ANSEV Meeting Presentations

Melbourne, Australia, 8 November 2023: INOVIQ Limited (ASX:IIQ) today advises that it will present new data on the effectiveness and utility of its proprietary exosome isolation technology, EXO-NET®, at the Annual Meeting of the Australia and New Zealand Society for Extracellular Vesicles (ANSEV) in South Australia from 7-10 November.

ANZEV is the leading Australian exosome scientific conference and provides further opportunity for INOVIQ to showcase these important advances to key opinion leaders in the extracellular vesicle field.

The first poster titled 'High-throughput isolation of extracellular vesicles and associated miRNAs' presents data on the first fully-automated high-throughput system for rapid and reproducible EXO-NET isolation of EVs and downstream RNA extraction on the Promega Maxwell® and Thermo Fisher Scientific KingFisher™ automated instruments.

The second poster titled 'A novel engineered protein bead-based matrix for enrichment of tumor derived extracellular vesicles' outlines data from a study that established the effectiveness of TEXO-NET in selectively capturing a subpopulation of EVs enriched with tumor-specific biomarkers.

INOVIQ CEO, Dr Leearne Hinch said: *"These presentations deliver new data to the scientific community supporting additional high-throughput and cancer applications for our rapid, efficient and reproducible EXO-NET technology. EXO-NET enables the translation of exosome diagnostics from the bench-to-clinic and delivery by large pathology laboratories."*

The poster presentations are appended to this release.

Authorised for release by Company Secretary, Mark Edwards.

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ABOUT INOVIQ LTD

INOVIQ Ltd (ASX:IIQ) (**INOVIQ**) is developing and commercialising next-generation exosome capture tools and precision diagnostics to improve the diagnosis and treatment of cancer and other diseases. The Company has commercialised 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, 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.

ANSEV POSTER PRESENTATIONS

1. **High-throughput isolation of extracellular vesicles and associated miRNAs**
   Poster Number: 82
   Presentation time 1:45PM - 3:00PM
   Presentation date: Wednesday, 8th November

2. **A novel engineered protein bead-based matrix for enrichment of tumor derived extracellular vesicles**
   Poster Number: 83
   Presentation time: 1:45PM - 3:00PM
   Presentation date: Wednesday, 8th November

High-throughput isolation of extracellular vesicles and associated miRNAs

Carlos Palma[1], Ramin Khanabdali[1], Michelle Mandrekar[2], Phuoc-an Vo[2], Rick Grygiel[2], Siena Barton[1], Sara Nikseresht [1], Sadman Bhuiyan [1], Mozhgan Shojaee [1], Kartini Asari [1], Susan Belzer [1], Kevin Kershner[2], Douglas Horejsh[2], Gregory Rice [1]

[1] INOVIQ Limited, Notting Hill, Victoria, Australia.
[2] Promega Corporation, Madison, Wisconsin, USA

