

EXOSOMES AS POTENTIAL BIOMARKERS FOR CANCER

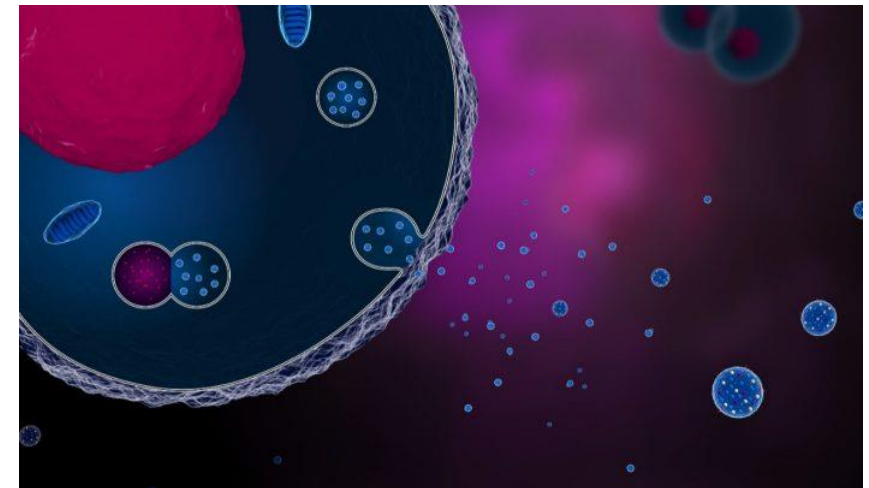
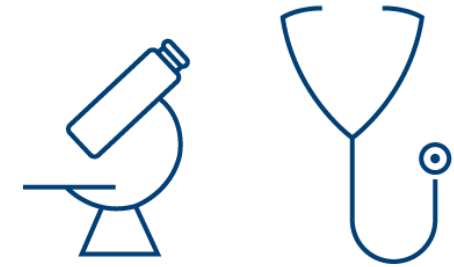
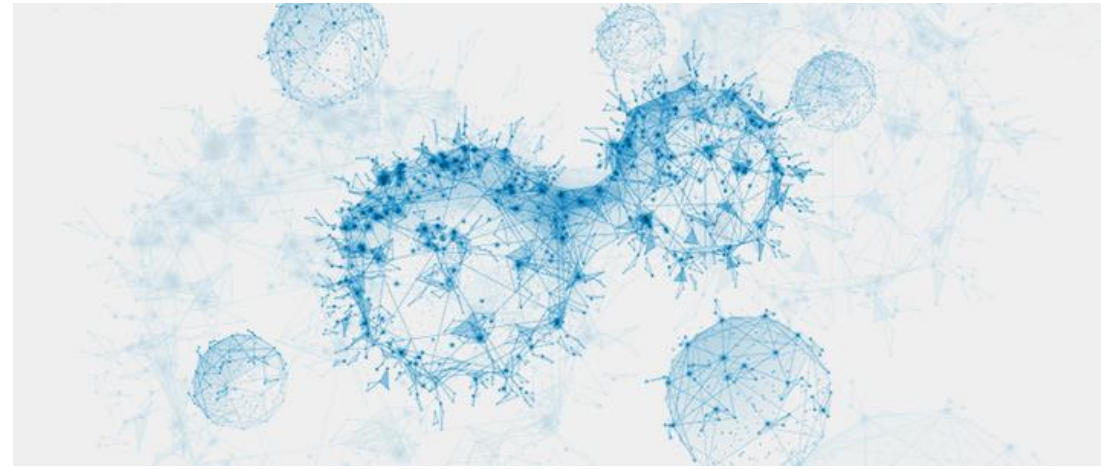
Natália Košútová ^a, Tomáš Bertók ^a, Katarína Kozics ^b, Alena Gábelová ^b, Jan Tkáč ^a

^a *Institute of Chemistry, Slovak Academy of Sciences, Dúbravská cesta 9, Bratislava 845 38, Slovak Republic*

^b *Biomedical Research Center, Slovak Academy of Sciences, Dúbravská cesta 9, 845 05 Bratislava, Slovak Republic*

