NLSEV

Abstract and Programme Book

Kick-off meeting of the

Netherlands Society for Extracellular Vesicles (NLSEV)

Friday Nov 17th 2017, Utrecht

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Program kick-off meeting of the Netherlands Society for Extracellular Vesicles (NLSEV)

Friday November 17th 2017

Lecture Hall ' Paard', Faculty of Veterinary Medicine, Utrecht University (Yalelaan 114, 3584 CM Utrecht)

9:45 – 10:00	Welcome by Marca Wauben	
10:00 – 10:20	University of Amsterdam (AMC)	Rienk Nieuwland
10:20 – 10:40	VU University (Medical Center)	Michiel Pegtel
10.40 – 10:55	Maastricht University (Medical Center)	Kasper Rouschop
10:55 – 11:05	Sponsored Presentation: Beckman Coulter	
11:05 – 11:40	Coffee break & Posters meet and greet	
11:40 – 11:50	Radboud University (Medical Center)	Fons van de Loo
11:50 – 12:00	Leiden University (Medical Center)	Elizabeth de Lange
12:00 – 12.15	STW-Perspectief Consortium Cancer-ID	Ton van Leeuwen
12:15 – 12:25	Sponsored Presentation: Particle Metrix	
12:25 – 13:30	Lunch & Posters meet and greet	
13:30 – 14:30	NLSEV General Assembly	
14:30 – 15:10	Keynote lecture: From gut immunity to live tracking in zebrafish: two decades of French-Dutch research on exosomes Guillaume van Niel (CPN INSERM, Paris)	
15:10 – 15:45	Coffee break & Posters meet and greet	
15:45 – 16:05	Erasmus MC	Guido Jenster
16:05 – 16:25	Utrecht University	Marca Wauben
16:25 – 16:45	UMC Utrecht	Joost Sluijter
16:45 - 17:45	Drinks	

ABSTRACTS

#1	
Authors	Arntz O.J., Pieters B.C.H., Thurlings R.M., Wenink M.H., van Lent P.L.E.M., Koenders M.I., van der Kraan P.M., van den Hoogen F.H.J., van de Loo F.A.J.
Affiliations	Radboudumc, Nijmegen
Poster Title	**IgM rheumatoid factor is detected on circulating extracellular vesicles in a subpopulation of rheumatoid arthritis patients with enhanced C-reactive proteins levels and global assessment of disease activity**
Abstract	**Objectives**: In this study we investigated if Rheumatoid Factor (RF), a predictor of more severe disease in rheumatoid arthritis (RA), is present on plasma derived EVs (pEVs) from RA-patients and related to disease. **Methods:** EVs were isolated from blood of RA patients and healthy controls (HC) by size exclusion chromatography (SEC). Particle concentration of pEVs was measured by Nanoparticle Tracking Analysis (NTA) and protein concentration determined by micro-BCA kit. IgM-RF was detected by ELISA. **Results:** Mean pEVs particle size, amount and protein content were not different between RA patients and HC. 12 out of 23 RA patients were IgM-RF positive and in 6 out of them IgM-RF was also detected on their pEVs. Disease activity score (DAS28), the tender (TJC) and swollen joint counts (SJC), blood C-reactive protein (CRP) level, erythrocyte sedimentation rate (ESR) and patient global health assessment by visual analog scale (VAS) were not significantly different between RF+ and RF- RA patients, however the CRP, ESR, DAS28 and VAS score were enhanced in RA patients with RF+ pEVs. **Conclusions:** This study shows that a subpopulation of RF+ RA patients with IgM RF positive pEVs have elevated CRP levels and VAS score, whereas plasma RF levels were not discriminative.

# 2	
Authors	F.C. Bach[1], S.F.W.M. Libregts[2], L.B. Creemers[3], B.P. Meij[1], K. Ito[3,4], M.H.M Wauben[2] and M.A. Tryfonidou[1]
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Poster Title	**Notochordal-cell derived extracellular vesicles exert regenerative effects on canine and human nucleus pulposus cells**
Abstract	During intervertebral disc ageing, chondrocyte-like cells (CLCs) replace notochordal cells (NCs). NCs have been shown to induce regenerative effects in CLCs. Since NC-released vesicles may be responsible for these effects, we characterized NC-derived extracellular vesicles (EVs) and determined their effect on CLCs. EVs were purified from porcine NC-conditioned medium (NCCM) through size exclusion chromatography, ultracentrifugation or density gradient centrifugation. Additionally, EVs were quantitatively analyzed by high-resolution flow cytometry. The effect of NCCM-derived EVs was studied on canine and human CLCs and compared with NCCM-derived proteins and unfractionated NCCM. Porcine NCCM contained a considerable amount of EVs, which exerted anabolic effects comparable to NCCM-derived proteins. However, unfractionated NCCM was more potent. The EV effects were not considerably influenced by ultracentrifugation compared with size exclusion-based purification. Upon ultracentrifugation, interfering glycosaminoglycans, but not collagens, were lost. Nonetheless, collagen supplemented to CLCs in a concentration as present in NCCM induced no anabolic effects. Thus, glycosaminoglycans and collagens appeared not to mediate regenerative EV effects. In conclusion, NC-derived EVs have regenerative potential, and their effects may be influenced by proteins present in NCCM. The optimal combination of NC-secreted factors needs to be determined to fully exploit the regenerative potential of NC-based technology.

# 3	
Authors	Birke J. Benedikter[1,2], Freek G. Bouwman[3], Alexandra C. A.Heinzmann[4], Tanja Vajen[4], Edwin C. Mariman[3], Emiel F. M. Wouters[2], Paul H. R. M. Savelkoul[1,5], Gernot Rohde[2,6], Rory R. Koenen[4], Rene van Oerle[4], Henri M. Spronk[4], Frank Stassen[1]
Affiliations	[1]Department of Medical Microbiology, NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University Medical Center (MUMC); [2]Department of Respiratory Medicine, NUTRIM School of Nutrition and Translational Research in Metabolism, MUMC; [3]Department of Human Biology, NUTRIM School of Nutrition and Translational Research in Metabolism, MUMC; [4]Department of Biochemistry, Cardiovascular Research Institute Maastricht (CARIM), Maastricht University, Maastricht, The Netherlands; [5]Department of Medical Microbiology & Infection Control, VU University Medical Center, Amsterdam, The Netherlands; [6]Medical clinic I, Department of Respiratory Medicine, Goethe University Hospital, Frankfurt/Main, Germany
Poster Title	**Cigarette smoke extract induces the release of pro-coagulant extracellular vesicles by bronchial epithelial cells**
Abstract	We have previously shown that cigarette smoke extract (CSE) triggers the release of extracellular vesicles (EVs) from BEAS-2B bronchial epithelial cells. Here, we applied nano LC-MS/MS to analyze the proteomic composition of EVs secreted by unexposed or CSE-exposed BEAS-2B cells: We identified 388 proteins of which 129 were differentially expressed in CSE-EVs. Functional enrichment analysis revealed that the CSE-EVs were highly enriched in proteins of the biological pathway hemostasis, including tissue factor. We confirmed increased expression and activity of tissue factor on CSE-EVs using bead-coupled flow cytometry and a factor Xa generation assay. Moreover, we showed by bead-coupled flow cytometry and prothrombinase assay that the membrane of CSE-EVs is enriched in the phospholipid phosphatidylserine. Calibrated automated thrombogram was used to demonstrate that CSE-EVs trigger thrombin generation in normal human plasma in a tissue factor- and phosphatidylserine-dependent manner. Taken together, bronchial epithelial cells respond to cigarette smoke by releasing procoagulant EVs. This may contribute to the elevated cardiovascular risk in smokers. Currently, we are investigating whether an increased abundance of tissue factor-expressing EVs can be detected in plasma samples from healthy smokers compared to healthy non-smokers.