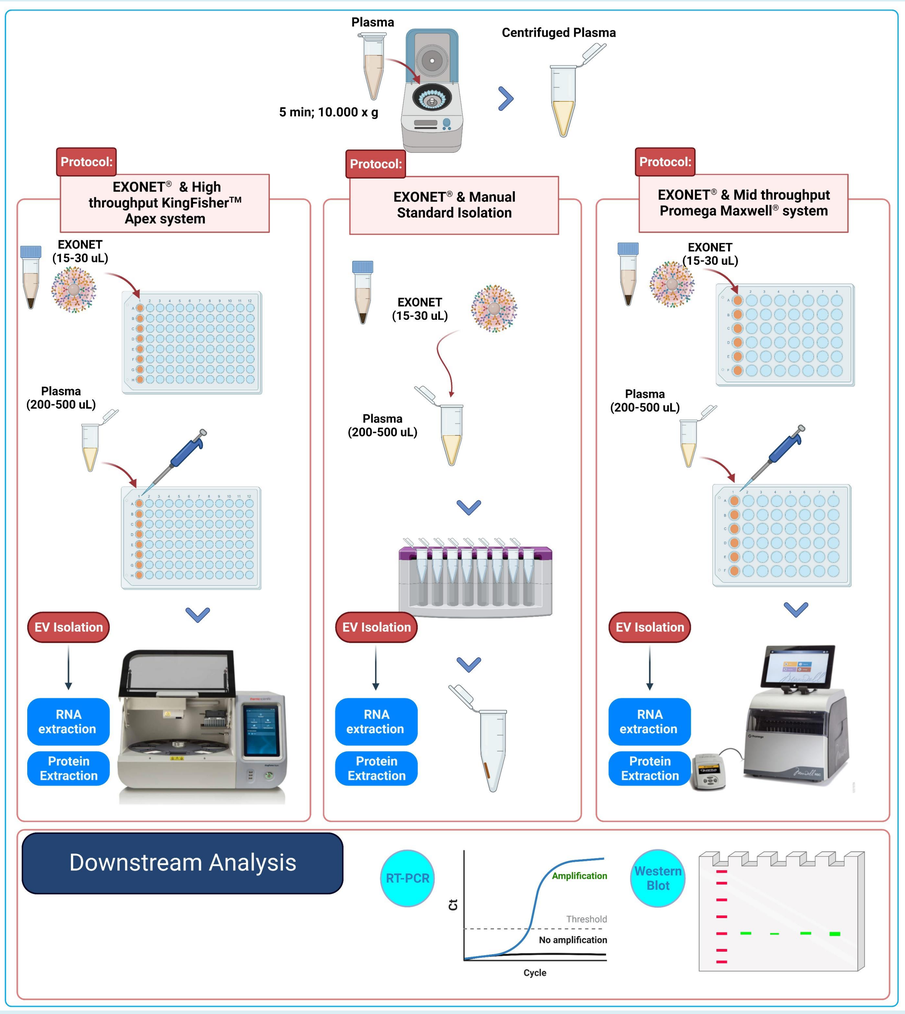
INTRODUCTION

Despite the growing number of disease-associated extracellular vesicle (EV) biomarkers being identified, the translation of EV diagnostics into routine clinical laboratory tests remains limited. The main challenge lies in the lack of simple, rapid, reproducible and high-throughput EV isolation and downstream analysis methods that can be easily integrated into clinical laboratory workflows. In this study, we evaluated a high-throughput bead-based immunoaffinity system (EXO-NET®) that captures a highly enriched subpopulation of EVs.

METHODS

EVs were isolated from human plasma samples using EXO-NET manually and two different automated systems. High-throughput (96 samples/run) EV isolation was performed on KingFisher™ Apex System, and mid-throughput (16–48 samples/run) was performed on Promega Maxwell® system. RNA or protein from isolated EVs were extracted on the same systems using Maxwell® RSC miRNA Plasma and Serum Kit or 1% SDS, respectively. For manual isolation, RNA extraction was carried out using manual ReliaPrep™ RNA Miniprep Systems and protein using 1% SDS.

