ASX Announcement

Melbourne, Australia, 08 November 2022

UPCOMING CONFERENCE PRESENTATIONS

- Poster Presentations at ANZSEV 2022 Conference:
  - "*Striving for Safety and Compliance with EV in vitro biological assays*" and
  - "*EV analysis using AF4 – 2+ birds, one stone*"

Genetic medicine and exosome-based drug-delivery company Exopharm Limited (ASX:EX1) releases notice of, and information to be covered in upcoming conference presentations, which contain new information.

Conference: Australia & New Zealand Society for Extracellular Vesicles (ANZSEV) 2022 Conference
Dates: 09-11 November 2022
Location: Voco, Gold Coast, Australia

Presentation 1

Title: "*Striving for Safety and Compliance with EV in vitro biological assays*"
Presenter: Anabel Silva, Head of Biochemical & Biological Analytics
Highlights:
The poster titled, "Striving for Safety and Compliance with EV in vitro biological assays" presents some experimental data supporting Exopharm's manufacturing technology, processes, and approach. These non-clinical results support the use of Exopharm's suite of proprietary technologies to develop EVs for therapeutic applications.

- The profile of a comprehensive set of biomarkers consistently found on our LEAP-purified HEK EVs.
- Endotoxin levels were below FDA-acceptable limits using an FDA-approved Endotoxin test.
- Co-incubating our LEAP-purified HEK EVs with cell lines in vitro were non-toxic to cells (i.e. no cytotoxicity).
- Co-incubating our LEAP-purified HEK EVs with primary human blood cells resulted in no activation of the immune system (T-cells) (i.e. no signals of immunogenicity).

Presentation 2

Title: "*EV analysis using AF4 – 2+ birds, one stone*"

Presenter: Sam Law/ Senior Research Scientist – Process Engineering

Highlights:

The poster titled, "EV analysis using AF4 – 2+ birds, one stone" covers some experimental data supporting the Company's analytical technology and manufacturing processes, focussing on its investment into the use of Asymmetric Flow-Field Flow Fractionation (AF4) and its utility to simultaneously perform multiple exosome characterization measures in one single analysis. Exopharm has demonstrated the efficiency of AF4 coupled with in-line detectors for unparalleled advantage in exosome analysis, providing information on free-protein, aggregation, particle analysis (from micron down to kilo-Dalton size range), and fluorescence tagging, all in one single analysis (in a 60 min run).

- Asymmetric Flow-Field Flow Fractionation (AF4) coupled with multiple detectors is a powerful analytical tool that can provide a comprehensive analysis of exosomes in one single measurement - including information on proteins, surface markers, small molecules, nanoparticles, and aggregates. This capability can help facilitate the product development process and support compliance with regulatory requirements for clinical/therapeutic applications.
- AF4 can provide information on exosome purity by measuring free protein and nanoparticles to guide technology development and process optimization.
- AF4 can be used to separate particle subpopulations, providing size distribution, and particle concentration (one example of application is for measuring the stability of exosomes subjected to different conditions).
- AF4 can be used to confirm the identity of fluorescence-tagged, in-house engineered exosomes.

By the Board - this announcement has been authorised for release by the Board.

COMPANY AND MEDIA ENQUIRIES:

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ABOUT EXOPHARM

Exopharm (ASX:EX1) is a leader in advancing Genetic Medicines and other exosome-based medicines using exosomes or extracellular vesicles (EVs) as a chassis for improved and non-viral drug-delivery.
Exopharm (ASX:EX1) is pursuing a product pipeline-driven platform strategy. Exosomes can be loaded with a variety of active pharmaceutical ingredients (APIs) and can be targeted to selected cell-types and tissue types, improving the safety-profile of the APIs and providing better treatments. Exosomes can be used to deliver small molecule drugs, mRNA, DNA and other types of APIs.
Exosomes are an alternative means of drug-delivery inside the body, alongside technologies such as lipid nanoparticles (LNP), cell-penetrating peptides, viral vectors and liposomes.
Exopharm's exosome technologies solve important needs for the success of exosome medicines – **LEAP** manufacturing technology, **LOAD** API loading technologies and **EVPS** tropism technologies.
Exosome-based medicines could improve the treatment of many chronic or inherited medical conditions.
Exopharm is making its proprietary technologies available to pharmaceutical and biotechnology companies that want to harness exosome-delivery for their own products.
In addition, Exopharm is using its technology platform to enable its own product development programs - each aimed at delivering a transformative medicine for an unmet medical need.