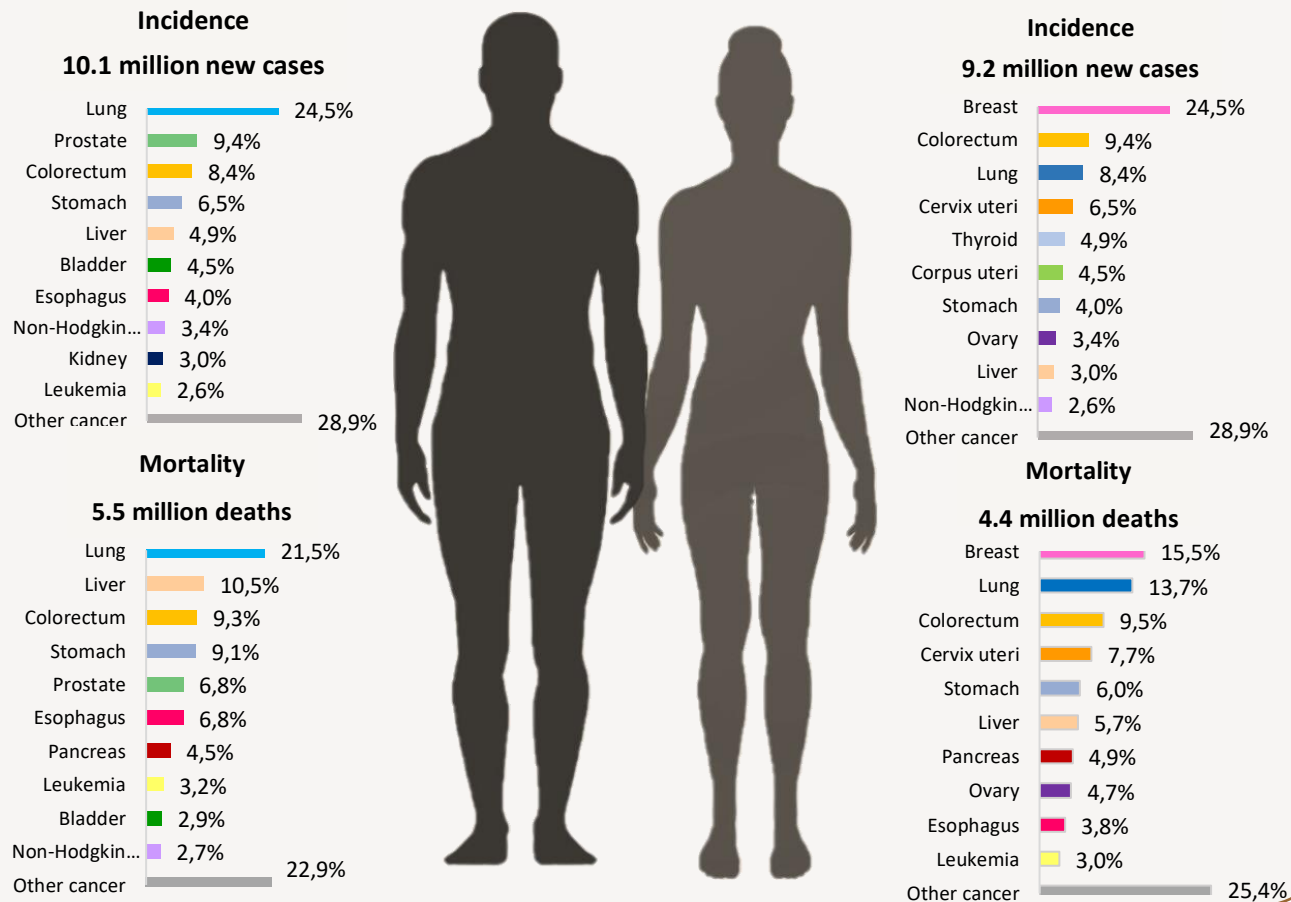
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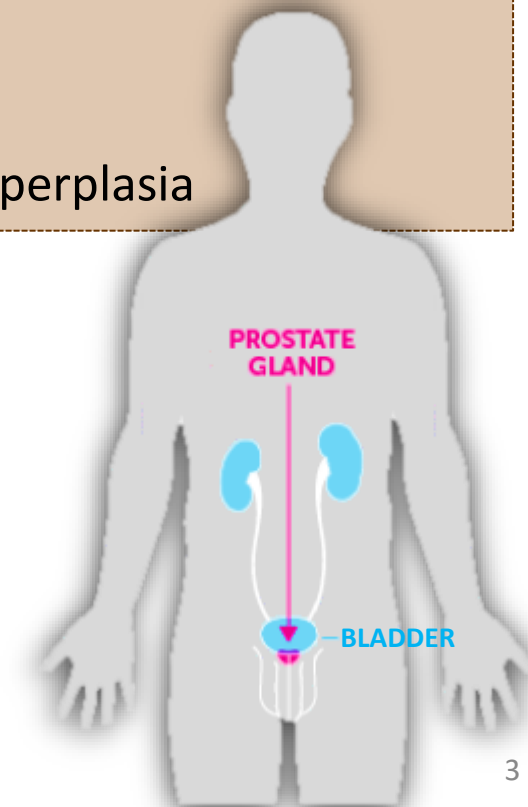
Cancer

- One of the most frequently diagnosed diseases in the world
- Detecting the disease during its early stage
 - Effective and mostly curable therapy