WORKFLOW



RESULTS

Analytical Validation

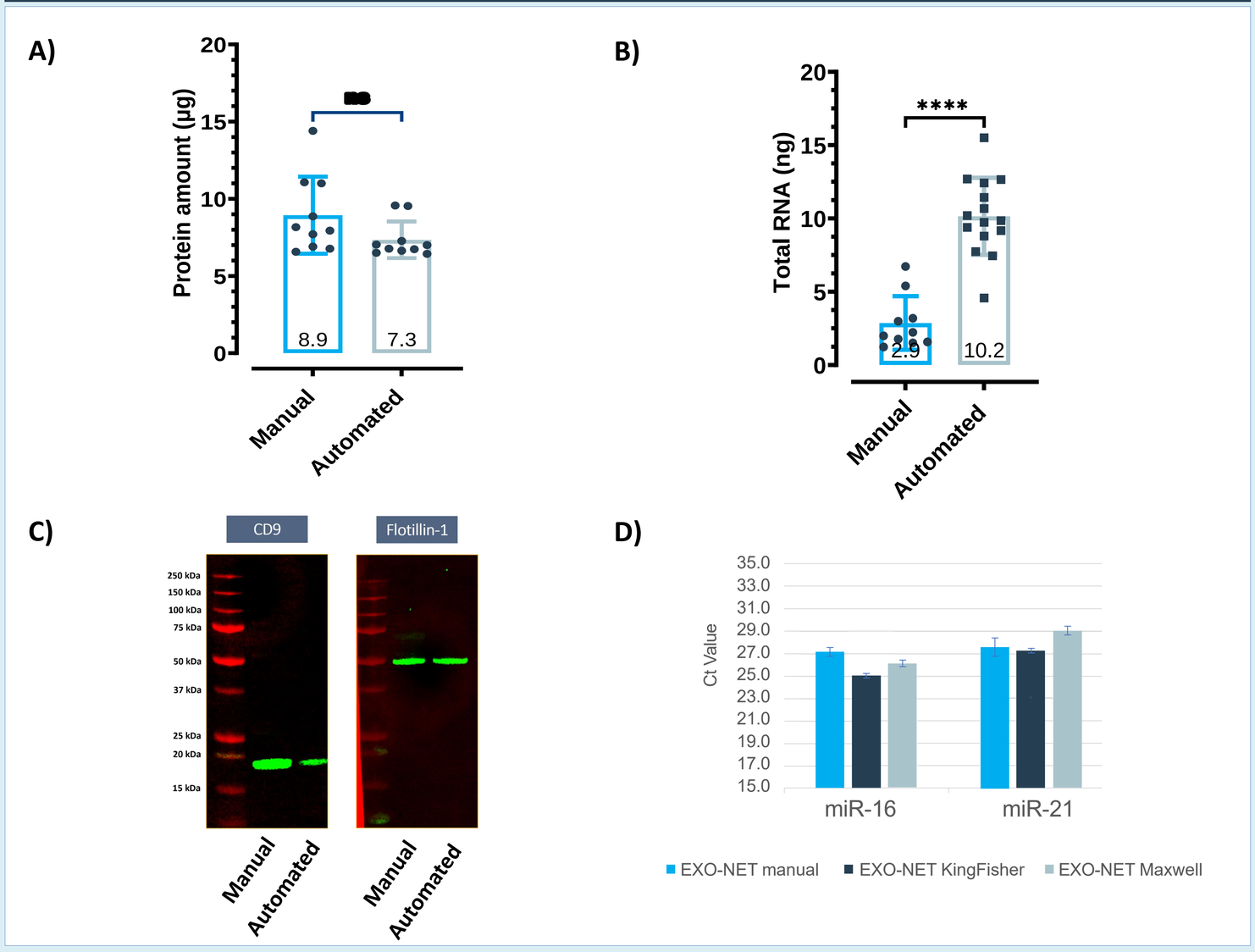


Figure 1. High throughput automated EV RNA and protein isolation and characterisation. EVs were isolated from plasma (200-500 µL) using EXO-NET (15-30 µL). A) Total protein yield analysis (n=10 each group) showed no significant differences between manual and automated system. C) Western blot analysis confirmed expression of CD9 and Flotillin-1 from isolated EV from both manual and automated systems. B) Total RNA yield analysis (n=10 each group) showed significantly higher yield of RNA on automated system than manual isolation. Unpaired *t-test* (**** p-value < 0.0001). Data presented as Mean ± SD. D) RT-PCR analysis showed higher expression of miRNAs (miR-16 and miR-21) on both automated systems compared with manual isolation.

