INOVIQ AND UNIVERSITY OF SYDNEY PRESENT NEW EXO-NET DATA AT ISEV

- **New data establishing the utility of EXO-NET® for On-Bead Analysis (OBA) of extracellular vesicles (EVs), using Fourier Transformed Infrared (FTIR) Spectroscopy was presented at the 2022 International Society for Extracellular Vesicles (ISEV) Annual Meeting held in Lyon, France**
- **Direct FTIR analysis of EVs captured by EXO-NET discriminated between EVs from different types of cancer cells and their response to stimulation**
- **EXO-NET provides a simple and rapid method for classifying cancer cell type and activation using low cost FTIR equipment**

Melbourne, Australia, 27th May 2022: INOVIQ Limited (ASX:IIQ) (**INOVIQ** or **the Company**) is pleased to announce that the results of a study conducted by the School of Chemistry and School of Medical Sciences at the University of Sydney were presented at the Annual Scientific Meeting of ISEV in France. The research establishes the utility for INOVIQ's EXO-NET EV isolation tool for on-bead FTIR analysis, thereby offering a simple, fast, and effective method for classifying cancer cells in a patient. A copy of the poster presenting the data at the conference accompanies this announcement.

FTIR Spectra for Profiling Extracellular Vesicles

Exosomes are a type of extracellular vesicle (EV) that are small particles (around 30-150 nm) released by most cells into biofluids such as blood, urine, and saliva. Exosomes contain different types of bioactive molecules such as DNA, RNA, proteins and lipids that convey important information about their parent cell that can be used for the identification of biomarkers, diagnosis, and treatment of disease.

FTIR spectroscopy is a method for identifying different classes of bioactive molecules and for generating unique fingerprints of EVs that can be used to differentiate between cell type of origin and their response to treatment.

Commenting on the research, INOVIQ Chief Scientific Officer Dr Greg Rice said, "*The direct On-Bead Analysis (OBA) of EVs attached to EXO-NET represents a very simple, rapid, and cost-effective method for generating FTIR spectra that provide information about disease states, including cancer. The clinical application of such data may include accurate disease classification and triage to treatment. FTIR OBA successfully classified different lung cancer cell types and the response to in vitro cytokine stimulation*".

ISEV is the leading professional society for researchers and scientists involved in the study of extracellularly secreted vesicles, with around 2,000 members. The 2022 Annual Meeting is being held in Lyon, France from 25-29 May 2022.

INOVIQ CEO, Dr Leearne Hinch said, "*This proof-of-concept study further demonstrates the application and advantages of INOVIQ's immunoaffinity capture tool EXO-NET for enabling translational and clinical research. We are delighted to be presenting this research at the 2022 ISEV*

Annual Meeting, as the event presents a valuable opportunity for INOVIQ to showcase EXO-NET and engage with Key Opinion Leaders in the field."

Authorised by the Company Secretary, Tony Di Pietro.

– ENDS –

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ABOUT INOVIQ

INOVIQ Ltd (ASX:IIQ) (INOVIQ) is developing and commercialising innovative diagnostic and exosome-based products to improve the diagnosis and treatment of cancer and other diseases.

The Company has commercialised the hTERT test used as an adjunct to urine cytology testing for bladder cancer and the EXO-NET pan-exosome capture tool for research purposes. Our cancer diagnostic pipeline includes blood tests in development for earlier detection and monitoring of ovarian, breast, prostate, and other cancers. For more information on INOVIQ, see www.inoviq.com.

FORWARDING LOOKING STATEMENTS

This announcement contains certain 'forward-looking statements' within the meaning of the securities laws of applicable jurisdictions. Forward-looking statements can generally be identified by the use of forward-looking words such as 'may', 'should', 'expect', 'anticipate', 'estimate', 'scheduled' or 'continue' or the negative version of them or comparable terminology. Any forecasts or other forward-looking statements contained in this announcement are subject to known and unknown risks and uncertainties and may involve significant elements of subjective judgment and assumptions as to future events which may or may not be correct. There are usually differences between forecast and actual results because events and actual circumstances frequently do not occur as forecast and these differences may be material. The Company does not give any representation, assurance or guarantee that the occurrence of the events expressed or implied in any forward-looking statements in this announcement will actually occur and you are cautioned not to place undue reliance on forward-looking statements.