FORWARD LOOKING STATEMENTS

This announcement contains forward-looking statements which incorporate an element of uncertainty or risk, such as 'intends', 'may', 'could', 'believes', 'estimates', 'targets', 'aims', 'plans' or 'expects'. These statements are based on an evaluation of current corporate estimates, economic and operating conditions, as well as assumptions regarding future events. These events are, as at the date of this announcement, expected to take place, but there cannot be any guarantee that such events will occur as anticipated or at all given that many of the events are outside of Exopharm's control or subject to the success of the Development Program. Furthermore, the Company is subject to several risks as disclosed in the Prospectus dated 6 November 2018.

Striving for Safety and Compliance with EV in vitro biological assays

Anabel Silva[1]; Sam Law[1]; Kylie Quinn[2]; Charles Tantuco[1]; Hansi Ranasinghe[1]
[1]Exopharm Limited, Australia; [2]RMIT/Monash University

Introduction

Extracellular vesicles (EVs) have demonstrated potential as therapeutic agents for a range of medical indications.
However, there is a need to develop guidelines at the regulatory level for their clinical use to ensure their safety and efficacy.

At Exopharm, we have a proprietary suite of technologies for the production of EVs:

Using this pipeline for our EV production, we are developing a comprehensive set of in vitro assays to evaluate the safety profile of our ***LEAP*** [1] purified EVs from Exopharm's McMaster Human Embryonic Kidney cell line (HEK).

Endotoxin levels below FDA acceptable limits found in Exopharm EV products

The low levels of endotoxin present post ***LEAP*** purification demonstrate our platforms are producing EV products that comply with safety standards for human administration.

Sample ID	Zetaview counts (p/mL)	Endotoxin (EU/mL)
1	3.4e10	1.04
2	7e10	<0.545

Table 1: Endosafe® Charles River Endotoxin Assay. This assay and all its reagents are licensed by the FDA for product release and are certified.

Successful surface marker profiling of HEK derived LEAP purified EVs by MACSPlex

MACSPlex analysis of our ***LEAP*** purified EV batches not only help us understand our product but also ensures we have consistency across different batches in our manufacturing and purification platforms.

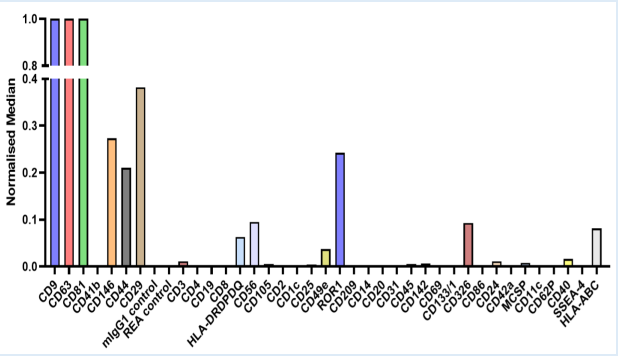


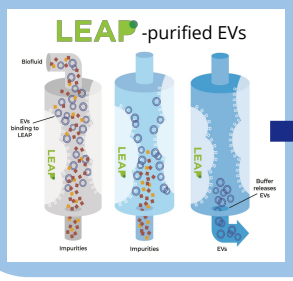
Figure 1: Relative EV marker levels normalised to the CD9, CD63 and CD81 tetraspanins

Conclusion

The in vitro assays described in this work not only contribute to filling the gap that exists at the regulatory level to ensure the safety of EV therapeutic products. The results also strengthens the safety support for Exopharm's proprietary suite of technologies for the production of EVs for therapeutics.
Our work demonstrate that Exopharm's manufactured HEK 293 EVs:

- Have low levels of Endotoxin
- Have a consistent profile of Surface markers
- Do not alter T cell activation in an MLR
- Do not cause cytotoxicity in vitro

Towards regulatory and safety compliance



This work establishes high-throughput assays that ensure low EV product batch-to-batch variation, including:

- Endotoxin testing within acceptable limits
- Constant surface marker profiling by MACSPlex
- Insignificant T cell activation by Mixed Lymphocyte Reaction (MLR)
- No cytotoxicity observed

No cytotoxicity observed when co-incubating EVs with cell lines in vitro

The presence of ***LEAP*** purified EVs does not alter the proliferation nor the viability of the cell lines tested in this in vitro cytotoxicity assay.