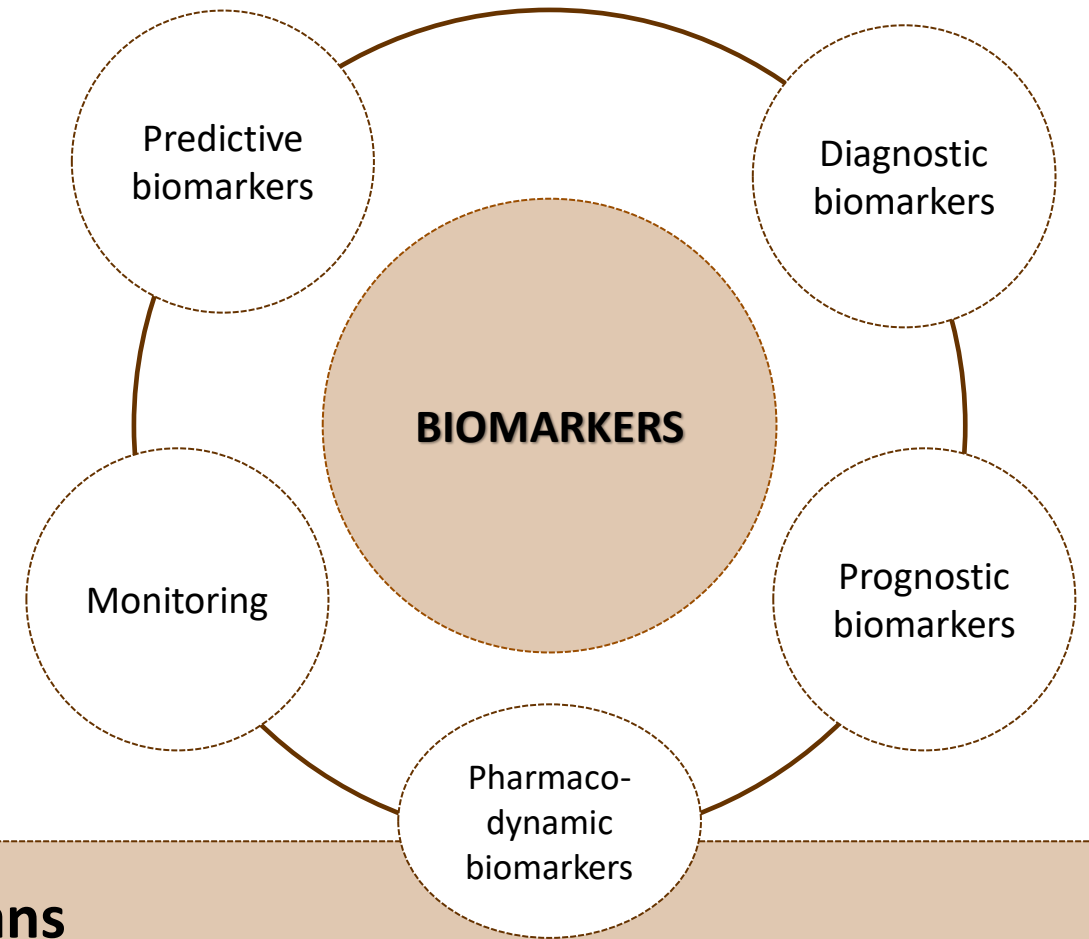
Prostate cancer

- One of the most common cancer in men
- Survival is related to tumor stage and grade
- **1.4 million new cases and 375,000 deaths**
- Prostate-specific antigen
 - Prostate cancer
 - Prostatitis
 - Benign prostatic hyperplasia



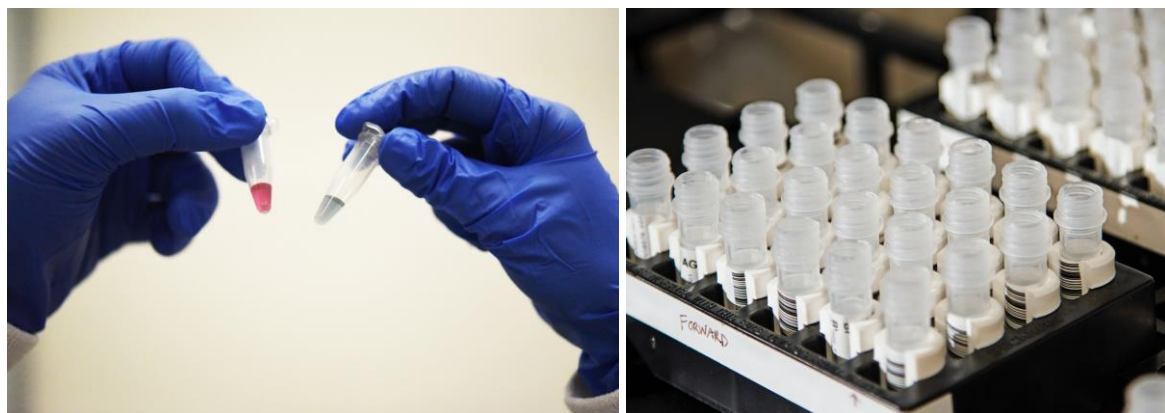
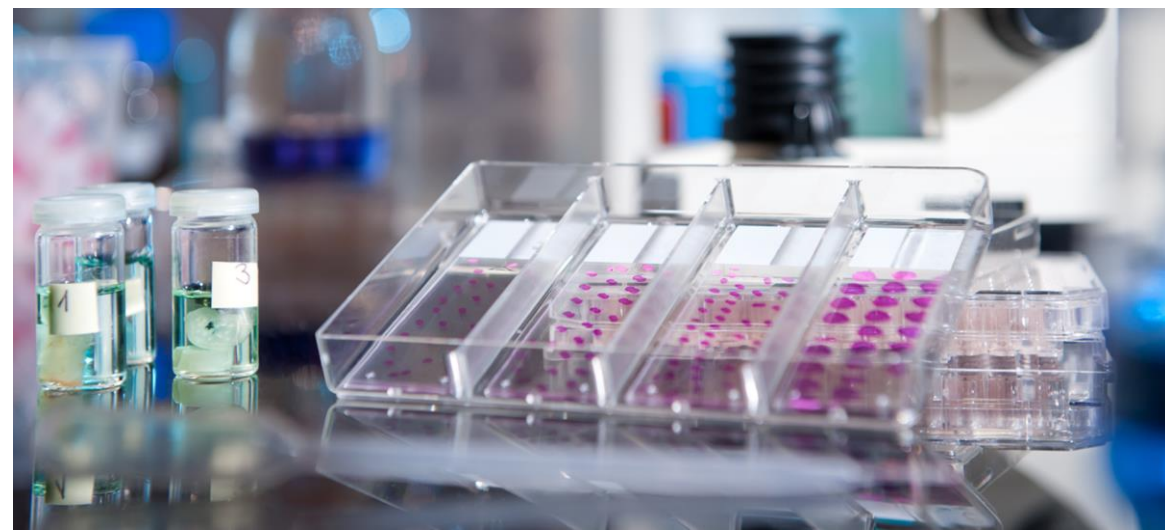
• Diagnosis

