



Platelet-derived extracellular vesicles:

The What, the Who & the Why?

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Supervisors: Dr. Allison Waters, Dr. Claire Wynne, Dr. Steve Meaney, Mr. Fabian McGrath

Partnership

- **First TU Dublin-IBTS Ph.D. project**
- Supervisors: *Dr. Allison Waters & Dr. Claire Wynne, Dr. Steve Meaney, Mr. Fabian McGrath*
- Collaboration with IBTS will support basic and applied sciences in transfusion and ultimately clinical outcomes- better blood products.



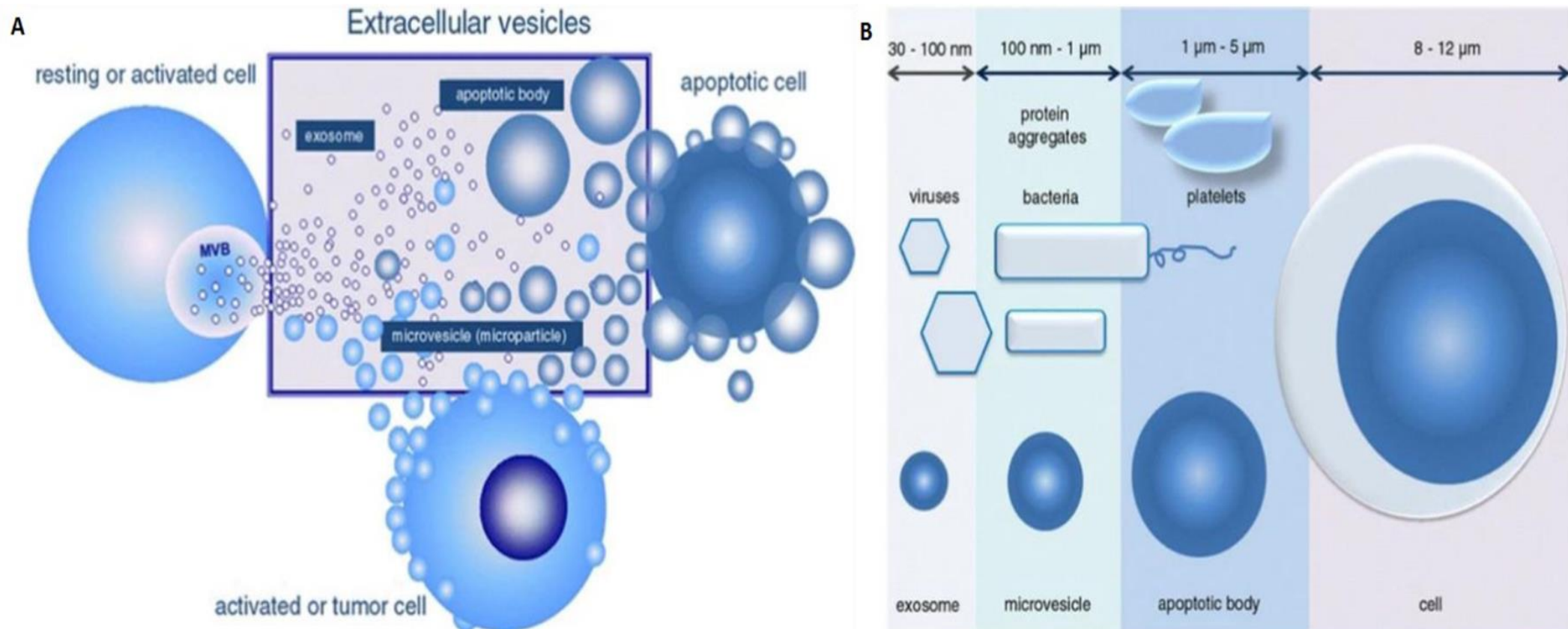


The What: What are Extracellular vesicles (EVs)?

- **Extracellular vesicles (EVs)** are biological particles, that are generated naturally and released in large amounts from cells after exposure to various stimuli, such as hypoxia, hunger, and oxidative stress.
- Found in blood, urine, saliva, synovial fluid, etc.

