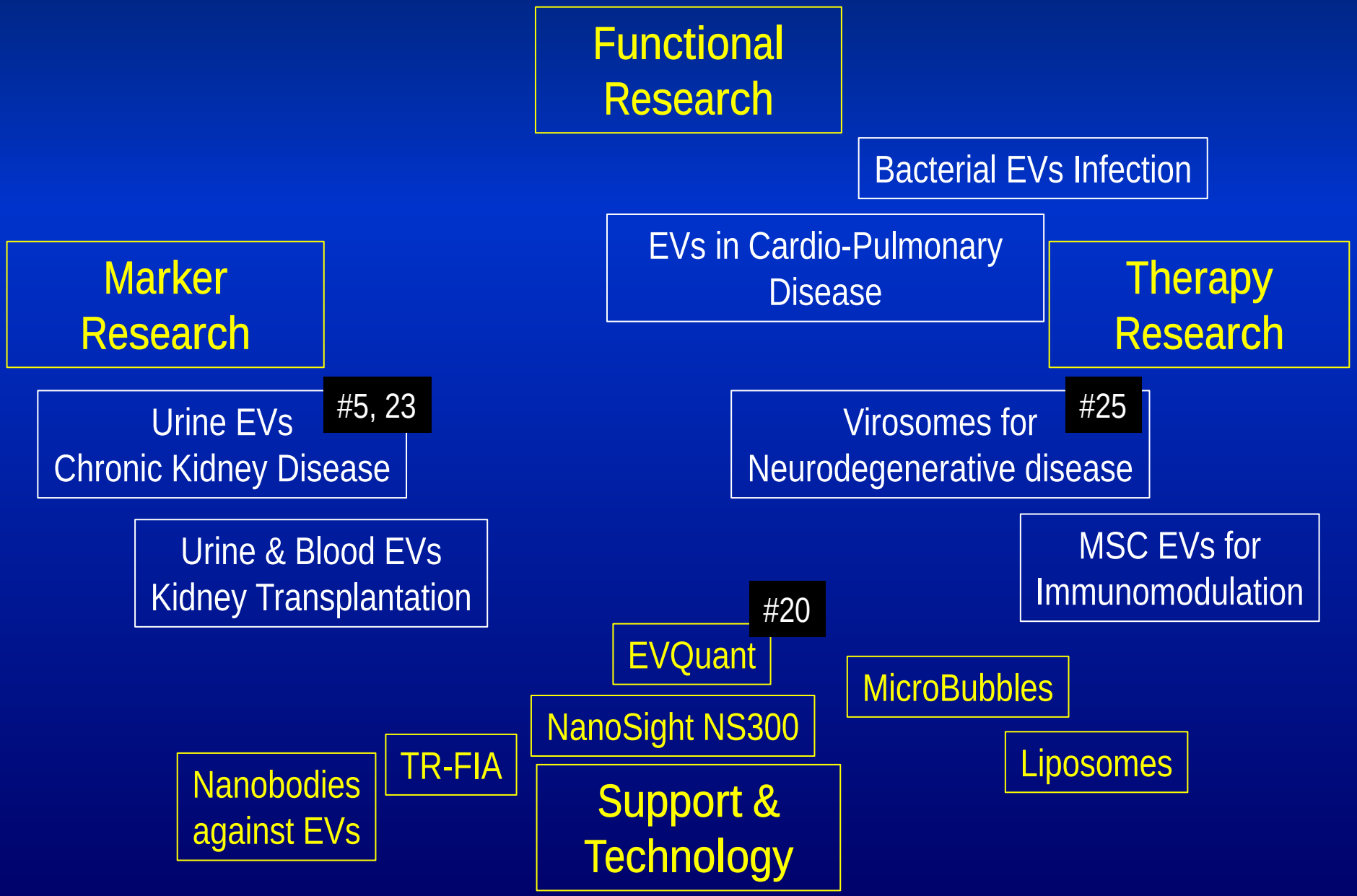
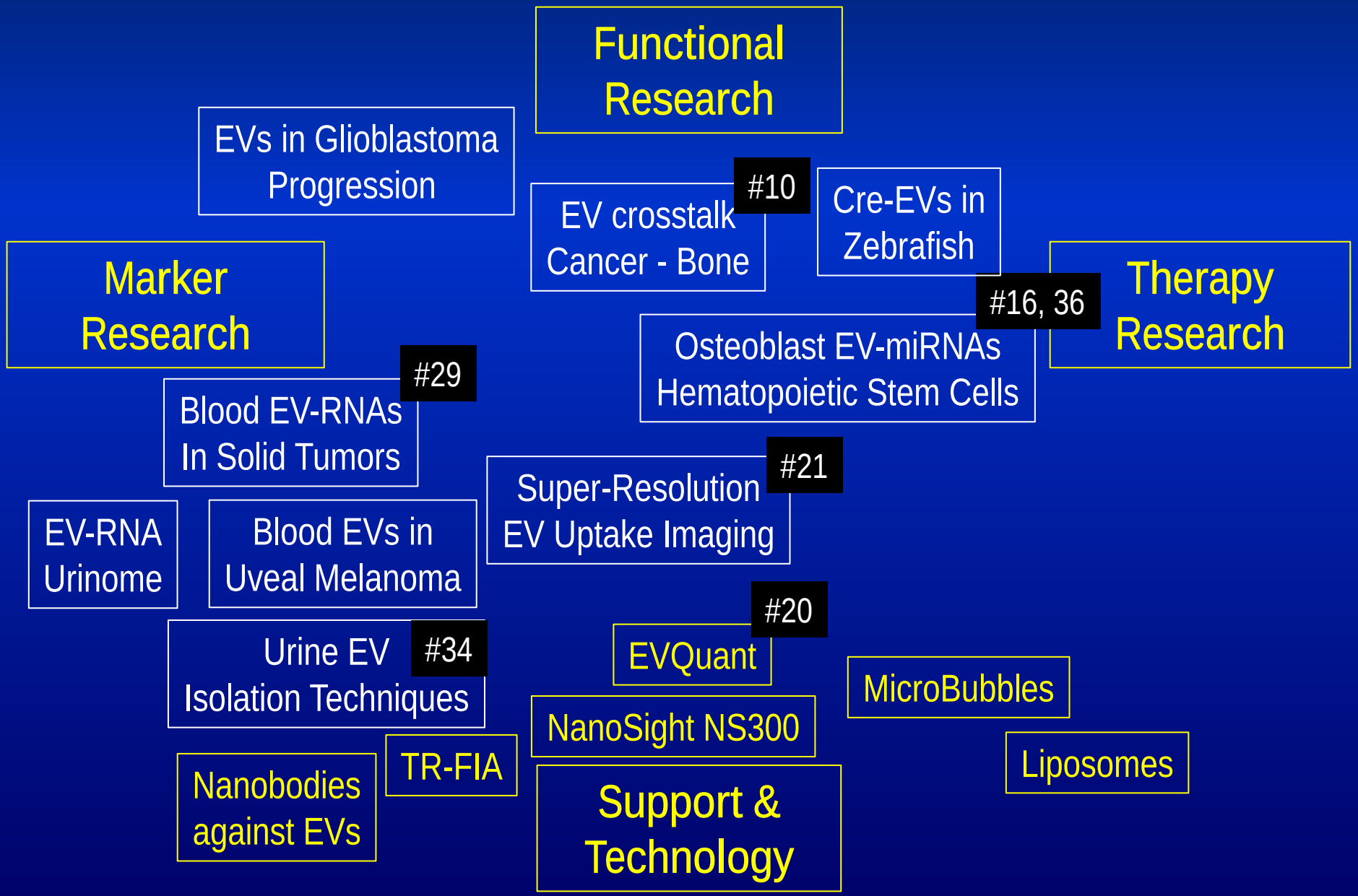
NLSEV Kick-off 2017: Erasmus MC Rotterdam