- Traditional biopsy
- Liquid biopsy

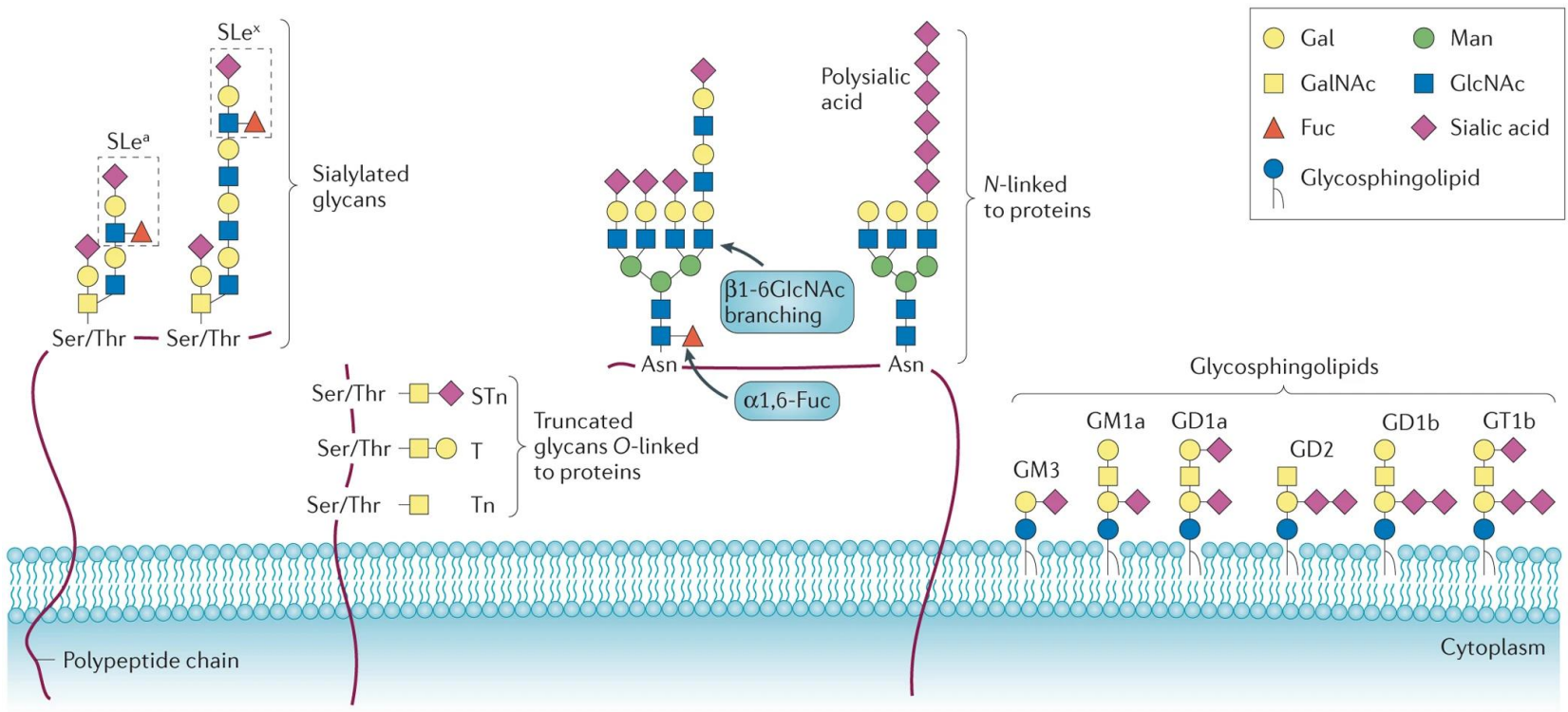
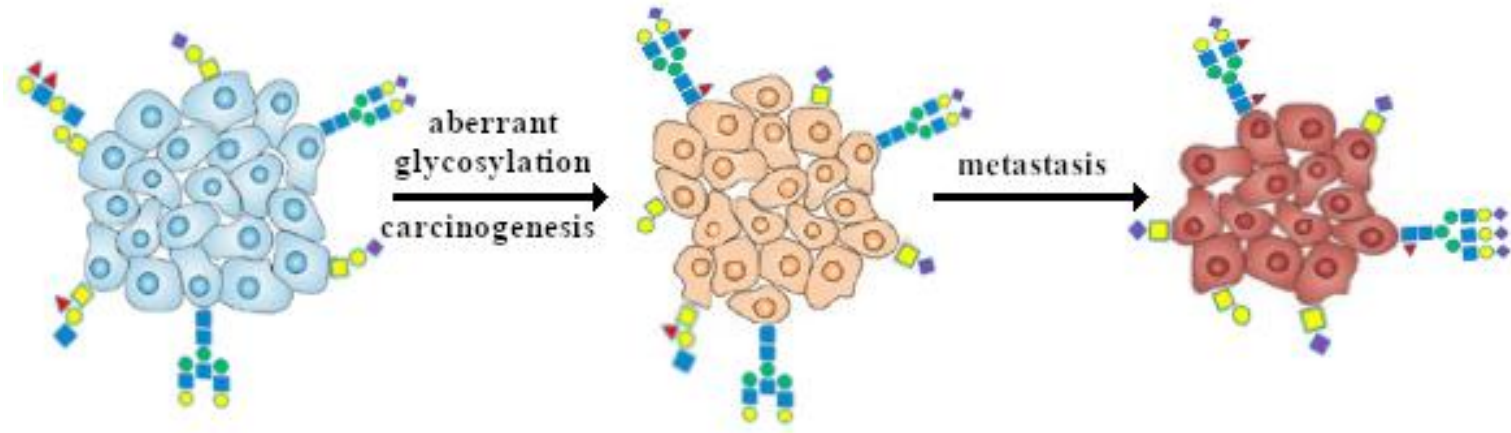


Glycans

- Complex carbohydrates
- Protective, stabilizing, organizational, and barrier functions
- Glycosylation
 - Essential role in physiological and pathological processes
 - N-glycans, O-glycans



- Aberrant glycans
 - Sialylation
 - Fucosylation
 - Lewis^x motives
 - Antennarity, branching ...