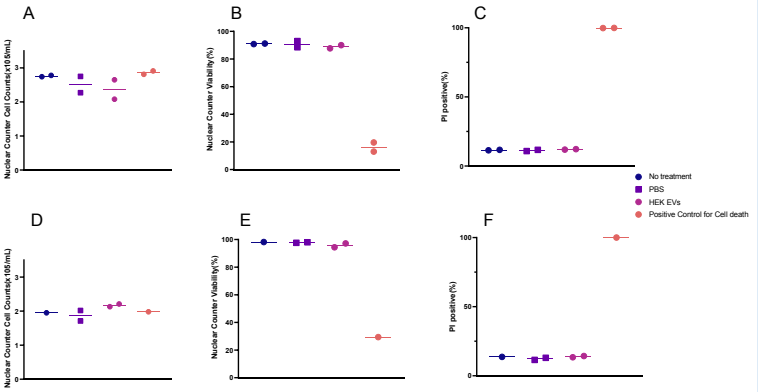
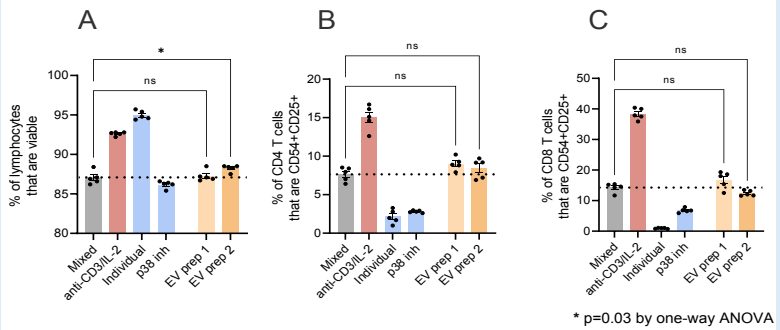


Figure 2. EV cytotoxicity on Human Embryonic Kidney Cells (HEKs) and Normal Human Dermal Fibroblasts (NHDFs)
2A. Cell Count of HEKs. **2B.** Cell Viability measured on NucleoCounter NC-200™
2C. Cell Viability measured by Flow Cytometry (% positive Propidium Iodide).
2D. Cell Count of NHDFs. **2E.** Viability measured on Nuclear Counter.
2F. Viability measured by Flow Cytometry (% positive Propidium Iodide).

EVs do not alter T cell activation in a Mixed Lymphocyte Reaction (MLR)

LEAP purified HEK EVs in Formulation H, Exopharm's patented formulation, do not alter the T cell activation in an MLR.



* p=0.03 by one-way ANOVA

Figure 3: MLR measured by Flow Cytometry [2].
9 individual PBMCs were mixed for the MLR reaction, the readout by flow detecting activated CD54+CD25+ T cells. Mixed group serving as the reference group for the MLR . Anti CD3/IL2 group; a positive control/extra stimulus for assay. Individual group; only one PBMC in the assay; negative control. p38 Inhibitor inhibits MLR reaction; negative control. EV prep 1 in PBS. EV prep 2 in Formulation H
3A. Cell viability. **3B.** CD54+CD25+ activated CD4 T cells.
3C. CD54+CD25+ activated CD8 T cells.

References

1. Law S, et al. 2021 BioProcess Technical
2. Rabea J. Madel, et al. 2020 bioRxiv

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EV analysis using AF4 – 2+ birds, one stone

Sam Q. K. Law, Melanie Schoppet, Md Al-Amin , Alex Hall , Owen Tatford
Exopharm Limited, Australia

Introduction

For extracellular vesicles (EV) to realize their potential as therapeutic products they need to be thoroughly characterized and meet a range of quality criteria.

Most analytical techniques are application-specific and incapable of providing a full characterisation of EV on their own (e.g. protein assays can only measure proteins, and particle-analyzers can only measure particles of certain size range). As a result, multiple analytical techniques are required for characterizing EV which can be cost- and time-intensive. As part of its therapeutic focus, Exopharm Ltd. has concentrated on developing a multi-technique analytical platform to characterize the quality of EV samples.

