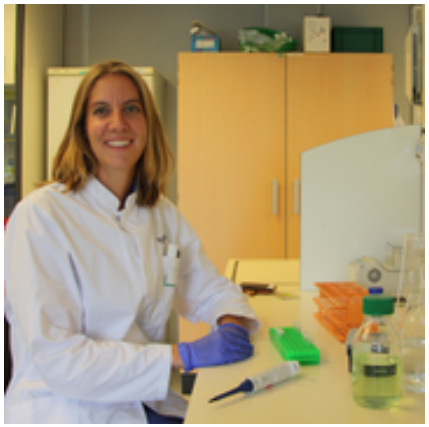
VUmc Cancer Center Amsterdam

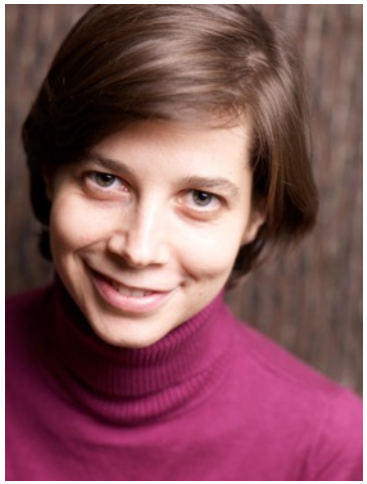
Rubina Baglio

Irene Bijnsdorp

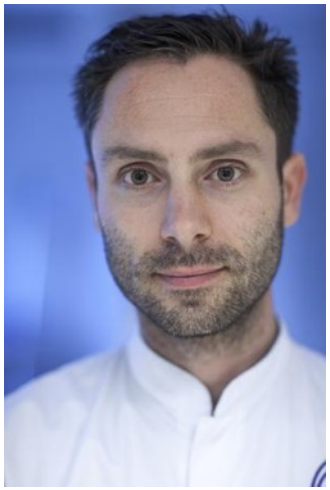


Jan vd Weering

Elisa Giovannetti



Michiel Pegtel



Tom Wurdinger

Connie Jimenez

Renee Musters

EV/ Exosome Proteomics VUmc

Clinical Proteomics @VUmc

Label-free Proteomics

Quality control
Careful study design

Extracellular Proteomics
Liquid biopsies

(Phospho) Proteomics
Solid biopsy

Integrative Analysis
Multi-Omics, Functional, Experimental, Clinical)

Non-invasive (early) diagnostics

Personalized (combination) therapy

Collaboration, Training, Dissemination, Valorisation

Bioinformatics, Biostatistics, Computational Biology

Shot-gun
Mass Spectrometry

Data-Independent
Acquisition
Mass Spectrometry

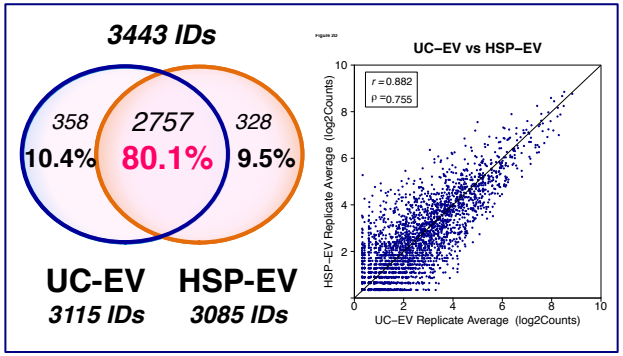
Connie Jimenez, c.jimenez@vumc.nl

www.oncoproteomics.nl

EV/ Exosome Proteomics VUmc

- EV proteome analysis of biomarker-rich proximal fluids:
  - Cancer cell & tumor tissue microenvironment (secretome)
  - Urine, CSF

- Novel HTP EV capture method bench-marked against ultracentrifugation for EV proteomics
  → HSP EV peptide capture method enables global exosome proteomics

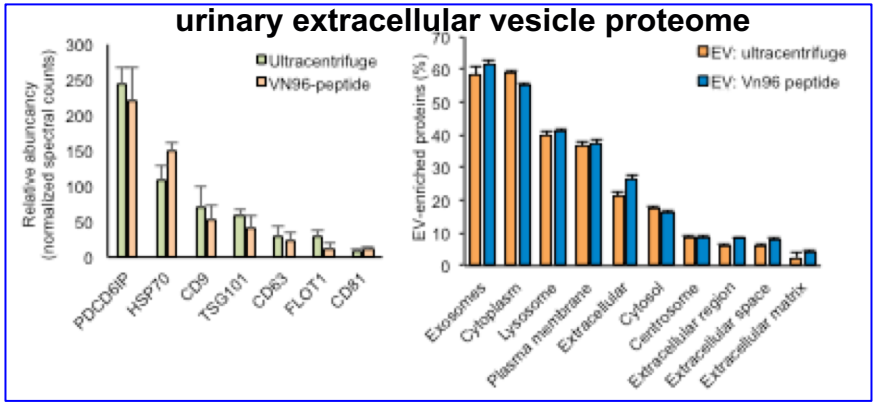


Knol, Jiménez et al. **Peptide-mediated 'miniprep' isolation of extracellular vesicles is suitable for high-throughput proteomics.** EuPA Open Proteomics Volume 11, June 2016, Pages 11–15

Bijnsdorp, Jimenez et al. **Feasibility of urinary extracellular vesicle proteome profiling using a robust and simple, clinically applicable isolation method.** *J Extracell Vesicles.* 2017; 6(1):1313091.

