ASX ANNOUNCEMENT

Explainer: The critical bottleneck of exosome isolation and the power of Exopharm's LEAP technology

27 March 2019

The Board of Exopharm Ltd ('Exopharm' or the 'Company') is pleased to provide an explanation of recent updates in the exosome field and how this relates to the LEAP technology.

Why Exopharm is poised to be a leader in the field of therapeutic exosomes.

Exosomes (also known as extracellular vesicles or EVs) from stem cells are poised to become a new form of regenerative medicine – described by some as the 'secret sauce' or 'cell juice' from stem cells. But the field has been handicapped by the absence of a suitable purification technology.

Here we describe recent expert publications in the exosome field that confirm the importance of Exopharm's LEAP Technology to remove the purification bottleneck that is holding the field back.

Recent animal studies highlight the regenerative power and safety of exosomes released by stem cells.

These exosomes are tiny packets of proteins and genetic material that have powerful regenerative properties that promote wound healing, brain injury recovery following stroke, and recovery from heart attack, to name just a few potential therapeutic applications.

In fact, the exosomes that stem cells release have now been shown to deliver the regenerative powers long associated with stem cells.

But as doctors seek to harness the regenerative power of exosomes in humans, they face the 'bottleneck' problem. The bottleneck problem is important to understand. Whilst cells produce trillions of exosomes naturally, the problem has been to purify these natural nano-scale particles as a proper drug product.

In 2019 experts from Johns Hopkins University USA published [1] "Despite these advances, a major bottleneck of MSC derived EV (MSC-EV)-based applications in clinics is the *inefficient production and purification of clinical-grade EVs*." (our italics)

[1] Liu, S., et al., Highly Purified Human Extracellular Vesicles Produced by Stem Cells Alleviate Aging Cellular Phenotypes of Senescent Human Cells. Stem Cells, 2019

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University College London researchers Daniel Bracewell, and Ivan Wall recently flagged that if attention is not given to developing drastically improved exosome isolation platforms, this exciting therapeutic could hit a major roadblock.[2]

To date the techniques used to isolate exosomes have been laborious, expensive, poorly reproducible and vulnerable to contamination. They are totally unsuitable, in other words, for producing a more purified, medical grade proprietary therapeutic product.

Bracewell and Wall argue. "There is an urgent need for technological advancements."

And by 'technological advancements', they certainly don't mean exosome isolation kits for use in the laboratory.

In a spin: the current bottleneck in exosome production

The standard lab-based approach to collect the exosomes produced by cultured cells has been by collecting the liquid cell culture medium and spinning it in an ultracentrifuge or passing it through a fine filter.

The challenge is that the cell culture liquid containing the exosomes is a complex mixture containing an incredible variety of biological components – and these contaminants need to be removed.

The most common technique used so far, the ultracentrifuge (UC), has major scalability limitations, including the high level of skill and manual labour required, the time-intensive nature of the process. UC has other limitations as well (e.g. damage to the vesicles) and is not a proprietary purification technology.

But the bigger issue is the lack of purity of the exosomes collected using UC. UC sorts the contents of cell culture medium by their density. Although the exosomes are concentrated by the removal of liquids, they could still be accompanied by all manner of other biological components present in the cell culture medium that happen to be a similar density to the exosome.

In short, ultracentrifugation is not sophisticated enough to purify an ideal therapeutic product.

Ultrafiltration (filters with very small pore size) or Size Exclusion Chromatography (SEC) are two other techniques that are being used and which purify based on size rather than density. These techniques have additional issues and are not proprietary technologies.

Another technique used in the laboratory is immunoaffinity chromatography (IAC). IAC relies upon identifying a certain molecule that is externally located on the membrane of the exosomes (Exosome Membrane Molecule or EMM). In IAC, an antibody (a complex

[2] Colao, I. L., Corteling, R., Bracewell, D., Wall, I. Manufacturing Exosomes: A Promising Therapeutic Platform. Trends in Molecular Medicine, 2018, 24, 242 https://doi.org/10.1016/j.molmed.2018.01.006

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biomolecule itself) that selectively binds to one EMM is loaded into an affinity chromatography column – and the binding interaction between the EMM and the antibody is used to purify vesicles containing the specific EMM. The problem with IAC is that exosomes/vesicles contain a wide range of surface molecules – yet vesicles may not contain any/many of the selected EMM [3]. So whilst IAC is well-suited to laboratory use it is not yet favoured by experts for making large quantities of therapeutic exosomes/vesicles.

LEAPing ahead

So the purification problem is still the holdup in applying purified exosomes as human therapeutics.

Thankfully, Exopharm has a different way to purify exosomes to overcome the limitations of other techniques.

Exopharm is using a chromatography based (but not immunoaffinity chromatography or IAC) purification method, we call LEAP (Ligand-based Exosome Affinity Purification).

Like a smart version of Velcro™, LEAP uses a patent-applied-for specific chemical (an inexpensive functionalised polymer) (we call this a LEAP Ligand) that is loaded into a chromatography column (like a maze for liquid) and sticks to the membrane surface of exosomes/vesicles. Liquid containing exosomes/vesicles flows through the chromatography column, and the LEAP Ligands bind to many of the passing vesicles whilst the other biological detritus in the cell culture medium is simply washed away. This has advantages over existing methods for achieving potential commercial scale production, as described in more detail below.