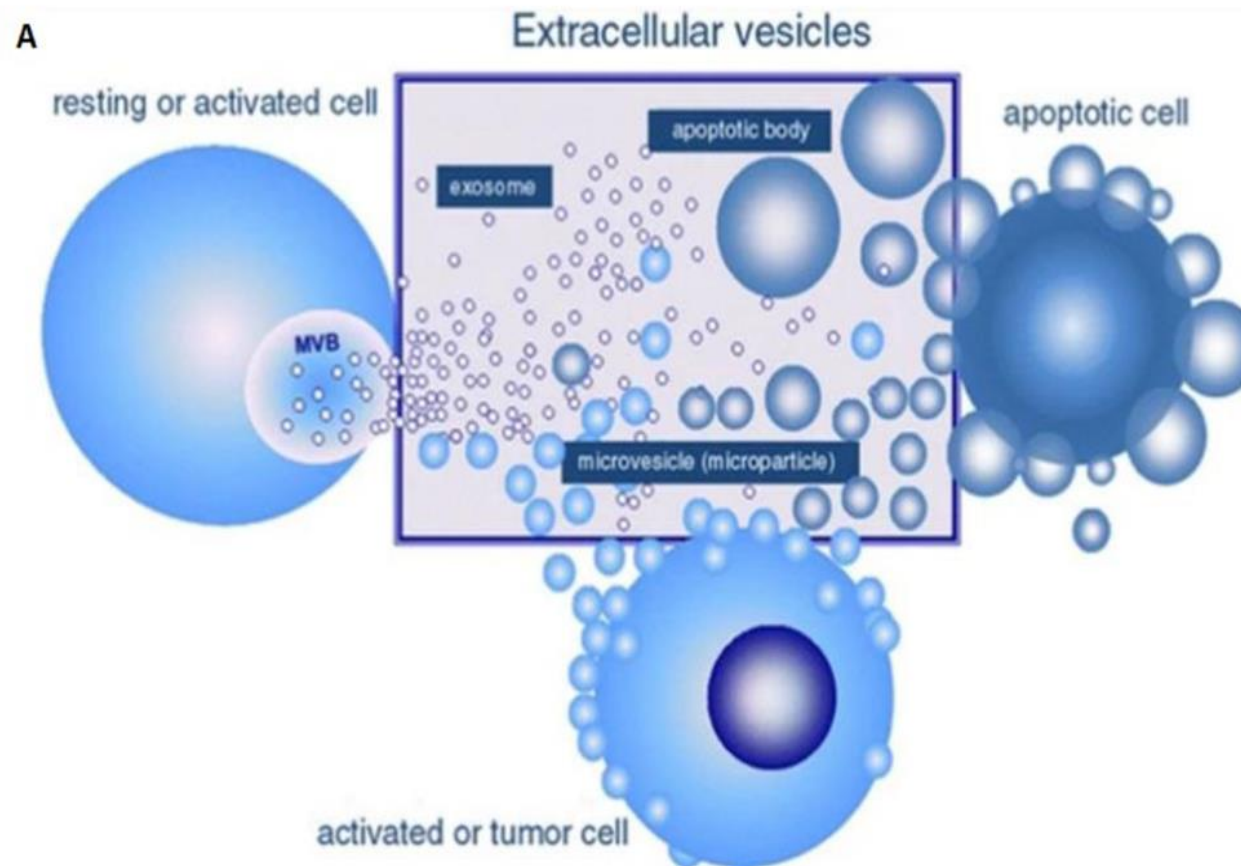


The What: *What are extracellular vesicles?*



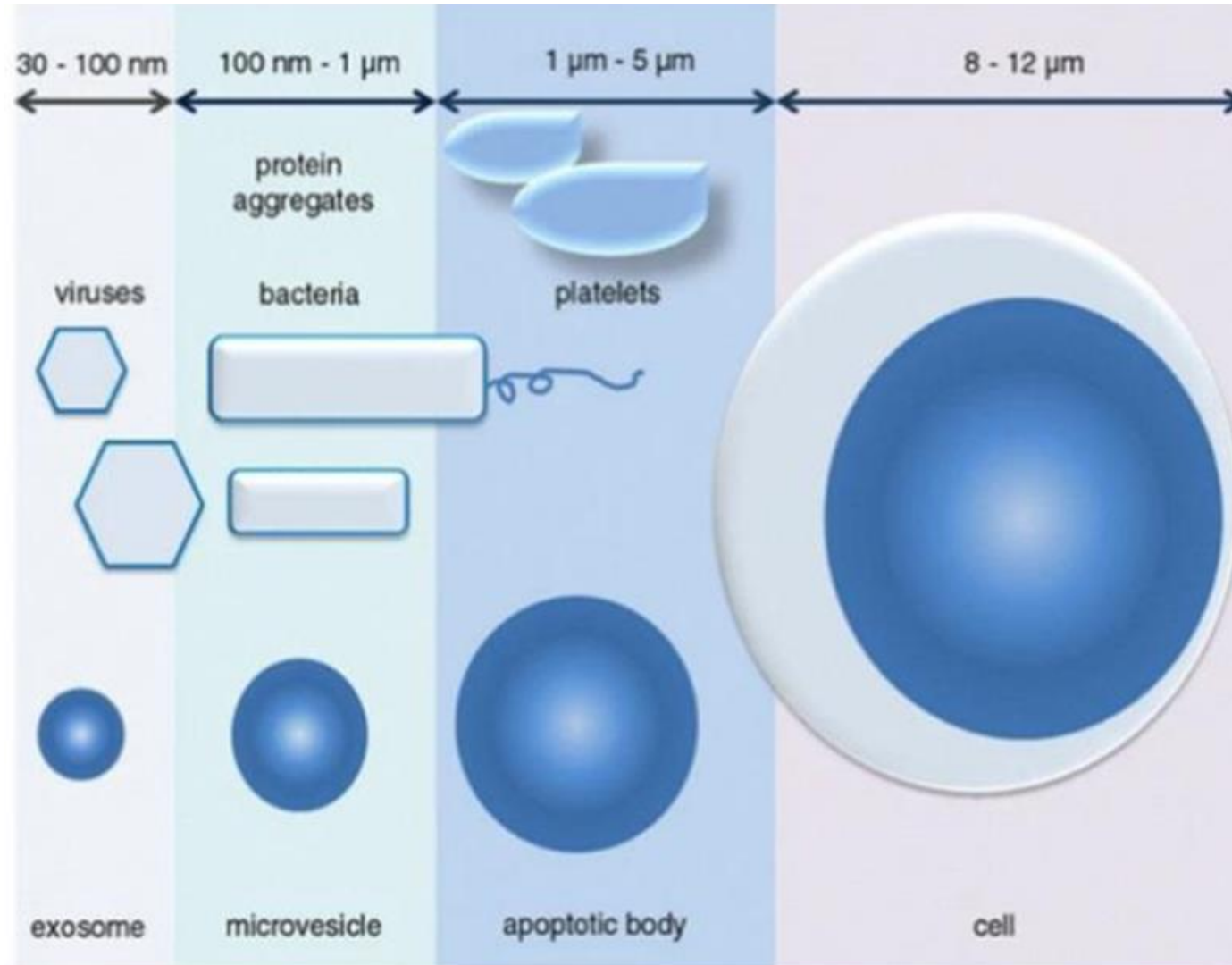


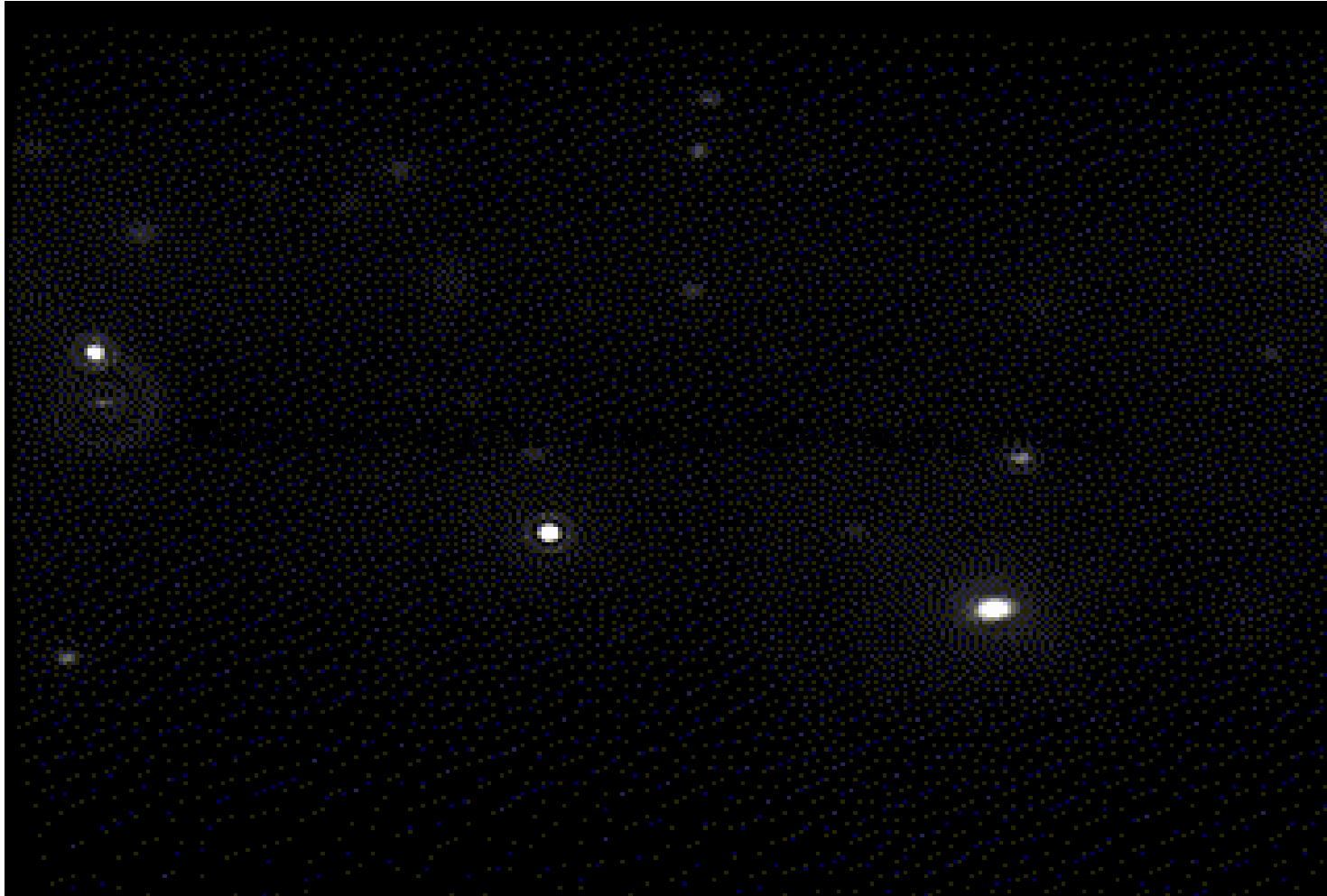
The What: *What are extracellular vesicles?*





Types of Extracellular vesicles





