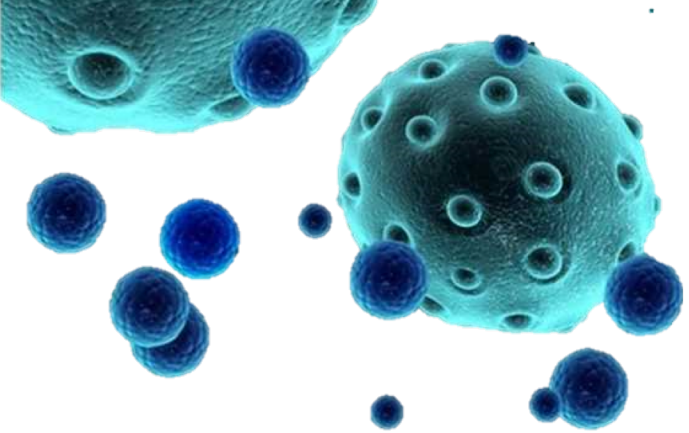
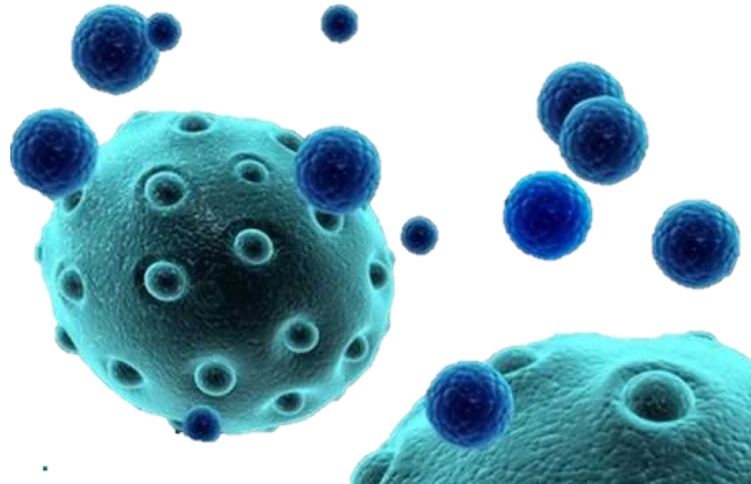