# 4	
Authors	L. Ayse Erozenci[1,2], Ning Wang[3], Allison Gartland[3], L. Leon[2], Jorn Mulder[1], Albert A. Geldof[1], Connie R. Jimenez[2] R. Jeroen van Moorselaar[1], Irene V. Bijnsdorp[1]
Affiliations	[1]Department of Urology, VUmc-Cancer Center Amsterdam, VU University Medical Center, The Netherlands; [2]OncoProteomics Laboratory, Department of Medical Oncology, VUmc-Cancer Center Amsterdam, VU University Medical Center, The Netherlands; [3]The Mellanby Centre for Bone Research, Department of Oncology and Metabolism, The University of Sheffield, Sheffield, UK
Poster Title	**Prostate cancer secreted microRNAs influence early steps in bone metastasis**
Abstract	**BACKGROUND:** Prostate cancer (PCA) metastases to bones affects approximately 70% of patients with advanced disease. PCA cells secrete extracellular vesicles (EVs), that contain microRNAs (miRNAs). We hypothesize that osteoclast-precursors scavenge PCA EVs, leading to a manipulation of their behavior, thereby driving metastases. **METHODS:** To test how EV-miRNAs affect metastases, we stably overexpressed these three metastasis-related miRNAs in PCA cells. Bone metastases were induced after intracardiac injection in nude mice and were monitored by F-luciferase signal (IVIS). Bones were analyzed by microCT. **RESULTS:** PC3 cells that overexpress two miRNAs accelerated the formation and size of early bone metastases compared to control PC3 cells. By contrast, one miRNA significantly decreased the number of metastases. To determine whether EVs secreted by PCA cells affect osteoclasts, mouse BM and human monocytes were allowed to differentiate to osteoclasts while exposing them to PCA EVs. EVs secreted by pro-metastatic PC3 cells enhanced osteoclast differentiation, while anti-metastatic cell derived EVs inhibited osteoclast differentiation. To test whether pro-metastatic miRNAs enhance metastases when mice osteoclast precursor are pre-stimulated (via EVs or soluble factors), subcutaneous tumours of pro-metastatic cells were grown for 2 weeks, after which metastases were induced. Trabecular bone volume was used as first indicator for metastases, other analyses are ongoing. In mice that were pre-stimulated with pro-metastatic PCA cells, the trabecular bone volume was decreased compared to non-prestimulated mice, indicative of an increased tumour burden. This demonstrates that a pre-stimulation of mice with a subcutaneous tumour enhanced metastases formation. **CONCLUSINON:** PCa miRNAs secreted via EVs affect osteoclasts, thereby driving or inhibiting the formation of metastases. Further investigation is needed to unravel novel pathways that can be targeted to prevent bone metastases.

# 5	
Authors	Charles J. Blijdorp[1], Thomas Hartjes[2], Martin E. van Royen[2], Guido Jenster[3], Robert Zietse[1], Ewout J. Hoorn[1]
Affiliations	[1]Department of Internal Medicine, Division of Nephrology & Transplantation; [2]Department of Pathology; [3]Department of Urology, Erasmus MC, Rotterdam, The Netherlands
Poster Title	**Quantification of urinary extracellular vesicles (uEVs)**
Abstract	**Background:** Urinary extracellular vesicles (uEVs) have emerged as a powerful non-invasive tool to study renal epithelial transport in humans. However, the optimal method to quantify and normalize uEVs remains unclear. **Methods:** Spot urines were collected of 4 healthy subjects, who underwent overnight thirsting followed by water loading. Subsequently, 4 uEV quantification techniques were compared: (1) nanoparticle tracking analysis (NTA), (2) uEV isolation by ultracentrifugation followed by immunoblotting of CD9, CD63, CD81, ALIX, and TSG101, (3) a timeresolved fluorescence immunoassay (TRFIA) that captures CD9+ uEVs, and (4) EVQuant, a novel technique which counts individual fluorescently labeled EVs after immobilization in a matrix. **Results:** The results of the 4 uEV quantification methods showed similar dynamics as urine osmolality and creatinine suggesting that uEV number changes in proportion to urinary concentration (r^2 for both 0.9, $P<0.0001$). Of interest, EVQuant identified 2.4 ± 0.6 times more uEVs than NTA. Using NTA as reference, the Bland-Altman analysis showed that EVQuant had the smallest standard deviation of bias (±16%) followed by TRFIA (±22%). **Conclusion:** uEV number is proportional to urinary concentration suggesting urine creatinine can be used to normalize spot urines. EVQuant is a promising alternative to NTA and appears more sensitive for uEV detection.

# 6	
Authors	Davide Chiasserini[1], Tim Schelfhorst[1], Sander R. Piersma[1], Thang V. Pham[1], Pier Luigi Orvietani[2], Lucilla Parnetti[3], Connie R. Jiménez[1].
Affiliations	[1]OncoProteomics Laboratory, Department of Medical Oncology, Cancer Center Amsterdam, VUmc, Amsterdam, the Netherlands; [2]Department of Experimental Medicine, section of Physiology and Biochemistry, University of Perugia, Perugia, Italy; [3]Department of Medicine, section of Neurology, University of Perugia, Perugia, Italy
Poster Title	**Proteomic analysis of extracellular vesicles from clinically available volumes of CSF: application to Alzheimer's disease**
Abstract	Cerebrospinal fluid (CSF) contains extracellular vesicles (EVs) with undisclosed biomarker potential for neurodegenerative diseases. However, current methods do not allow the proteomic analysis of EV isolated from clinically available volumes of CSF (0.5-1 mL). Here we explored the use of a peptide affinity method (Vn96 peptide) to isolate an EV-enriched fraction from CSF and we used this approach to identify potential Alzheimer's disease (AD) biomarkers in CSF EVs. EV isolation was carried out on 1 mL of CSF using the peptide-affinity method that precipitates EVs based on binding to heat shock proteins on the vesicle surface. Mass spectrometry was used to characterize the proteome of CSF EVs. In total, 936 proteins were consistently identified in all the CSF EV samples obtained via the peptide-affinity method. More than 60 frequently identified exosomal proteins were found in CSF EVs, including proteins involved in exosomes biogenesis, heat shock proteins, and tetraspanins. Substantial overlap (63%) was found between the CSF EV proteome isolated by the new method in comparison to gold-standard ultracentrifugation. When applied to CSF from AD and control subjects, we found 39 proteins up-regulated in AD EVs, while 25 were down regulated. Up-regulated proteins in AD EVs were related to extracellular matrix organization and cell adhesion, while down-regulated proteins were associated with immune response and B-cell signaling. The peptide-affinity method allows the isolation of EVs from small volumes of CSF and is suitable for the analysis of CSF EV proteomes from patients with neurodegenerative diseases.

# 7	
Authors	Aleksandra Gąsecka[1,2,3] , Edwin van der Pol[2,3,4], Anita Böing[2,3], Najat Hajji[2,3], Auguste Sturk[2,3], Pia Siljander[5,6], Paul Harrison[7], Krzysztof J. Filipiak[1], Grzegorz Opolski[1], Rienk Nieuwland[2,3]
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Poster Title	**P2Y1 and P2Y12 antagonists mediate the release and composition of platelet extracellular vesicles**
Abstract	**Background:** Activated platelets contribute to inflammation and thrombosis by release of platelet extracellular vesicles (PEV). Because the P2Y1 and P2Y12 receptors for adenosine diphosphate (ADP) regulate platelet activation, we hypothesized that inhibition of these receptors affects the concentration and composition of PEV. **Aims:** To investigate the role of platelet ADP receptors in the release of PEV. **Methods:** Platelet-rich plasma from 6 healthy volunteers was incubated with saline, P2Y1 antagonist MRS2179 (100 µM), and/or P2Y12 antagonist ticagrelor (1 µM). Platelets were activated by ADP (10 µM) under stirring conditions at 37 °C. The reactivity was assessed by impedance aggregometry. Concentrations of PEV exposing CD61, CD62p and phosphatidylserine (PS) were determined by flow cytometry (A60-Micro, Apogee). **Results:** ADP-induced aggregation was decreased 73% by P2Y1 antagonist, 86% by P2Y12 antagonist, and 95% by both antagonists ($p<0.001$ for all).The release of $CD61^+/CD62p^-/PS^-$PEV was inhibited by 50% in presence of both antagonists ($p=0.003$). The release of $CD61^+/CD62p^+/PS^+$ PEV was decreased 62% by P2Y12 antagonist ($p=0.04$) and 72% by both antagonists ($p=0.03$). **Conclusions:** Inhibition of P2Y1 and P2Y12 receptors affects the release of distinct subpopulations of PEV upon activation by ADP. Patients treated with P2Y12 antagonists may have decreased concentrations of proinflammatory and prothrombotic PEV.