Blood-based biomarker

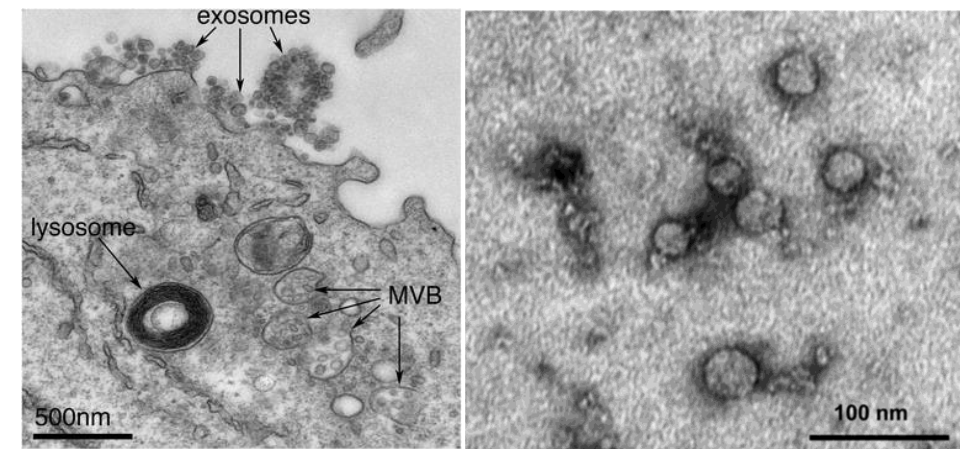
vs.

Exosomes

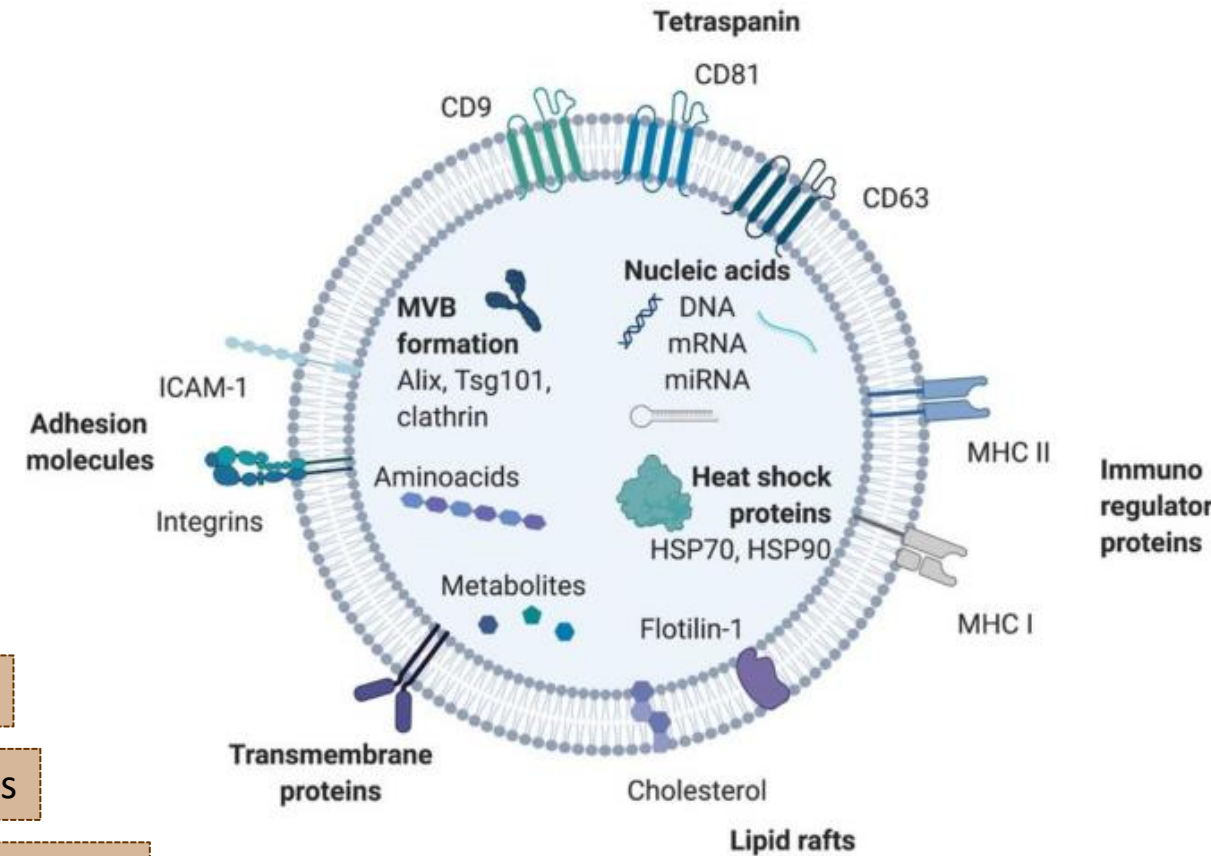
- Composition of plasma
- Low concentrations
- Low specificity