Platelet-derived EVs from Pooled platelet Pack – Nanoparticle tracking analysis



What do we know so far?

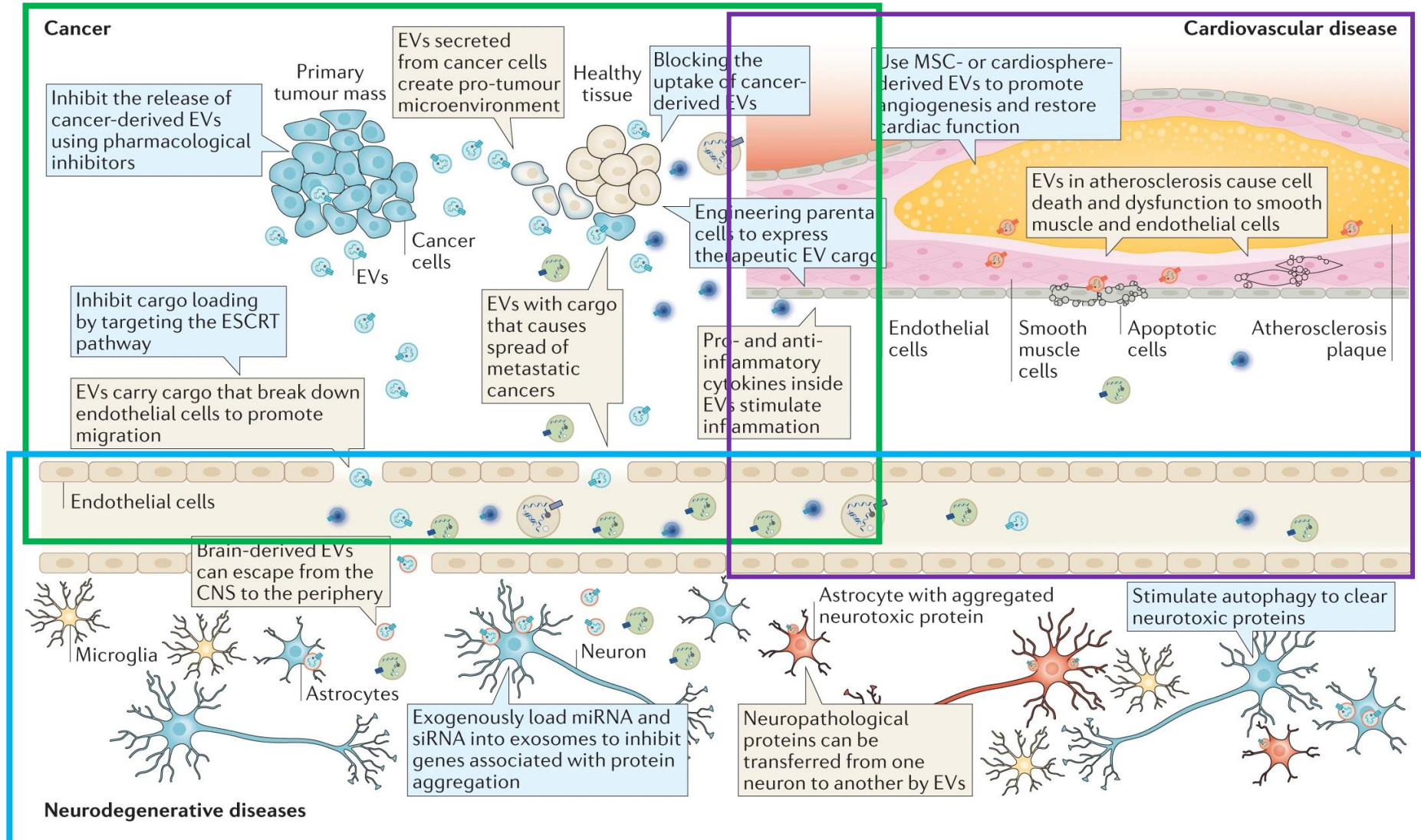
EVs are released and may partake in *intercellular communication*

Affect processes such as:

- Coagulation and thrombosis
- Angiogenesis
- Immunomodulation
- Inflammation

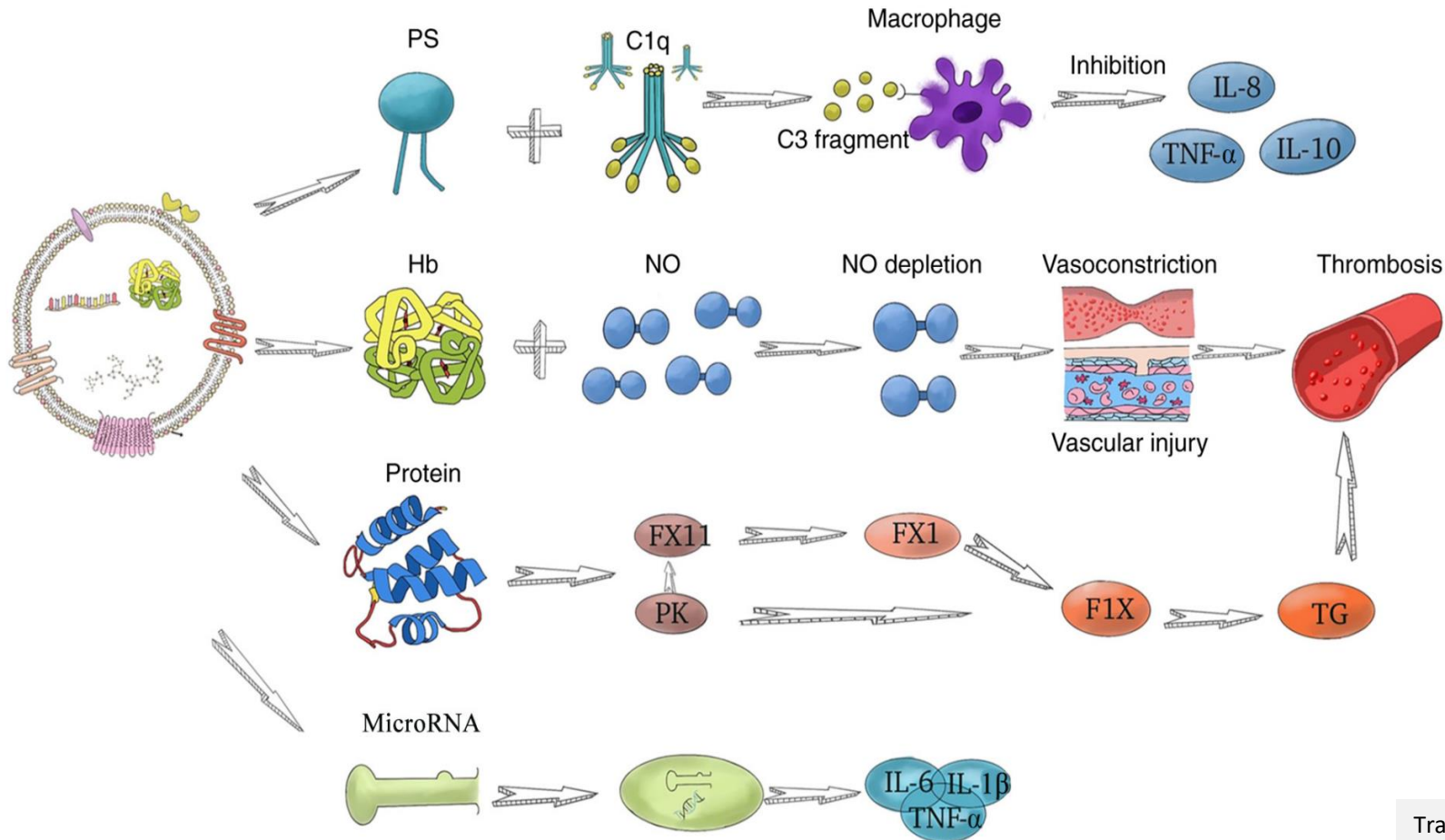


THE ROLE OF EVS IN CANCER, NEURODEGENERATIVE DISEASES AND CARDIOVASCULAR DISEASE.





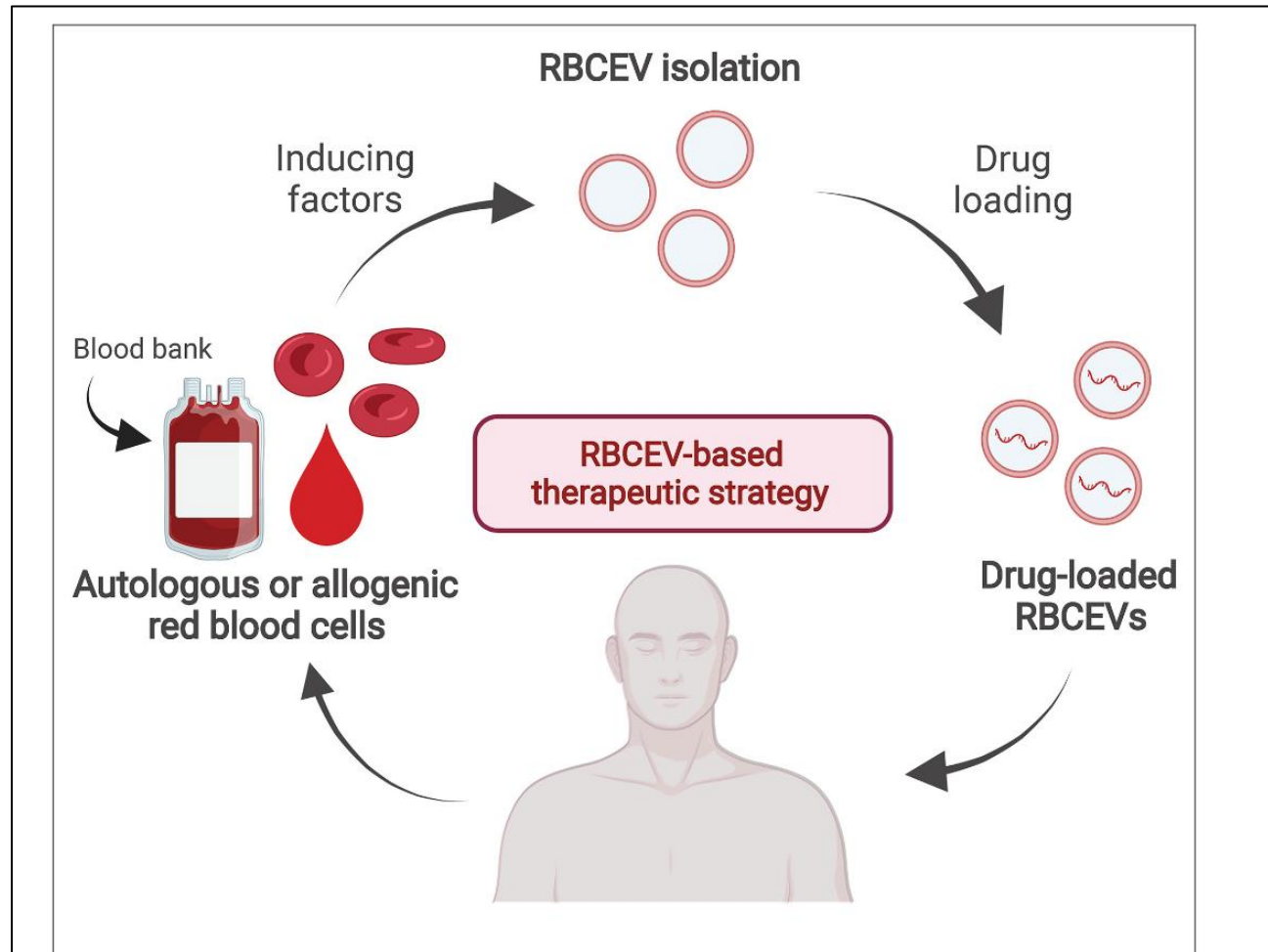
Mechanism of stored RBC-derived EVs in TRIM in patients with cancer receiving transfusions



