VI° CONVEGNO del DIMED
Padova, 15 novembre 2024

DIFFERENCES IN SKELETAL INVOLVEMENT BETWEEN INDOLENT SYSTEMIC MASTOCYTOSIS AND PRIMARY OSTEOPOROSIS

*Gaetano Paride Arcidiacono**

Background

- Indolent Systemic Mastocytosis (ISM) is characterized by excessive mast cell accumulation in organs and tissues, and skeletal fragility presenting with multiple vertebral fractures (VFx) may be a common manifestation.
- Given the rarity of the disease and the nonspecificity of other clinical manifestations, skeletal fragility in patients with ISM is often misdiagnosed as primary osteoporosis (OP).
- This study aimed to explore the role of vertebral morphometry in identifying ISM and differentiating it from OP.

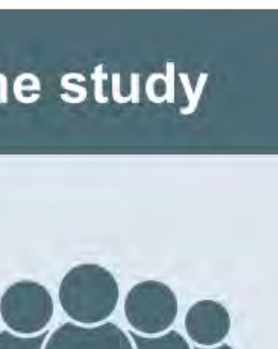
The study

Patients aged ≥ 18 years

with

≥ 2 VFx

Two study groups

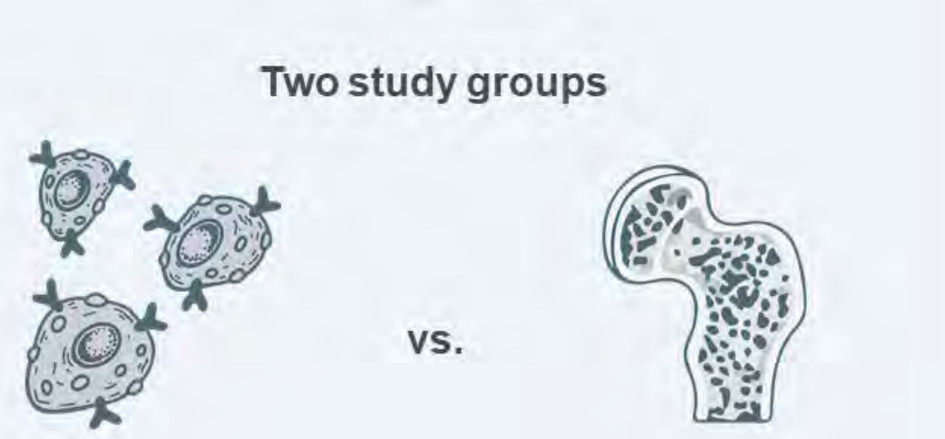


ISM
15 patients

vs.