Microproteomics workflow for exosome biomarker discovery

Enabling single mouse analysis on longitudinal models

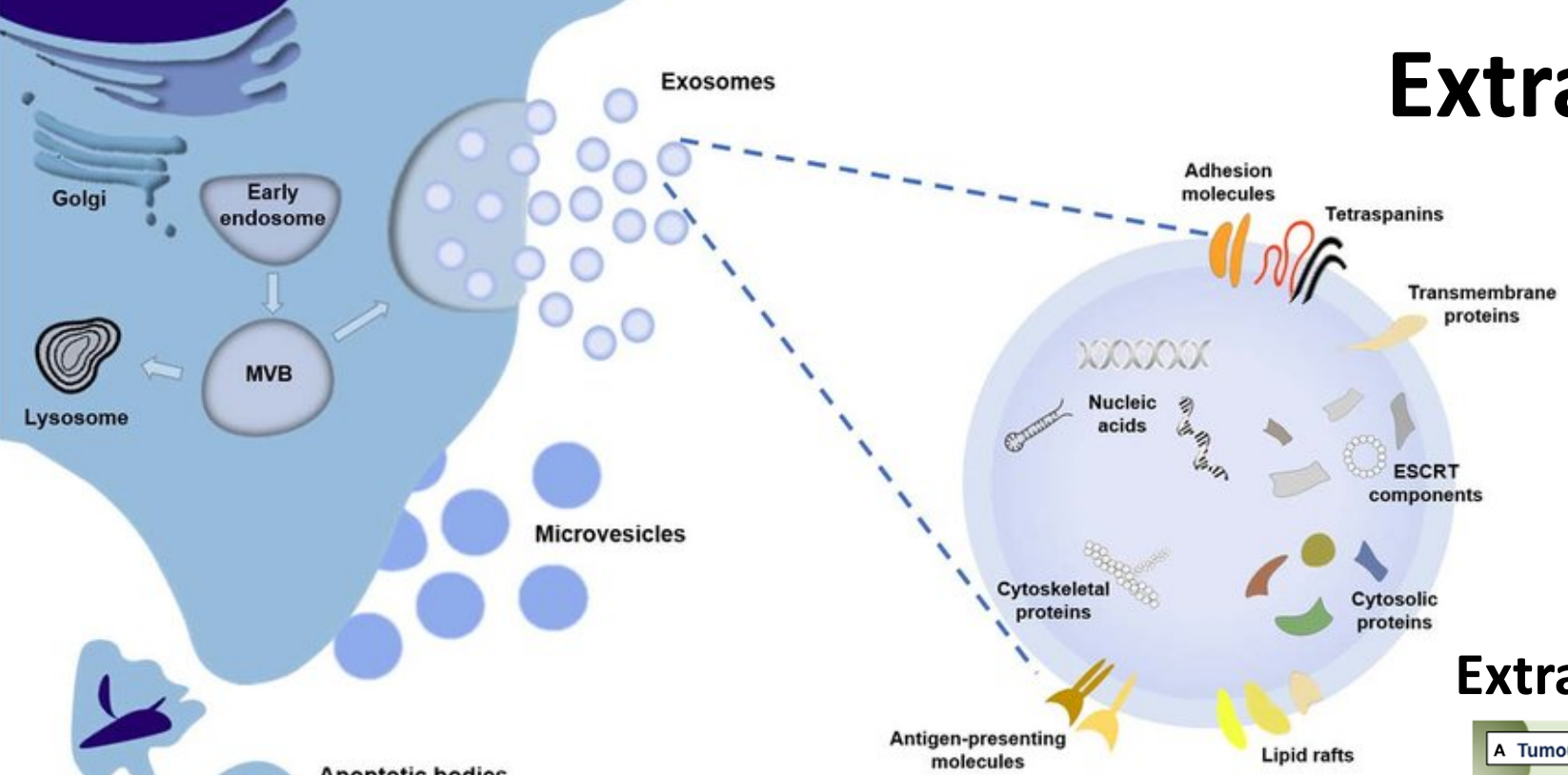
Federica Anastasi

PhD Student



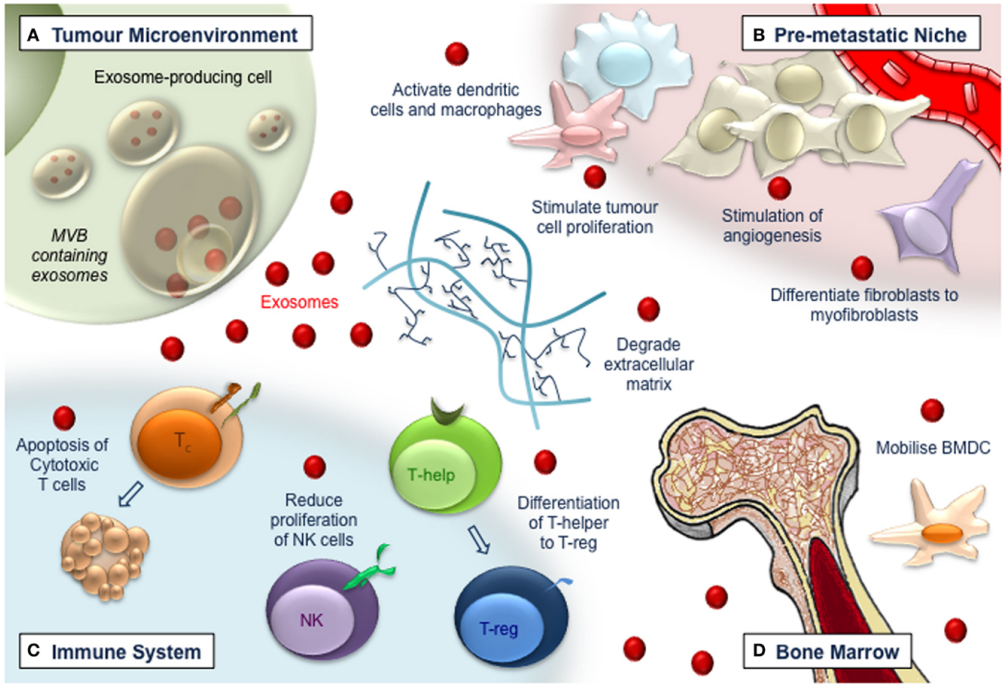
17/10/2019

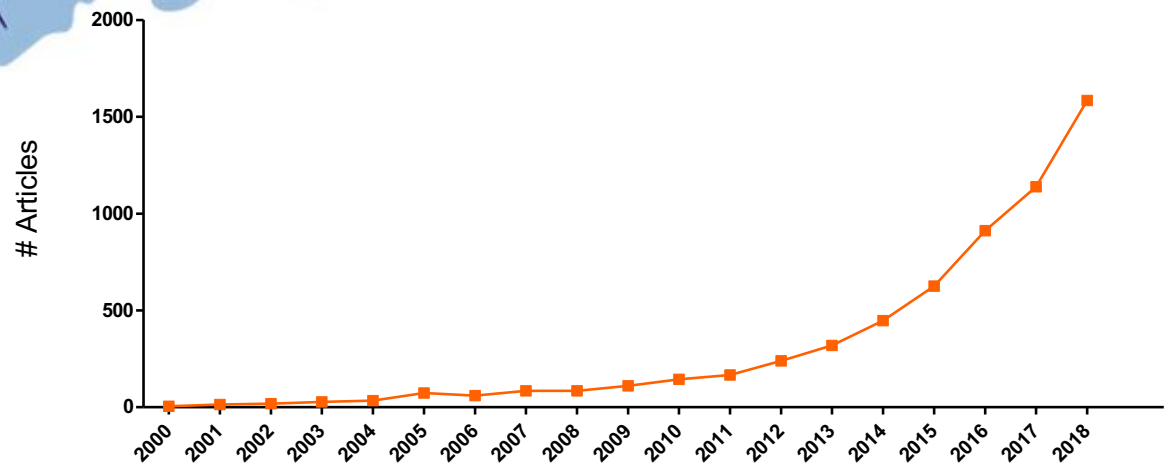
Extracellular vesicles