Exosomes

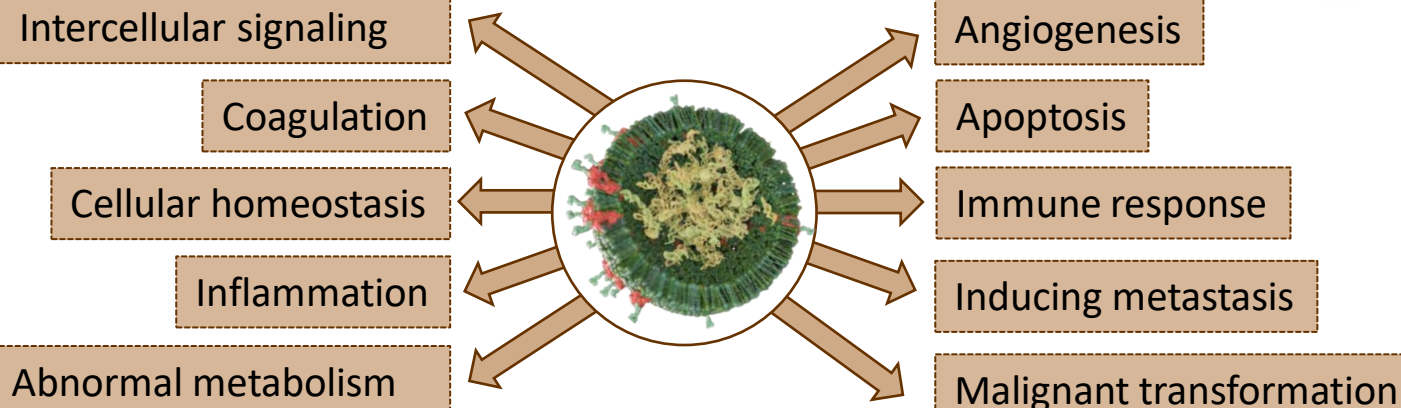
- Naturally produced biological nanoparticles [30 - 200 nm]
- Blood, saliva, urine, cerebrospinal fluid, breast milk
- Very rich range of contents
 - 9,000+ proteins, 6,000+ RNAs and 1,000+ different lipids
- Diagnosis and therapy
 - Biomarker
 - Carrier for specific biomarkers



Muller et al, 2016.




Valencia et al., 2021



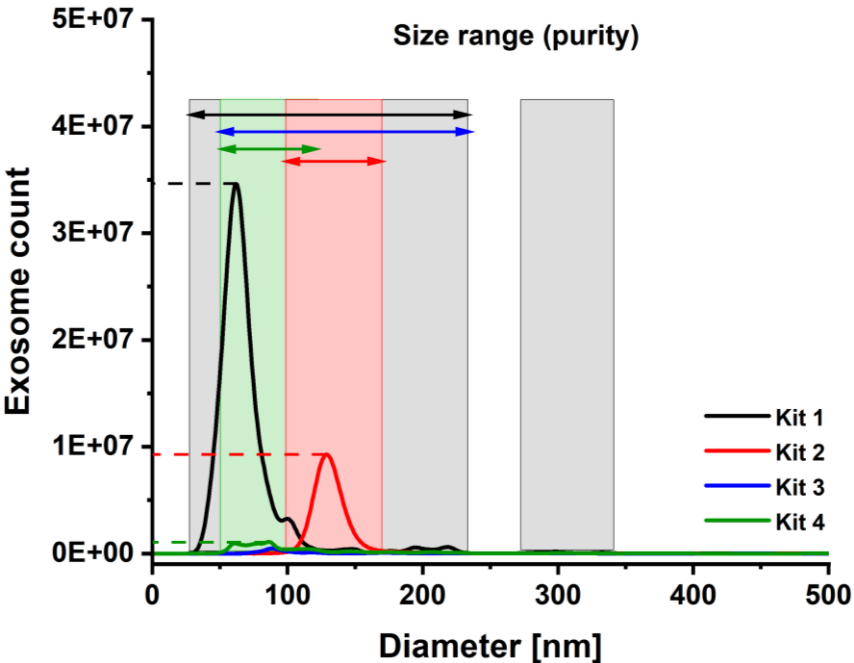
Isolation of exosomes



- Ultracentrifugation (time-consuming) 
- Four different commercially available kits
 - filtration, magnetic beads enrichment, extraction and precipitation



| | Kit 1 | Kit 2 | Kit 3 | Kit 4 |
|--------------------------------------------------|--------------|-----------------|-------------|--------------------|
| Commercial name | Capturem EIK | Fuji MagCapture | PureExo EIK | SBI ExoQuick Ultra |
| Principle | Filtration | Magnetic beads | Extraction | Precipitation |
| Input volume (μl) | 5,000 | 1,000 | 2,000 | 1,500 |
| Output volume (μl) | 500 | 100 | 90 | 950 |
| Time (h) | 1.5 | 5 | 2 | 2 days (o/n) |
| Price (from highest to lowest, i.e. from 1 to 4) | 4 | 2 | 3 | 1 |
| Yield / purity | ☑ | ☑ | ☒ | ☒ |
| Error prone protocol | ☒ | ☒ | ☑ | ☑ |
| Low sample volume | ☒ | ☑ | ☒ | ☑ |

