NLSEV-2020
Online Symposium

Presented by
the Netherlands Society for Extracellular Vesicles (NLSEV)

Monday October 26th 2020

Europe:	**9:30-13:00 AM CET**
USA:	**4:30-8:00 AM EDT**
Australia:	**7:30-11:00 PM AEDT**

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Program

9:15	**Meeting room opens**
9:30 - 9:40	**Welcome and opening** *Marca Wauben and Rubina Baglio*
9:40 - 10:00	**Extracellular vesicles for drug delivery to the brain** *Inge Zuhorn (University Medical Center Groningen)* *Moderator: Rubina Baglio*
10:00 - 10:20	**The urinary EV proteome as a promising source for prostate- and pan-cancer biomarkers** *Ayse Erozenci (Vrije Universiteit Amsterdam)* *Moderator: Sander Kooijmans*
10:20 - 10:40	**Unraveling EV-mediated cardioprotection: EV-dependent and -independent mechanisms?** *Marieke Roefs (University Medical Center Utrecht)* *Moderator: Irene Bijnsdorp*
10:40 - 10:55	**Break and Q&A session 1** *Chat with the presenters in parallel breakout rooms*
10:55 - 11:35	**Keynote lecture:** **Differential glycosylation of parasite-derived extracellular vesicles and consequences for functional interactions with host immune cells** *Cornelis H. Hokke (Leiden University Medical Center)* *Moderator: Bas van Balkom*
11:35 - 11:55	**Direct measurement of extracellular vesicles in unprocessed human plasma by imaging flow cytometry** *Wouter Woud (Erasmus University Medical Center Rotterdam)* *Moderator: Sander Kooijmans*
11:55 - 12:15	**Procoagulant EVs released by airway epithelial cells – mediators of pulmo-vascular inflammation?** *Birke Benedikter (Maastricht University Medical Center)* *Moderator: Michiel Pegtel*
12:15 - 12:20	**Closing remarks** *Marca Wauben*
12:20 - 13:00	**Q&A session 2 (optional)** *Chat with the presenters in parallel breakout rooms*

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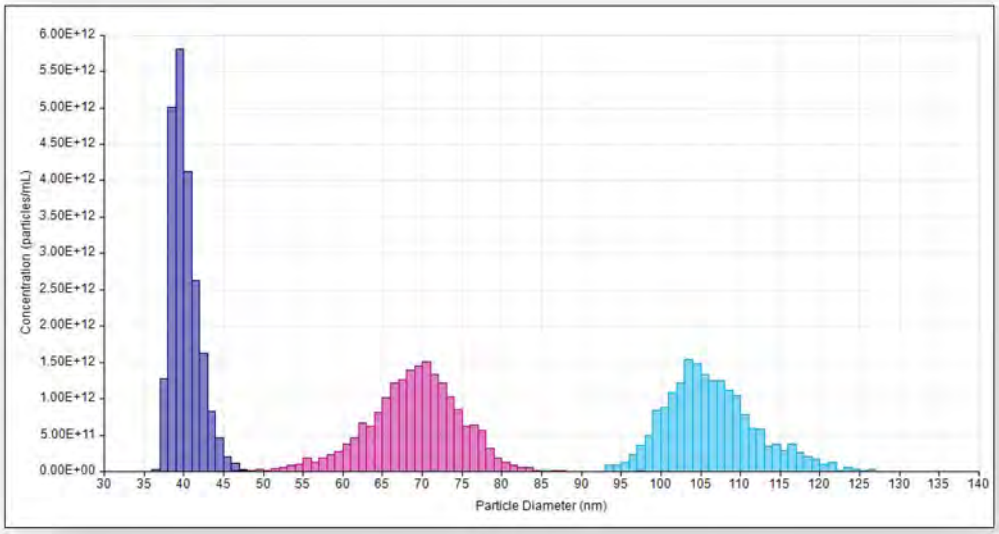

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Keynote lecture: Cornelis H. Hokke (10:55 – 11:35)