Key players in intercellular communication

Extracellular vesicles' role in cancer





Clarivate Analytics
WEB OF SCIENCE™

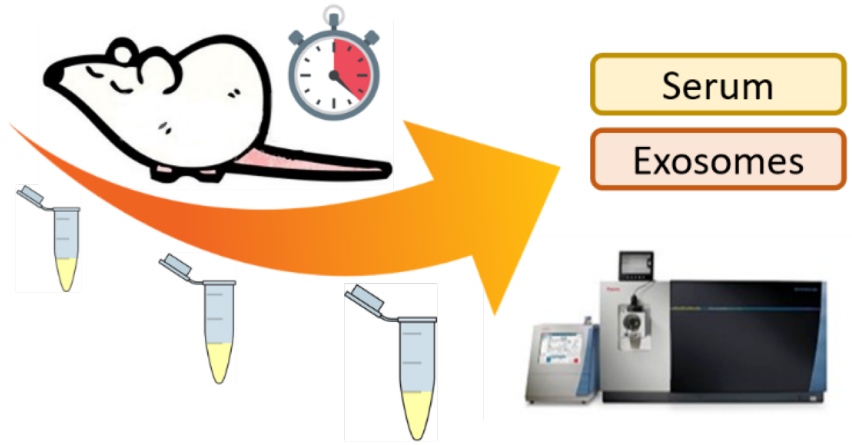
PhD Project aim

Microproteomics workflow for low amount serum exosome analysis

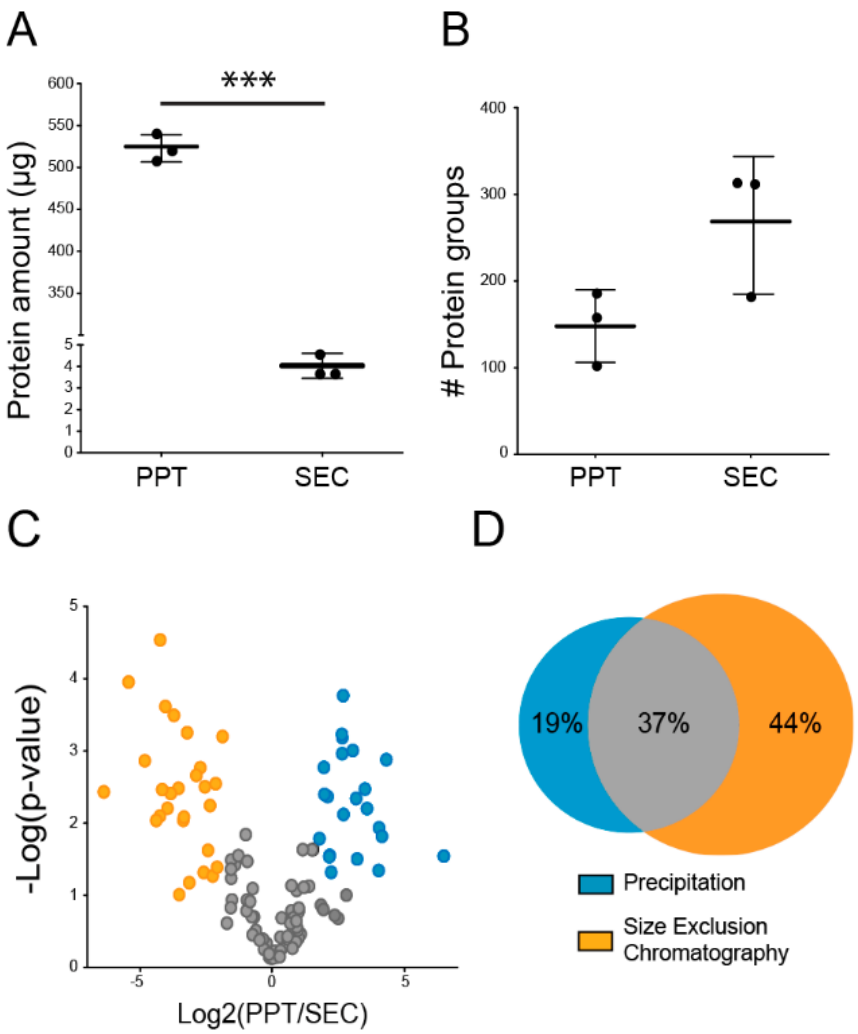