Prof. Guido Jenster
Erasmus MC
Urology
g.jenster@erasmusmc.nl


Functional Research
Bacterial EVs Infection
EVs in Cardio-Pulmonary Disease
Marker Research
Therapy Research
Urine EVs Chronic Kidney Disease
#5, 23
Virosomes for Neurodegenerative disease
#25
Urine & Blood EVs Kidney Transplantation
MSC EVs for Immunomodulation
#20
EVQuant
MicroBubbles
NanoSight NS300
TR-FIA
Liposomes
Nanobodies against EVs
Support & Technology

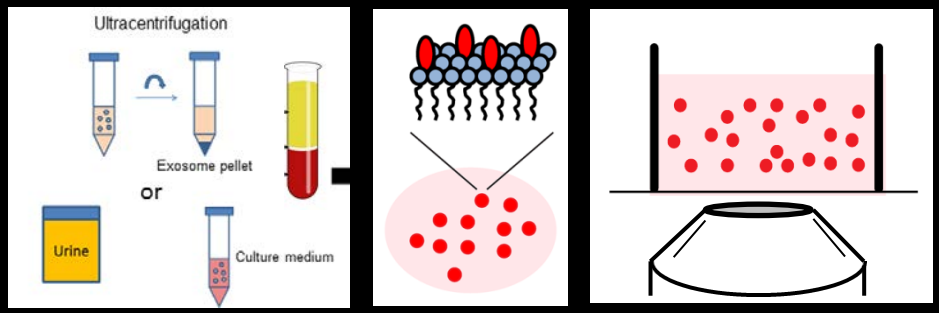
EMC EV Research Project Cloud: Cancer Research



EVQuant:

Fast and versatile EV quantification and characterization assay

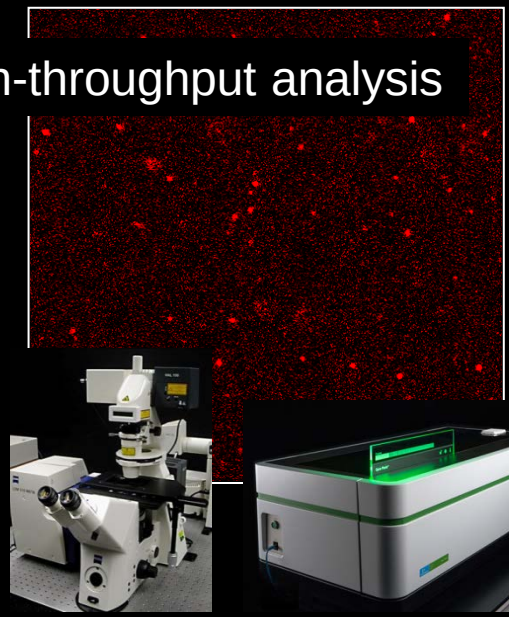
m.vanroyen@erasmusmc.nl



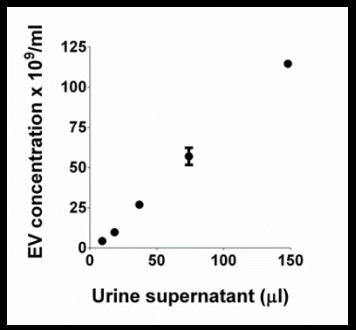
Large variety of sample types
No EV isolation/purification

Detection of small EVs

High-throughput analysis

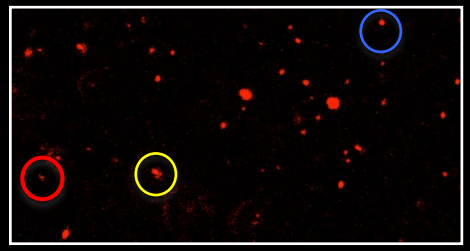


EVQuant

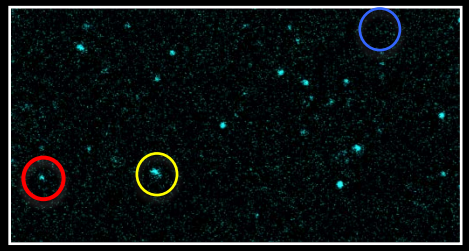


Research and clinical samples

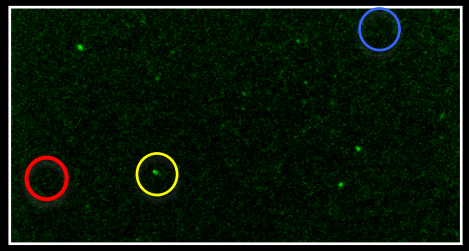
Detect multiple biomarkers on individual EVs