The LEAP technology fits with the prediction of experts "Downstream processing needs to transition from traditional ultracentrifugation methods to combinations of filtration and chromatographic-based methods that can achieve consistent and reproducible purification at scale."[4]

The LEAP Ligand technology has four key advantages over other approaches:-

1. it appears to capture the vesicles by binding to the membrane of the vesicle (and not specific molecules 'decorating' the vesicle);
2. the ligand itself is not expensive to make;
3. it gives Exopharm a proprietary step in the process; and
4. it uses affinity chromatography equipment – which is a standard technique in biomanufacturing

[3] Helwa, I. et al. A Comparative Study of Serum Exosome Isolation Using Differential Ultracentrifugation and Three Commercial Reagents. PLoS ONE 2017, 12, e0170628. doi:10.1371/journal.pone.0170628
[4] Zeringer, E., Barta, T., Li, M., Vlassov, A.V. Strategies for isolation of exosomes. Cold Spring Harb Protoc. 2015 Apr 1;2015(4):319-23. doi: 10.1101/pdb.top074476

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The LEAP technology is referred to as a 'platform technology' – because the one technology can be used to make a range of products. Platform technologies can be especially important and valuable once a few example products have derisked the platform.

Exopharm's LEAP platform technology opens the way to manufacturing a range of products for treating a broad range of clinical indications.

The product is the process – LEAP gives Exopharm exclusivity

The LEAP Technology is a core manufacturing technology that both protects and enhances Exopharm's position in this emerging industry.

Exosomes/vesicles are likely regulated as biologic products. In biologic products there is an adage 'the product is the process – the process is the product'. This means that a change in the process to make a biologic product also changes the product – and two seemingly similar products made by different processes are seen as distinct products (with potentially distinct safety profile and potency).

What this means is that even if two companies used the same cells and bioreactor to produce exosomes – but one purified the product with ultracentrifuge and the other used the LEAP technology – the actual final products would be considered to be different (i.e. not biosimilar).

Using the example above, each company would be required to conduct separate clinical trials, and later seek separate registration of the product before sales.

The company using ultracentrifuge would not normally be able to rely upon the safety or efficacy data from the second company (or vice versa).

So Exopharm's LEAP technology includes the potential to exercise exclusivity over the exosome products purified with LEAP technology - justifying Exopharm's investment into product manufacture, clinical trials and product registration.

Other companies may utilise standard purification technologies (e.g. ultracentrifuge, tangential flow filtration, size exclusion chromatography) – but those standard manufacturing steps are more likely to deliver a biosimilar product that competitors can copy and compete with. Furthermore, if a non proprietary biosimilar product is safe (like aspirin) then anyone can make and sell it.

LEAPing ahead

Exopharm sees LEAP as the key to solving the bottleneck problem that is delaying the production and purification of clinical-grade exosomes/EVs.

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With the LEAP technology to break the purification bottleneck, Exopharm is positioned to be a worldwide leader in the commercialisation of therapeutic exosomes and exosome production.

Exopharm's proprietary LEAP (patent-applied-for) technology should help fend off emerging competition and attract potential partners.

ENDS.

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ABOUT EXOPHARM

Exopharm Limited ("Exopharm" or the "Company") (ASX:EX1) is an Australian regenerative medicine biopharmaceutical company seeking to develop and commercialise exosomes as therapeutic agents – initially a product called Plexaris™ and later a product called Exomeres™.

These products are exosomes that are derived from human platelets in relation to Plexaris, and adult stem cells in relation to Exomeres, and purified using the LEAP Technology and referred to as biologic products.

As its primary focus, Exopharm aims to be a leader in the field of human therapeutics using exosomes as regenerative medicine products for heath span related conditions.

The Initial Development Program: The Company's main objectives for the next 12 months are to complete the following stages of its Development Program:

1. manufacturing - make and supply clinical grade autologous Plexaris product and development of the Exomere manufacturing process;
2. clinical use - initial small-scale human clinical studies of autologous Plexaris in wound healing as a demonstration program;
3. supporting research and development activities - conducting research activities to support the ongoing Development Program and the development of related intellectual property; and
4. to also investigate other LEAP Technology Opportunities.

CORPORATE SNAPSHOT

Exopharm Limited is listed on the Australian Securities Exchange (ASX:EX1).

EXOPHARM: EXTENDING HEALTH SPAN THROUGH CELL FREE REGENERATIVE MEDICINE

BOARD: Non-Executive Chairman: MR JASON WATSON; Founder, Managing Director DR IAN DIXON; Non-Executive Director and Company Secretary MR DAVID PARKER

ISSUED CAPITAL: 80,500,000 Fully Paid Ordinary shares on issue.

For more information please visit: exopharm.com or contact Dr Ian Dixon on (03) 9111 0026 or via email, info@exopharm.com.

FORWARD LOOKING STATEMENTS

Exopharm Limited ("Exopharm" or the "Company This announcement contain 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.

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