In this study, the analysis of EV using Asymmetric Flow-Field Flow Fractionation (AF4), coupled to multi-angle light scattering (MALS), dynamic light scattering (DLS) ultraviolet (UV), fluorescence (FLD) and refractive index (RI) detectors, is demonstrated. This analytical technique combines the robust separation properties of AF4, particle sizing and count using in-line MALS/DLS detector and biomolecules concentration derived from RI and UV detectors. The addition of fluorescence detector further extends the flexibility of the platform by allowing detection of tagged EV. Hence multiple characterization can potentially be performed simultaneously in one single analysis, providing a deeper understanding of purified naïve- as well as engineered-EV.

How AF4-UV-MALS-DLS-dRI works[1]

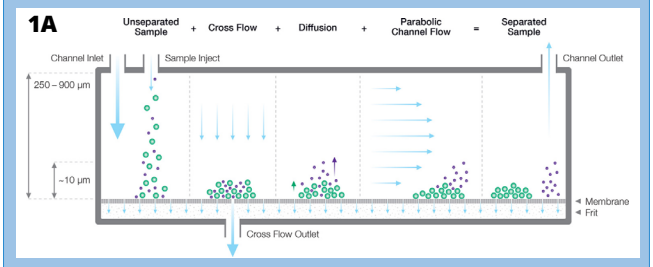


Figure 1A: AF4 separates a sample with a mixed population based on sizes.
1B: By coupling the AF4 outlet to multiple detectors (EV, MALS, DLS, FLD and dRI), in-depth characterization of each separated subpopulation can now be performed.

AF4 separates and measures both small and large biomolecules in heterogenous samples

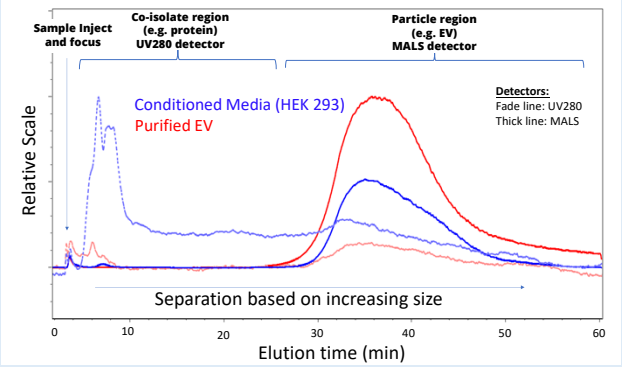


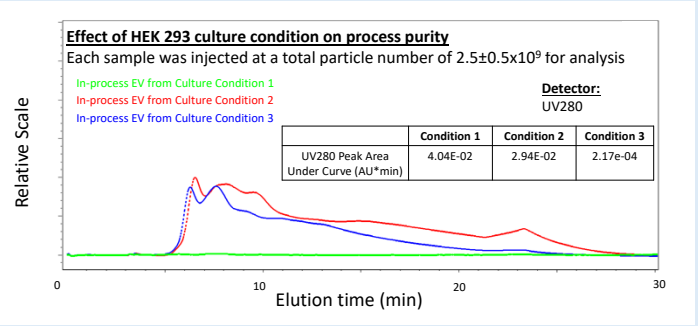
Figure 2: Analysis of HEK293 EV using AF4 coupled to UV, MALS, DLS, FLD and dRI detectors.
The samples were injected and focused on the membrane for 3 min before separation occurs – hence the peak in the first 3 min after sample injection is considered artifact (usually non-significant). Smaller biomolecules (e.g. free proteins) elute between 3 to 20 min, and larger biomolecules (e.g. EV) elutes at 30-50 min.

Conclusion

Here, we demonstrated that AF4 coupled with in-line detectors provided an unparallel advantage in EV analysis, providing information on free-protein, aggregation, particle analysis (from micron down to kilo-Dalton size range), and fluorescence tagging, all in one single analysis (in a 60 min run).

When further combined with other analytical techniques, this technique can further facilitate compliance with regulatory requirements for full characterisation of products, especially when intended in clinical/therapeutic applications.