# 8	
Authors	Tom Groot Kormelink (1), Susanne G. van der Grein (1), Ger J.A. Arkesteijn (1, 2), Esther N.M. Nolte-'t Hoen (1), Marca H.M. Wauben (1)
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Poster Title	**Depending on the activation stimulus, mast cells release distinct subsets of extracellular vesicles that can have discrete immunomodulatory capacities**
Abstract	Besides their well-known effector function in allergic disorders, mast cells (MC) can significantly contribute to adaptive immune responses against microorganisms, and to chronic inflammations characterized by unbalanced T cell responses. A possible mechanism by which MC accomplish this is by releasing immunomodulatory extracellular vesicles (EV). We here analyzed the release of EV from differentially activated murine MC, and their ability to activate dendritic cells and modulate naive T helper cell development. In contrast to unstimulated MC which release CD9+ EV, degranulation results in the release of high numbers of CD63+ EVs in a comparable rate of release to that of soluble mediators. These CD63+ EVs are either found in heparin-based clusters that are pelleted at 10.000g, or as relatively small single EVs that are pelleted at 100.000g. TLR triggering instead did not change the number of CD9+ EV released from MC, although these EV did induce dendritic cell activation. Strikingly, EV released from TLR1/2-activated MC did differentially modulate subsequent T cell responses compared to TLR4 activated MC. These data indicate that concerning the strategic tissue distribution of MC and the presence of activated MC in various (patho)physiological conditions, MC-EV should be considered as potentially important immune-regulators.

# 9	
Authors	Martijn J. C. van Herwijnen[1], Marijke I. Zonneveld[1,2], Soenita Goerdayal[3], Arianne van Bruggen - de Haan[4], Esther N. M. Nolte – 't Hoen[1], Johan Garssen[2,5], A.F. Maarten Altelaar[3], Gerbrich N. van der Meulen[4], Ruurd M. van Elburg[5,6], Frank A. Redegeld[2], Marca H. M. Wauben[1]
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Poster Title	**Milk-derived extracellular vesicles from non-allergic and allergic mothers differ in T cell modulatory capacity and have a distinct protein composition**
Abstract	While breastfeeding may aid in the prevention of allergies, it has been suggested that the breast milk from allergic mothers can negatively influence the infant's immunity. However, the molecular mechanism underlying this effect has not been solved. In this study, we compared the protein composition and functional T cell modulatory capacity of milk-EV derived from allergic and non-allergic mothers. For this, milk-derived EV were isolated via differential centrifugation followed by density gradient-based separation of human milk from allergic or non-allergic mothers. Functionality was tested in vitro by co-culturing EV with stimulated CD4+ T cells. Additionally, proteomic analysis was performed to compare the milk-EV proteomes, followed by pathway analysis. Results showed that general T cell activation was inhibited in the presence of milk-derived EV. Remarkably, EV from allergic mothers inhibited T cell activation to a lesser extent than EV from non-allergic mothers. By comparing the proteomes of milk-derived EV we found quantitative differences in proteins between allergic and non-allergic mothers. These individual proteins linked specifically to signaling pathways affecting cell proliferation. These data show that milk-derived EV differ in their T cell modulatory capacity depending on the allergic status of the mother, which might explain the effects of breastfeeding.

# 10	
Authors	Joëlle Klazen, Iris Robbesom, Hans van Leeuwen and Marjolein van Driel
Affiliations	Department of Internal medicine, Erasmus MC, Rotterdam
Poster Title	**The role of extracellular vesicles in the crosstalk of cancer cells to bone**
Abstract	Bone is a preferential site for cancer metastases, especially from prostate and breast cancer. Here, metastasized cancer cells are resistant to current therapies and create a vicious circle of negative influences on bone metabolism to stimulate cancer cell growth. The mechanism is complex and involves cross-talk between cancer cells and cells from the bone. Compounds that restore bone integrity are promising therapies, such as vitamin D (1□,25-OH2D3). Extracellular vesicles (EVs) may be important messengers in bone metastases and may be part of the mechanism of the beneficial effects of vitamin D. We developed a human co-culture model of differentiating bone forming cells (osteoblasts) and metastatic prostate cancer cells to study cellular crosstalk and therapeutic interference. We aim to answer 3 main questions: Is there a role for EVs in the direct interaction of metastatic cancer cells with bone cells? And/or in preparing the metastatic niche in the bone? What is role of EVs in the therapeutic effects of vitamin D in bone metastases? Ongoing research shows that vitamin D treatment increases the number of EVs produced by bone metastatic prostate cancer cells (PC-3) and possibly affects their function in interaction with osteoblasts.

# 11	
Authors	S.A.A. Kooijmans, J.J.J.M. Francois, R.M. Schiffelers, P. Vader
Affiliations	Department of Clinical Chemistry and Haematology, UMCU, Utrecht, The Netherlands
Poster Title	**Recombinant phosphatidylserine-binding nanobodies for targeting of extracellular vesicles to tumor cells: A plug-and-play approach**
Abstract	Extracellular vesicles (EVs) are increasingly being recognized as candidate drug delivery systems due to their ability to functionally transfer biological cargo between cells. However, manipulation of EV tropism through engineering of the producer cells can be challenging. As a novel approach to confer tumor targeting properties to isolated EVs, recombinant fusion proteins of nanobodies against the epidermal growth factor receptor (EGFR) fused to phosphatidylserine (PS)-binding domains of lactadherin (C1C2) were expressed in HEK293 cells and purified to near-complete purity. Fusion proteins specifically bound PS and showed no affinity for other EV membrane lipids. C1C2 fused to anti-EGFR nanobodies (EGa1-C1C2) bound EGFR with high affinity and competed with binding of its natural ligand EGF, as opposed to C1C2 fused to control nanobodies (R2-C1C2). C1C2-nanobodies readily self-associated onto membranes of EVs derived from distinct sources without affecting EV integrity. EV-bound R2-C1C2 did not influence EV-cell interactions, whereas EGa1-C1C2 dose-dependently enhanced specific EV binding and uptake by EGFR-overexpressing tumor cells. In conclusion, a novel strategy to efficiently and universally confer tumor targeting properties to isolated EVs was developed, circumventing the need to modify EV-secreting cells. This strategy may also be employed to decorate EVs with other moieties, including imaging probes or therapeutic proteins.