OP
45 patients

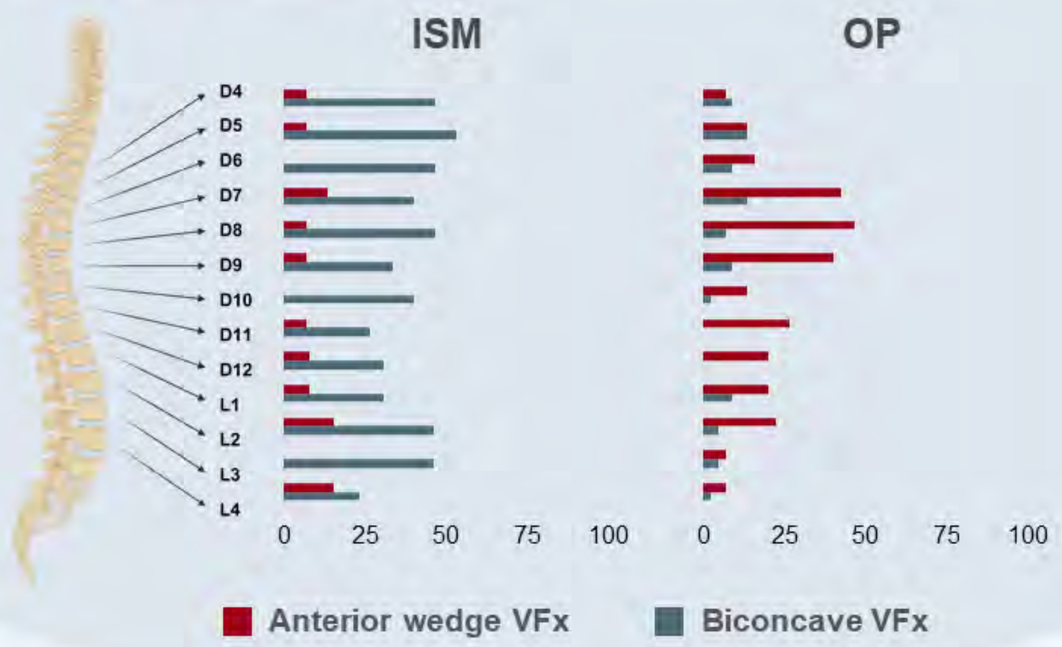
VFx assessment



X-ray of D4-L4 segment

Findings

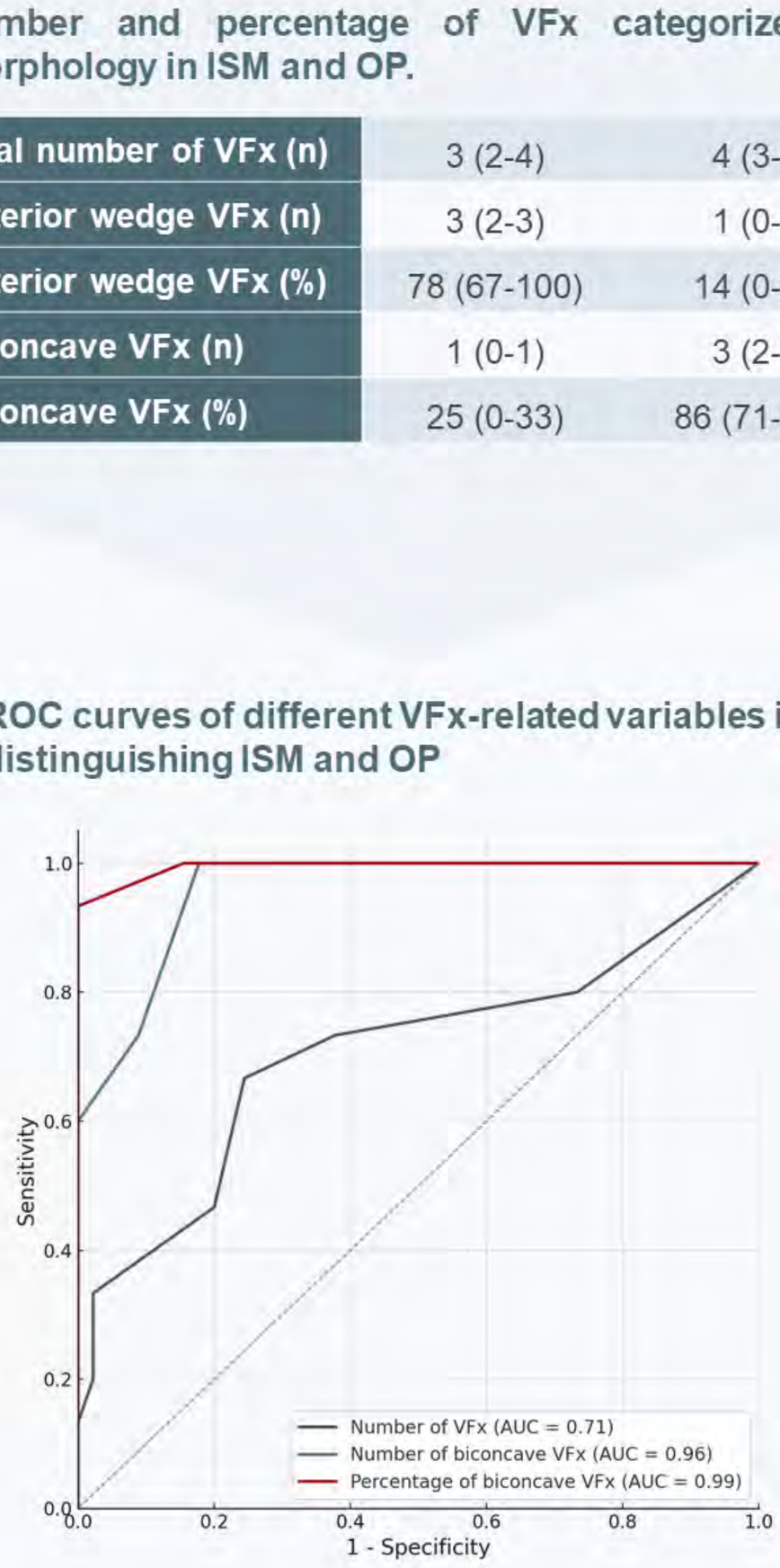
Number of VFx per 100 individuals categorized by morphology in ISM and OP.