Differential glycosylation of parasite-derived extracellular vesicles and consequences for functional interactions with host immune cells

Cornelis (Ron) H. Hokke is professor of Glycobiology of Host-Pathogen Interactions at the Department of Parasitology, Leiden University Medical Center. His main focus is on basic and translational research of glycosylation at the host-parasite interface. Building on a background in analytical biochemistry he has applied mass spectrometry, glycan microarrays and other molecular approaches to investigate the structure and function of glycans in parasitology and immunology, in particular in the context of helminth (worm) infections. Over the last two decades, his work has contributed to the development of diagnostic and vaccine targets for control and elimination of schistosomiasis, as well as the identification of parasite-derived glycoproteins that are crucial in immunomodulatory mechanisms associated with helminth infections. Recently, Hokke and collaborators have taken up studies of extracellular vesicles (EV) secreted by *Schistosoma mansoni*, one of the major parasites of humans, aiming to unravel the molecular mechanisms by which these EV play a role in infection and parasite-host communication[1,2].

References

1. Kuipers ME, Nolte-'t Hoen ENM, van der Ham AJ, Ozir-Fazalalikhan A, Nguyen DL, de Korne CM, Koning RI, Tomes JJ, Hoffmann KF, Smits HH, Hokke CH. DC-SIGN mediated internalisation of glycosylated extracellular vesicles from *Schistosoma mansoni* increases activation of monocyte-derived dendritic cells. J Extracell Vesicles. 2020; 9:1753420.
2. Nowacki FC, Swain MT, Klychnikov OI, Niazi U, Ivens A, Quintana JF, Hensbergen PJ, Hokke CH, Buck AH, Hoffmann KF. Protein and small non-coding RNA- enriched extracellular vesicles are released by the pathogenic blood fluke *Schistosoma mansoni*. J Extracell Vesicles. 2015; 4:28665.

Inge Zuhorn (9:40 – 10:00)

Extracellular vesicles for drug delivery to the brain

Bhagyashree S. Joshi[1], Marit A. de Beer[2], Ben N.G. Giepmans[2], Sameh A. Youssef[3], Alain de Bruin[3], Harm H. Kampinga[2], Inge S. Zuhorn[1]

1. University of Groningen, University Medical Center Groningen, Department of Biomedical Engineering, Groningen, The Netherlands; 2. University of Groningen, University Medical Center Groningen, Department of Biomedical Sciences of Cells and Systems, Groningen, The Netherlands; 3. University of Utrecht, Department of Biomolecular Health Sciences, Utrecht, The Netherlands

Extracellular vesicles have advantageous properties for drug delivery applications, including non-immunogenicity and homing capacity. Important parameters for efficient drug delivery to the brain using (natural) nanocarriers are efficient crossing of the blood-brain barrier (BBB) and spatiotemporal control of cargo release. Here we show that EVs derived from neural stem cells (NSCs) are taken up by brain microvascular endothelial cells following binding to heparan sulfate proteoglycans, and efficiently cross the BBB.

In addition, we developed an analytical methodology, combining state-of-the-art molecular tools and correlative light and electron microscopy to demonstrate that EV cargo release occurs from endosomes and lysosomes.

Finally, we have explored the potential of neural stem cell derived EVs enriched with DNAJB6 as a therapeutic intervention for Huntington's disease (HD). HD is a neurodegenerative disorder characterized by aggregation of the huntingtin (HTT) protein containing expanded polyglutamine (polyQ) tracts. DNAJB6, a DNAJ chaperone, has been reported to efficiently inhibit polyQ aggregation in vitro, in cell models and in vivo, in HD animal models. Administration of DNAJB6-containing EVs to cells expressing expanded polyQ tracts suppressed HTT aggregation. Furthermore, intrathecal injection of DNAJB6-enriched EVs into R6/2 transgenic HD mice significantly reduced mutant HTT aggregation in the brain. Taken together, our data suggest that EV-mediated molecular chaperone delivery may be an effective way to reduce polyQ aggregation and potentially treat polyQ diseases, including HD.