On-going:
Large-scale application to urine of prostate cancer patients

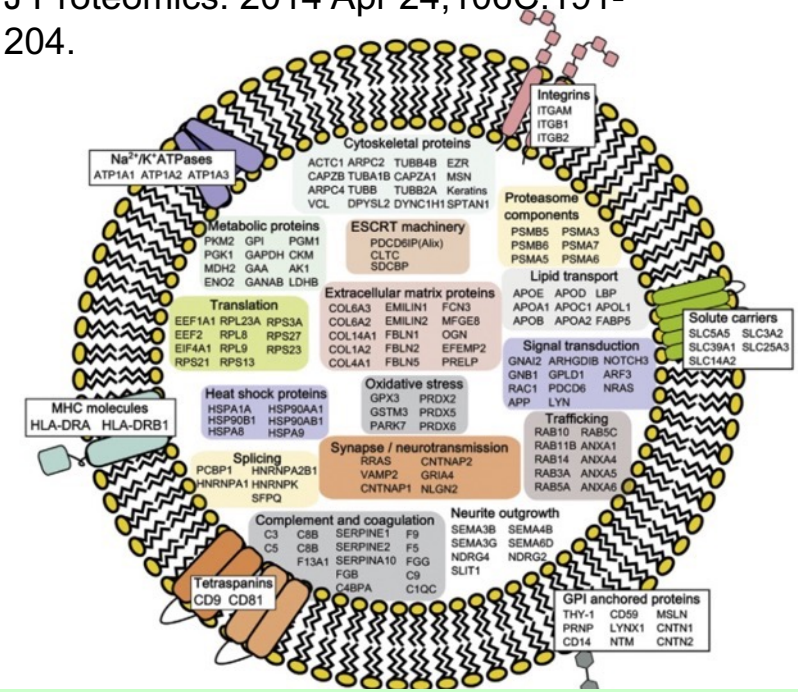
IMMPROVE



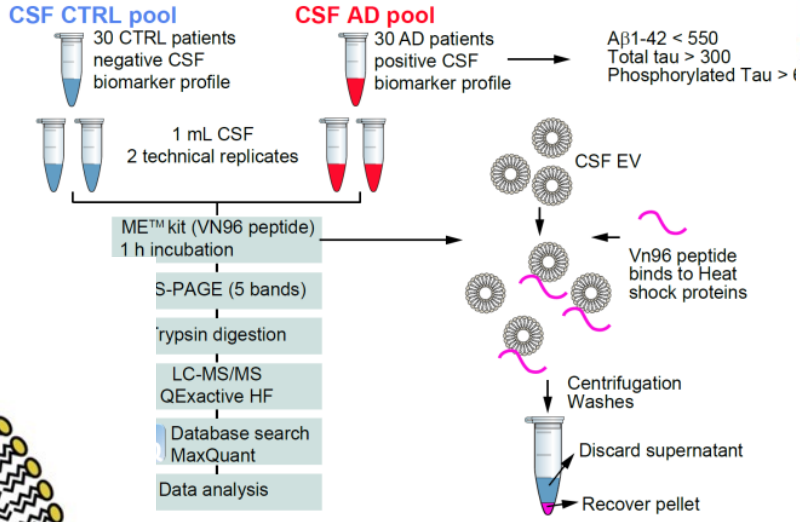
EV/ Exosome Proteomics VUmc

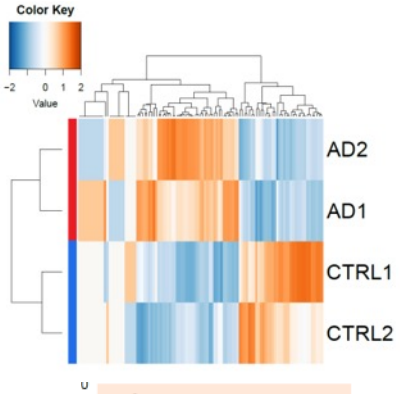
First CSF EV proteome

Chiasserini, Jiménez et al.
Proteomic analysis of cerebrospinal fluid extracellular vesicles: A comprehensive dataset.
J Proteomics. 2014 Apr 24;106C:191-204.



