EV ARRAY

High-throughput Multiplexed Phenotyping of Extracellular Vesicles (EVs)

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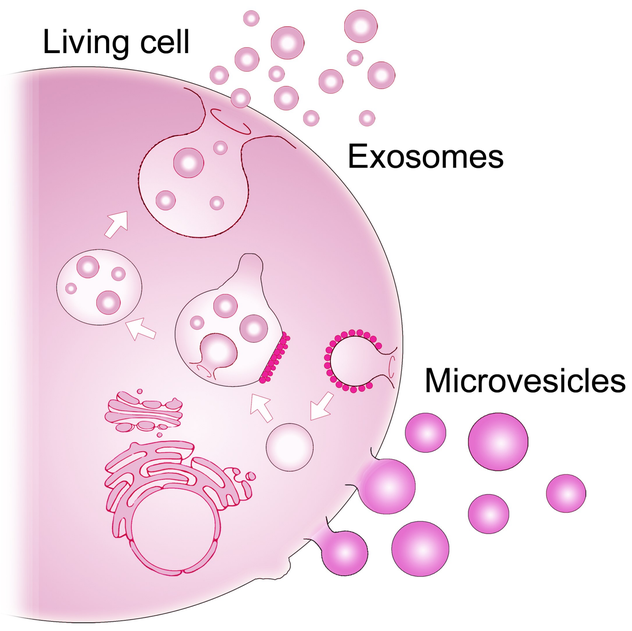


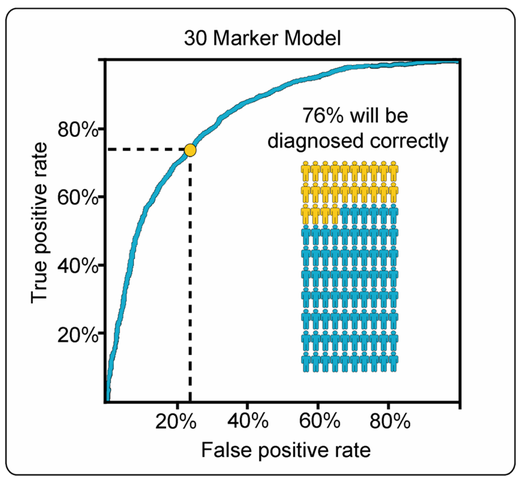
Extracellular Vesicles

All living cells produce extracellular vesicles (EVs), which are **nano-sized compartments**. They are considered as a pivotal part of the intercellular environment and act as important players in cell-to-cell communication. The fact, that EVs are involved in the development and progression of several diseases, has formed the basis for the use of EV analyses in a **clinical setting** and envisions a great potential for using EVs as **disease-related biomarkers.**

EVs are a heterogeneous population of membrane -enclosed vesicles that can be divided into a number of subpopulations based on specific characteristics such as size, biogenesis, cellular origin, protein composition, and biological function. The two major subtypes of EVs are exosomes and microvesicles.

Diagnostic and Prognostic Tool for Lung Cancer (NSCLC)

The **diagnostic power** of the EVs to identify lung cancer patients can be visualized by the receiver operating characteristics (ROC), which shows the models' sensitivity, specificity and accuracy. The data presented was obtained by analyzing EVs from 10 µL of plasma from 109 lung cancer patients (**non-small cell lung cancer, NSCLC**) together with 110 lung diseased patients (non-cancerous). A panel of 37 different EV markers were analyzed simultaneously and a multivariate data analysis was performed. Using the results from 30 of the variables it was possible to distinguish lung cancer patients from other lung diseased patients with a specificity of 79% and a sensitivity of 73% with an accuracy of 76%.



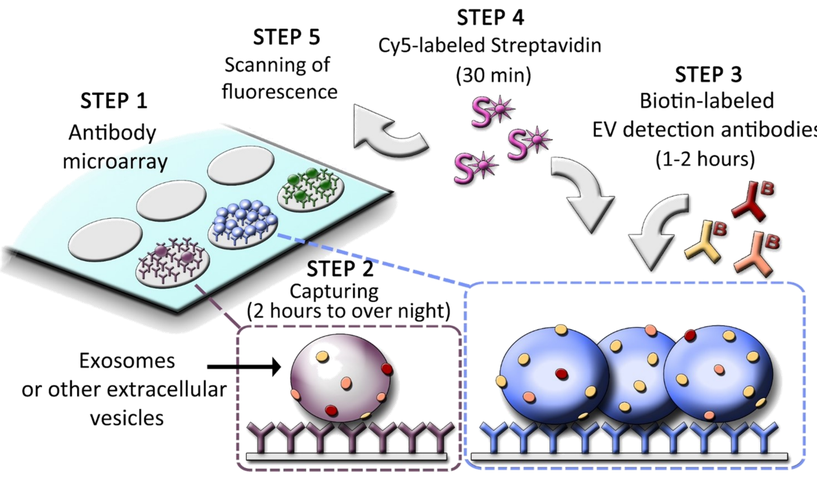
Principle of the Extracellular Vesicle Array (EV Array)

Step 1: The EV Array is composed of different capture antibodies printed on a micro-array slide.

Step 2: 10-100 µL plasma or other body fluids (urine, saliva, BALF, etc.) are applied in a 96-well setup and incubated 2 hours to overnight.