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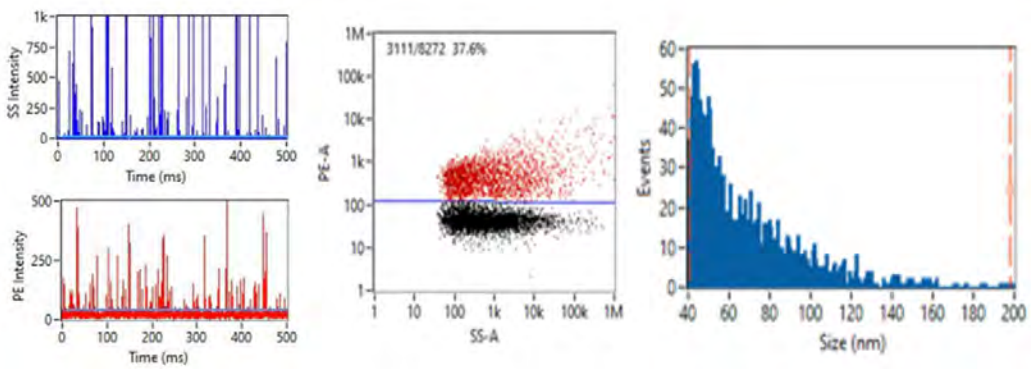
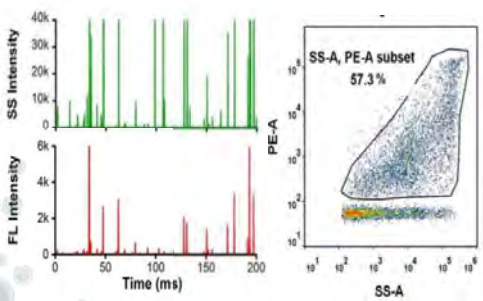
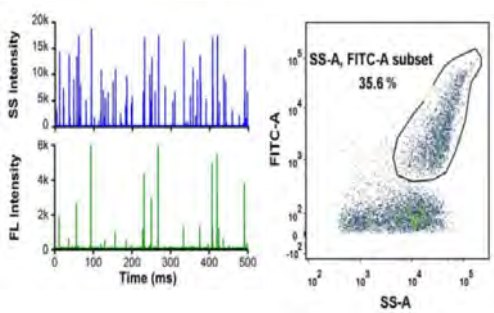


LIPID ANALYSIS with PKH67



RNA ANALYSIS with SYTO RNAselect



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Ayse Erozenci (10:00 – 10:20)

The urinary EV proteome as a promising source for prostate- and pan-cancer biomarkers

L. Ayse Erozenci[1,2], Sander Piersma[2], Thang Pham[2], Fenna Feenstra[2], R. Jeroen van Moorselaar[1], A. Vis[1], Guido Jenster[3], Jack Schalken[4], Gerald Verhaegh[4], Irene Bijnsdorp[1], Connie R. Jimenez[2]

1. Department of Urology, Amsterdam UMC, Vrije Universiteit Amsterdam, The Netherlands; 2. OncoProteomics Laboratory, Department of Medical Oncology, Amsterdam UMC, Location VUmc-Cancer Center Amsterdam, The Netherlands 3. Department of Urology, Erasmus MC, Rotterdam, The Netherlands, 4. Department of Urology, Radboud UMC, Nijmegen, The Netherlands (IMMPROVE consortium)

Introduction: Cancer cells secrete extracellular vesicles (EVs) in biofluids, which can end up in urine. For (the proximal to urine) prostate cancer (PCa) this is known, however recent developments also indicate that distant cancer type-secreted proteins and/or EVs can be found in urine. EVs harbor molecular components of their cell-of-origin; therefore proteomics profiling of urinary EVs may provide a sensitive and accurate source for the detection of PCa, and also distant cancers.