Number and percentage of VFx categorized by morphology in ISM and OP.

Total number of VFx (n)	3 (2-4)	4 (3-6)
Anterior wedge VFx (n)	3 (2-3)	1 (0-1)
Anterior wedge VFx (%)	78 (67-100)	14 (0-29)
Biconcave VFx (n)	1 (0-1)	3 (2-5)
Biconcave VFx (%)	25 (0-33)	86 (71-100)

ROC curves of different VFx-related variables in distinguishing ISM and OP



Conclusions

Our study shows different prevalent morphologies of VFx between ISM and OP. Further research is needed to identify the distinct pathophysiological mechanisms between the two conditions and to explore the clinical implications.

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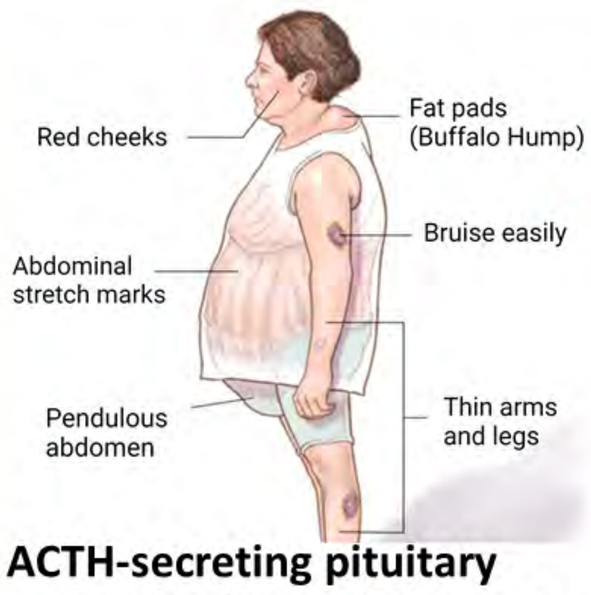
European Reference Network on Rare Bone Diseases (ERN BOND)

GAETANO PARIDE ARCIDIACONO
Clinica Medica 1

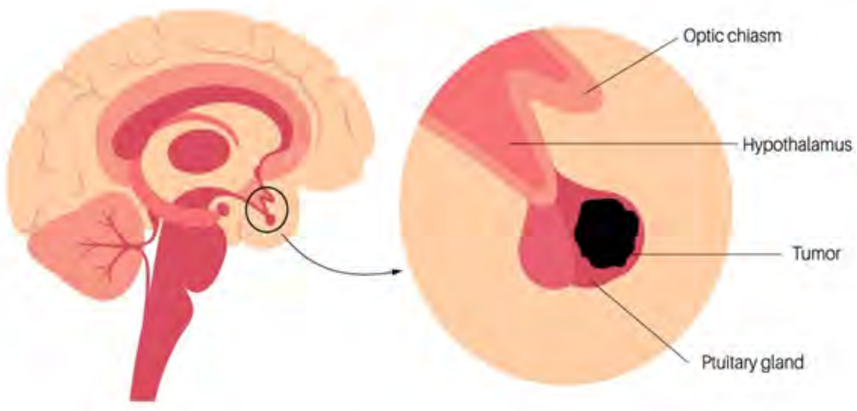
VI° CONVEGNO del DIMED
Padova, 15 novembre 2024