Clinical Validation

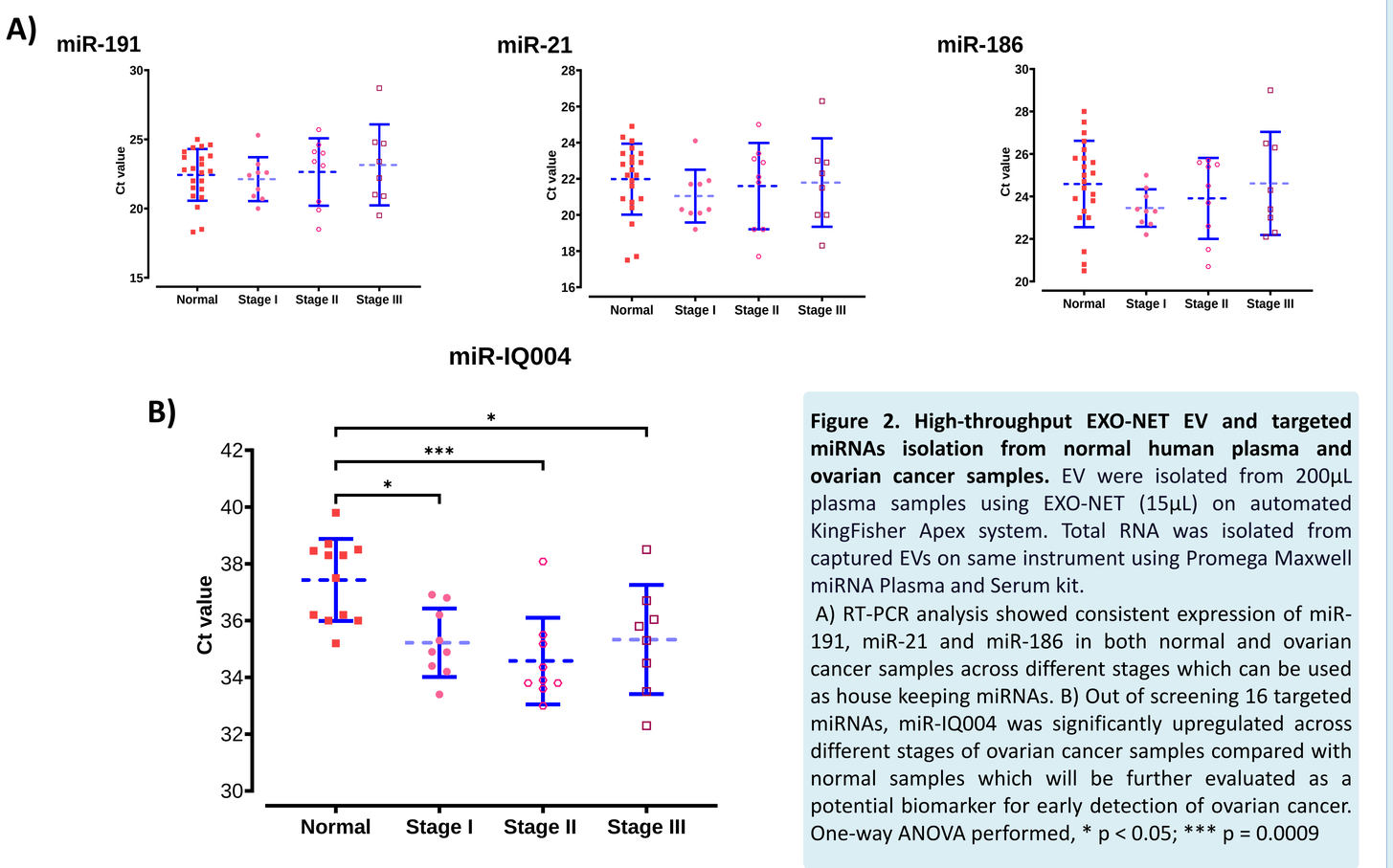


Figure 2. High-throughput EXO-NET EV and targeted miRNAs isolation from normal human plasma and ovarian cancer samples. EV were isolated from 200µL plasma samples using EXO-NET (15µL) on automated KingFisher Apex system. Total RNA was isolated from captured EVs on same instrument using Promega Maxwell miRNA Plasma and Serum kit.
A) RT-PCR analysis showed consistent expression of miR-191, miR-21 and miR-186 in both normal and ovarian cancer samples across different stages which can be used as house keeping miRNAs. B) Out of screening 16 targeted miRNAs, miR-IQ004 was significantly upregulated across different stages of ovarian cancer samples compared with normal samples which will be further evaluated as a potential biomarker for early detection of ovarian cancer. One-way ANOVA performed, * $p < 0.05$; *** $p = 0.0009$

CONCLUSIONS

The combination of high-throughput EV isolation using EXO-NET and Promega Maxwell high-throughput miRNA Plasma and Serum kit represents the first fully-automated high-throughput system for rapid, efficient and scalable enrichment of EVs. This approach holds great promise for facilitating the integration of EV diagnostics into routine clinical practice.