Transfusion-related immunomodulation in patients with cancer:
Focus on the impact of extracellular vesicles from stored red blood cells
(Review) XINGYU MA, YANXI LIU, QIANLAN HAN, YUNWEI HAN, JING
WANG, and HONGWEI ZHANG, DOI: 10.3892/ijo.2021.5288



Strategy of drug-loaded RBC EVs therapy





The Who: *Platelet-derived EVs from apheresis donors*

- Blood and blood-derived products are a scarce resource
 - must be used judiciously for the best clinical outcomes.
- During storage
 - deleterious changes- may harm the patient.
- Changes include the **release of extracellular vesicles (PEV)**
 - poorly understood.



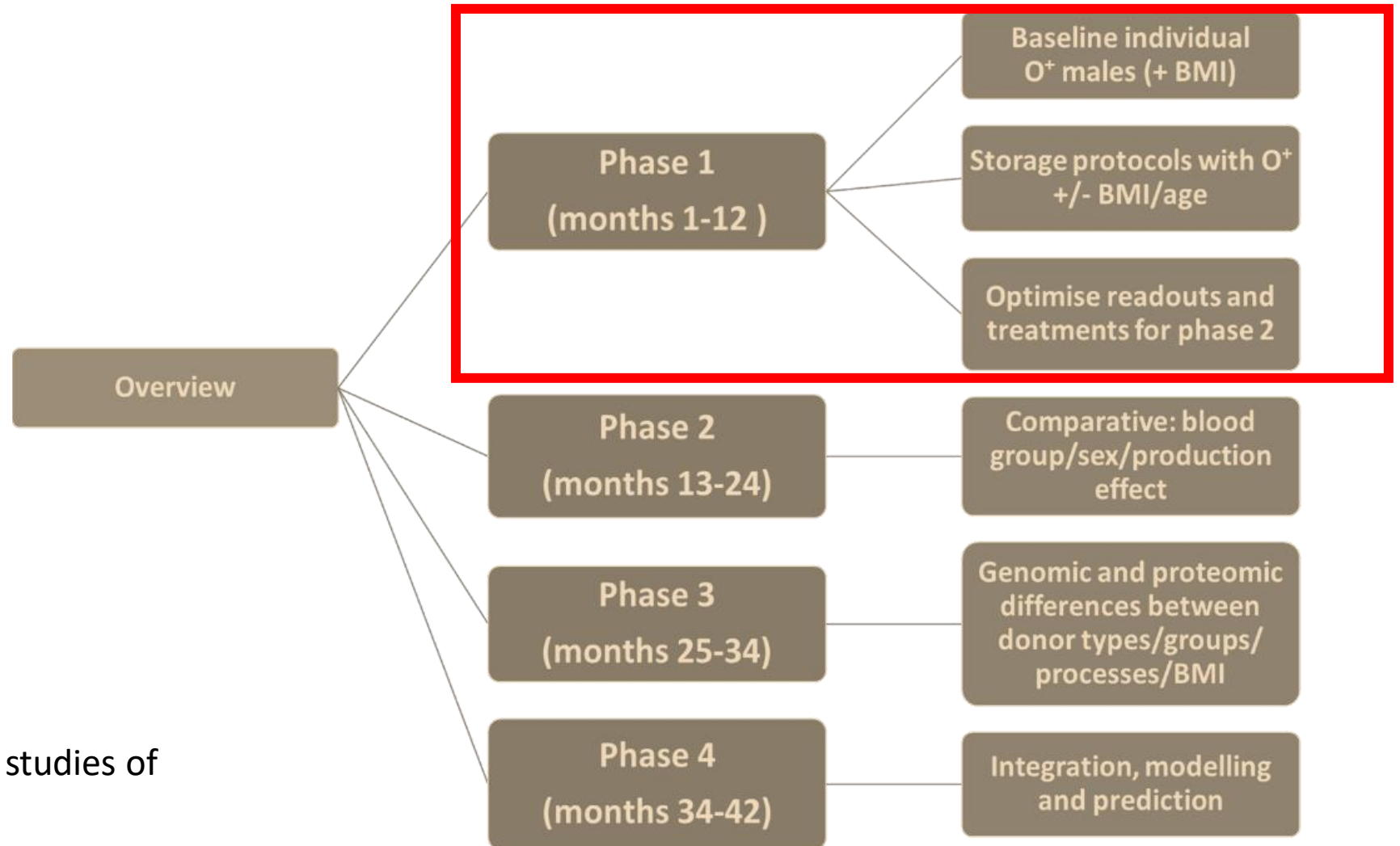
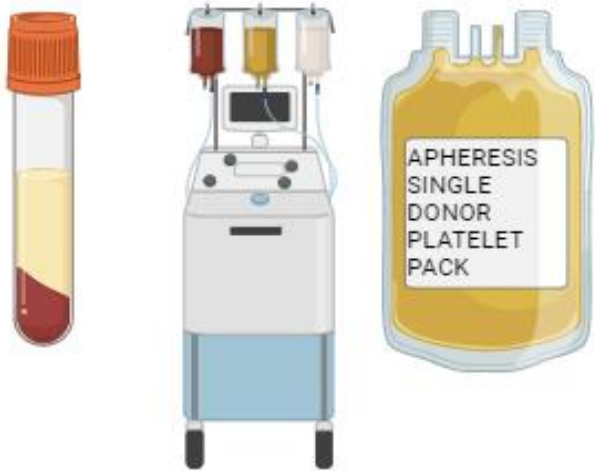
The Why: *Research Question*

What biochemical and immunohaemostatic changes occur in platelet-derived EVs due to the preparation, processing and storage of blood components?