S. Avallone[1,2], E. Santillo[2], D. Regazzo[2], P. Simioni[2] and M. Barbot[2]
1 – Department of Biology – DIBIO, Padova, Italy
2 – Department of Medicine – DIMED, Padova, Italy

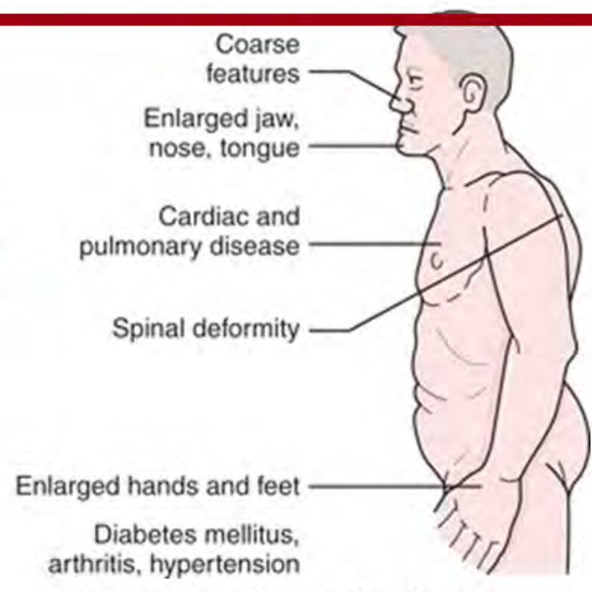
Establishing 3D Culture Models for Pituitary Adenomas



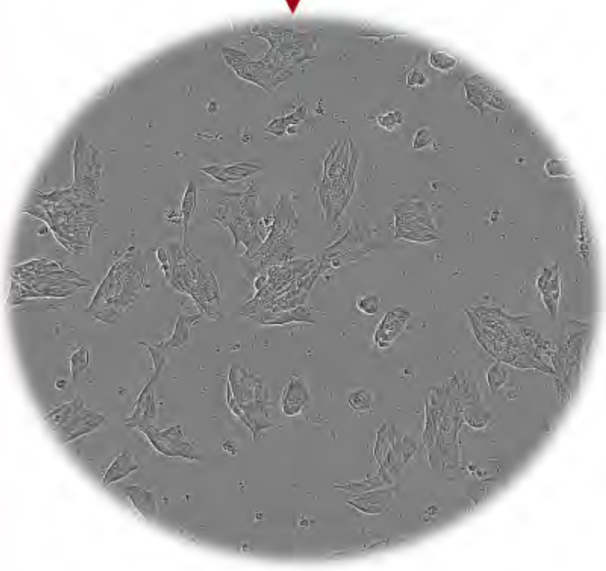
ACTH-secreting pituitary adenoma → Cushing Disease