A novel engineered protein bead-based matrix for enrichment of tumor derived extracellular vesicles

Sara Nikseresht [1], Amirah N. Fitri[1], Sadman Bhuiyan[1], Ramin Khanabdali[1], Gregory Rice [1]

[1] INOVIQ Limited, Notting Hill, Victoria, Australia.

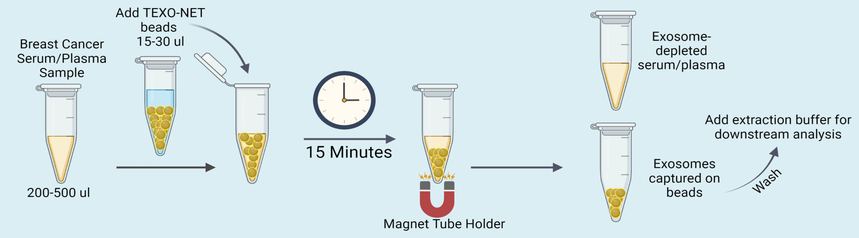
INTRODUCTION

Glycolylneuraminic acid (Neu5Gc) incorporation in tumor-derived glycoconjugates is an evident feature of many cancer biomarkers and is often associated with tumor growth, metastasis and immune evasion in neoplastic transformation. Cancer cells release extracellular vesicles (EVs), and these small messengers express tumor-specific epitopes on their surface, including Neu5Gc-glycans. SubB2M is a genetically engineered lectin with enhanced affinity for Neu5Gc. SubB2M may recognize EVs containing Neu5GC and be of utility in discriminating between cancer-derived and non-cancer EVs.

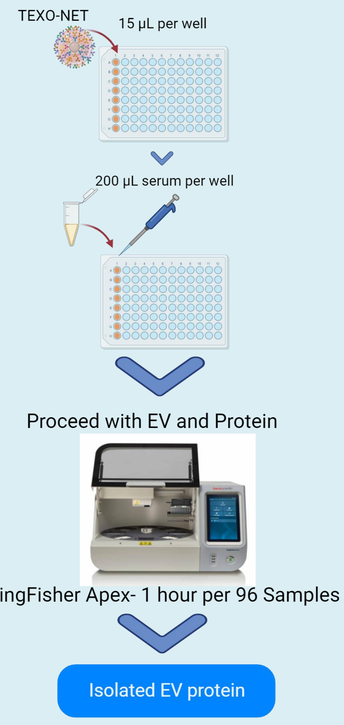
In this study, we have evaluated the utility of SubB2M immobilized on paramagnetic nanoparticles (TEXO-NET) to isolate Neu5Gc enriched subpopulation of tumor-derived EVs for diagnostic application.