- SAMPLE PRE-TREATMENT
- EXOSOME PURIFICATION
- EXOSOME LYSIS
- PROTEIN QUANTIFICATION
- PROTEIN DIGESTION

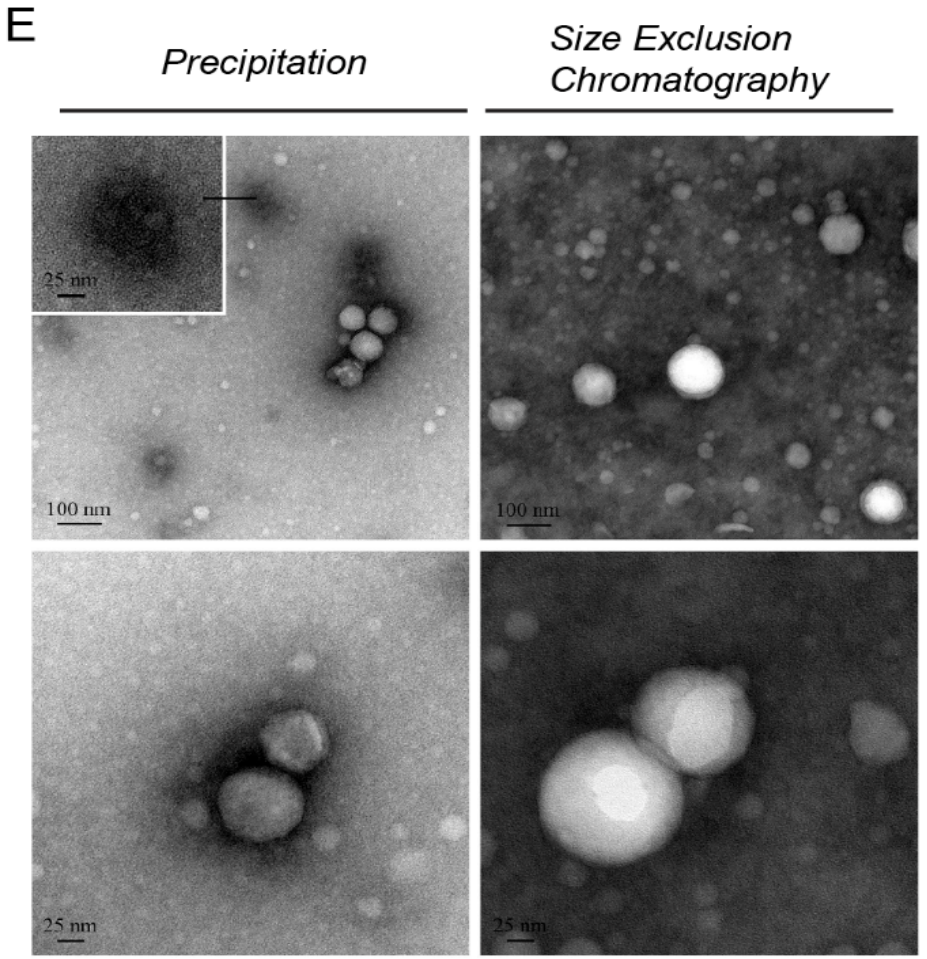
Scaling down to 50 µl **enables parallel analysis of total serum proteome and exosome proteome** on single mouse serum sample for longitudinal studies