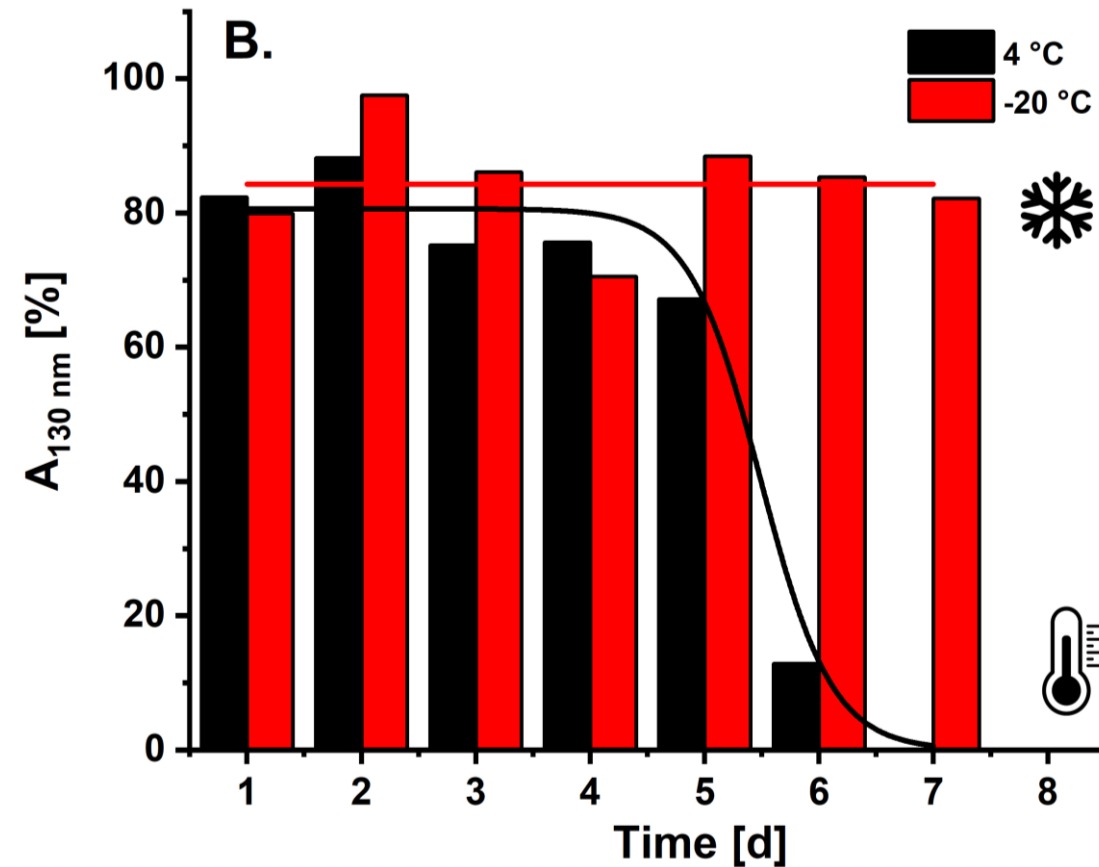


- The size of isolated exosomes - similar compared to other published studies

Stability – temperature and storage of isolated exosomes

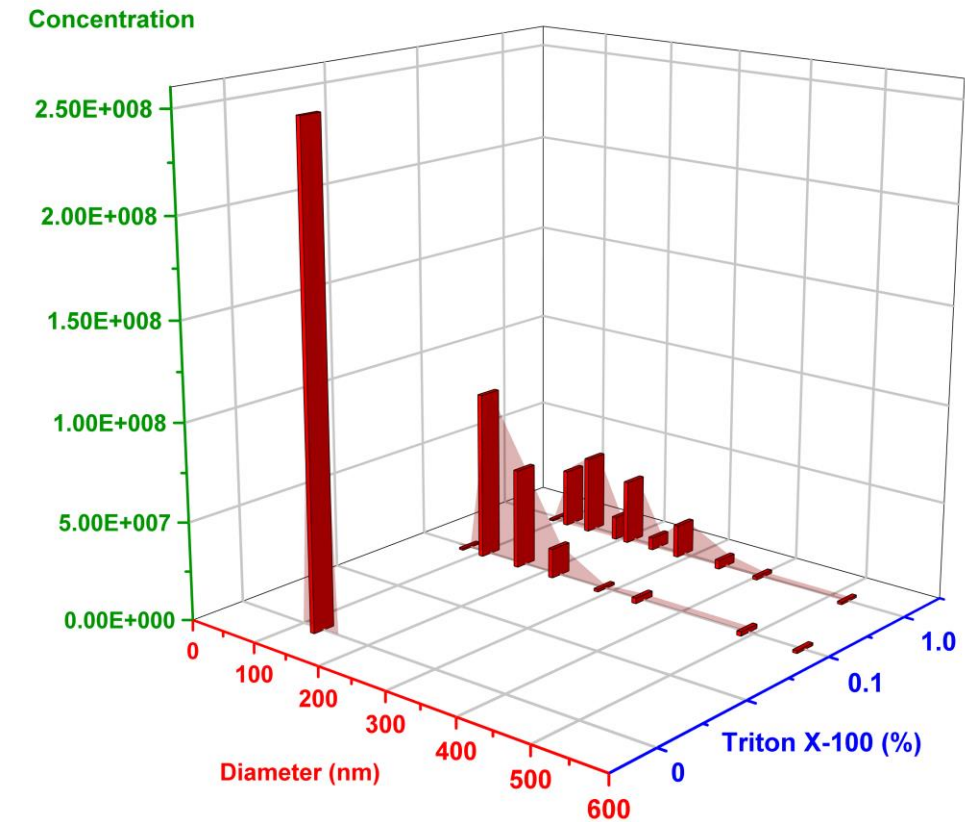
- Investigation of storage conditions
- Change of the exosome level

- Exosomes are stable at 39°C for at least 90 min
- at 4°C – exosomes were stable for 5 days
 - 7 days of storage - no exosome level
- at -20°C – exosomes were stable in the time frame examined
- at -20°C (7 freeze/thaw cycles) - exosomes were stable for 7 days



Disintegration of exosomes

- The effect of 0.1 and 1% Triton X-100 on exosome size
- 3-step process:
 - Diffusion into lipid bilayer
 - Vesicle expansion (dependent on detergent concentration)
 - Micellization