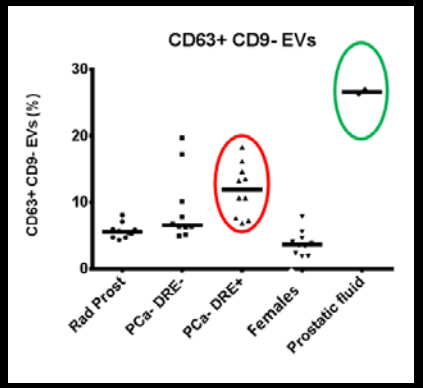
Membrane



CD9-alexa647

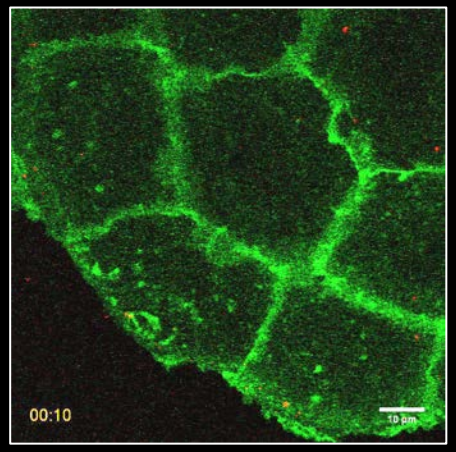


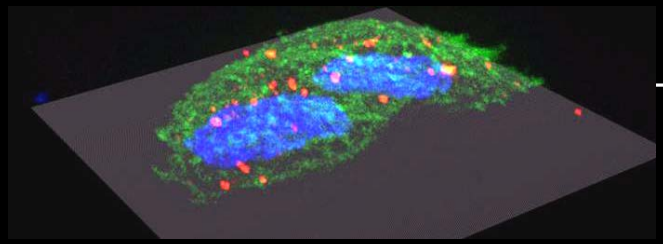
CD63-alexa488



Visualization of uptake and further processing of EVs

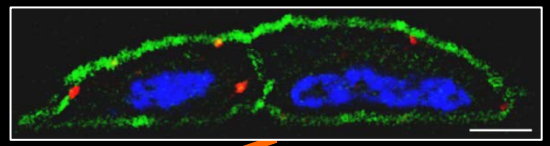
m.vanroyen@erasmusmc.nl





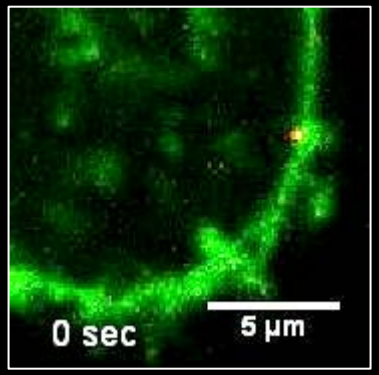
Pi microscopy

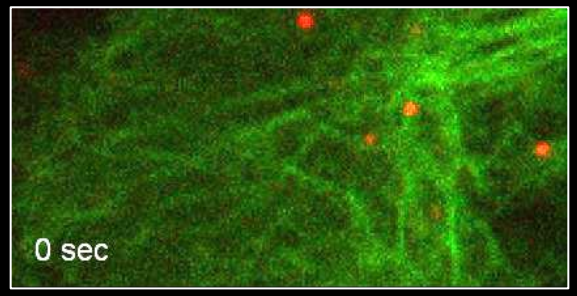
Confocal microscopy



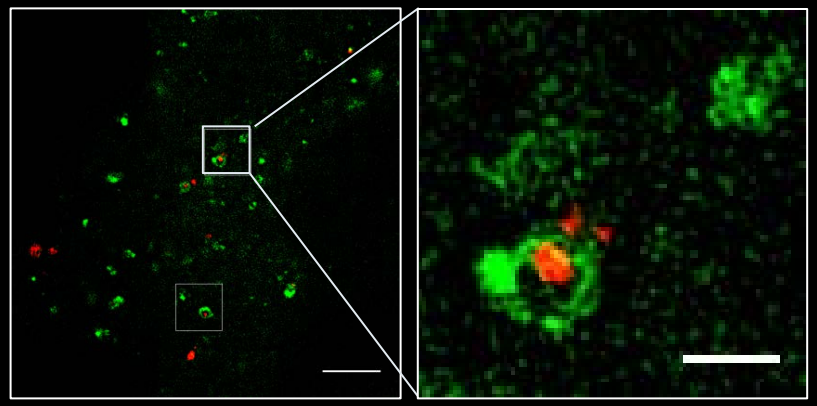
Exosomes
Cell membrane
Cell nucleus

Seconds **Minutes** **Hours** **Days**





Spinning disk microscopy

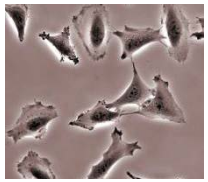


Structured illumination microscopy (SIM)

EV-Nanobodies

Joke Veldhoven, Janne Leivo, Guido Jenster

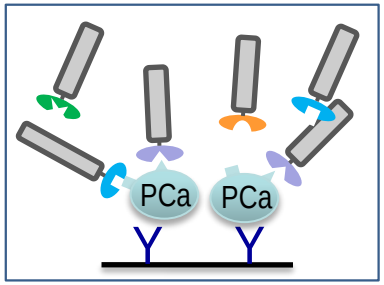
Urinary EVs from men with and without PCa