Methods: Urine was collected after signed informed consent, and was approved by the local medical ethical committee. EVs were isolated using the ME-kit, based on the expression of heat-shock proteins at the outer-surface of EVs. Protein profiles were measured using LC-MS/MS proteomics. The urinary EV proteome was measured of multiple cancer patients, including prostate (n=43 and n=94), lung (n=15), colorectal (CRC, n=15), cervical (n=15) and bladder cancer (n=6) to investigate cancer-specific patterns. We subsequently validated the expression of 4 PCa-protein-candidates by ELISA on the urinary EVs (n=20). In parallel, we also measured *in vitro* the EV-surface proteome using LC-MS/MS.

Results: We identified >3000 proteins in the urinary-EVs datasets. PCa-related urinary EV proteome was enriched for known cancer-related pathways including vesicle-secretion, motility, immunity and metabolism. PCa-candidate proteins were selected based on multiple filters including significance, fold change, involvement in (prostate)cancer development/growth, and supporting EV surface and multi-cancer urinary EV datasets. 4 candidates underwent validation using ELISA in urinary EVs where they showed promising results. Importantly, cancer-specific patterns were detected in urinary EVs derived from different cancer-patients, underlining the potential of urinary EVs for the detection of multiple distant cancers.

Conclusions: In conclusion, urinary EVs hold great potential for the detection of cancer protein biomarkers.

Marieke Roefs (10:20 – 10:40)

Unraveling EV-mediated cardioprotection: EV-dependent and -independent mechanisms?

Marieke T. Roefs[1], Pieter Vader[1,2,*], Joost P.G. Sluijter[1,*]

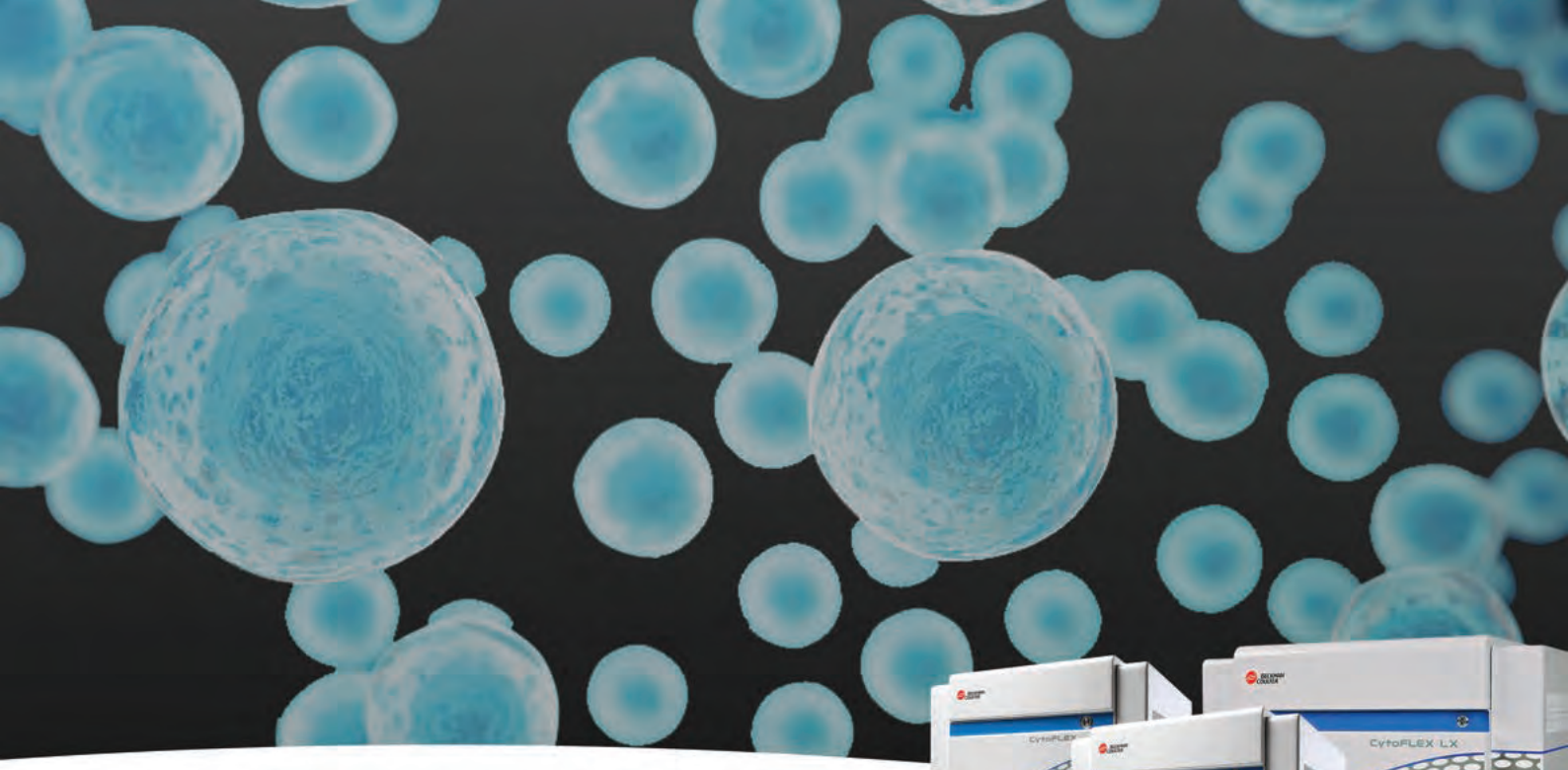
1. Department of Experimental Cardiology, University Medical Center Utrecht, Utrecht University, The Netherlands; 2. CDL Research, University Medical Center Utrecht, The Netherlands; *Equal contributions