EV proteomics pilot on Alzheimer's disease CSF





1. Cellular localization, folding

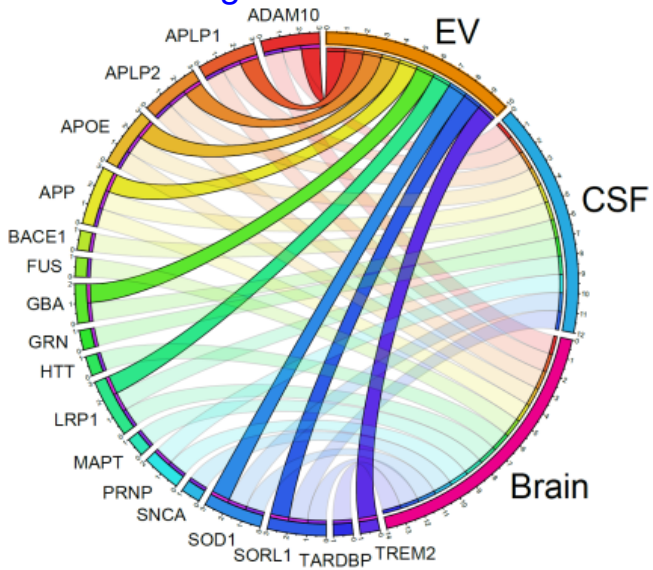
2. Extracellular structure organization, neuron differentiation, vesicle trafficking

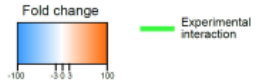
3. Proteoglycan metabolic process, dermatan-sulfate metabolic process

4. Extracellular matrix organization, biological adhesion

5. Protein N-glycosylation, N-glycan processing

CSF EV proteins involved in neurodegenerative diseases





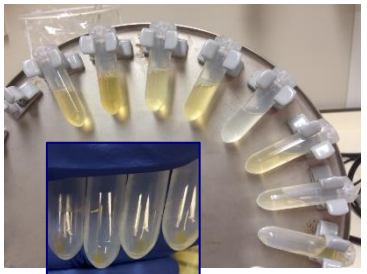
1. Complement activation, defense response

2. Response to ROS, oxidative stress response

3. Neuron projection development

4. NS

On-going:
Optimization of HSP EV capture for CSF exosome proteomics to enable large scale profiling in brain diseases



IMMPROVE

Prostate cancer EV research

Department of Urology, Irene Bijnsdorp

1. Proteomics profiling or urinary EVs:

- Diagnostic & prognostic testing
- Developed a clinical applicable urinary EV-capture method
- *Bijnsdorp et al, J Extracell Vesicles, 2017*
- Large biobank: urine and blood sample collection
- Current: validation of markerpanel

Alpe d'HuZes
opgevenisgeenoptie.nl

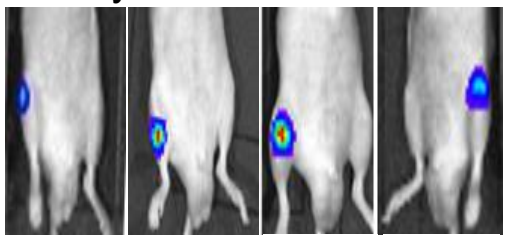
Consortium: Jimenez, Jenster and Schalken)

2. Biological role of prostate cancer EVs in metastasis:

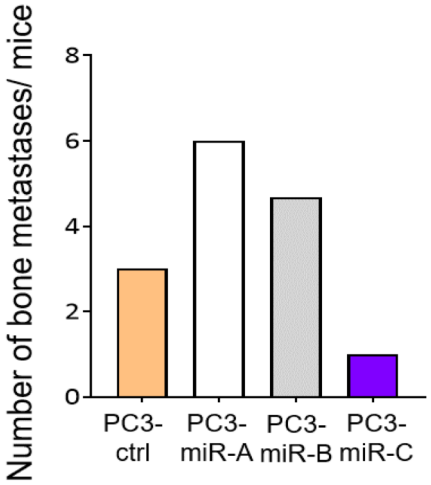
- Focus on preventing bone metastasis
- Mouse studies demonstrated that cancer EVs influence early metastasis: formation and type of bone metastases