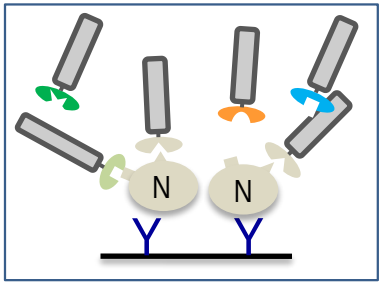


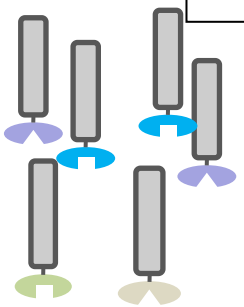
Immunize

Llama

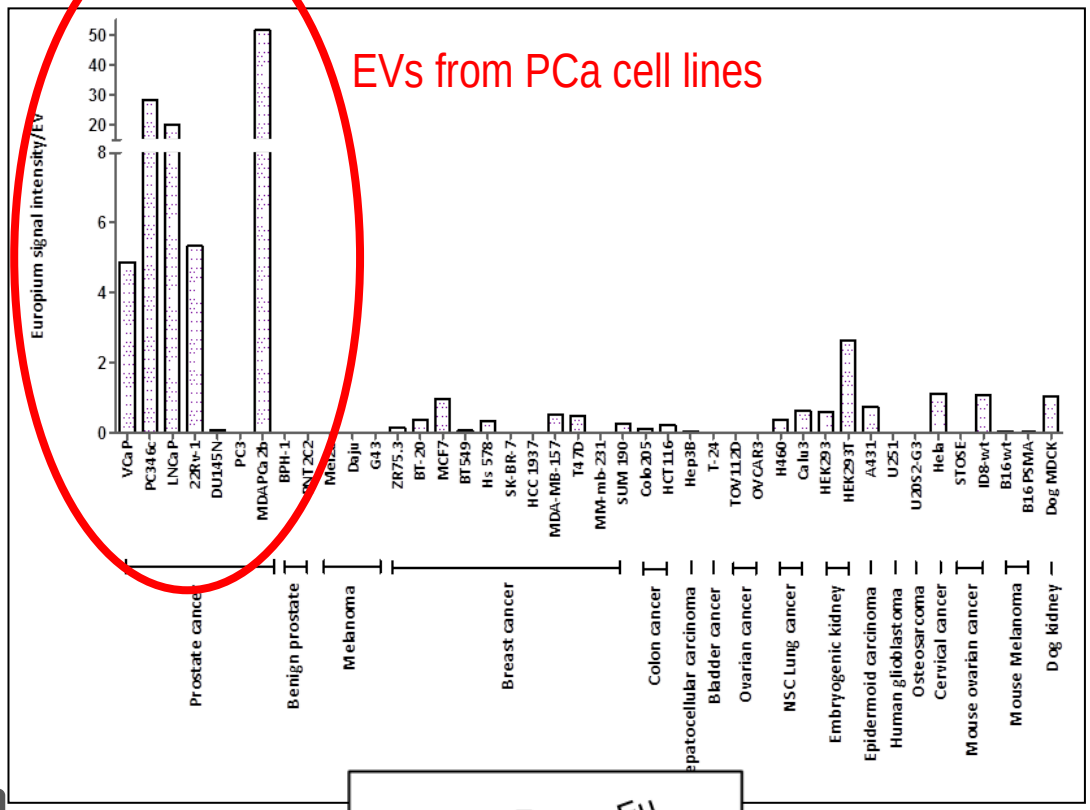
Nanobody Library

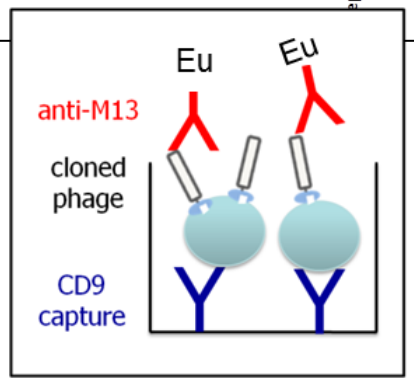






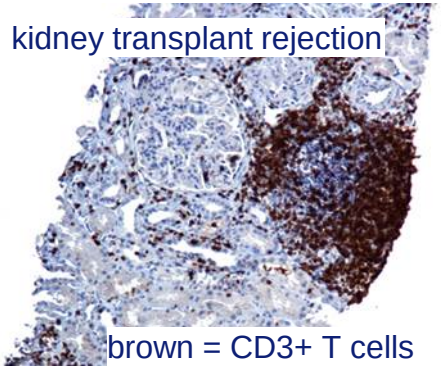
EVs from PCa cell lines





TR-FIA: Time-Resolved Fluorescence ImmunoAssay

LIquid BIopsies for Detection of Acute Rejection -> 'LIBIDAR'



Biopsy as gold standard for acute rejection after kidney transplantation

Drawbacks:
- invasive procedure with risk of complications
- impossible in combination with anti-coagulation treatment
- sampling error (small part of kidney)
- subject to interobserver variability

→ Is it possible to diagnose kidney transplant rejection early, and minimally invasive in blood or urine via detection of donor-specific EVs?

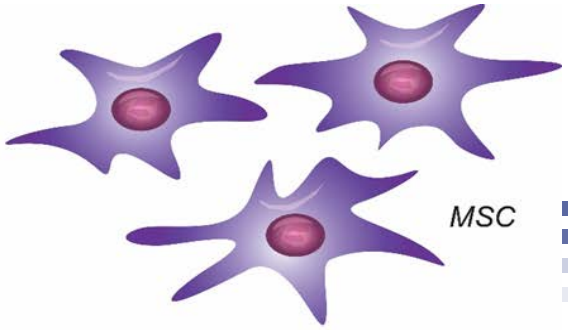
- Do EVs play a significant role in the anti-donor immune response after transplantation?

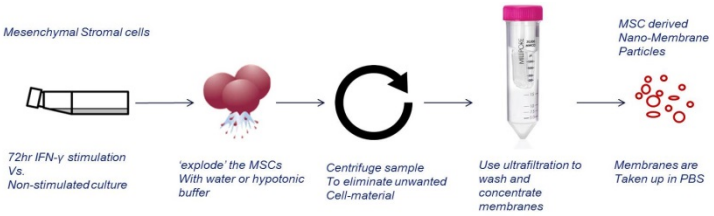
Internal Medicine
Nephrology and Transplantation

Erasmus MC

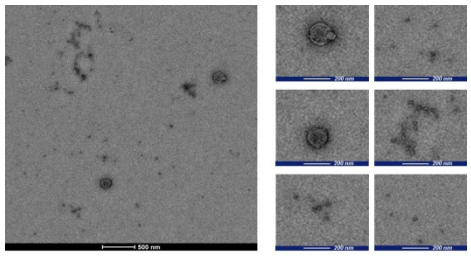
Objective: Characterize Membrane Particles generated from Mesenchymal Stromal Cells cultured with and without IFN-γ, analyze their immunomodulatory properties, and their interaction with the immune system.

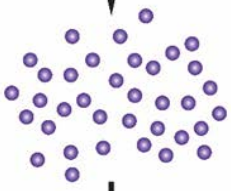
Ana Merino & Martin Hoogduijn

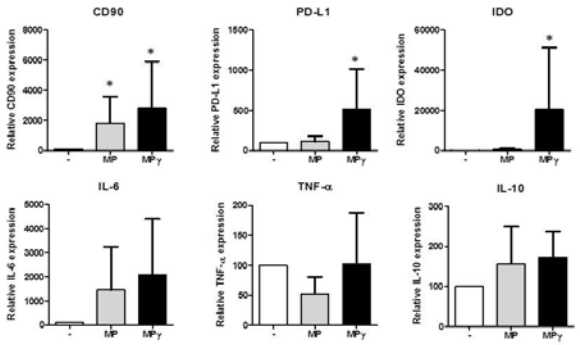




Membrane particle generation

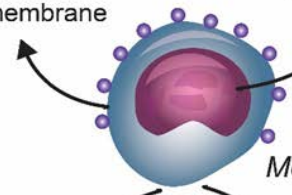




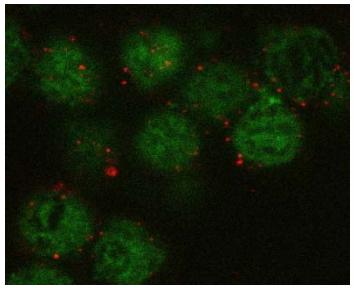


Bind to the surface membrane

Effects on gene expression

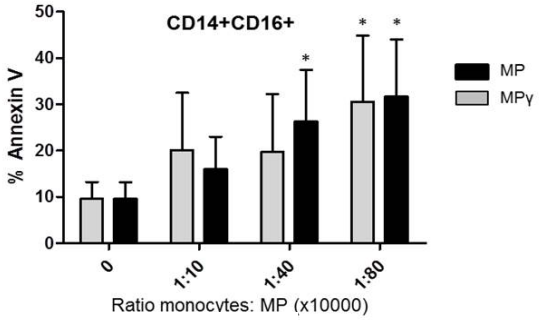


Monocyte



Immune modulation

Apoptosis of proinflammatory monocytes



Conclusion: Membrane particles generated from MSC modulate immune responses by selective targeting of pro-inflammatory monocytes

Urinary Extracellular Vesicles (uEVs)