# 12	
Authors	M.E. Kuipers[1,2], E.N.M. Nolte-'t Hoen[2], D.L. Nguyen[1], A.J. van der Ham[1], K.F. Hoffmann[3], H.H. Smits[1]*, C.H. Hokke[1]*
Affiliations	[1]Department of Parasitology, LUMC, Leiden, Netherlands; [2]Department of Biochemistry & Cell Biology, Utrecht University, Utrecht, Netherlands; [3]IBERS, Aberystwyth University, Aberystwyth, United Kingdom (*These authors contributed equally)
Poster Title	**Characterisation of glycans of extracellular vesicles from Schistosoma mansoni juvenile worms and their involvement in vesicle-DC binding**
Abstract	Cells release extracellular vesicles (EV) which contribute to intercellular communication via their molecular cargo. Recently, it has been shown that the parasitic worm Schistosoma mansoni also secretes EV. However, the EV composition and interaction with the host immune system are largely uncharacterised. As schistosome glycans are important in host-parasite interaction, we focused on the glycans of S. mansoni juvenile worm (schistosomula) EV and their capacity to bind to human monocyte-derived dendritic cells (moDC). Mass spectrometric glycosylation analysis, performed on EV obtained from cultured schistosomula, revealed dissimilarity of the composition of N-glycans on the in- and outside of the EV. Western blot analysis of EV confirmed the presence of these glycans. To investigate binding of EVs by host immune cells, moDCs were incubated with fluorescently labelled EVs. Subsequent flowcytometric analysis showed a dose-dependent binding, which was reduced after preincubation with EGTA or mannan. This suggests the involvement of C-type lectin receptors DC-SIGN or mannose receptor, which is in agreement with the glycan analysis.

# 13	
Authors	Wooje Lee[1], Afroditi Nanou[2], Linda Rikkert[2,3,4], Frank A.W. Coumans[4,5], Leon W.M.M. Terstappen[2] and Herman L. Offerhaus[*1]
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Poster Title	**Characterization of Extracellular Vesicles using Raman Spectroscope for Label-free Prostate Cancer Detection**
Abstract	Extracellular vesicles (EVs) constitute a mechanism of intercellular communication transporting a wide range of biomolecules. The transported cargo varies depending on the EV origin. This fact implies that the chemical composition and signature of EVs derived from diseased cells could be used as a biomarker to detect the respective diseases. In this report, we demonstrate that EVs is a promising a biomarker to detect prostate cancer. To identify cancer-cell-derived vesicles, Raman spectra were obtained and analyzed by principal component analysis (PCA). Herein, we collected EVs from two different prostate cancer cell lines (PC3 and LNCaP) and EVs found in blood products of healthy donors (specifically red blood cell- and platelet-derived EVs). All EV subtypes were measured using a spontaneous Raman spectroscope and the obtained data was analyzed by a prediction model, the PCA to investigate whether prostate cancer EVs can be separated by the rest of the EVs and used as a biomarker to diagnose cancer.

# 14	
Authors	Lucienne A. Vonk[3], Sanne F. J. van Dooremalen[1;2], Nalan Liv1, Judith Klumperman[1] , Paul J. Coffer[1;2], Daniël B.F. Saris[3;4;5] and Magdalena J. Lorenowicz[1;2]
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Poster Title	**Mesenchymal stromal/stem cell-derived extracellular vesicles promote human cartilage regeneration**
Abstract	Osteoarthritis (OA) is a rheumatic disease leading to chronic pain and disability with no effective treatment available. Recently, allogeneic human mesenchymal stromal/stem cells (MSC) entered clinical trials as a novel therapy for OA. Increasing evidence suggests that therapeutic efficacy of MSC depends on paracrine signalling. Here we investigated the role of bone marrow MSC-derived extracellular vesicles (BMMSC-EVs) in cartilage repair and show that BMMSC-EVs promote cartilage regeneration *in vitro*. Treatment of OA chondrocytes with BMMSC-EVs induces production of proteoglycans and type II collagen and promotes proliferation of these cells. MSC-EVs also inhibit the adverse effects of inflammatory mediators on cartilage homeostasis. Our data show that BMMSC-EVs downregulate tumor necrosis factor alpha (TNF-alpha) induced expression of pro-inflammatory cyclooxygenase-2, pro-inflammatory interleukins and collagenase activity in OA chondrocytes. The anti-inflammatory effect of BMMSC-EVs involves the inhibition of NFκB signaling, activation of which is an important component of OA pathology. Thus, our findings indicate that BMMSC-EVs have ability to promote human OA cartilage repair by reducing the inflammatory response and stimulation of OA chondrocytes to produce extracellular matrix, the essential processes for restoring and maintaining cartilage homeostasis. Taken together, our data demonstrate that MSC-EVs can be important mediators of cartilage repair and hold great promise as a novel therapeutic for cartilage regeneration and osteoarthritis.

# 15	
Authors	MSc Emma A. Mol[a,d], Prof. Marie-José Goumans[d], Prof. Pieter A. Doevendans[a,b,c], Prof. Joost P.G. Sluijter[a,b,c], Dr. Pieter Vader[e]
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Poster Title	**Stem cell-derived extracellular vesicles: towards clinical application**
Abstract	Stem cell-derived extracellular vesicles (EVs) are regarded as potential cell-free therapeutics for organ repair, including the myocardium. To progress towards clinical application, many challenges have to be overcome, including development of scalable and robust EV isolation protocols and sustained EV delivery in the damaged myocardium. Recently, we have shown that isolating EVs using size-exclusion chromatography (SEC-EVs) yields EVs with higher functionality as compared to ultracentrifugation (UC-EVs). Western blot analysis revealed higher pERK/ERK ratios in endothelial cells after stimulation with SEC-EVs compared to UC-EVs. One promising biomaterial for controlled EV release is the fourfold hydrogen bonding supramolecular ureido-pyrimidinone (UPy) unit coupled to poly(ethylene glycol) chains. In vitro, EV release from UPy-gel was followed with flow cytometry after capturing EVs with CD63 antibody-coated beads and staining with fluorescent detection antibodies. EVs were gradually released from the UPy-gel in vitro, with 10% being released after 4 days. One hour after intramyocardial injection of UPy-gel loaded with EVs in pigs, EVs could be detected in the heart using fluorescent imaging. In conclusion, SEC is an improved method for EV isolation for functional studies and EV delivery may be prolonged using UPy-gels, which could contribute to higher therapeutical effects upon local delivery.

# 16	
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Poster Title	**Osteoblast-secreted extracellular vesicles stimulate the expansion of CD34$^+$ human umbilical cord blood cells**
Abstract	Osteolineage cells represent one of the critical bone marrow niche components that regulate self-renewal and differentiation of hematopoietic stem and progenitor cells (HSPCs). Recent studies demonstrated that extracellular vesicles (EVs) regulate stem cell development via horizontal transfer of bioactive cargo. In the present study, we focused on the characterization of human osteoblast-derived EV-miRNAs and investigated their implications on HSPC-osteolineage-cell crosstalk. We used human pre-osteoblasts (SV-HFO cells) to isolate EVs by a series of ultracentrifugation steps. Using next-generation sequencing we show that osteoblast-EVs contain highly abundant miRNAs specifically enriched in EVs, including critical regulators of hematopoietic proliferation (e.g., miR-29a). EV treatment of human umbilical cord blood-derived CD34+ HSPCs alters the expression of candidate miRNA targets, such as *HBP1*, *BCL2* and *PTEN*. Furthermore, EVs enhance proliferation of CD34+ cells (2-fold, $P < 0.01$) and their immature subsets (> 2-fold, $P < 0.005$) in growth factor-driven *ex vivo* expansion cultures. Importantly, EV-expanded cells retain their differentiation capacity *in vitro* and successfully engraft *in vivo*. In this study, we demonstrate a novel osteoblast-derived EV-mediated mechanism for regulation of HSPC proliferation and expansion. These discoveries provide a foundation for the utilization of EV-miRNAs for the development of cord blood-derived HSPC expansion strategies to treat hematological disorders.