Differential detection of cancer-derived extracellular vesicles using combined antibody functionalized magnetic beads and infrared spectroscopy

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INTRODUCTION

- Extracellular vesicles (EVs) paly a pivotal role in cellular functions including signaling pathways involved in the maintenance of normal physiological processes (1).
- EV surface antigens and molecular cargoes are altered in response to challenges to cellular homoeostasis and may contribute to disease pathogenesis (2).
- Rapid and simple methods for isolating enriched populations of EVs and characterizing their cell-specific biomarkers and molecular cargo are requisite to realizing their diagnostic potential.
- Non-destructive label-free vibrational spectroscopies definitively inform about the relative concentrations of biomolecular classes and, in some cases, specific biomolecules.
- Several advantages over current "omics" technologies, i.e., ability to assess relative concentrations of classes of biomolecules, e.g., lipids, proteins, DNA, RNA, etc., and to analyze the content of individual EVs and identify conformational changes within these biomolecules.
- FTIR has been successfully used to differentiate between case and control clinical samples (3-5) and *in vitro* cell phenotypes (6-8).

STUDY AIMS

- To compare the FTIR spectral signatures of EVs released from different cancer cell lines and phenotypes, and isolated by differential centrifugation or bead-based immunoaffinity capture.
- To model the future detection,in plasma from cancer patients, EV were isolated from the cell-conditioned of two different cancer cell lines: the human epithelial carcinoma A549; and the SV-40 immortalized mesothelial cell line MeT-5A. To model the inflammatory state of tumor micro-environment, the effects of IFN-ɣ stimulation of A549 EVs was determined.

METHODS

- ***Cell Culture:*** Lung cancer cell line, A549, and the SV40-immortalized mesothelial cell line, MeT-5A, were seeded at 1x10^4 cells/mL in tissue culture dishes and cultured for 4 d in RPMI-1640 with 10% fetal bovine serum. Cell-conditioned medium was collected and stored at -80^{0}C until analyzed. A549 cells were further incubated with IFN-ɣ (100 ng/mL) for 48 h.
- ***EV Isolation by Immunoaffinity Isolation****:* EVs were isolated from cell-conditioned medium (500 µL) using immunoaffinity magnetic bead capture (50 µL EXO-NET®, INOVIQ Ltd, Figure 1).

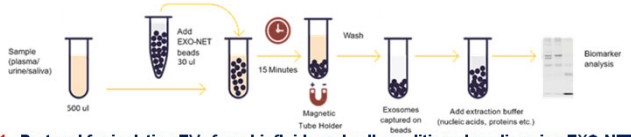

Fig 1. Protocol for isolating EVs from biofluids and cell-conditioned media using *EXO-NET®*

- ***EV Isolation by Differential Centrifugation of Medium***: 2,000 g for 10 min, 10,000 g for 45 min, 18,000 g for 45 mins and then 100,000 g for 1 h at 4°C in 30% sucrose-deuterium oxide (D_2O).
- ***FTIR Spectroscopy:*** Mapping of air-dried aliquots (5 µL) on a CaF_2 window (0.5 x 0.5 cm) used a Bruker Lumos FTIR microscope (transmission mode; 20 x 4 transverse maps.
- Spectra from whole dried aliquots on CaF_2 used the Bruker Alpha: aliquots (5 µL) with a Platinum ATR module over the region of 4000-400 cm^{-1} (attenuated total reflection mode with the coaddition of either 64 or 256 scans.
- Alternatively, spectra from whole air-dried aliquots (1 µL) deposited on a Si 96-well plate were measured on a Bruker Tensor HTS-XT instrument
- Spectra compensated for atmospheric moisture and CO_2 interference, vector-normalized to the 1800-1050 cm^{-1} region and subjected to smoothing using the Savitsky-Golay algorithm with 13 smoothing points (Bruker OPUS software).
- Further smoothing with nine smoothing points of second derivatives (Savitsky-Golay algorithm, The Unscrambler X software), then Principal Component Analysis (PCA) was applied.