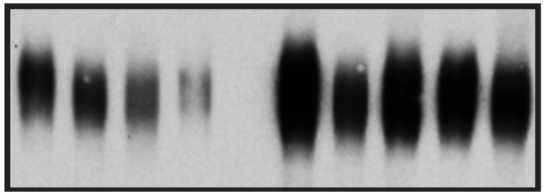
Department of Internal Medicine, Division of Nephrology & Transplantation

Aim 1 *Quantification and normalization of uEVs*
Aim 2 *Identification of disease markers in uEVs*

Example 1

Sodium transporters in uEVs as marker for hypertensive disease

Phosphorylated NCC

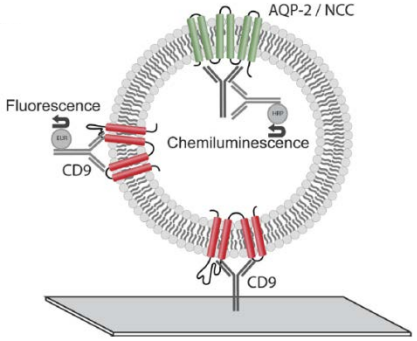


Essential hypertension | Primary aldosteronism

Van der Lubbe *et al.*, Hypertension 2012

Example 2

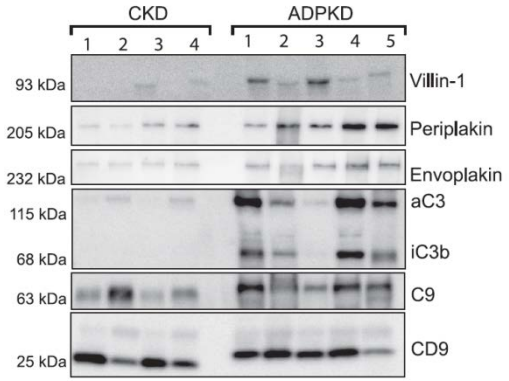
Development of immunoassay for tubular transport proteins



Salih *et al.*, Am J Physiol Renal Physiol 2016

Example 3

Proteomic identification of disease markers for polycystic kidney disease

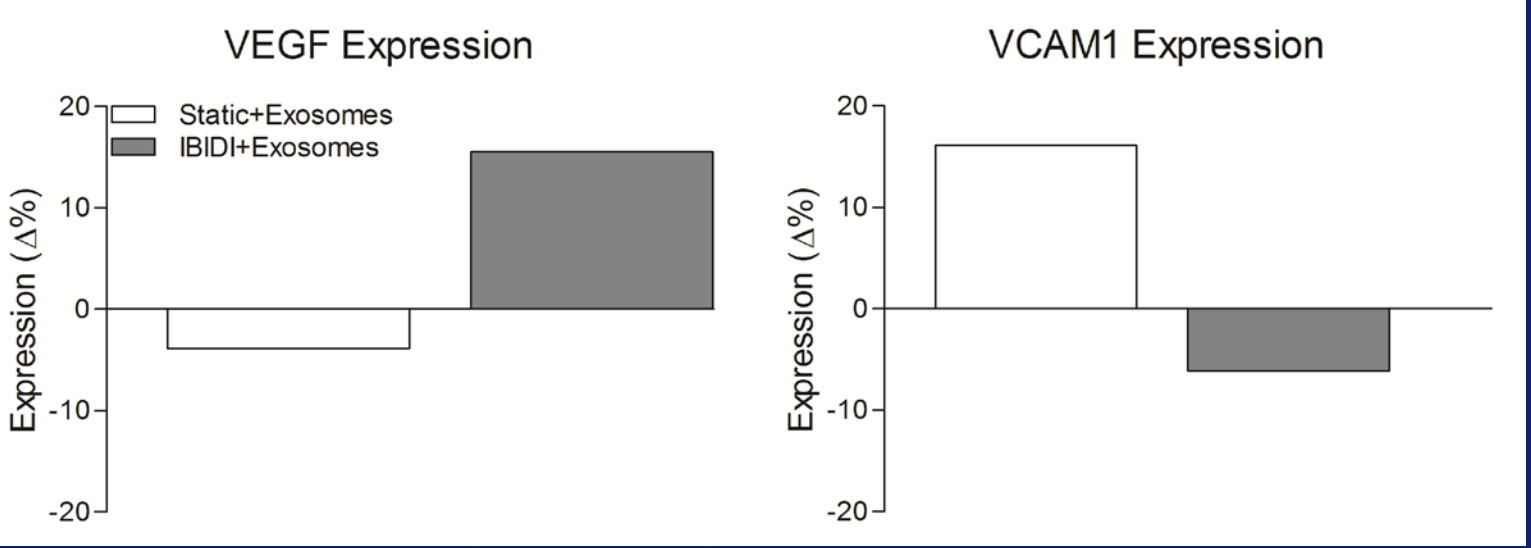


Salih *et al.*, J Am Soc Nephrol 2016

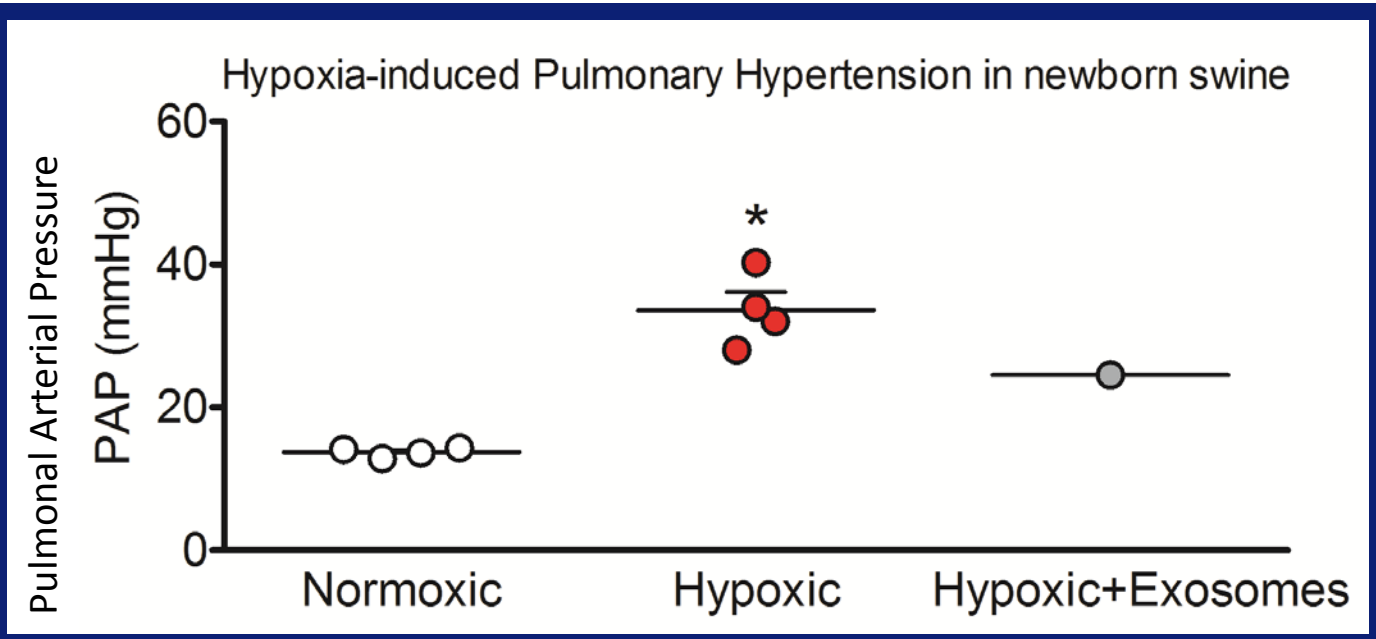
Welcome at today's poster by Charles Blijdorp (abstract no. 5)!