# 17	
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Poster Title	**Cell stimulation-induced plasticity in the small non-coding RNA transcriptome of extracellular vesicles from dendritic cells**
Abstract	Cell-derived extracellular vesicles (EV) contain unique combinations of lipids, proteins, and genetic material, and have been implicated in various (patho)physiological processes. The molecular composition of EV, and thereby their signaling function to target cells, is regulated by cellular activation and differentiation stimuli. Changes in RNA-composition of EV in body fluids are also in the limelight as biomarkers for disease. While changes in EV-derived microRNAs (miRNA) have been intensively studied, it is unknown whether cell stimulation affects other classes of small non-coding RNA in EV. We employed primary immune cells, different immune-related stimuli, and methods yielding highly pure EV to show that external stimuli affect the levels of some EV-associated RNA classes, while leaving others unchanged. Prominent changes were found in the miRNA, snoRNA and Y-RNA content of EV from differentially stimulated dendritic cells, whereas tRNA and snRNA levels were much less affected. Importantly, EV-RNA levels only partly reflected changes in cellular RNA, implicating that the EV-transcriptome not necessarily predicts RNA levels in EV-producing cells. The acquired knowledge can guide discovery and understanding of non-coding RNA types beyond miRNAs as functional entities in EV and as EV-RNA-based biomarkers.

# 18	
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Poster Title	**Characterization of extracellular vesicles by transmission electron microscopy**
Abstract	Transmission electron microscopy (TEM) is a high-resolution imaging technique capable to distinguish extracellular vesicles (EVs) from similar-sized non-EV particles. However, TEM sample preparation protocols are diverse and have never been compared directly to each other. We compare commonly applied negative staining protocols for their efficacy to detect EVs. Four negative staining protocols were selected from literature, which differ in fixation of the EV sample and mounting of EVs to a TEM grid. These protocols were applied to a single sample of cell-free human urine. Images were taken at one selected image location and five predefined locations of the grids. The obtained images were compared for their qualitative and quantitative usefulness with respect to: morphology, EV count, and quality of the obtained TEM images. EVs were detectable by all four protocols. However, at predefined locations, the EV recovery varied by two-fold between protocols. Evaluation of image quality by four different researchers active in the EV field demonstrated a difference in image quality and suitability for EV research. In conclusion, EV sample preparation protocols have a large influence on the TEM image quality. The sample protocol without fixation, carbon coated grids, blotting after sample mounting and short incubation with uranyl acetate was preferable over the other evaluated protocols based on numerical evaluation and overall image quality.

# 19	
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Poster Title	**Comparison of generic fluorescent dyes for detection of extracellular vesicles by flow cytometry**
Abstract	**Background:** Because extracellular vesicles (EVs) in plasma are potential biomarkers of disease, a generic fluorescent dye specifically staining EVs is desirable. Here we evaluated five commonly used generic dyes for flow cytometry. **Methods:** EVs from MCF7-conditioned culture medium and human plasma were stained with calcein AM, calcein violet, CFSE, di-8-ANEPPS, or lactadherin. The concentration of EVs detected by generic dyes was measured by flow cytometry (A60-Micro, Apogee). EVs were identified by immunostaining EpCAM for MCF7-EVs, and CD61 for platelet EVs. Scatter triggering was applied as a reference, and the influence of non-EV components was evaluated. **Results:** Di-8-ANEPPS, lactadherin and side scatter detected 100% of EpCAM+ MCF7-EVs. In plasma, di-8-ANEPPS inefficiently stained EVs due to protein binding, which improved by protein removal. Lactadherin and side scatter detected 33% and 61% of CD61+ EVs, respectively. Because all generic dyes stained proteins, the overall sensitivity to detect platelet EVs in plasma was 33% at best. Calcein AM, calcein violet and CFSE were either inefficient at detection of EVs in both samples, or suffered from swarm detection and/or insufficient event rates. **Conclusions**: None of the generic dyes detected all and only EVs in plasma. Side scatter triggering detected the highest concentration of plasma EVs on our flow cytometer, followed by lactadherin. The choice between scatter or lactadherin primarily depends on the sensitivity of the flow cytometer used.

# 20	
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Poster Title	**EVQuant: Combined quantification and characterization of individual extracellular vesicles in experimental and clinical samples**
Abstract	Extracellular vesicles (EVs) are an important biomarker source, but quantification in clinical samples remains challenging. We provide a microscopy based assay (EVQuant) to quantify and characterize individual EVs without isolation/purification. EVs are labelled using a membrane-dye and/or immunofluorescent antibodies and immobilized in a transparent medium to enable detection of individual EVs in conditioned culture media and minimally processed urine and serum. Multicolour imaging allowed detection of a large variation in the general markers CD9 and CD63 on EVs from different cell lines. It showed no direct correlation to the total marker levels, suggesting variation in CD9 and CD63 molecules on individual EVs. Surprisingly, differences in EV percentages with CD9 and CD63 markers are also found in urines from men, men with radical prostatectomy and females. In conclusion, EVQuant is a rapid, robust, assay which enables detection of small (~50nm) vesicles in (clinical body) fluids, and does not require EV isolation or purification limiting the loss of EVs. Moreover, biomarker detection on individual EVs allows identification of organ or disease specific EV sub-populations. The possibility of EV-analysis in high-throughput makes EVQuant a suitable candidate for implementation in a clinical setting. This project is funded by PC-UK(G2012-36) and KWF(EMCR 2015-8022).

# 21	
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Poster Title	**Super-resolution imaging of extracellular vesicle transfer in prostate cancer**
Abstract	Tumor cells influence their microenvironment to enhance tumor progression and metastasis. Tumor-derived extracellular vesicles (EVs) play an important role in this communication between tumor cells and surrounding stromal and epithelial cells. We have developed imaging tools to visualize these EV-mediated mechanisms in detail. Uptake and further processing of fluorescently labeled prostate cancer-derived EVs by prostate epithelial cells is followed on different time scales, from seconds to multiple hours, by live cell imaging using confocal microscopy and high-speed spinning disk microscopy. Several stages of EV uptake and processing are visualized in more detail using high-resolution 4Pi microscopy and structured illumination super-resolution microscopy (SIM). Time-lapse imaging, 3D 4Pi microscopy, and high-speed spinning disk microscopy shows that EVs are internalized as a whole and are dynamically transported via microtubules. Super-resolution co-localization studies between EVs and markers for the endocytic pathway show a clear shift in co-localization from clathrin to early endosomes to late endosomes and lysosomes in time. Together, the variety of imaging approaches enabled us to visualize and study in detail the subsequent steps and dynamics of EV uptake and further processing by target cells. This project is funded by Prostate Cancer UK (G2012-36).

# 22	
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Poster Title	**Naked virions, extracellular vesicles, vesicle-enclosed virions: it's a small world**
Abstract	**Background:** Picornaviruses classically believed to release non-enveloped progeny through the induction of cell lysis, were recently shown to also exit from intact cells inside extracellular vesicles (EV). Enclosure of virus particles inside EV may have a large impact on viral dissemination or antiviral immunity and therefore on the pathology of many infectious diseases. To better understand the function of picornavirus-induced EV we performed in-depth analysis of EV release dynamics during infection and of host- and virus-derived components enclosed in these EV. **Methods:** EV released by picornavirus-infected cells were separated into subpopulations using differential ultracentrifugation and density gradient purification. EV and viral particles were quantified using high-resolution flow cytometry and end-point titration, and viral or host-derived EV contents were analyzed by western blot and qPCR. **Results:** We found that early after viral infection, before cell lysis occurs, picornavirus triggers the release of several distinct EV populations. Small EV pelleted at 100,000x*g* and floating to low-density fractions contained mature infectious virus particles. In addition, EV pelleted at 10,000x*g,* which likely represent larger EV, also enclosed viral particles. The release of these distinct populations of virus-containing EV is induced at different time points after infection. In the pre-lytic phase of infection virus-containing EV constitute a prominent portion of the released infectious particles. Interestingly, picornavirus also induces changes in EV lacking viral products. **Conclusions:** Picornavirus infection induces major changes in the repertoire of EV released by cells. Moreover, the release dynamics of virus-containing EV and other virus-induced EV is tightly regulated. These different EV types may each play a distinctive role in virus propagation or host protection, contributing to the continuous battle between virus and host.