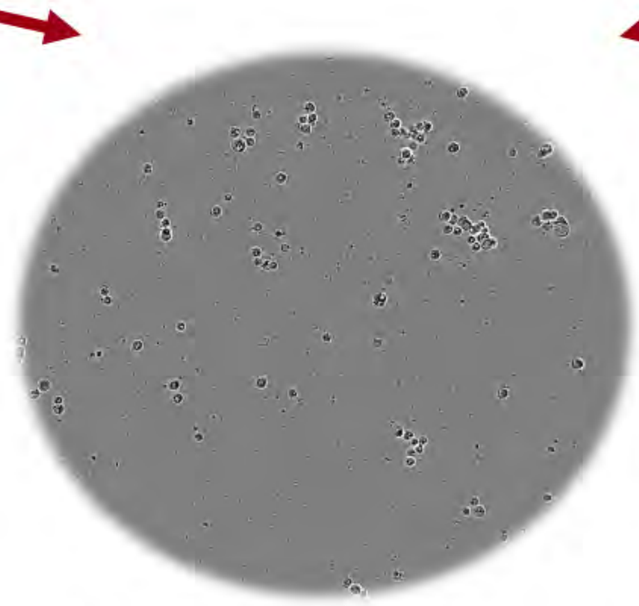
Pituitary adenoma



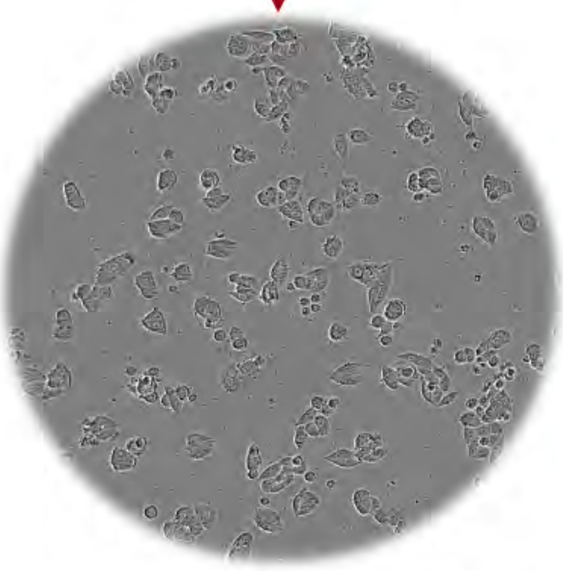
GH-secreting pituitary adenoma → Acromegaly