METHODS

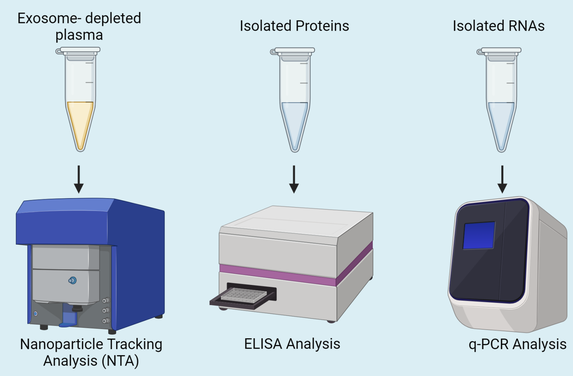
A) Manual TEXO-NET EV isolation



B) High-throughput TEXO-NET EV isolation



C) Characterization of TEXO-NET Isolated EVs



RESULTS

TEXO-NET captured EVs is blocked by pre-incubation with Neu5Gc

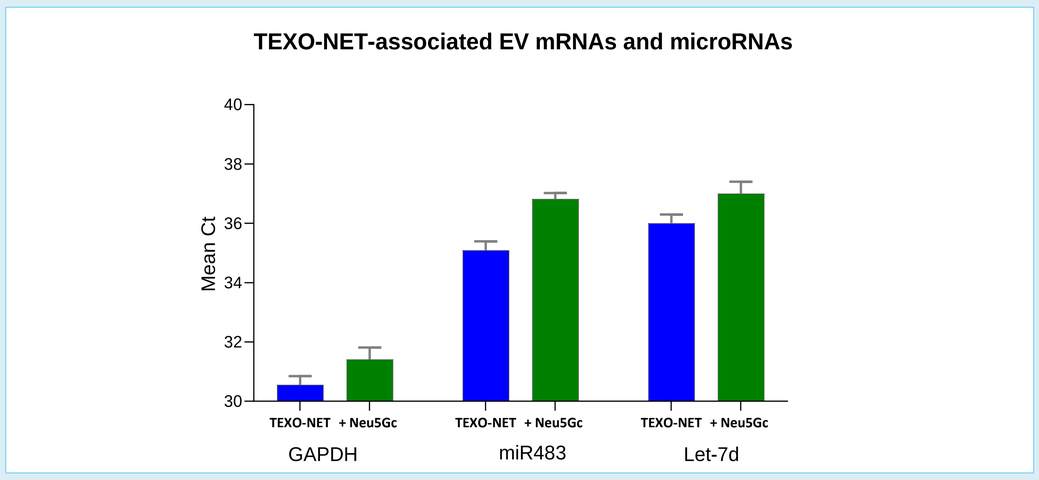


Figure 1. RT-PCR analysis for EV-associated GAPDH, miR483 and Let-7d. TEXO-NET was preincubated with or without 1 mM Neu5Gc for 15 min, washed with PBS four times and then 30 µL of the beads were used to capture EVs from 500 µL of normal human Plasma. The captured EVs were subjected to RNA extraction and qPCR analysis.

TEXO-NET captures EVs containing ovarian and breast cancer markers

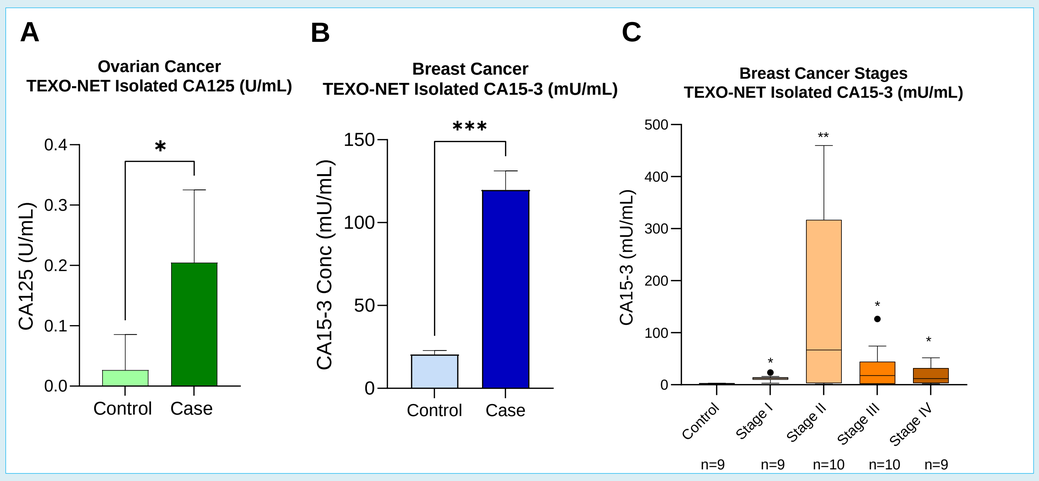


Figure 3. TEXO-NET isolates EVs containing CA125 and CA15-3. Panel A and B: EVs were isolated from 500 µL pooled ovarian or breast cancer serum and match controls by TEXO-NET. Panel C: EV-associated CA15-3 isolated from serum obtained from healthy women and women with breast cancer (Stages I-IV). EVs were isolated from 200 µL of serum using 15µL of TEXO-NET.