# 23	
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Poster Title	**Urinary extracellular vesicles as a tool to study renal salt reabsorption in hypertension**
Abstract	Animal studies have shown that the calcineurin inhibitors (CNIs) cyclosporine and tacrolimus activate the renal thiazide-sensitive NaCl-cotransporter (NCC). A common side effect of CNIs is, therefore, hypertension. Renal salt transporters such as NCC are excreted in urinary extracellular vesicles (uEVs) after internalization into multivesicular bodies. In this study, we investigated the effects of CNIs on the abundance of total NCC (tNCC) and phosphorylated NCC (pNCC) in uEVs, and assessed whether NCC abundance in uEVs predicts the blood pressure response to thiazide diuretics. Our results show that in kidney transplant recipients (KTR) treated with CNIs (n=32), the abundance of tNCC and pNCC in uEVs is 4-5 fold higher than in CNI-free KTR (n=13) or healthy volunteers (n=6). In hypertensive KTR, higher abundances of tNCC and pNCC prior to treatment with thiazides predicted the blood pressure response to thiazides. During thiazide treatment, the NCC abundance in uEVs increased in responders (n=10), but markedly decreased in non-responders (n=8). Thus, our results show that CNIs increase the abundance of tNCC and pNCC in uEVs, and these increases correlate with the blood pressure response to thiazides. This implies that assessment of NCC in uEVs could represent an alternate method to guide anti-hypertensive therapy in KTR.

# 24	
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Poster Title	**Characterization of plasma microvesicles in patients with diabetic nephropathy**
Abstract	Diabetic nephropathy (DN) is a major complication of diabetes and is characterized by a proinflammatory and procoagulant state. Both conditions are known to change MV numbers, size, cellular origin, composition and function. In this study, we characterized plasma MV in type 2 diabetes mellitus (T2DM) patients with albuminuria. We divided 103 T2DM patients 3 groups based on 24h urinary albumin levels; normoalbuminuria (<30mg/day), microalbuminuria (30-300mg/day), and macroalbuminuria (>300mg/day). MV from citrated plasma were stained with Calcein violet AM (CV), and simultaneously labeled for cell surface molecules of monocytes, erythrocytes, t-cells, leukocytes, platelets, activated platelets, endothelial cells, activated endothelial cells, granulocytes. The number of double positive MV were determined using flow cytometry (A60-Micro, Apogee). The total number of CV-positive MV, erythrocyte-derived MV, and leukocyte-derived MV were significantly higher in both micro- and macroalbuminuria patients, compared with the diabetic controls. Platelet-, granulocyte-, activated endothelium derived were elevated in either micro- or macroalbuminuria patients. Our data show that MV from T2DM patients with micro- and macroalbuminuria display different profiles of cellular origin, which possibly reflects the procoagulant and proinflammatory state that accompanies DN.

# 25	
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Poster Title	**Development of VIROSOMES (extracellular vesicle – virus hybrids) for treatment of neurodegenerative diseases**
Abstract	Extracellular vesicles (EVs) have great potential to serve as vectors for delivery of (gene) therapeutics. In contrast to viral vectors, EVs supposedly have "stealthy" characteristics (not detectable by neutralizing antibodies), display wide-spread distribution *in vivo*, and are capable to penetrate deep into tissues. We are developing and testing VIROSOMES, EVs loaded with viral genomes, for treatment of neurodegenerative diseases. One of our VIROSOME types, consisting of EVs loaded with adeno-associated virus (AAV) expressing green fluorescent protein (GFP), demonstrated efficient transduction of neuroblastoma cells and (to a low degree) protection against AAV-neutralizing antibodies. To translate towards gene therapeutic applications for neurodegenerative diseases of the eye, we screened different types of VIROSOMES on a *retina-in-a-dish* model, which identified VIROSOMES with superior transgene delivery to retinal neurons (outperforming non-packaged, naked viruses). In parallel, we are developing gene therapeutic VIROSOMES for treatment of brain cancer. Our first assessments in a glioblastoma mouse model have revealed remarkable characteristics of VIROSOMES, with regards to vector spread and transgene delivery. Currently, our VIROSOMES are further characterized (incl. content omics) and optimized ("picking the best performers") on cell cultures and animal models for neuropathies (in particular optic neuropathies caused by mitochondrial deficiency) and brain cancer.

# 26	
Authors	Tzviya Zeev-Ben-Mordehai
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Poster Title	**Membrane protein enriched extracellular vesicles: a platform for the structure determination of membrane proteins by cryo electron tomography**
Abstract	Membrane proteins are a central subclass of the proteome. Structure determination of membrane proteins has fallen behind partially due to the inherent challenges in protein production and crystallization. In particular, understanding the mechanism of membrane proteins at the molecular level requires studying their structure in the context of membranes. We have developed a system that display natively anchored, topologically correct membrane proteins on isolated cell derived membrane vesicles and used this system to study the structure and function of fusion proteins. This system enabled the visualization of highly-elusive, membrane fusion intermediates by electron cryo microscopy and tomography. We have further determined the structure of the proteins by applying sub-volume averaging.

# 27	
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Poster Title	**Validation of a plasma vesicle-miRNA assay for longitudinal therapy-response monitoring in classical Hodgkin lymphoma**
Abstract	**Introduction:** We previously demonstrated that Extracellular Vesicles (EVs) in plasma is a potential biosource for miRNA biomarkers to monitor treatment response in Hodgkin lymphoma (HL) patients. Now we collected a cohort of longitudinally plasma cHL samples before, during and after treatment, and compared the EV-miRNA assay with serum TARC levels and metabolic tumor activity. **Methods:** EV-associated extracellular RNA (exRNA) fractions are isolated with standardized size-exclusion chromatography (SEC) from plasma of cHL patients (n=25, 120 samples and healthy subjects (n=19) at multiple timepoints. qRT-PCR is used to quantify miRNAs. sTARC-levels were measured by ELISA. EV-miRNA and sTARC-levels were correlated with clinical parameters at presentation, platelet numbers and treatment outcome, determined by FDG-PET. **Results:** Matched serial monitoring of EV-miRNA levels in patients before, directly after treatment and during long-term follow-up revealed robust, stable association with treatment results observed by FDG-PET, both for responders (n=21) and non-responders (n=4). **Conclusion:** cHL-related miRNA levels in circulating EVs reflect the presence of vital tumor tissue and show a promising potential for future development of a liquid biopsy test capable for therapy-response and relapse monitoring in cHL patients.

# 28	
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Poster Title	**Towards Extracellular Vesicles-Based Biomarker Detection Using High-Resolution Flow Cytometric Analysis of Rare Events**
Abstract	Extracellular vesicles (EVs) are submicron-sized particles that are released by cells and play an important role in intercellular communication. Since their molecular content may contain important information regarding presence of disease, disease state or efficiency of medical intervention, EVs could serve as promising biomarkers. Isolation, detection and characterization of EVs is however hampered by their size, their heterogeneity, and the complexity of biological fluids. Moreover, disease-related EV are likely to appear as rare events and could be obscured by the bulk of EVs and other nano-sized particles present in biological fluids. By adapting a commercially available high-end flow cytometer (BD Influx) and developing a generic fluorescent staining protocol, we previously established a high-resolution flow cytometric approach that allows multi-parameter analysis and sorting of individual EVs. By performing spike-in experiments we are currently investigating whether high-resolution flow cytometry allows for the detection of rare populations of EVs in biological fluids.