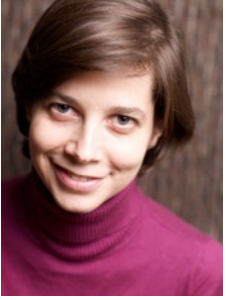
worldwide cancer research

Early metastasis formed



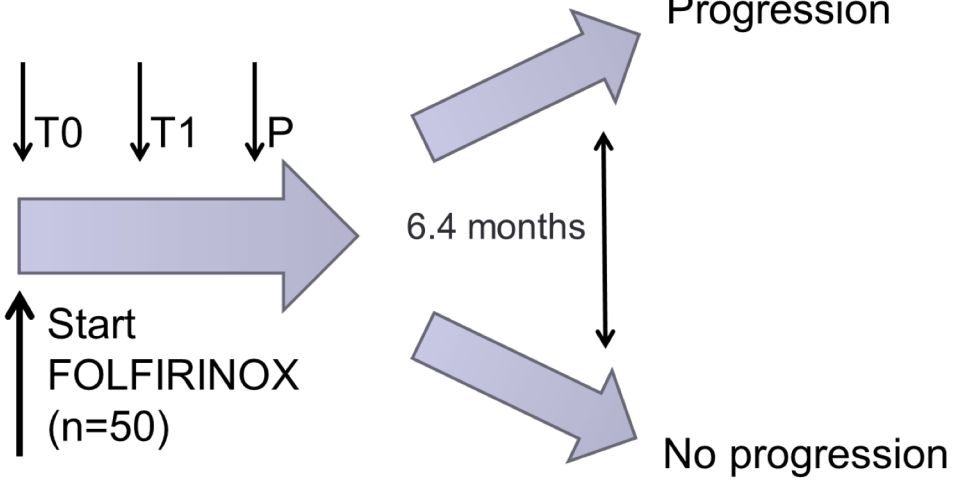
PC3-ctrl miR-A miR-B miR-C

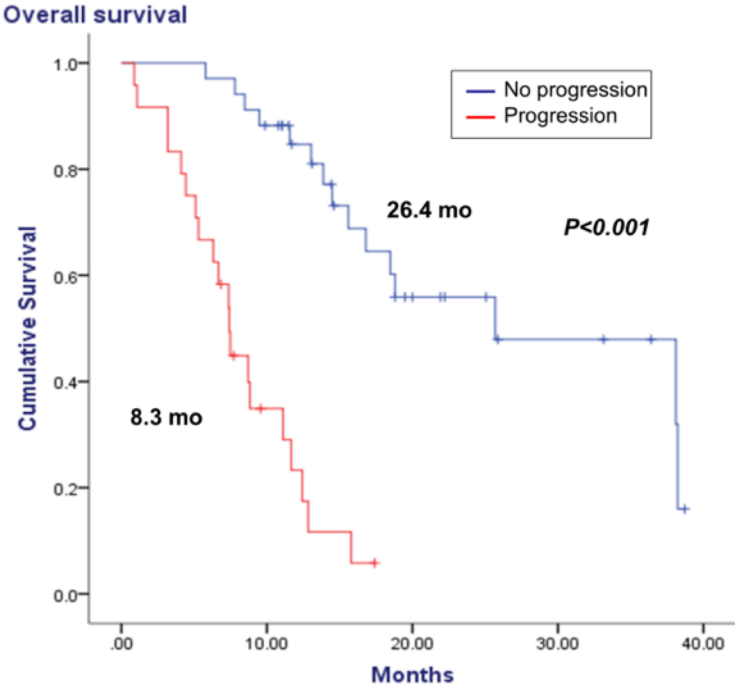




Predictive and monitoring biomarkers are essential to guide patient therapy in pancreatic cancer

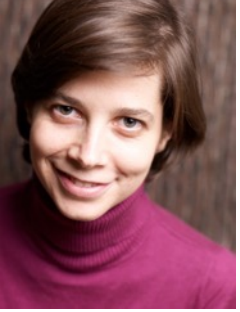
Methods: PCR array





→ Find predictive and monitoring miRNAs

Circulating microRNAs as dynamic biomarkers of response to treatment with FOLFIRINOX combination therapy in advanced pancreatic ductal adenocarcinoma. *Laura L Meijer, Adam E Frampton, Ingrid Garajová, Chiara Caparello, Tessa Y S Le Large, Niccola Funel, Enrico Vasile, Justin Stebbing, Jonathan Krell, Geert Kazemier, Elisa Giovannetti* http://www.thelancet.com/pdfs/journals/lancet/PIIS0140-6736(17)30464-6.pdf



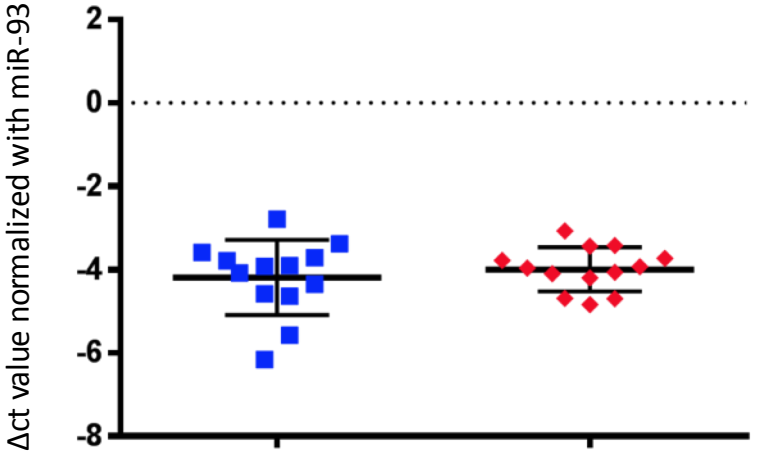
miR-29a is significantly upregulated in EVs after treatment in non-progressive patients