Introduction: Cardiac progenitor cell (CPC)-derived extracellular vesicles (EVs) have been shown to protect the myocardium against ischemia/reperfusion injury. However, the underlying mechanisms for CPC-EV-mediated cardioprotection remain elusive. By exploring protein-mediated effects of CPC-EVs, we discovered that crude EV preparations activate recipient endothelial cells through EV-dependent and –independent pathways.

Methods: CPCs were stimulated with calcium ionophore (ca ion-EVs) or vehicle (control-EVs) for 24 hours and crude EVs were isolated using size exclusion chromatography (SEC). EV concentration and size was assessed using NTA and proteomic composition profiled using mass spectrometry. Following SEC, Optiprep gradient ultracentrifugation was used to separate EVs from free proteins. EV and protein fractions were functionally characterized based on endothelial cell activation assays.

Results: Endothelial cells displayed enhanced phosphorylation of ERK1/2 and AKT and increased wound closure after stimulation with control-EVs, but not with ca ion-EVs. Proteomic analysis identified multiple proteins uniquely expressed or enriched in control-EVs compared with ca ion-EVs. Surprisingly, when investigating the contribution of individual candidate proteins, the extent of endothelial cell activation was found to be influenced by the purity of the EV preparations. EVs isolated using Optiprep gradients lost part of their ability to activate endothelial cells compared with either free proteins or crude EV preparations. In addition, several candidate proteins were found to be present in the free protein fraction instead of EV fraction. This hints towards a co-stimulatory role of co-isolated proteins in recipient cell activation.

Conclusions: A specific set of EV proteins is identified that may be functionally responsible for the activation of endothelial cells upon exposure to CPC-EVs. It is important to identify if these proteins are EV-bound or represent co-isolated factors that contribute to endothelial cell activation. This may lead to a better mechanistic understanding of CPC-EV-mediated cell activation and translation of EV-mediated therapeutics.