RESULTS

- ***Method of EV Isolation:*** EVs isolated using a differential centrifugation contained a residual concentration of sucrose that absorbed intensely in the IR region and overwhelmed the detector and signal from the extracellular vesicles, which prevented meaningful analysis.
- Samples isolated using EXO-NET were successfully analyzed using IR spectroscopy Lumos with individual points across a dried sample (Fig 2) or averaged spectra for all points across two separate samples (Fig. 3.)
- Subsequent studies, therefore, were performed using EXO-NET isolated EVs.
- ***Cell and Phenotypic Spectral Signatures: S***pectra from whole dried spot were measured using the Tensor instrument, which operates with 96 well-plates, (Fig. 4), or using the ATR mode using an inexpensive Bruker Alpha (Fig. 5), EV from both cell lines and unstimulated vs stimulated A549 cells were clearly differentiated from one another and the beads alone.
- Distinct clustering of the spectra by sample type indicated that the different varieties of cell-derived exosomes were differentiated using several vibrational spectroscopic methods.

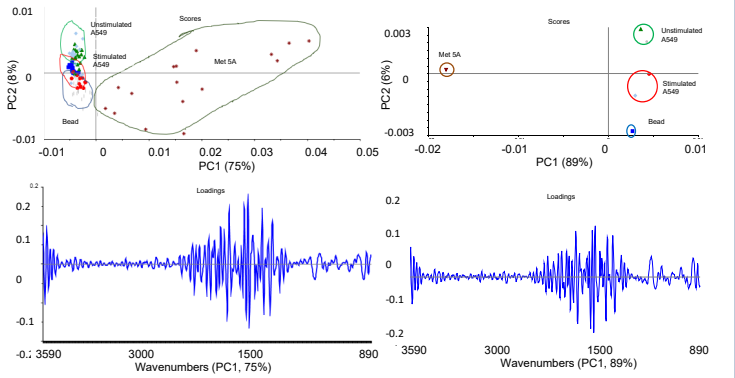

Fig 2. PCA scores and loadings plots of second derivative spectra collected from unloaded beads and beads loaded with exosomes; stimulated A549 cells **(1)**, unstimulated A549 cells **(2)** and MeT-5A cells **(4)**.

Fig 3. PCA scores and loadings plots of the average spectra collected from beads loaded with the three cell varieties and unloaded beads, showing distinct grouping by cell variety.

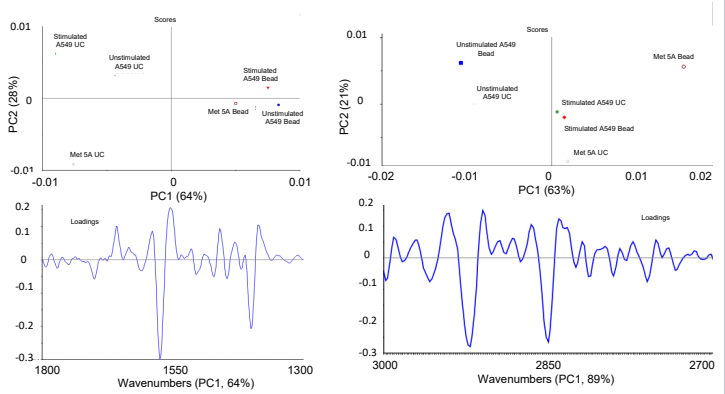

Fig 4. PCA scores (top) and loadings (PC1, bottom) plots for second derivative spectra collected on the Bruker Tensor (1800-1300 cm^{-1}). Spectra are separated by isolation method of the relevant EVs along PC1.

Fig 5. PCA scores (top) and loadings (PC1, bottom) plots for second derivative spectra collected on the Bruker ALPHA, over 3000-2700 cm^{-1} range. The MeT-5A and IFN-γ stimulated A549 cells are separated along PC1 from the regular A549 samples.

CONCLUSIONS

1. Bead-based immunoaffinity capture (EXO-NET) represents a simple and rapid method for preparing enriched subpopulations of EVs for direct, on-bead FTIR spectroscopic analysis.
2. On-bead analysis using vibrational spectroscopy provides a rapid and simple method for determining the relative changes in EV biomolecular contents from different cancer cell types.
3. Distinct clustering of the spectra by sample type occurred, indicating that the different subpopulations of cell-derived exosomes could be differentiated using this vibrational spectroscopic method.
4. Data obtained support the hypothesis that on-bead FTIR analysis of EV differentiates cancer cell type from position in PC1 and the phenotype from position in PC2. Both parameters are determinants of disease classification accuracy and triage to treatment.

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