AtT-20

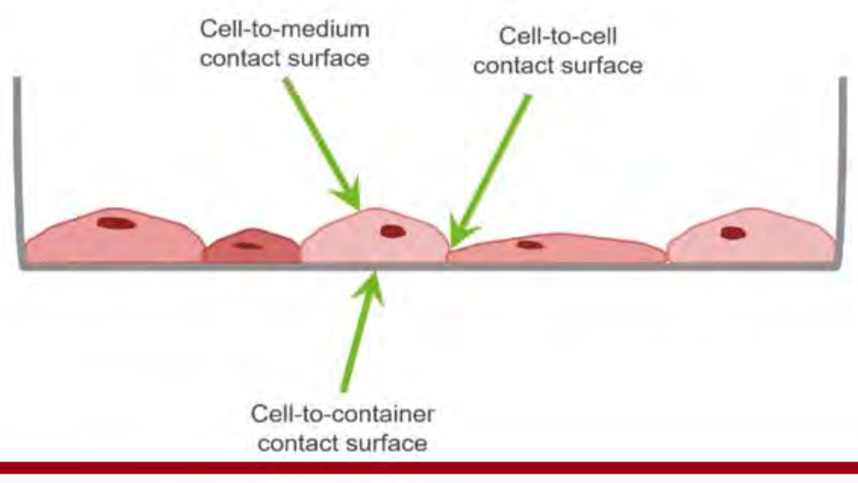


Primary cell cultures from surgically removed tumors

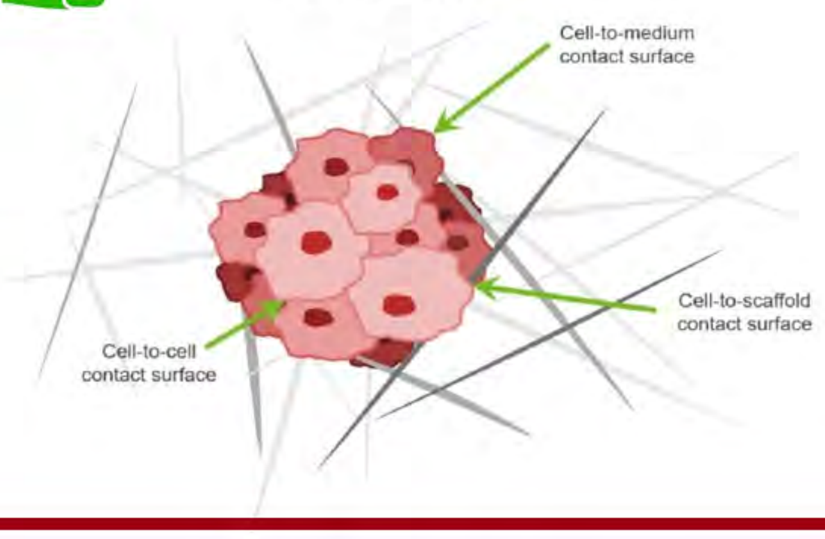


GH3

2D Culture

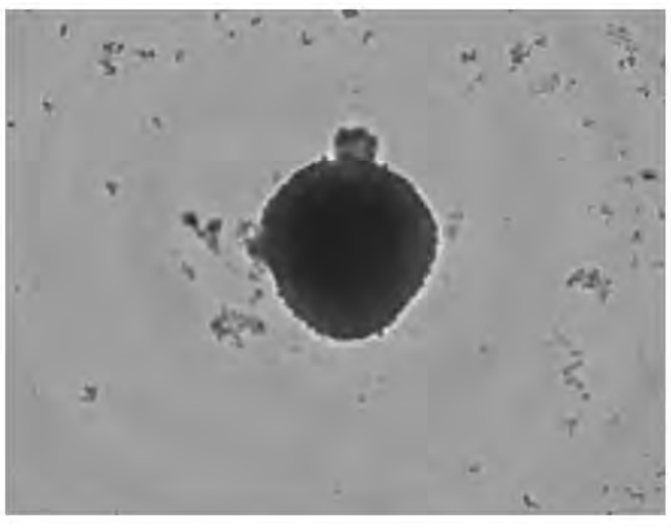