# 29	
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Poster Title	**A workflow to detect wild-type and mutant transcripts in blood- derived extracellular vesicles from patients with solid cancers**
Abstract	**Background:** Extracellular vesicles (EVs) are secreted by various cell types into body fluids and contain nucleic acids and proteins of the cells they originate from. The goal of the current study was to define a workflow for EV mRNA (EV-RNA) expression profiling and characterization to detect mutant transcripts in EVs derived from blood plasma of solid cancer patients with circulating tumor DNA. **Materials & Methods:** A workflow to isolate and characterize EVs was established using conditioned medium from 6 breast cancer cell lines. EV-RNA was isolated using the exoRNeasy Kit (Qiagen). Cell line mRNA and EV-RNA were evaluated in duplicate by RT-qPCR for a 96-gene expression panel and by digital PCR (dPCR) for mutant and wild-type transcripts in target genes. dPCR was performed on cell-free DNA (cfDNA) isolated from about plasma of 20 patients with metastatic cancer isolated within 24 hours following drawing blood collected in EDTA, BCT and CellSave tubes. All patients harbored a known mutation in their primary tumor which were for 10 patients also detected in their cfDNA. **Results:** After normalization on 3 reference genes, EV-RNA and matched parental cell line mRNA had similar expression profiles for 55 genes, whereas 8 genes were significantly upregulated in EV-RNA. However, principal component analyses showed that the EV-RNA profile cluster with matched parental cellular mRNA profile. Furthermore, cellular mRNA and matched EV-RNA demonstrated comparable variant allele frequencies for mutations in PIK3CA, KRAS, and BRAF. Reference and target gene mRNA copies were detected for all patients in EV-RNA of plasma derived from EDTA blood but not from BCT or CellSave blood. In three of twenty patients with mutations detected in cfDNA, target mRNA copies contained also mutant transcripts, i.e. a lung cancer patient with an EGFR T790M mutation (VAF: 24% vs 65% in the primary tumor or metastatic lesion) and two colon cancer patients with a KRAS G13D mutation: (VAF: 15% and 2.2% in EV-RNA vs VAF: 60% and 22% primary tumor or metastatic lesion, respectively). **Conclusions:** Our study shows that EV-RNA can be used to perform expression analysis of multiple genes. Moreover, cell line derived EV-RNA harbor the same mutant transcripts as detected in their matched cellular mRNA. In line with this, we detected somatic mutations in patient plasma samples. However, EV-RNA analyses showed a lower sensitivity compared to cfDNA. Nonetheless, our data suggests that tumor EV-RNA in

	patients' blood mimics tumor-specific characteristics, opening new perspectives for the use of liquid biopsies.

# 30	
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Poster Title	**Extracellular vesicles as Liquid Biopsy in Alzheimer's disease- search for early translation of molecular phenotype from rat to human**
Abstract	Extracellular vesicles (EV's) are released into body fluids. Preclinical species provide the possibility to investigate the biological system at and relationships between brain tissue changes in neurodegenerative diseases and body fluid exosomes contents. For humans this is more restricted to body fluids, such as CSF and blood. Therefore, paralleled investigations in preclinical species and patients is anticipated to be of high value in translational research on biomarker fingerprints of exosomes to diagnose different stages of neurodegenerative diseases and potentially as novel readouts for pharmacological interventions. We aim to exploit the cargo of exosomes in *Alzheimer's disease (AD).* The cargo of blood and CSF EV's will be investigated in samples obtained from an AD rat model and from AD patients, at different ages and stages of the disease. The relation between age, disease stage and EV's cargo will be established using mathematical modeling, including multivariate statistics. This will provide (clinically relevant) biomarker fingerprints of the AD disease stage, which is essential for early diagnosis of AD, for enrolment into clinical trials as well as for the evaluation of pharmacological interventions.

# 31	
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Poster Title	**GABARAPL1 is required for secretion of pro-angiogenic extracellular vesicles during hypoxia**
Abstract	Hypoxia is a hallmark of solid tumours and is associated with tumour progression and therapy resistance. In response to hypoxia, cells secrete pro-angiogenic factors to induce blood vessel formation and restore oxygen supply to the tumour. EVs are emerging as mediators of intercellular communication in the tumour microenvironment. Recently, we identified the LC3/GABARAP protein family member GABARAPL1 as a hypoxia-induced protein. Here, we show that GABARAPL1 is required for the secretion of pro-angiogenic EVs during hypoxia. GABARAPL1 is expressed on the surface of EVs and can be targeted. Silencing GABARAPL1 perturbs EV secretion and results in decreased tumour growth due to decreased vascularisation and enhanced necrosis. Additionally, targeting GABARAPL1 directly after tumour irradiation resulted in enhanced tumour growth delay. Here we show that hypoxic tumour cells secrete a unique EV subset, marked by GABARAPL1 expression and controls tumour progression, these EVs are targetable and are interesting to pursue as therapeutic target.

# 32	
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Poster Title	**Involvement of $\alpha_{IIb}\beta 3$ integrin on release of extracellular vesicles**
Abstract	Platelets represent a major source of extracellular vesicles (EV) in which EV release occurs spontaneously or after stimulation of platelets. Platelet activation leads to active $\alpha_{IIb}\beta 3$ integrin, which enables ligand binding and subsequent outside-in signalling. Gemmell *et al*. showed that $\alpha_{IIb}\beta 3$ integrin is involved in platelet EV release. However, the exact mechanism behind the involvement of $\alpha_{IIb}\beta$b3 integrin in the release of platelet EV. We aim to identify the underlying mechanisms of platelet EV release by activated platelets. Isolated platelets were stimulated with different agonist and EVs were isolated. Release of platelet EV were determined by prothrombinase-based assay and Nanoparticle tracking analysis. The mechanism of extracellular release was studied with several inhibitors. Platelet EV release is dependent on platelet activation pathway, in which convulxin-activated platelets reached the highest level of EV release. Pre-treatment with $\alpha_{IIb}\beta 3$ inhibitor, eptifibatide, cytochalasin D and Gα13 inhibitor, reduced platelet EV release triggered by convulxin. Inhibition of PTPN1 resulted in increased EV release. These data conclude that the $\alpha_{IIb}\beta 3$ integrin is involved in release of platelet EV. Furthermore, cytoskeletal rearrangement is important for EV release in activated platelets. Interestingly, PTPN1 might have a regulatory role in EV release

# 33	
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Poster Title	**Delivery of membrane-bound CD39/CD73 by extracellular vesicles (EVs) for treatment of inflammatory disease**
Abstract	I**ntroduction:** The imbalance between pro-inflammatory extracellular ATP and anti-inflammatory adenosine in the synovial compartment of rheumatoid arthritis patients contributes to ongoing inflammation. CD39 is a membrane-associated ATPase that converts ATP and ADP into AMP, while membrane-associated CD73 converts AMP into adenosine. Removal of the transmembrane domain of CD39 reduces its activity by >90%. Thus we assessed the potential of extracellular vesicle (EV)- mediated delivery of these membrane-bound enzymes as a novel treatment for inflammatory disease. **Methods:** We performed a large scale purification (~50L) of CD39/CD73-EVs from the supernatant of a stably transfected HEK293 cell line overexpressing CD39 and CD73. EVs were concentrated by tangential flow filtration and precipitation and subsequently purified by size exclusion chromatography. **Results**: The purity of isolated EVs was 5.8E10 EVs per µg protein. Specific enzymatic activity (phosphate/min/µg protein) of CD39/CD73-EVs was 20.5-fold (CD39) and 4.5-fold (CD73) higher when compared with their soluble counterparts. CD39/CD73-EVs were ~10-fold more potent than the soluble enzymes in reducing pro-inflammatory cytokine production in *in vitro* human cell-based inflammation assays. **Summary/Conclusion:** Engineered EVs are a promising tool to deliver membrane-bound, biologically active therapeutic enzymes and may have great potential for the treatment of inflammatory disease, including rheumatoid arthritis.