miR-29a in EVs

P=0.0024

Before treatment After treatment

Cell-free miR-29a



→ Validation to explore the monitoring potential

→ Functional experiments

Circulating microRNAs as dynamic biomarkers of response to treatment with FOLFIRINOX combination therapy in advanced pancreatic ductal adenocarcinoma. *Laura L Meijer, Adam E Frampton, Ingrid Garajová, Chiara Caparello, Tessa Y S Le Large, Niccola Funel, Enrico Vasile, Justin Stebbing, Jonathan Krell, Geert Kazemier, Elisa Giovannetti* http://www.thelancet.com/pdfs/journals/lancet/PIIS0140-6736(17)30464-6.pdf

VUmc Exosome Research group
Cancer Center Amsterdam

ERG Research

- Small RNA sorting into EVs and functional transfer
  - Pegtel et al., PNAS 2010
  - Koppers-Lalic et al., Cell Reports 2014

- Viral RNAs and EVs in autoimmunity
  - Pegtel PNAS 2014
  - Baglio et al., PNAS 2016
  - Van Dongen et al., MMBR 2016

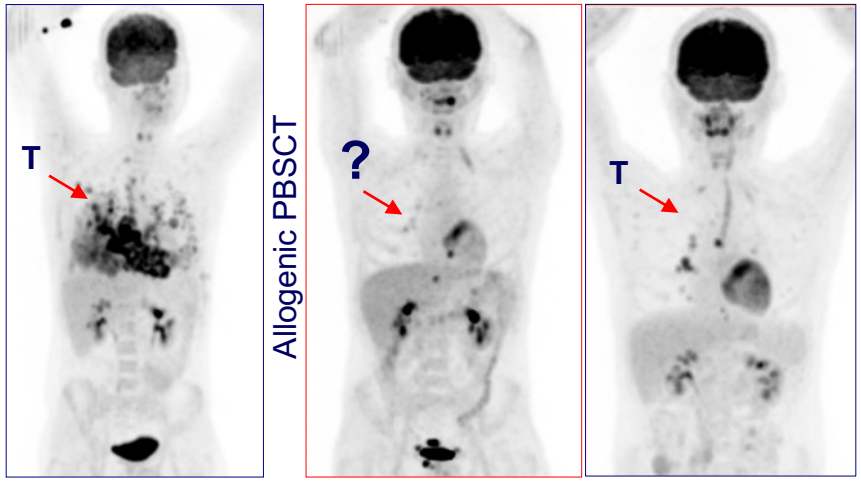
- EV small RNAs for Liquid biopsy approaches
  - Van Eijndhoven et al., JCI insight 2016

- Exosome biogenesis and release
  - Verweij et al., EMBO 2011
  - Verweij & Bebelman et al., JCB accepted

- Baglio group: EVs in the tumor-microenvironment
  - Baglio et al., Clin Canc Res 2017