Is there a link between donor characteristics & platelet EV function?



Study Design

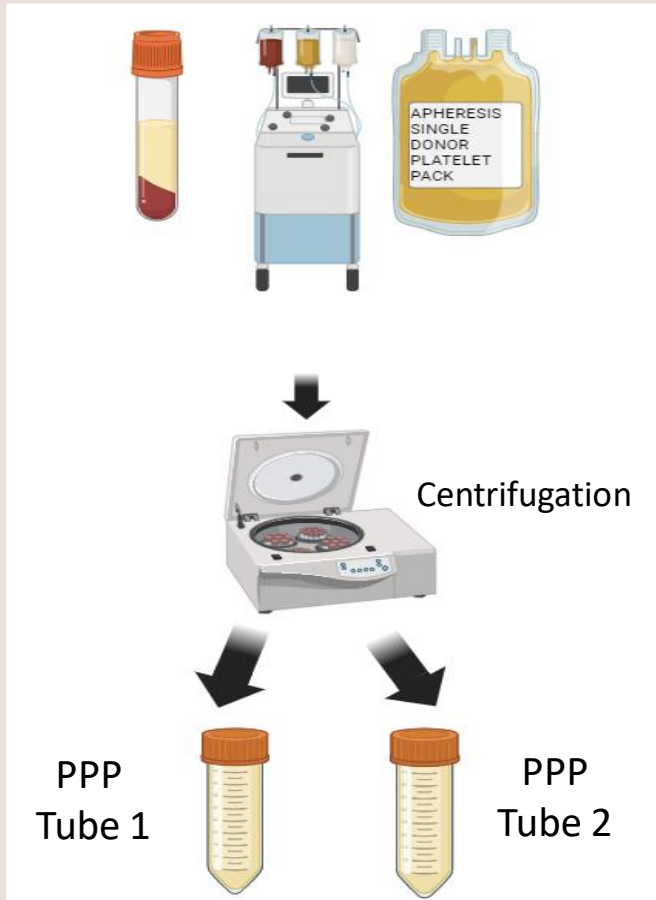


MISEV: Minimal information for studies of extracellular vesicles(2018)

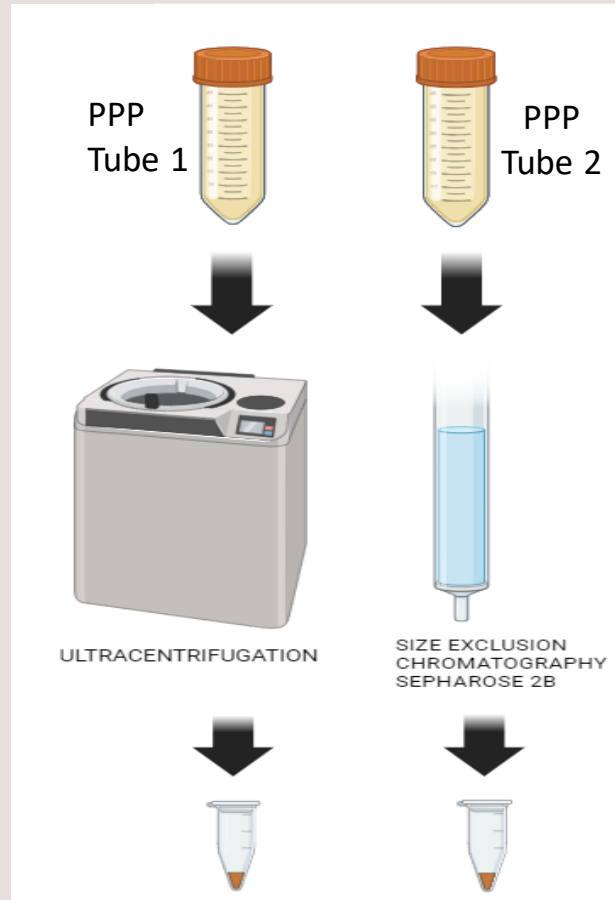


Experimental Design

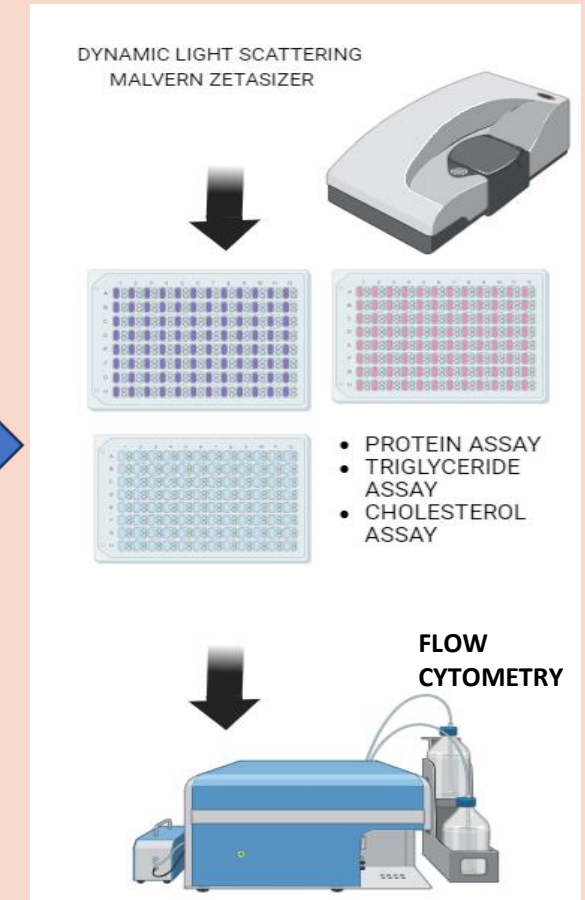
Preprocessing to obtain PPP



EV Isolation



EV Characterization





Results - Donor Full Blood Counts

Phase one cohort (n=18)

- Healthy males (24-56 y/o)
- O positive
- Apheresis donors

Parameter	Range	Median
Haemoglobin (g/dL)	12.4 - 16.3	14.8
HCT (L/L)	0.369 – 0.472	0.445
MCV (fL)	84.7 - 96.3	89.6
Platelets (x 10 ⁹ /L)	180 - 322	253
MPV (fL)	8.2 - 10.7	9.9
WBC (x 10 ¹² /L)	4.8 - 9.1	6.1