People: Prof. Ewout Hoorn (PI), prof. Bob Zietse, Charles Blijdorp, Mahdi Salih
Contact: e.j.hoorn@erasmusmc.nl

Exosome Therapy for Cardio-Pulmonary Disease



In vitro Swine endothelial cells incubated with human EVs without and with flow (IBIDI)



Experimental Cardiology

Principal Investigator
Daphne Merkus

Postdoc
André Uitterdijk

PhD-Candidate
Jarno Steenhorst

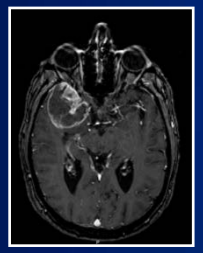
Research Technician
Esther van de Kamp

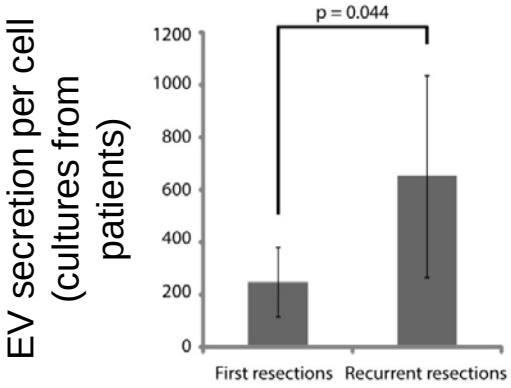
d.merkus@erasmusmc.nl

***Jeroen de Vrij** et al.*

Dept. of Neurosurgery, Erasmus MC, NL

❖ EVs to monitor glioblastoma progression and resistance to therapy





Primary → **Recurrent**

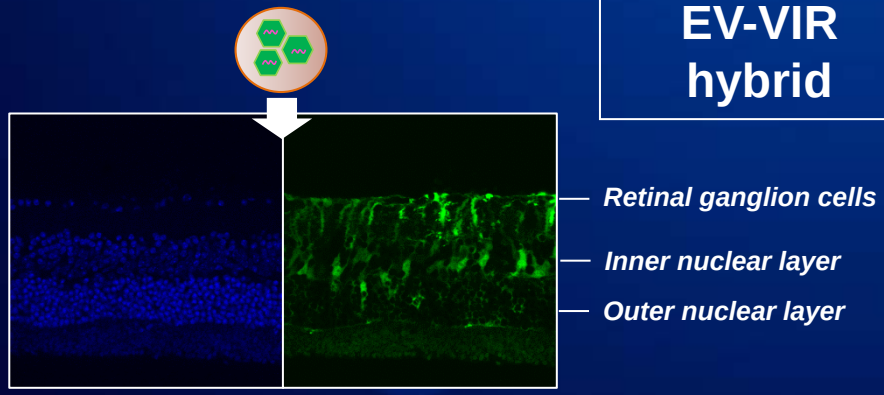
Chemo/RT sens. → Chemo/RT resist.

- EV secretion increases
- Per EV increased aggressiveness on stromal cells → different contents?

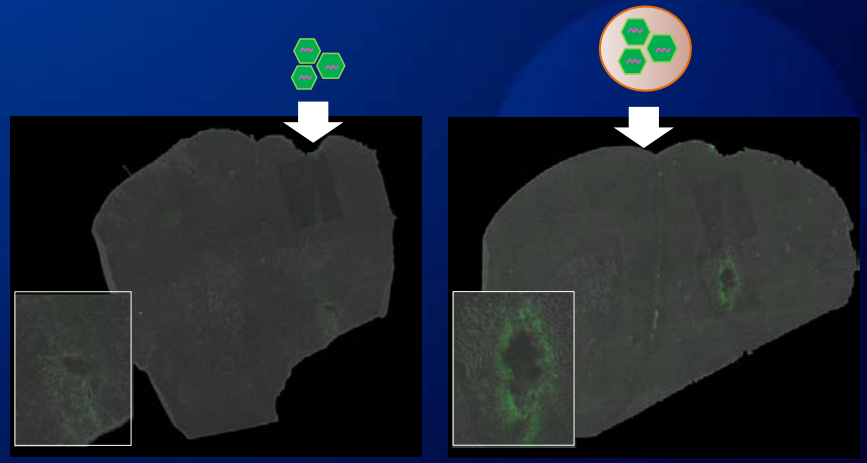
From De Vrij, Maas, Broekman. Int J Cancer, 2015

❖ "Virosomes" for improved (cancer) gene therapy

EV-VIR hybrid



→ **Deep penetration into retinas**

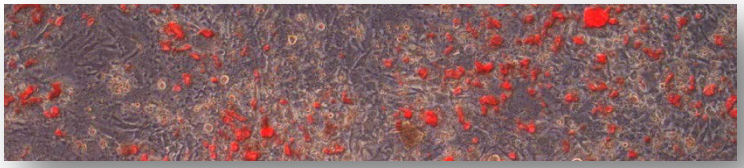


→ **Delivery to brain tumors**

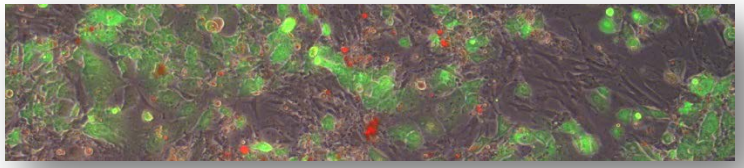
Role of extracellular vesicles in crosstalk of cancer cells to bone

1 Role in the direct interaction of metastatic cancer cells with bone cells?

Transfer of factors regulating bone destruction and cancer cell growth



+ cancer cells^{GFP} →



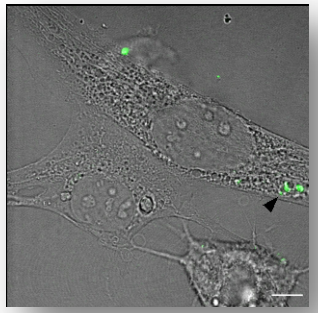
Bone cells: mineralization (orange)

Affected mineralization, survival of cancer cells

2 Role in preparing a pre-metastatic niche in bone?

+ Subsequent change in bone cell behaviour?