- Integratie beeldvorming & moleculaire analyse voor een complete diagnose

FDG-PET

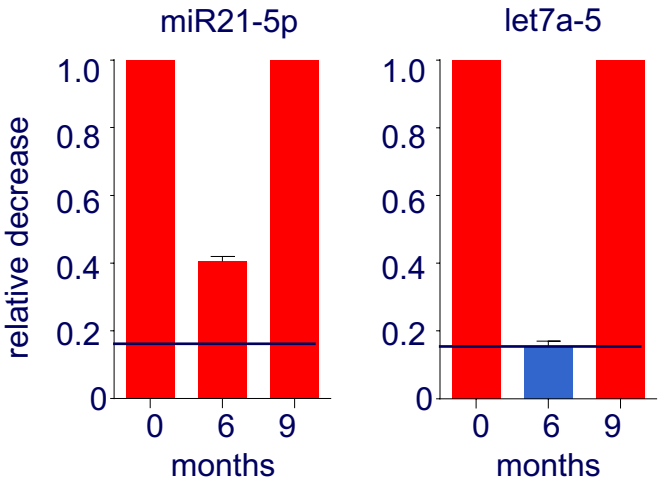


At presentation; t=0 months

End of treatment; t=7 months

Follow up; t=9 months

Bloed test

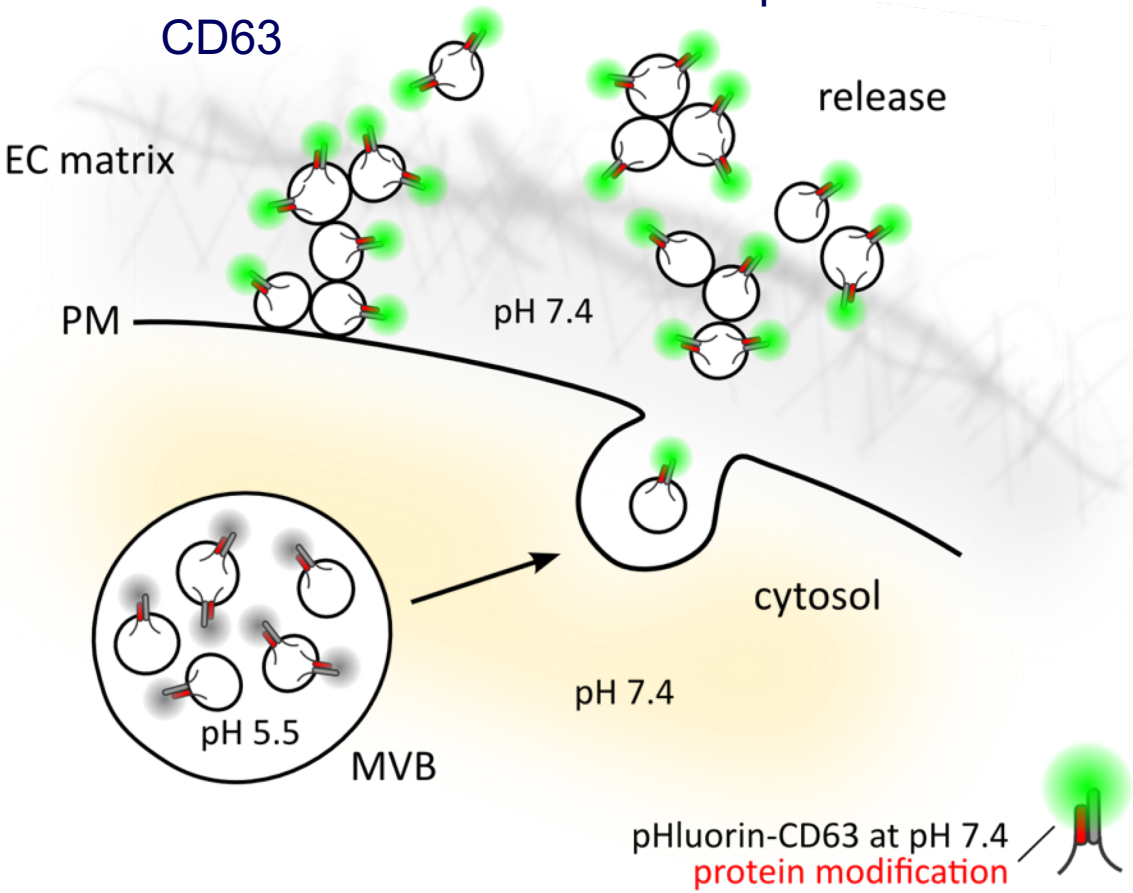


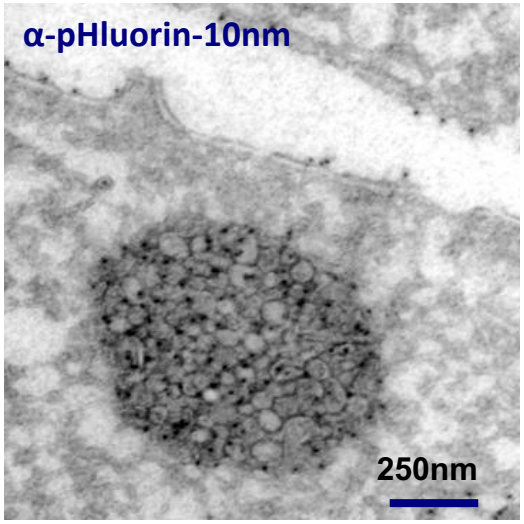
Van Eijndhoven et al., JCI insight 2016

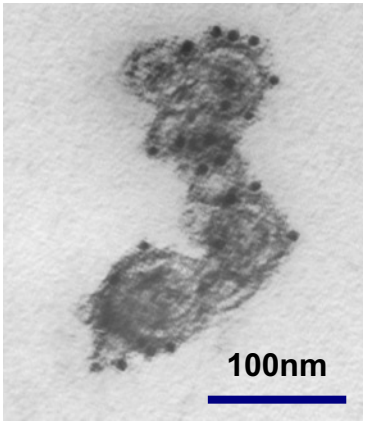
• Development of reporter for visualization of exosome release from living single cells