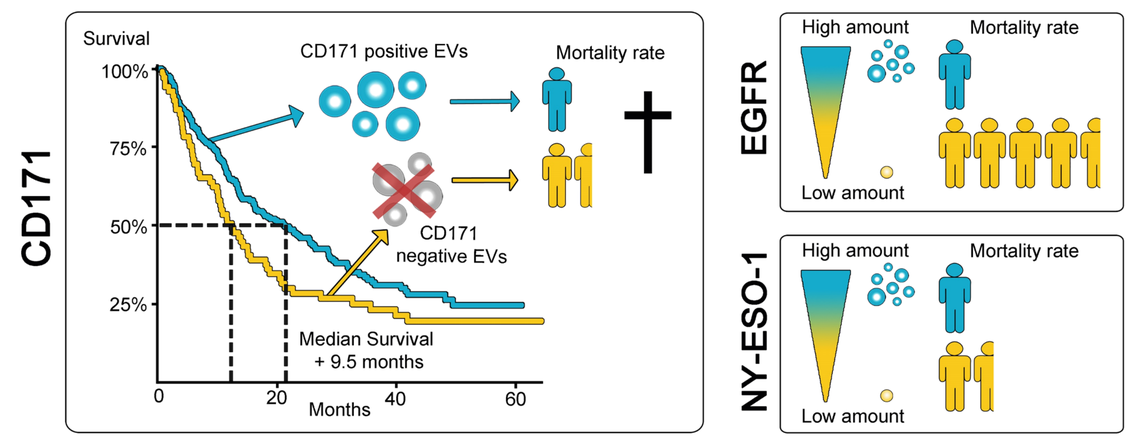
Step 3: The EVs are detected with a cocktail of biotinylated antibodies.

Step 4/5: The presence and thereby phenotype of EVs is visualized after incubation with Cy5-labeled Streptavidin using a fluorescence scanner.



Prognostic biomarkers are needed to improve patient selection for **optimal treatment**. We have evaluated EVs as a prognostic biomarker in NSCLC analyzing EVs in plasma from 276 NSCLC patients using the EV Array. Several markers were found to correlate to the overall survival (OS) of the patients. As shown on the survival curve, patients with CD171 positive EVs (blue curve) had a better **overall survival** with a 9.5 months longer median survival compared to patients without CD171 on their EVs (yellow curve).

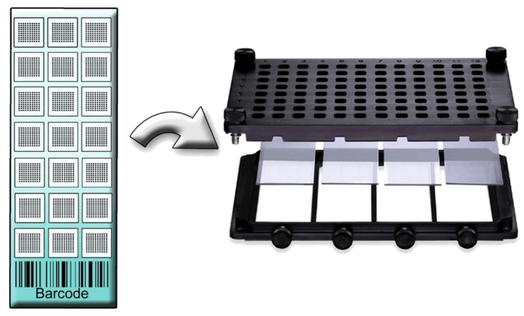
Other markers, as e.g. EGFR and NY-ESO-1, were found to influence the overall survival in a concentration-dependent manner. Patients with high levels of EVs positive for any one of these markers were found have a significantly lower mortality rate, with up to 4.6-times for EGFR. This indicates that high levels of specific EVs are protecting the patients against the cancer.



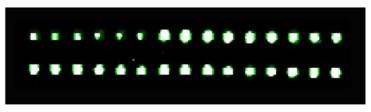
EV Array in Practice

A Glass slide printed with antibodies

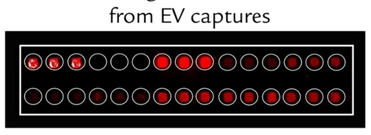
B High-throughput format (84 samples/96-well format)



C Procedure includes preanalytical quality control



D Scanning of fluorescence from EV captures



A. Barcoded microarray glass slides (7.5 x 2.5cm) printed in a 21-well setup.
B. Printed slides are placed in multiwell-cassettes for either one glass slide (up to 21 samples) or four glass slides (up to 84 samples).
C. The procedure is validated by a quality control of the printed spots, which includes positive and negative controls.
D. Scanning after capture and detection of EVs can be performed on a normal laboratory fluorescence scanner or on a specialized microarray scanner for maximum sensitivity.

Detailed information about the technology can be found at:
WWW.EVARRAY.DK

Value proposition / USP

EV Array is an **unique biomarker platform** that can exploit the intercellular trafficking in various biofluids to provide a non-invasive method for diagnostics and prognostics.

Business Opportunity / Objective / Commercial Perspectives

The apparent role of EVs in a vast number of biological processes, forms the basis of extending EV analysis beyond basic research and into clinical and therapeutic context. The applications of this type of analysis include the areas of diagnostics and prognostics, as well as drug therapy, regenerative medicine, and vaccines. The analysis of EVs could be incorporated as a screening tool and applied to confirm a diagnosis. Furthermore, the EV Array analysis has the potential to become an element in treatment surveillance and companion diagnostics.

Technology Description / Technology Summary

The EV Array is based on the technology of protein microarray and allows for the detection and phenotyping of EVs from unpurified starting material in a high-throughput manner. The technology was developed to perform multiplexed phenotyping of EVs in an open platform. The EV Array has been optimized for plasma, but can analyze urine, saliva, ascites, bone marrow, and cerebrospinal-, synovial- and bronchoalveolar fluids as well.

Development Phase / Current state

The current stage of the EV Array technology is a **fully developed analysis** optimized for a small-to-medium laboratory research scale. Several studies has demonstrated the EV Array efficiency in detecting cancers, lung-related diseases (pneumonia, COPD), venous thrombosis, multiple sclerosis and malaria.

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