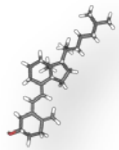
Prostate cancer cell derived EVs:
uptake in very few osteoblasts



3 Role in the therapeutical effect of vitamin D in bone metastases?

Are EVs part of Vitamin D working mechanism?

↓



- Vitamin D effect on cancer cell EVs?
- Vitamin D effect on cancer cell EV interaction with bone?
- Vitamin D incorporated in EVs?

Joëlle Klazen, Iris Robbesom, Hans van Leeuwen, Marjolein van Driel

Department of Internal medicine, Erasmus MC, Rotterdam

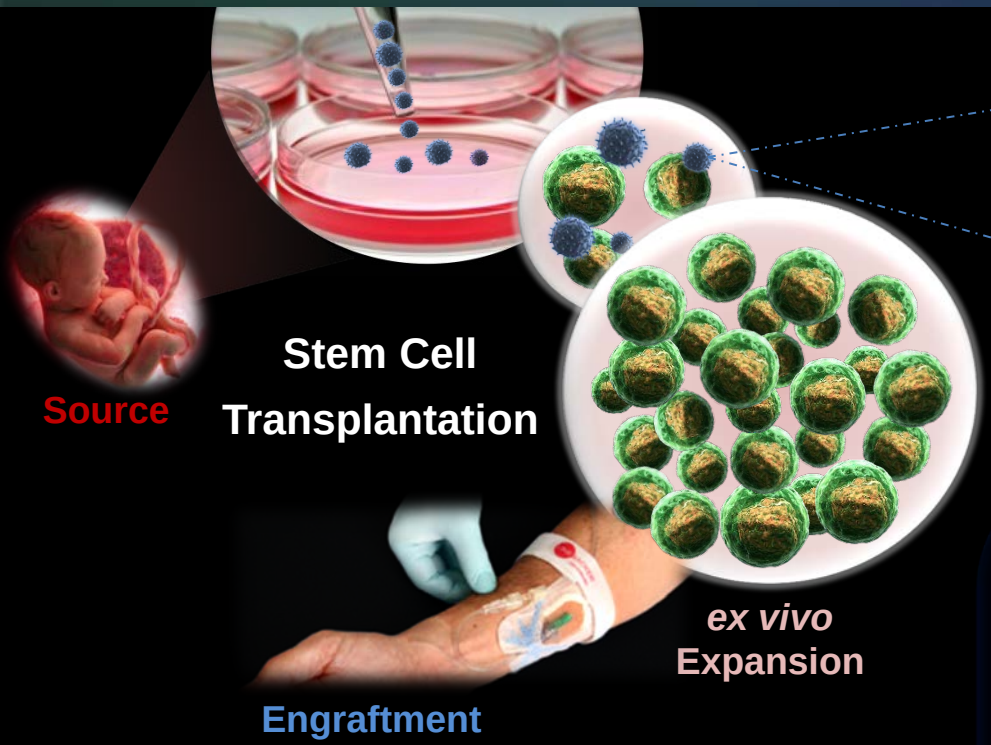
Collaborations Erasmus MC: Thomas Hartjes, Martin van Royen (Pathology, Optical Imaging Center)

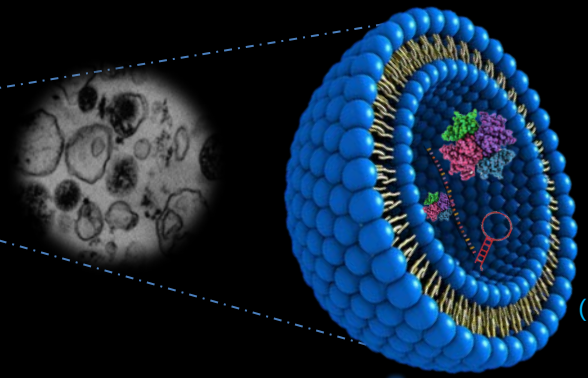
Erasmus MC
Universitair Medisch Centrum Rotterdam

j.morhayim@erasmusmc.nl

Hematology & Internal Medicine

Osteoblast-Derived Extracellular Vesicles

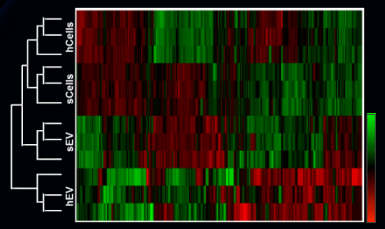


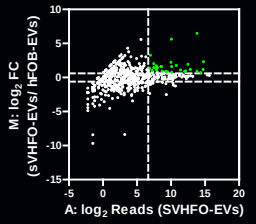


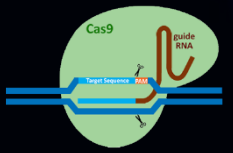
Proteins
(FASEB J. 2015)

miRNAs
(Sci. Rep. 2016)

mRNAs
(BBA Mol. Cell Res. 2017)



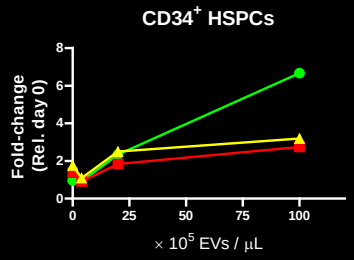




Osteoblast-EVs promote growth factor-driven expansion of cord blood-derived hematopoietic stem and progenitor cells (HSPCs)

EV-expanded HSPCs retain their differentiation capacity *in vitro* and *in vivo*

EVs from different osteolineage sources do not have same expansion potential



EV-miRNA sequencing analysis shows significant differences between stimulatory and less-stimulatory EVs

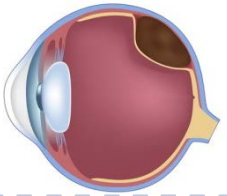
Ongoing studies

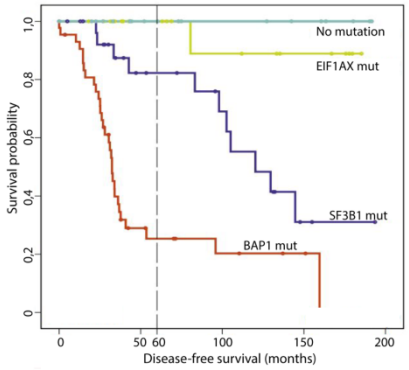
- Selection of candidate EV-miRNAs that are known regulators of hematopoiesis
- Candidate miRNA knock-out with CRISPR/Cas9 technology