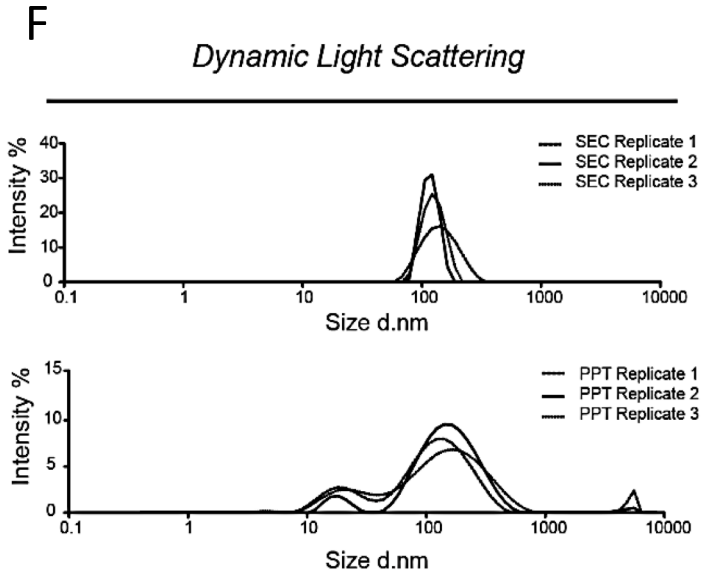
OPTIMIZATION → **SCALING DOWN**



Exosome purification procedures comparison: Size Exclusion Chromatography vs. Precipitation







	Precipitation (nm)	SEC (nm)
MAX	118	135
MIN	15	17
AVE	37	46
ST.DEV	19	24

n=142

Comparison between three replicates. *: p<0.05, **: p<0.01, ***: p<0.001. Volcano plot: t-test on filtered for all valid values matrix: n=111

Procedure scale down to 50 µl and digestion optimization

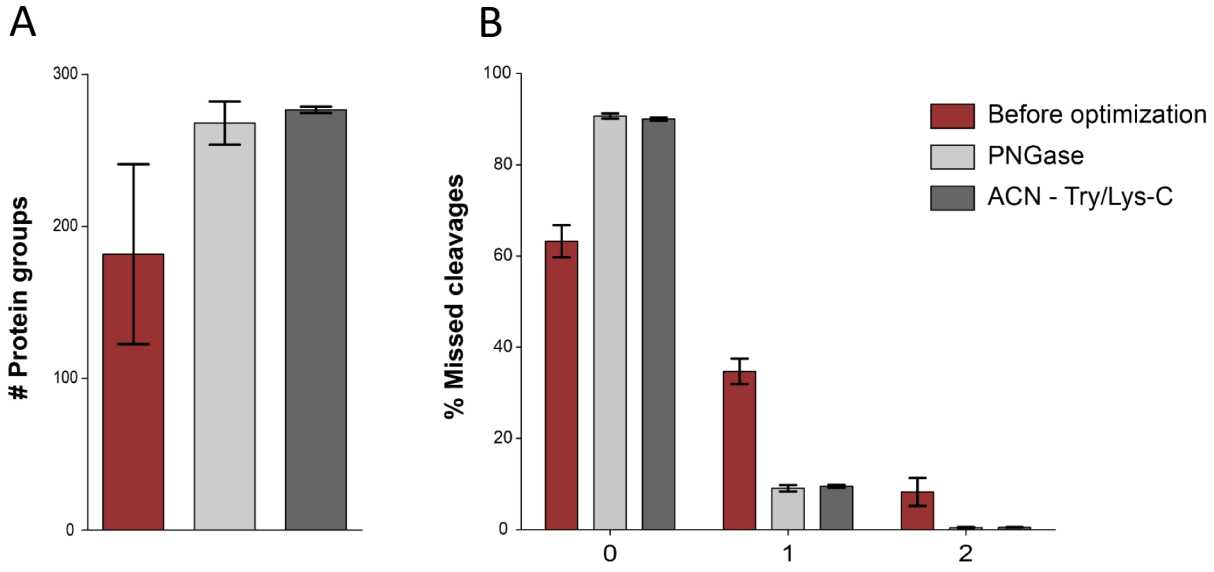
	Protein amount (µg)	# Protein groups
100 µl	4 ± 0.58	334 ± 38
50 µl	2.6 ± 0.26	182 ± 59