CD63-pHluorin

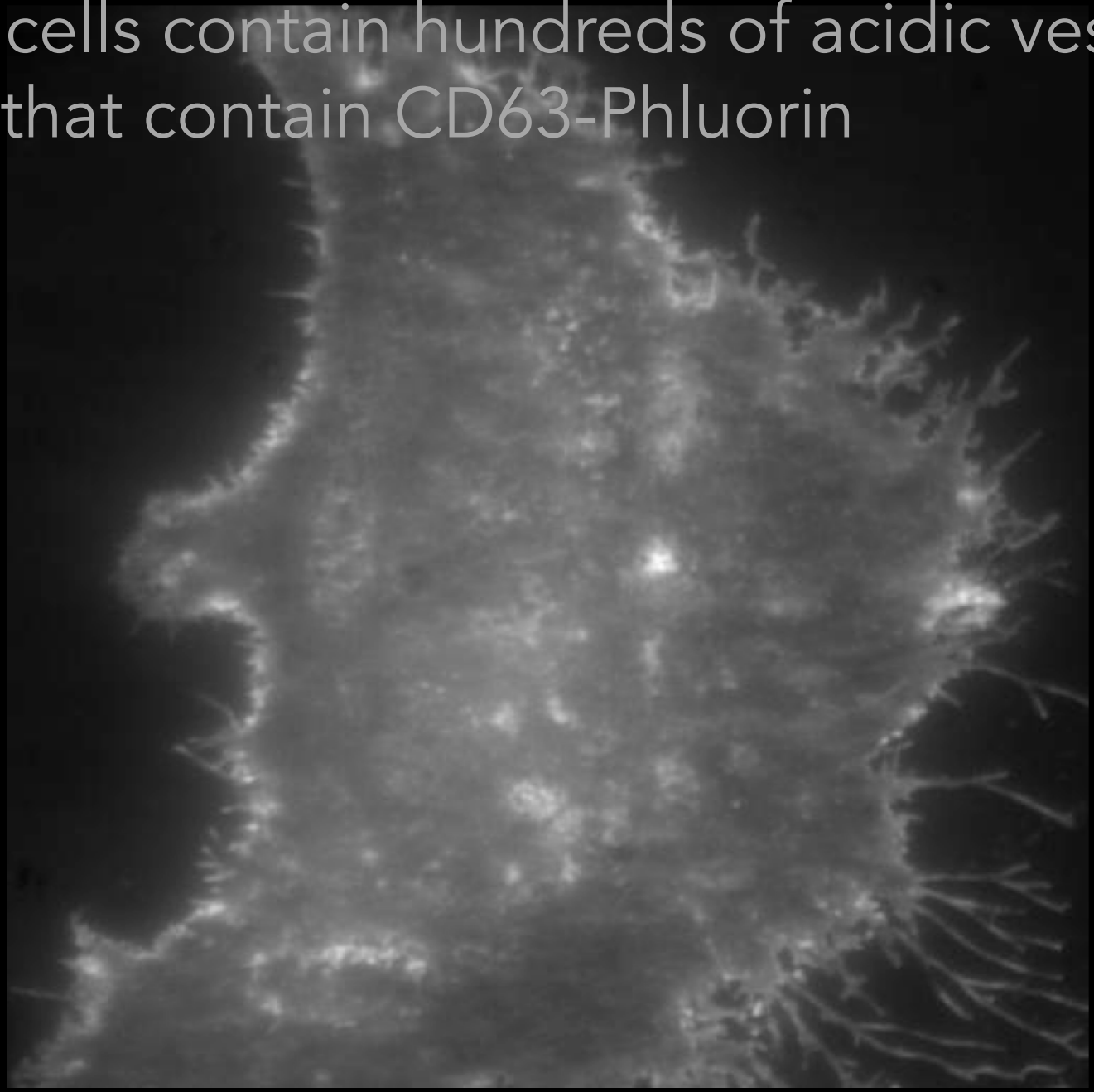
- pHluorin: pH-sensitive GFP variant
- Fused in 1st extracellular loop of CD63







Cancer cells contain hundreds of acidic vesicles (MVBs) that contain CD63-Phluorin