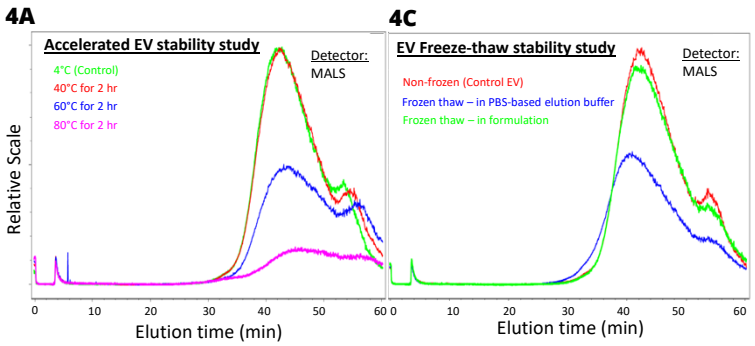
AF4 provides information on presence of co-isolates to guide process optimization decision



	Condition 1	Condition 2	Condition 3
UV280 Peak Area Under Curve (AU*min)	4.04E-02	2.94E-02	2.17e-04

Figure 3: Co-isolate analysis (3-22 min) of in-process HEK EV samples by AF4 (note that the additional peak at 23-24 min was due to sharp change in flow disrupting the UV detector). It was demonstrated that different culture parameters (e.g. flask types in this example) can have a significant impact on the purity (e.g. free non-EV-associated proteins) of the in-process samples, highlighting the utility of applying AF4 to EV process development and to drive critical process optimization decisions.

AF4 separates particles based on size, thus providing information on size distribution and particle concentration



4B

	4°C (control)	40°C 2hr	60°C 2hr	80°C 2hr
Peak radius [nm]	97.4	97.9	97.7	101.4
Peak conc. [p/mL]	8.38E+04	8.11E+04	4.07E+04	1.03E+04

4D

	Control EV (non-frozen)	Frozen (no formulation)	Frozen (in formulation)
Peak radius [nm]	1.20E+05	2.48E+05	9.22E+04
Peak conc. [p/mL]	80.3	67.9	80.5

Figure 4: As a result of its ability in separating and resolving mixed population based on size, AF4 can be a valuable technique at monitoring stability studies. Here, two examples of EV stability studies utilizing AF4 are presented.
4A: The assessment of EV stability at various temperatures. Each sample was held at test temperature for 2 hours before analysis on AF4. Table **4B** shows that EV particle size and concentration remained largely unchanged at 40°C but quickly degraded at higher temperature as expected (particle number drops and peak size increases indicating aggregation).
4C: The stability of EV samples through one cycle of freeze-thaw was assessed via AF4-MALS. Table **4D** shows that without formulation (containing cryo-protective), EV are ruptured after one freeze thaw cycle, generating small debris.

Fluorescence detector extends the capability of AF4 by allowing detection of tagged EV

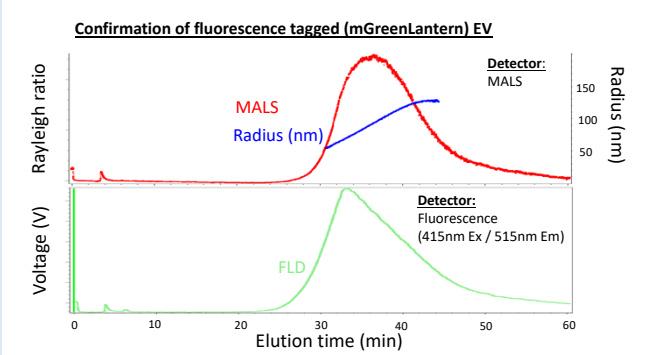


Figure 5: Coupling of AF4-MALS to a fluorescence detector further extends the capability of the AF4 technique. In this example, an in-depth AF4 analysis was performed on an EV sample purified from in-house engineered HEK 293 cells expressing green fluorophore (mGreenLantern) on the EV surface. The AF4 chromatogram showed a good overlap between MALS and fluorescence signal, with minimal fluorescence detected in the co-isolate region, suggesting high mGreenLantern expression in the EV and minimal free non-EV associated fluorescent protein.

Reference

[1] TN6006 (Rev B) - Eclipse & VISION Handbook, p. 11 (Wyatt)

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