Digestion before optimization:
18 h – Try/Lys-C 1:25

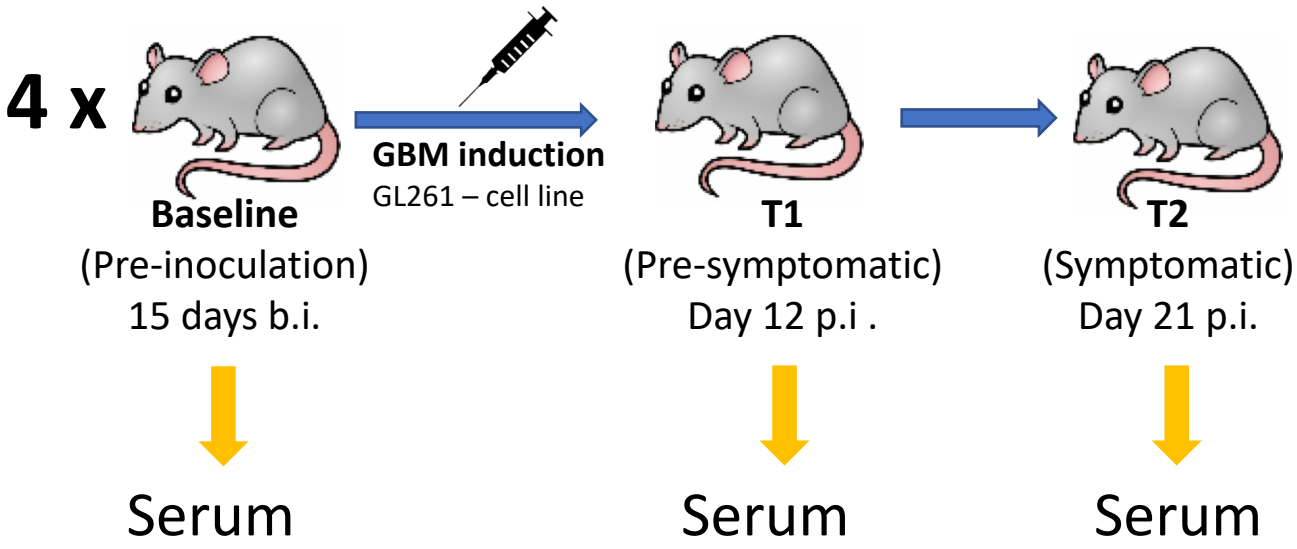
Two different digestion condition tested:

1. **16h** – Try/Lys-C 1:25
   + 2h PNGase 1:20

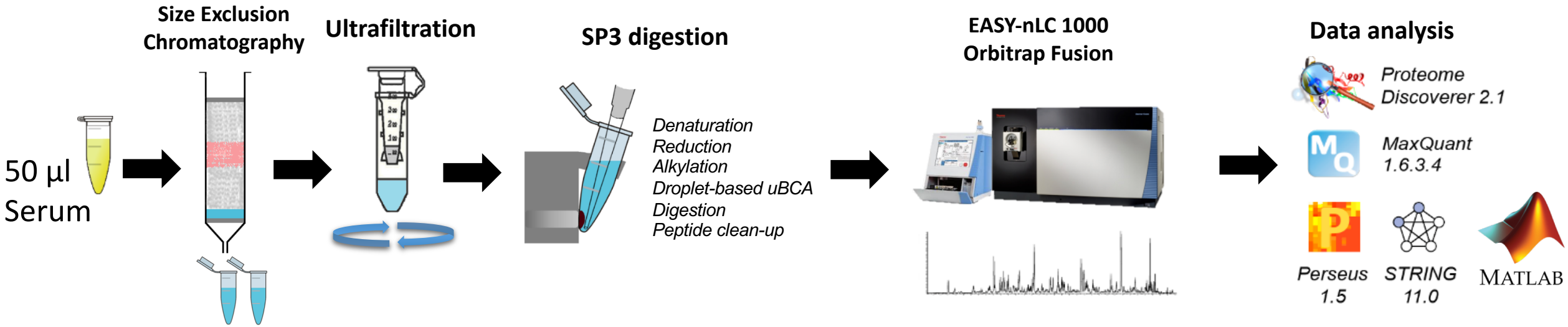
2. **16h** – Try/Lys-C 1:25
   + 2h Try/Lys-C 1:75 - 60%ACN