Neu5Gc releases EVs from TEXO-NET

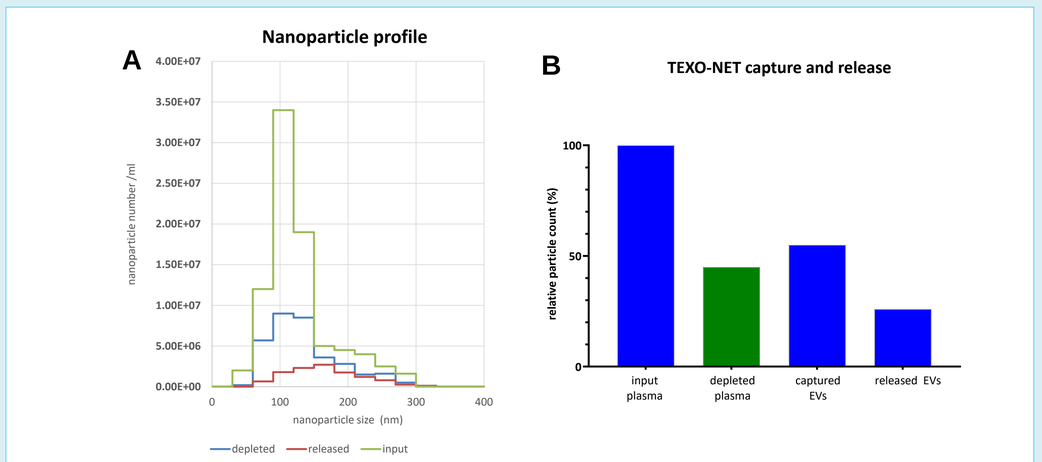


Figure 2. Neu5Gc release of EVs from TEXO-NET. Plasma (500 µL) was incubated with 30 µL TEXO-NET for 15 min. Panel A: The concentration and size of EVs present in plasma before and after incubation with TEXO-NET and following incubation with 1 mM Neu5Gc for 1 min were determined by ZetaView analysis. Panel B: Percent of EVs captured and released from TEXO-NET.

High-throughput isolation of EVs using TEXO-NET

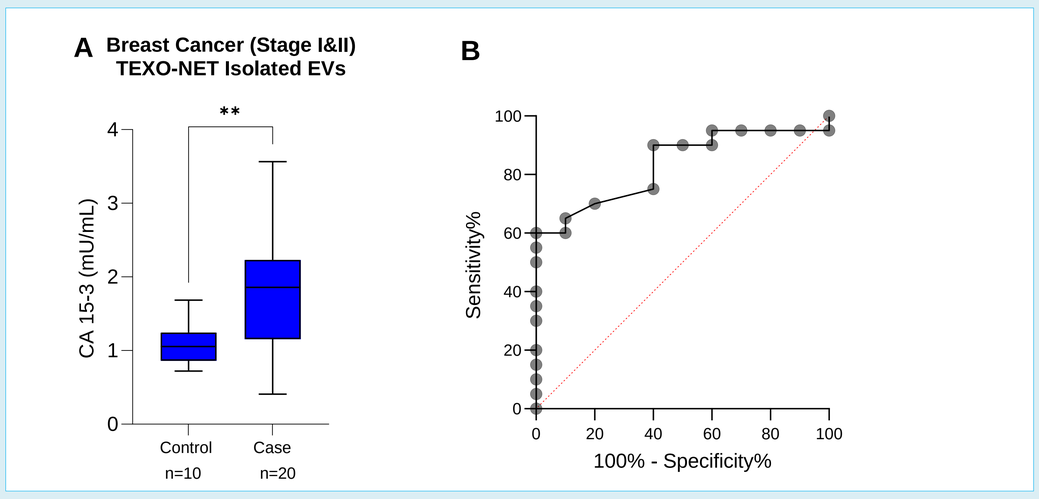


Figure 4. High-throughput isolation of EVs using TEXO-NET. Panel A: EVs were isolated from healthy women and women with early-stage breast cancer (Stages I-II). EVs and protein were isolated from 200 µL of serum incubated with 15µL of TEXO-NET using automated KingFisher Apex. Panel B: TEXO-NET ROC curve to diagnosis early stages of breast cancer (Area= 0.83).

CONCLUSIONS

We have established a high-throughput system to isolate EVs, protein and RNA for biomarker discovery and diagnostic applications. The data obtained confirm that TEXO-NET captures a subpopulation of EVs that is enriched in tumor-specific biomarkers which can potentially be used for identification of diagnostics biomarkers of tumor onset, progression, triage to treatment, and treatment response.