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Wouter Woud (11:35 – 11:55)

Direct measurement of extracellular vesicles in unprocessed human plasma by imaging flow cytometry

Wouter W. Woud[1], Karin Boer[1], Erik Mul[2], Martin J. Hoogduijn[1], Carla C. Baan[1], Ana Merino[1]

1. Nephrology and Transplantation, Department of Internal Medicine, Erasmus MC, University Medical Center Rotterdam, The Netherlands; 2. Department of Blood Cell Research, Sanquin Research and Landsteiner Laboratory, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands

Introduction: The interest in EVs as potential biomarker in health and disease is still increasing. However, their characterization is hampered by their small size, low epitope copy number and the use of different isolation methods which may modify the EVs of interest. Analysis of EVs in human plasma is even more complicated due to the molecular complexity of plasma (e.g. lipoproteins and soluble factors). In recent years, Imaging Flow Cytometry (IFCM) has emerged as a potential technique that is sensitive enough to discriminate and analyse single EVs. Here we present a protocol including settings of IFCM to accurately quantify, phenotype and visualize single human plasma derived small EVs (sEVs) while excluding potential artefacts.

Methods: Platelet-Poor Plasma (PPP) from healthy individuals was stained directly *without* prior purification / isolation of EVs with CFSE and antibodies directed against proteins of interest including tetraspanins and CD31. A strict set of controls were used for each sample to allow for the accurate detection of sEV signatures. Acquisition was performed by running each sample for 3 minutes on an ImageStream^X (IS^X) MkII IFCM.

Results: Fluorescent background levels of the IFCM were established and a gating strategy to accurately analyse sEVs was developed. sEV signatures were identified as double-positive events expressing a tetraspanin marker (CD9/CD63/CD81) in conjunction with enzyme activity (CFSE+) or presence of a cellular origin marker (CD31+). Detergent lysis was performed to enable discrimination of biological signals from artefacts. Validation of sEV analysis was achieved through serial dilution.

Conclusions: We successfully developed a method to discriminate, identify and quantify sEVs in complex mixtures such as human plasma without prior purification or isolation of EVs. In this work, we propose a set of criteria for events to be classified as true sEVs by IFCM.

Birke Benedikter (11:55 – 12:15)

Procoagulant EVs released by airway epithelial cells – mediators of pulmo-vascular inflammation?

Birke J. Benedikter[1,2], Freek G. Bouwman[1], Jacco J. Briedé[1], Pascalle H. van Eijck[1], Gert E. Grauls[1], Guido R.M.M. Haenen[1], Alexandra C.A. Heinzmann[1], Rory R. Koenen[1], Theo M. de Kok[1], Carmen Lopez-Iglesias[1], Edwin C. Mariman[1], Rene van Oerle[1], Niki L. Reynaert[1], Gernot G.U. Rohde[1], Paul H.M. Savelkoul[1], Bernd Schmeck[2], Henri M. Spronk[1], Tanja Vajen[1], Charlotte Volgers[1], Antje R. Weseler[1], Emiel F.M. Wouters[1], Frank R.M. Stassen[1]

1. Maastricht University Medical Center, Maastricht, The Netherlands; 2. Philipps-University Marburg, Marburg, Germany'

Introduction: Longterm inhalation of harmful substances such as cigarette smoke strongly increases the risk for various and often coinciding inflammatory disorders, including chronic obstructive pulmonary disease (COPD) and thrombosis. We explore if airway epithelial EVs contribute to this effect.

Methods and results: By bead-based flow cytometry and TRPS, we show that treatment of BEAS-2B airway epithelial cells with cigarette smoke extract (CSE) induces release of tetraspanin-positive small EVs via thiol reactive components. CSE-induced EVs were isolated using ultrafiltration followed by size exclusion chromatography and subjected to proteomics analysis by nanoLC-MS/MS. Procoagulant proteins, including tissue factor (TF), were strongly enriched in EVs after CSE-treatment. EV-TF was functionally active as shown by activation of the blood clotting cascade upon EV addition to healthy human plasma.

Conclusions: Besides resulting in blood clotting, TF and downstream products of its enzymatic activity can activate inflammatory signalling by cleaving the extracellular domain of protease activated receptors (PAR). Therefore, EV-TF may mechanistically link lung inflammation and clotting risk in individuals with a history of inhalational exposure. This is currently under investigation using a combination of functional in vitro experiments and EV-TF measurements in clinical specimen.