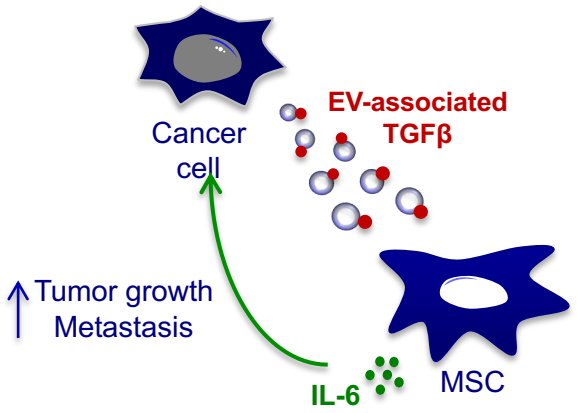


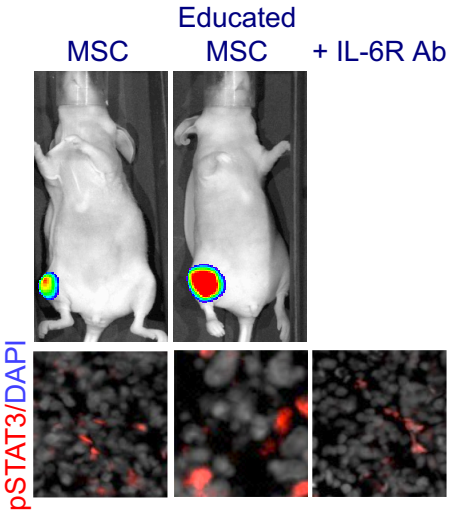
Verweij & Bebelman et al, JCB accepted

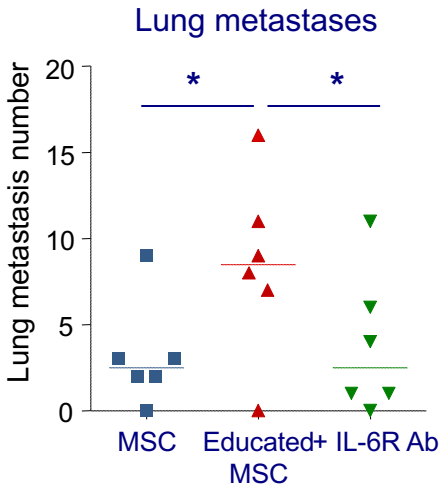
Tumor microenvironment and inflammation:

Preclinical mouse models to define cancer EV-induced alterations of the tumor microenvironment

- Bone cancer cells release **EVs** that "educate" **mesenchymal stem cells (MSC)** to favor cancer growth and lung **metastasis formation**
- Alterations of the MSC **cytokine expression profile** can be revoked using anti-inflammatory agents in xenograft models





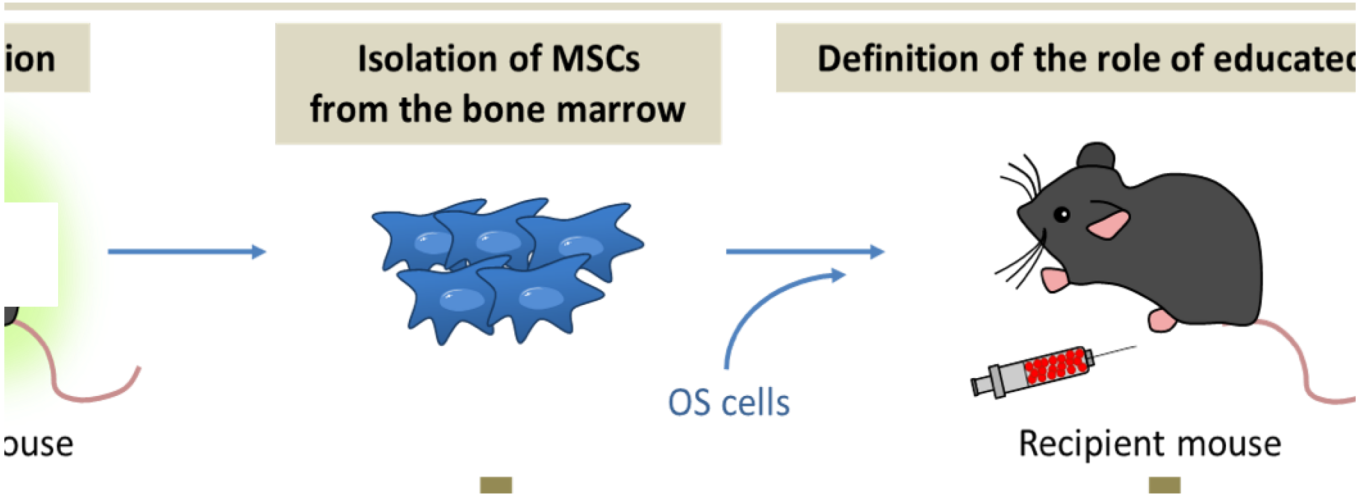


Baglio et al. Clin Cancer Res, 2017

Tumor microenvironment and inflammation:
Preclinical mouse models to define cancer EV-induced alterations of the tumor microenvironment

Using in vivo tumor models and next-generation techniques we aim to:

- Globally define the **immune profile alterations** induced by cancer EVs
- Evaluate the efficacy of specific combinations of **immunomodulatory drugs** to block the pro-metastatic effects of cancer EVs
- Identify circulating EV-associated biomarkers for treatment response prediction



We are currently looking for PhD candidates, visit www.exosomes.nl or www.werkenbijvumc.nl

KWF KANKER BESTRIJDING