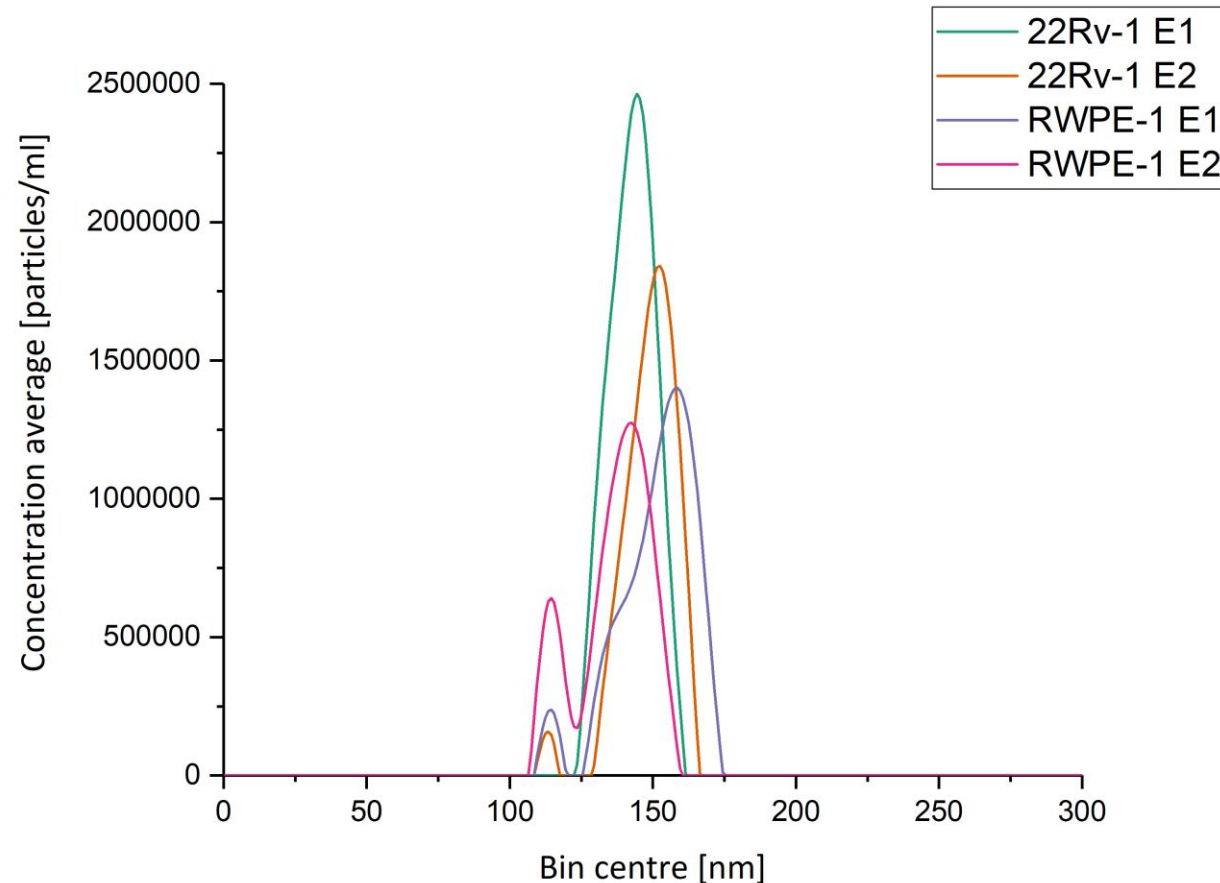
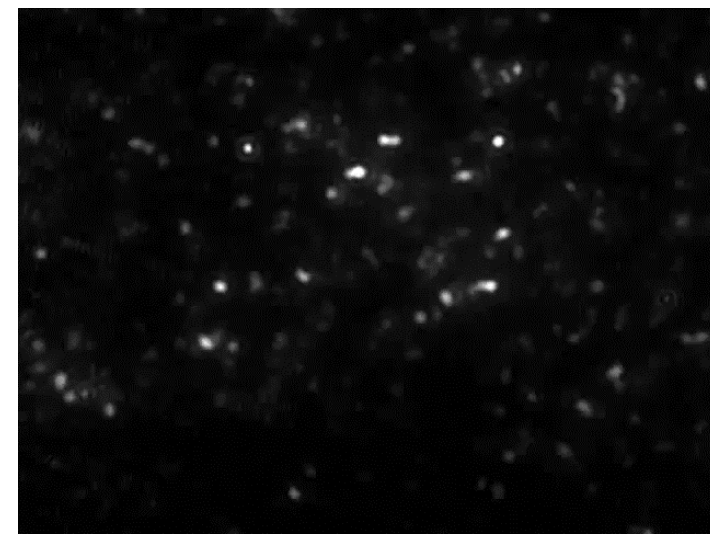


| Exosome size [nm] | Level of exosomes in 1×10^8 particles | | |
|-------------------|------------------------------------------------|------------------|----------------|
| | 0% Triton X100 | 0.1% Triton X100 | 1% Triton X100 |
| 0-100 | 0 (0%) | 0.01 (0.6%) | 0.01 (0.6%) |
| 100-200 | 2.50 (100%) | 1.39 (85.2%) | 0.83 (55.9%) |
| 200-300 | 0 (0%) | 0.17 (10.2%) | 0.57 (38.4%) |
| 300-400 | 0 (0%) | 0.03 (1.7%) | 0.05 (3.1%) |
| 400-500 | 0 (0%) | 0.02 (1.4%) | 0.02 (1.2%) |
| 500+ | 0 (0%) | 0.01 (0.9%) | 0.01 (0.9%) |
| Total | 2.50 (100%) | 1.63 (100%) | 1.48 (100%) |

- ↑ concentration of Triton X-100
- ↓ level of exosomes
- ↑ level of particles with aggregates

Nanoparticle tracking analysis

- Simultaneous, multiparameter analysis of nanoparticles [10 – 1 500 nm]
- Size distribution, concentration, direct and real-time visualization
- Brownian motion of particles analyzed in real time



Summary

- Investigate 4 different exosome isolation kits based on different principles
 - The best one - the kit based on magnetic beads
- Comparison of storage conditions of isolated exosomes
 - Storage at -20°C is a preferential option over storage at 4°C
 - An option to work with frozen exosomes
 - Exosomes can be isolated from frozen serum samples
- A decrease in the overall level of exosomes with an increased concentration of Triton X-100
- No significant difference in size of exosomes for the cell line RWPE1 and cell line 22Rv1



Thank you for your attention

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