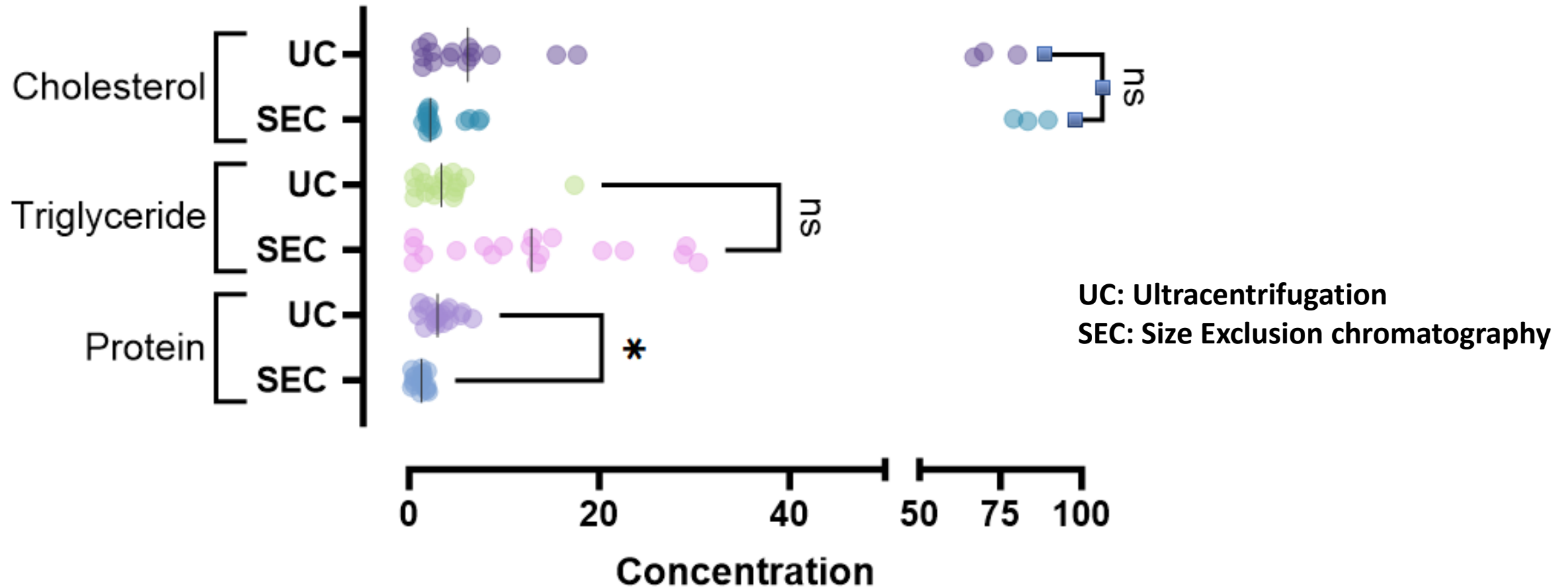


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Biochemical characteristics of platelet EVs



