreover, several members involved in this study have contributed to the position papers indicating guidelines for EVs analysis (Thery et al, JEV 2018 ; Welsh et al, JEV 2023).

This study was launched in April 2022, according to a methodology decided within the consortium:

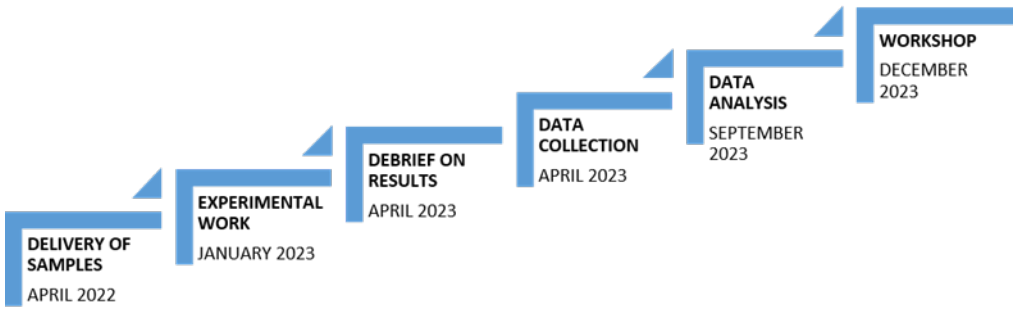

Figure 2: *Chronological organization of the Interlaboratory EV study project.*

Several techniques are used, with a majority of measurement approaches in solution, and also characterizations by microscopy.

We have worked in 4 sub-groups:

- Nanoparticle Tracking Analysis “NTA” subgroup
- Tunable Resistive Pulse Sensing “TRPS” subgroup
- Videodrop subgroup
- Microscopy subgroup

to discuss efficiently the results obtained under each category of techniques.

Biomaneo compagny (Dijon) is involved in the program to manage data collection (respecting nomenclatures), developing a specific database called MIDAAS, and to carry out the analysis of intra-group results, then inter-groups ones, meaning therefore inter-laboratory results.

The results of this interlaboratory study will be shared with the scientific community through a forthcoming publication.

About the consortium:

Laboratories: Institut FEMTO-ST UMR 6174 CNRS (Besançon), CGFL (Dijon), UMR 7369 CNRS MEDyC (Reims), URCATech-URCACyt – Université de Reims Champagne-Ardenne (Reims), INRAE, USC 1383 (Nantes), LIBio and UMR 7365 CNRS - Université de Lorraine IMoPA (Nancy), IBPS/CNRS UMR 8256 (Paris), Curie/U932 (Paris), MSC UMR7057 (Paris), Institut Galien Paris-Saclay, INSERM UMR-S1197 (Paris), UMR-5250 (Grenoble), ICMG FR-2607, CNRS (Grenoble), C2VN AMU-INSERM 1263 (Marseille), INRAE UMR 1019 - Unité de Nutrition Humaine (Clermont-Ferrand), ICGM -UMR5253 CNRS UM (Montpellier), Laval INRS, Québec, Laval (Canada), INRAE, PF MIMA2 (Jouy-en-Josas).

Compagnies: EVerZom (Paris), Evora Biosciences (Paris), Biomaneo, " A Shimadzu Group Compagny", (Dijon), Malvern Panalytical (Lyon), Myriade (Paris), NanoFCM (Nottingham, UK), Excilone (Elancourt), Abbelight (Paris).

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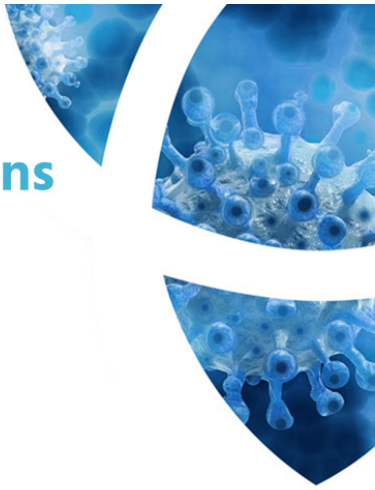
Immunotherapies & Innovations for Infectious Diseases

8th Edition– 27 & 28 November 2024 - Lyon, France



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LYONBIOPOLE AUVERGNE-RHÔNE-ALPES

Informations and Registrations: **WWW.I4ID.ORG**

About MabDesign

MabDesign, the French biotherapy industry association, aims at structuring the biopharmaceutical industry in France from its R&D phases to bioprocessing and marketing. Its objectives are to promote the emergence of innovative start-ups & academic spin-offs, to increase the global visibility of the national industry, to support the development and competitiveness of companies, and to stimulate innovation and collaborations in the field. Created in 2014, MABDESIGN's board of administrators is composed of representatives from ABL Europe, Biomérieux, DBV Technologies, Institut Pasteur, Lyonbiopôle, Pierre Fabre, Sanofi, Thermo Fisher Scientific, TreeFrog therapeutics and 3 independent field experts.

MABDESIGN OFFERS

Operational since September 2015, MabDesign currently has more than 280 member companies, including pharmaceutical and biotech companies, service providers, training organizations, high-tech equipment suppliers and specialized consultants.

Next on Watch

- **ImmunoWatch - Immuno-Oncology**
- **BioprocessWatch - USP and DSP for viral vectors**

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