# 34	
Authors	E.S. Martens-Uzunova, N. Dits, M. Vredenbregt-van Den Berg, T. Hartjes, M van Royen, G. Jenster
Affiliations	Erasmus MC, Rotterdam
Poster Title	**Systematic evaluation of techniques for the isolation and detection of small non-coding RNA from urine-derived extracellular vesicles**
Abstract	The ability to stratify prostate cancer patients in a non-invasive manner, into these who benefit from radical treatment versus these who can be enrolled in an active surveillance or watchful waiting program, would answer a currently unmet clinical need. A promising solution to this clinical problem is the use of the minimally invasive “liquid biopsy” approach, which aims at the detection of tumor biomarkers in body fluids such as blood and urine. Over the last years, extracellular vesicles (EVs) emerged as a novel promising source of cancer-related biomarkers. Tumor cell originating EVs can be used as a rich source of protein and RNA biomarkers. In this study, we aimed to evaluate currently available methods for the extraction and quantitation of small noncoding RNAs present in urinary EVs in order to evaluate their use as minimally invasive PCa biomarkers. We tested 11 different combinations of direct and stepwise methods for EV isolation and RNA extraction and quantitated the content of previously established by us small RNAs with high biomarker potential in PCa by two different qPCR techniques. To obtain high amounts of uniform quality starting material, urine samples from healthy donors where depleted from native EVs by ultracentrifugation protocol and spiked in with known amount of EVs isolated from prostate cancer cells. The amount of spiked EVs was equivalent to the amount of removed vesicles. Subsequently, EVs were captured by four different techniques, i.e. ultrafiltration, precipitation, size exclusion chromatography, and affinity capture. Total RNA was isolated either directly from the captured EVs or after EV recovery using two different kits, with or without phenol-chloroform extraction. The amounts of small noncoding RNAs (miRNAs, isoMiRs, tRNA fragments, snoRNA and snoRNA fragments) were measured by quantitative real time PCR (qPCR) either with a SyBR Green technique and LNA-based primers or with a probe-based technique.

# 35	
Authors	Melissa C. Piontek and Wouter H. Roos
Affiliations	University of Groningen
Poster Title	**Extracellular vesicles and their mechanical properties investigated by atomic force microscopy**
Abstract	Known for having a strong potential in therapeutics and diagnosis, the interest in extracellular vesicles (EVs) has substantially increased. EVs are biological nanoparticles secreted by different cell types, which are present in many body fluids, e.g. urine, breast milk and plasma. They differ in size, lamellarity, function, synthesis, morphology as well as in their cargo. Therefore, a characterization of EVs on various levels is indispensable. To investigate these particles mechanically, we use atomic force microscopy (AFM). With its ability to image with nanometer-accuracy and to be operated in near-to physiological conditions, AFM provides access to the single particle level, and thus, the particle to particle variability can be studied. Performing nanoindentation experiments, information about the particle's deformability, stiffness and bending modulus can be extracted. After optimization of the experimental conditions, e. g. concentration and particle size, the extracted parameters could be used as a characterization of EVs. Furthermore, the possible difference in the mechanical properties of EVs secreted by different cells and different donors could then serve as a potential biomarker. Consequently, the approach could be a noninvasive, early-state diagnosis method of cancer.

# 36	
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Poster Title	**Ex vivo expansion of umbilical cord blood-derived hematopoietic stem cells using extracellular vesicles of bone marrow mesenchymal stromal cells origin**
Abstract	Umbilical cord blood (UCB) has emerged as an attractive alternative source of hematopoietic stem and progenitor cells (HSPCs) for allogeneic hematopoietic stem cell transplantation in addition to bone marrow or mobilized peripheral blood. However, the total number of HSPCs from one UCB graft is often insufficient to successfully promote the rapid engraftment and hematopoietic recovery of patients eligible for stem cell transplantation. A promising approach to overcome this issue represents ex vivo expansion of UCB-derived HSPCs. Mesenchymal stromal cells (MSCs) represent one of the critical bone marrow niche components that support maintenance of HSPCs and based on recent studies that indicate that extracellular vesicles (EVs) regulate stem cell development via transfer of bioactive lipids, proteins and RNA, we hypothesize that MSC-derived EVs can support ex vivo expansion of the HSPCs. Our observations showed that human MSCs-derived EVs improve the ex vivo expansion of UCB-derived CD34+ HSPCs. MSCs-derived EVs enhance the proliferation of UCB-derived CD34+ HSPC and their immature subset (CD45RA-CD38-CD34+CD90+ cells), while retaining their progenitor capacity, resulting in myeloid rather than erythroid lineage. In conclusion, our findings indicate the potential use of MSC-derived EVs in development of a novel clinically relevant expansion strategy for UCB-derived HSPCs.

# 37	
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Poster Title	**Real-time quantification of multivesicular body-plasma membrane fusion reveals modulation of exosome release by G protein-coupled receptor signaling**
Abstract	**Introduction***:* Exosomes are endosome-derived small extracellular vesicles (EVs) implicated in cell-cell communication and secreted by Multivesicular Bodies (MVBs) fusing with the plasma membrane. Current techniques to study exosome physiology are based on isolation procedures post-secretion, precluding direct dynamic insight into the mechanics of exosome biogenesis and the regulatory mechanisms involved in exosome release. Here we propose real-time visualization of MVB-PM fusion to overcome these limitations. **Methods***:* We designed tetraspanin-based optical reporters that spot MVB-PM fusions using live Total Internal Reflection Fluorescence (TIRF) microscopy and dynamic Correlative Light-Electron Microscopy (CLEM). **Results***:* Detailed single-cell analysis demonstrates that MVB-PM fusion activity is reduced by depletion of the tSNAREs SNAP23 and Syntaxin-4 and can be induced by stimulation of the Histamine H1 Receptor (H1HR). Activation of this G protein-coupled receptor in HeLa cells increases Ser110 phosphorylation of SNAP23 promoting MVB-PM fusion. **Conclusion**: Using this single-cell live imaging approach, we highlight the modulatory dynamics of MVB exocytosis that will increase our understanding of

	exosome physiology and help identify druggable targets in exosome-associated pathologies.

# 39	**SPONSORED ABSTRACT**
Authors	Chad Schwartz, Oh.D., Zach Smith, M.S.
Affiliations	Beckman Coulter Inc.
Poster Title	**To the Standardization of Exosome Isolation and Characterization**
Abstract	Research involving extracellular vesicles (EVs) is rapidly expanding due to evidence suggesting a role in cancer metastasis and cell-to-cell communication, with a vast increase in the quantity of publications over the last 5 years. An improved and more efficient isolation and characterization protocol for EVs is critical to advancing this exciting field and experts have recently called for the establishment of standardized methods. EV isolation is particularly tedious, requiring several rounds of differential centrifugation and a density gradient centrifugation step to obtain highly pure vesicles. Downstream challenges involve a standardized method for genetic profiling of encapsulated miRNA. Here, we describe a workflow using automated Biomek methods for centrifugation layering and fractionation, total RNA extraction, and cDNA amplification and clean-up for next generation sequencing. NGS results are reported on benign and cancerous colon cell lines.

# 40	**SPONSORED ABSTRACT**
Authors	Carley D. Ross, Aliaksandr Kachynski and Thomas Ramin
Affiliations	Beckman Coulter Inc., *Life Sciences Development Center*
Poster Title	**Extracellular Vesicle Isolation by Flow Cytometric Sorting and Characterization by Analytical Ultra-Centrifugation and Dynamic Light Scatter**
Abstract	Hypothesis: Extracellular vesicles may be sorted via flow cytometry and the sorted populations may be analyzed via analytical ultra-centrifuge and dynamic light scatter. Conclusion: MoFlo Astrios EQ is capable of sorting exosomes for further downstream analysis. AUC may be used to determine EV population composition on the interference and absorbance with 10-20 nm resolution for a 100 nm exosome and the Delsa Max measures exosome population sizes.