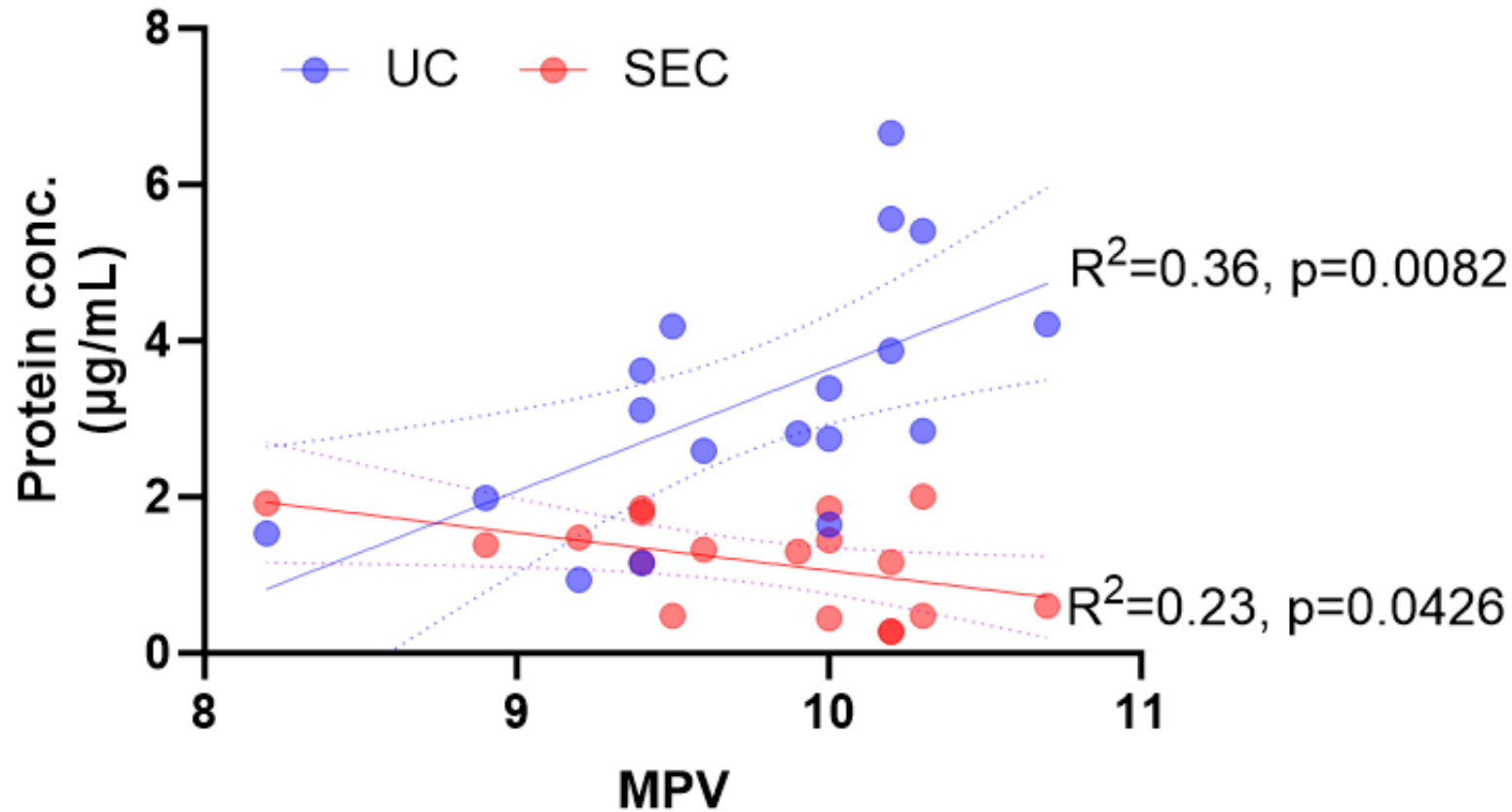


Key Findings- Donor characteristics

r	P-value	UC Protein		SEC Protein		UC Cholesterol		SEC Cholesterol		UC Triglycerides		SEC Triglycerides	
Age		0.29926	0.227665	-0.171788	0.495488	0.290322	0.242529	0.266426	0.285219	0.042886	0.865824	-0.103101	0.683931
BMI		0.205904	0.412387	-0.110036	0.663816	-0.245562	0.326009	-0.21355	0.394853	-0.320318	0.19501	-0.413913	0.087714
Systolic Blood Pressure		0.019699	0.040676	0.129079	0.763512	-0.273387	0.788222	-0.23637	0.744826	0.272414	0.913015	0.049908	0.73499
Diastolic Blood Pressure		0.178294	0.581403	-0.039186	0.653789	-0.310907	0.835107	-0.265422	0.811866	-0.158291	0.510683	-0.315302	0.618018
Total collection time(min)		0.179583	0.475823	-0.348481	0.156418	0.240842	0.335687	0.234787	0.348344	-0.308719	0.212589	-0.265351	0.287241

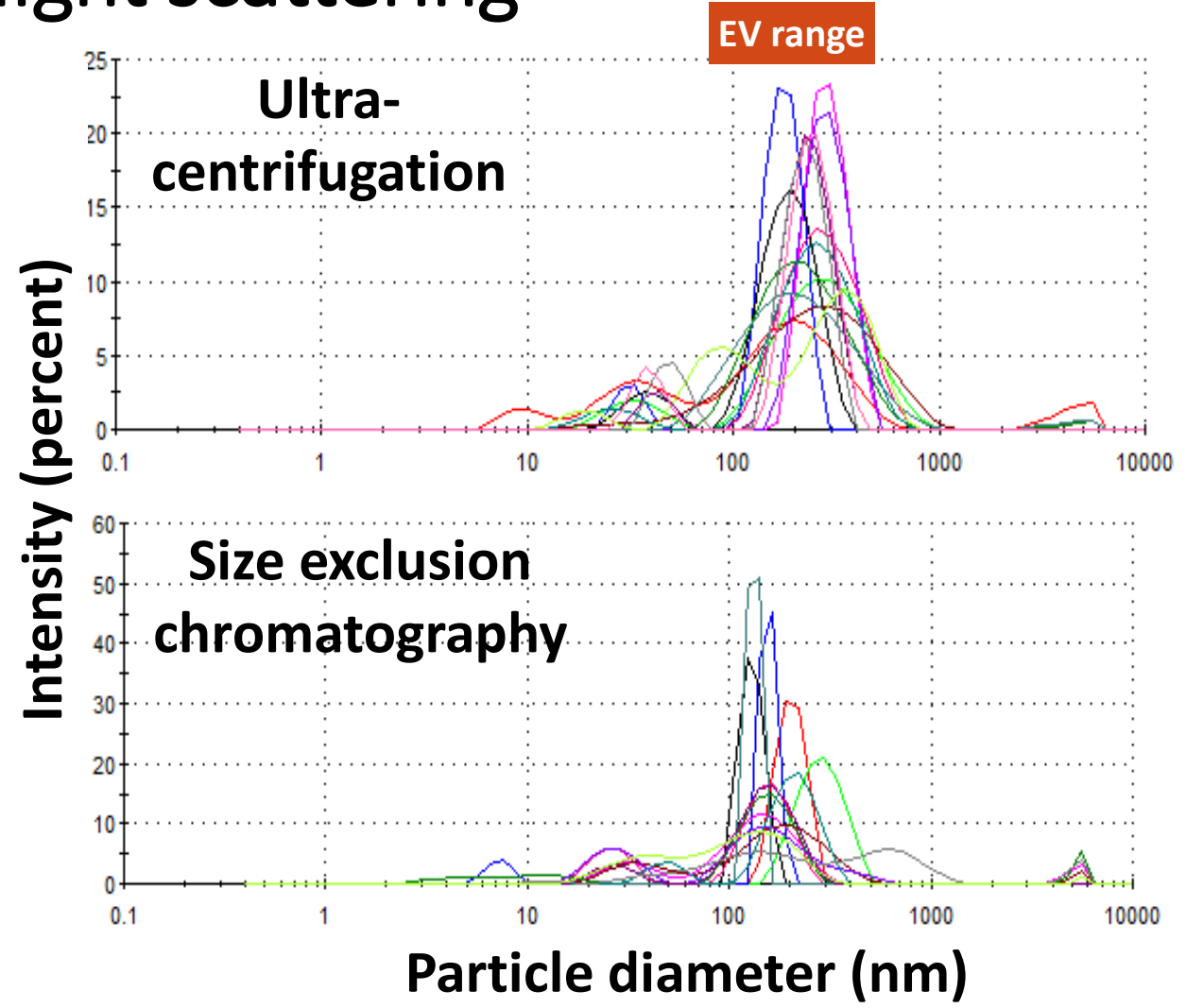


Donor mean platelet volume appears correlated with protein content of isolated EVs





Physical characterization of platelet Evs- Dynamic light scattering





Next Steps

- **Size:** Nanoparticle tracking analysis (NTA)
- **Structure:** Transmission electron microscopy (TEM)
- **Surface characteristics:** Flow cytometry – Cd62p, Annexin V
- **Functional Assays:** Thrombin generation assay
- **Immunomodulatory activity:** Multiplex cytokine assays
- **Relationship between EVs and miRNA:** GeneChip miRNA Assay
- **Proteomics profiling:** Trapped ion mobility time of flight mass spectrometer connected to a nano-LC chromatography.



Future Impact

- Insight into using platelet-derived EVs as a **quality marker** for platelet packs.
- Insight into **mechanisms that influence blood components** from the point of donation through to transfusion.
- Better understanding of **physiological and pathological implications** of platelet-derived EVs.
- Characterise the **immunohaemostatic properties** of platelet-derived EVs and **donor variability** in platelet packs which could be directed to various clinical situations (eg. procoagulant EVs to bleeders rather than cancer patients).



Thank You.
Go raibh maith agaibh.

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