3D Culture

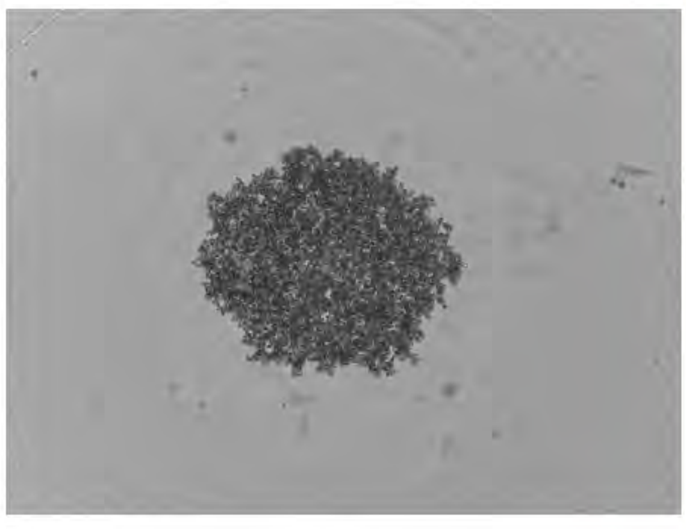


Now

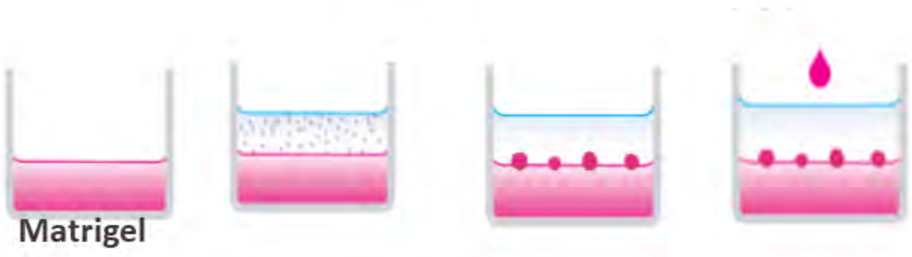
AtT-20



GH3



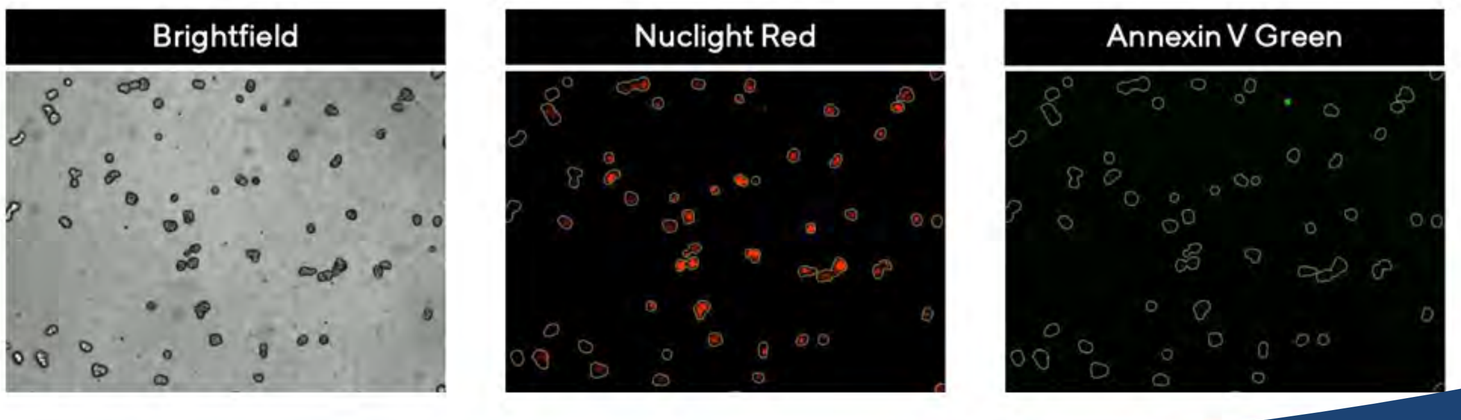
Future steps