ROMS
Rotterdam center of expertise uveal melanoma

Exosomes in Uveal Melanoma

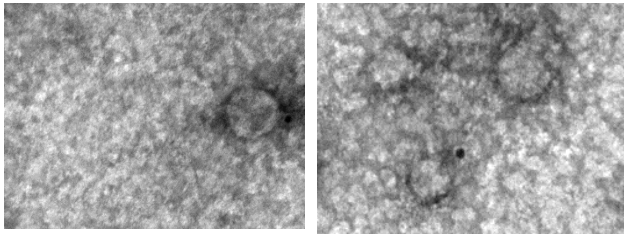
prognostication of UM patients by analyzing exosome content

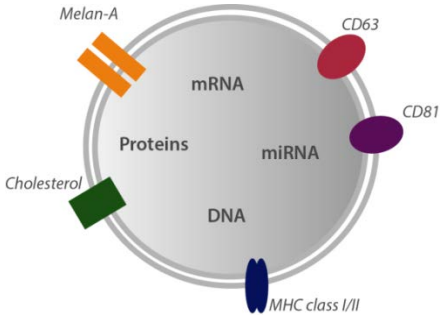




Three different risk groups are observed in UM:
(low, intermediate and high metastatic risk)

UM exosomes contain melanocyte specific proteins on their surface





Exosomal content is currently being analyzed by NGS, to determine if it can be used as a non-invasive biomarker

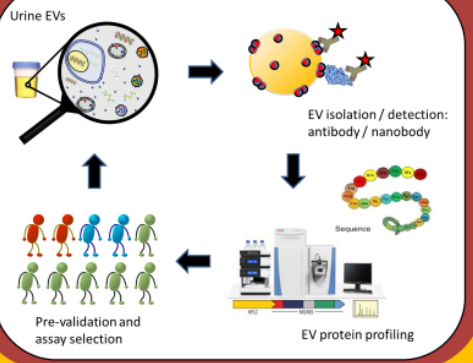
Department of Clinical Genetics & Ophthalmology
Principal investigators: Annelies de Klein & Emine Kiliç
PhD student: Kyra Smit

Erasmus MC
Universitair Medisch Centrum Rotterdam

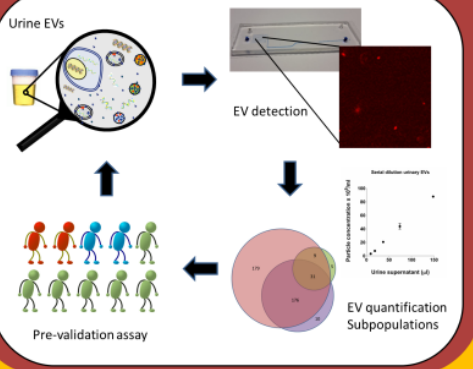
Innovative Measurements and Markers for PROstate cancer diagnosis and prognosis using extracellular VEsicles

g.jenster@erasmusmc.nl

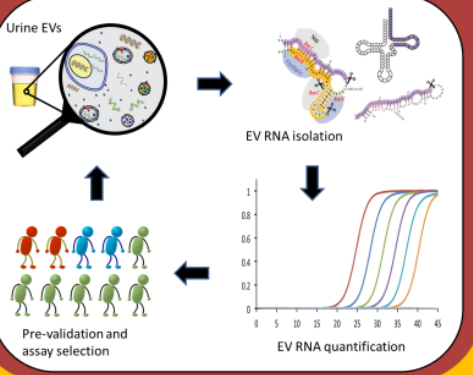
EV protein markers



EV quantification



EV RNA markers

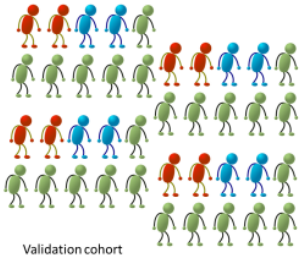


- Enrichment assay for prostate (cancer) EVs
- Pre-validated candidate markers

- Microfluidics assay development
- Quantification method for prostate (cancer) EVs and EV subpopulations
- EVQuant pre-validation assay

- Optimized assay for RNA measurement
- Pre-validated RNA candidate markers

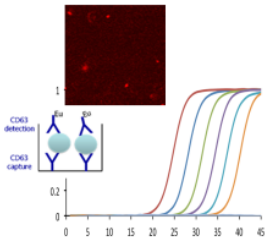
Translating into clinical practice – Validation trial


Validation cohort

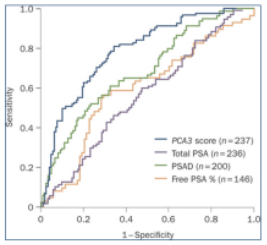
Sample Type	WP4 Validation (n)
Urine control groups	
PSA <1 AND no biopsies	100
PSA<10 AND biopsies negative AND no rising PSA in 2 years	100
Urine indolent PCa group	
PSA <10 AND biopsies: Gleason 6 AND low volume disease	200
Urine significant PCa group	
Gleason 4+3 or higher OR upgrading after surgery	100

Characteristics of patient and control samples

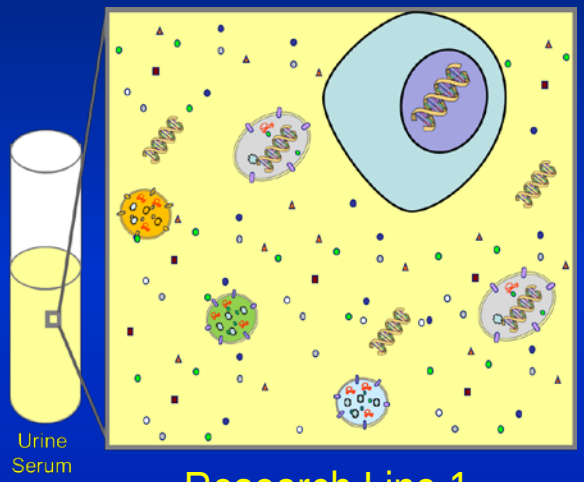
Urine biobank



Best performing EV assays



Comparison to current gold standards in clinical practice

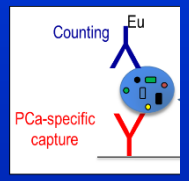


Research Line 1
EMC & VUmc cohorts

Purify EVs
Task 3.1.1

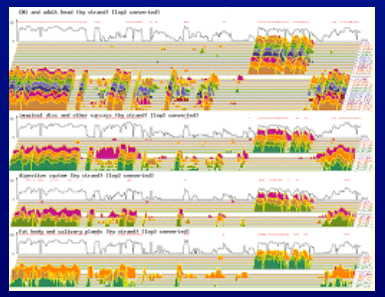
Filtration

Affinity purification



Small & long RNAseq
Task 3.1.2

RNAseq database
Task 3.1.3



Develop & Validate
RNA profiles
Task 3.1.4

- Novel profiles
- ExosomeDx profile
- PCA3 & TMPRSS2-ERG
- sdRNAs and tRFs
- PCa-associated RNAs
- miQ: miRNAs