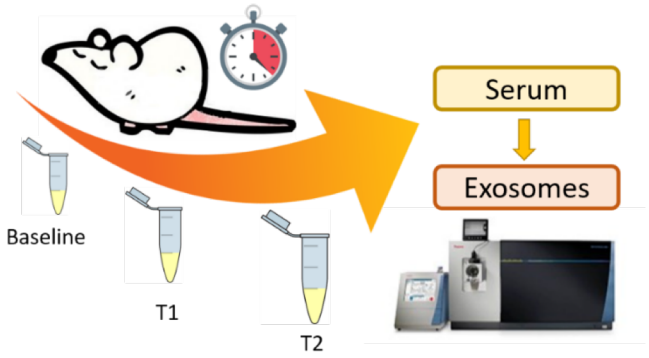
Clinical case: glioblastoma mouse model



- Sample amount: 0.5 – 2.5 protein µg
- 0.5 µg were analysed
- raw files were processed by MaxQuant searching against the UniProt Mus Musculus protein database (January 2018)



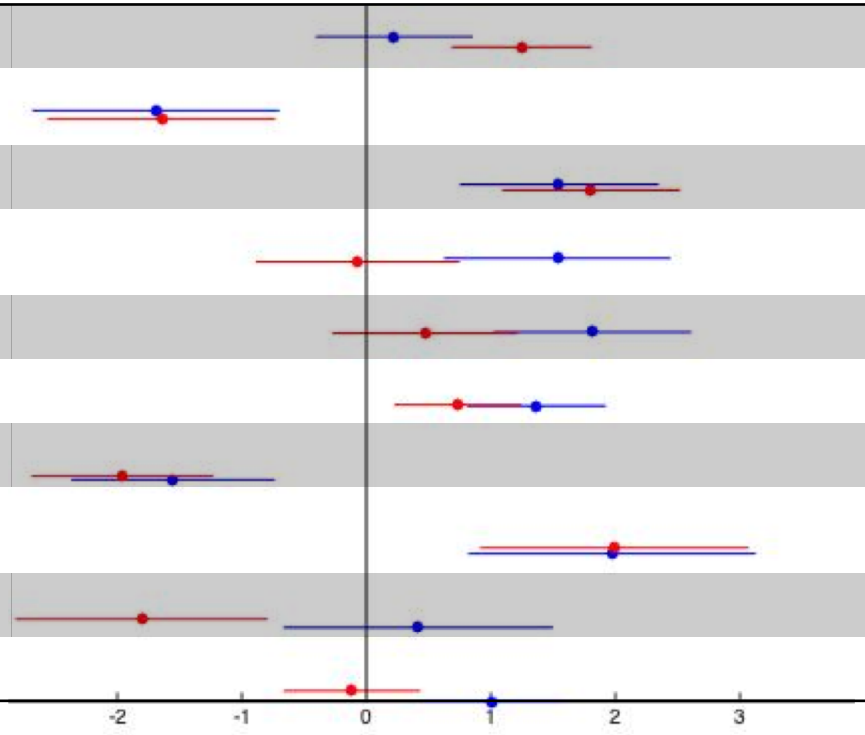
Longitudinal data analysis



Longitudinal data deals with the problem of non-independence of the levels of the time factor → **ANOVA test is not applicable**

Linear mixed effect model (LME) was applied to the longitudinal study since it accounts for the variance due to the different subjects

Accession #	Protein name	Regression coefficient and CI 95%	p-value
A2AVA0	Sushi, von Willebrand factor type A		0.0266
E9Q414	Apolipoprotein B-100		0.0498
O08538	Angiopoietin-1		0.0266
O54890	Integrin beta-3		0.0488
P29788	Vitronectin		0.0266
Q02105	Complement C1q subcomponent subunit C		0.0266
Q61268	Apolipoprotein C-IV		0.0266
Q61704	Inter-alpha-trypsin inhibitor heavy chain H3		0.0498
Q62351	Transferrin receptor protein 1		0.0266
Q99K41	EMILIN-1		0.0266



Gene ontology

Biological processes upregulated in GBM samples	
Biological processes	**FDR**
cell-substrate adhesion	5.19E-05
cell adhesion	0.00023
positive regulation of cell adhesion	0.00053
cell-matrix adhesion	0.00053
Molecular Function	**FDR**
integrin binding	0.0003
vascular endothelial growth factor receptor binding	0.0003
extracellular matrix binding	0.0021
signalling receptor binding	0.0078
Cellular Component	**FDR**
Extracellular region	0.00038
Integrin complex	0.0018
Collagen trimer	0.0062
microvillus	0.0073

Three upregulated proteins are extracellular activators of **PI3K-AKT signaling pathway :**

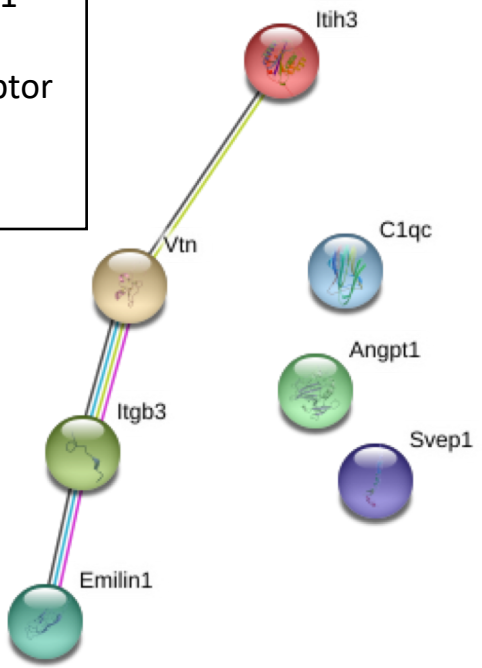
- **Vitronectin**
- **Integrin β-3**
- **Angiopoietin-1**

Ang-1 is expressed in GBM tissues and Ang-1 mRNa is present in GBM vessels, Ang-1 is involved in neurogenesis through Tie-2 receptor activity

Journal of Neuroscience 31 March 2010, 30 (13) 4573-4584
Arterioscler Thromb Vasc Biol. 2001 Apr;21(4):536-41

Vtn is glycoprotein known in neuroblastoma for its adhesive role in processes such as cell growth, angiogenesis and metastasis

*BMC Cancer2019**19**:479*



Gene ontology analysis performed with STRING software

Others projects and collaborations

OPTIMIZED
Microproteomics workflow for exosome proteome analysis

Longitudinal GBM mouse model analysis for biomarker discovery
GLIOMICS Project
Nanobiomarker Project

Manuscript in preparation

SAMPLE PRE-TREATMENT

SEC

ON-FILTER LYSIS

MODIFIED SP3 PROTOCOL

OPTIMIZED DIGESTION

UTRECHT future collaboration:
6 months visiting PhD student
Maarten Altelaar
Procedure application on a malignant melanoma clinical case (200 human serum samples)

FPS – synergies

1. **Exosome membrane proteome characterization** (*Giovanni Signore, Giulia Matteoli*)
2. **Exosome proteomics on GBM organoids** (*Serena Barachini, Giovanni Signore, Chiara Maria Mazzanti*)
3. **Apo E3/4 exosome analysis** (Maria Teresa Dell'Anno)

CNR – collaboration:
Laura Poliseno
melanosome analysis

CNR- IFC collaboration:
Renata Del Carratore characterization of neuronal exosomes from AD, PD, and SLA patients.

Acknowledgments

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Valentina Cappello
Mauro Gemmi

NANOBIOMARKER
Grant RST 148/16

GLIOMICS
FAS Salute 2014

This work will be presented at the first Italian Society of Extracellular Vesicles Symposium – November 6th-9th , Palermo

Part of this work has been presented at**:**
HPLC 2018, Washington DC and **IMSC 2018**, Florence