Cell lines

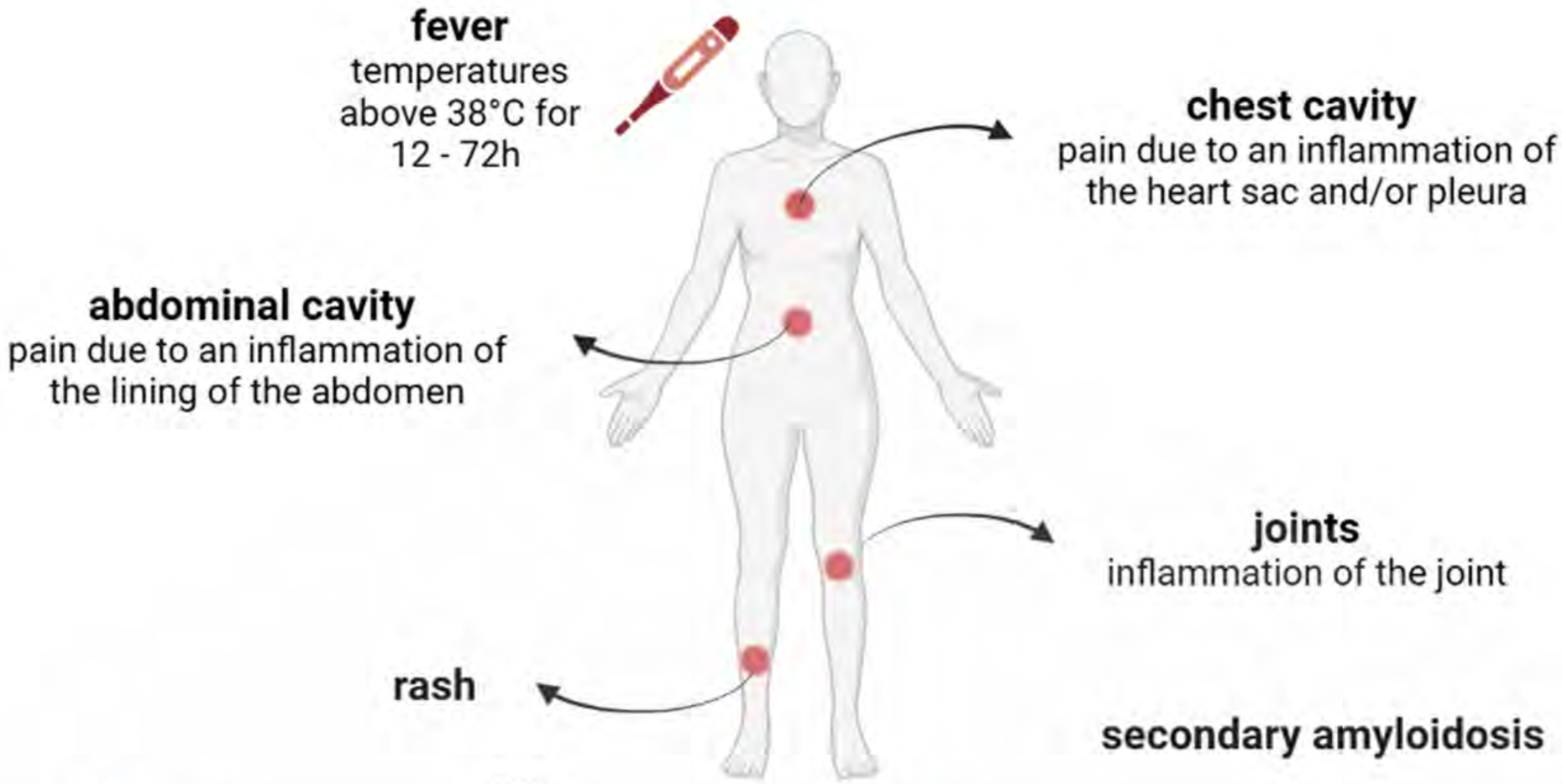
Primary cell cultures

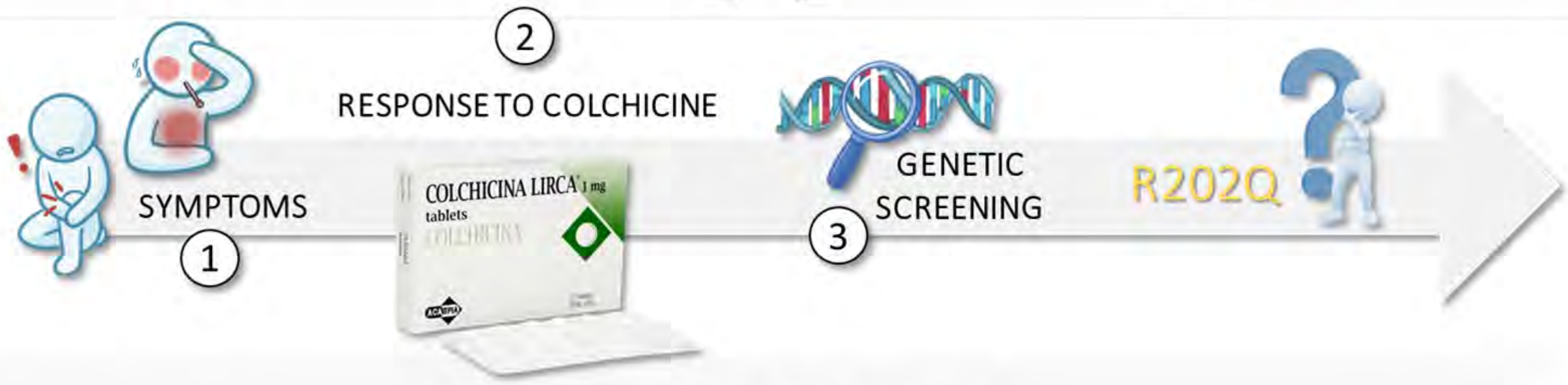
Treatments with new and currently used drugs



SERENA AVALLONE
Endocrinologia

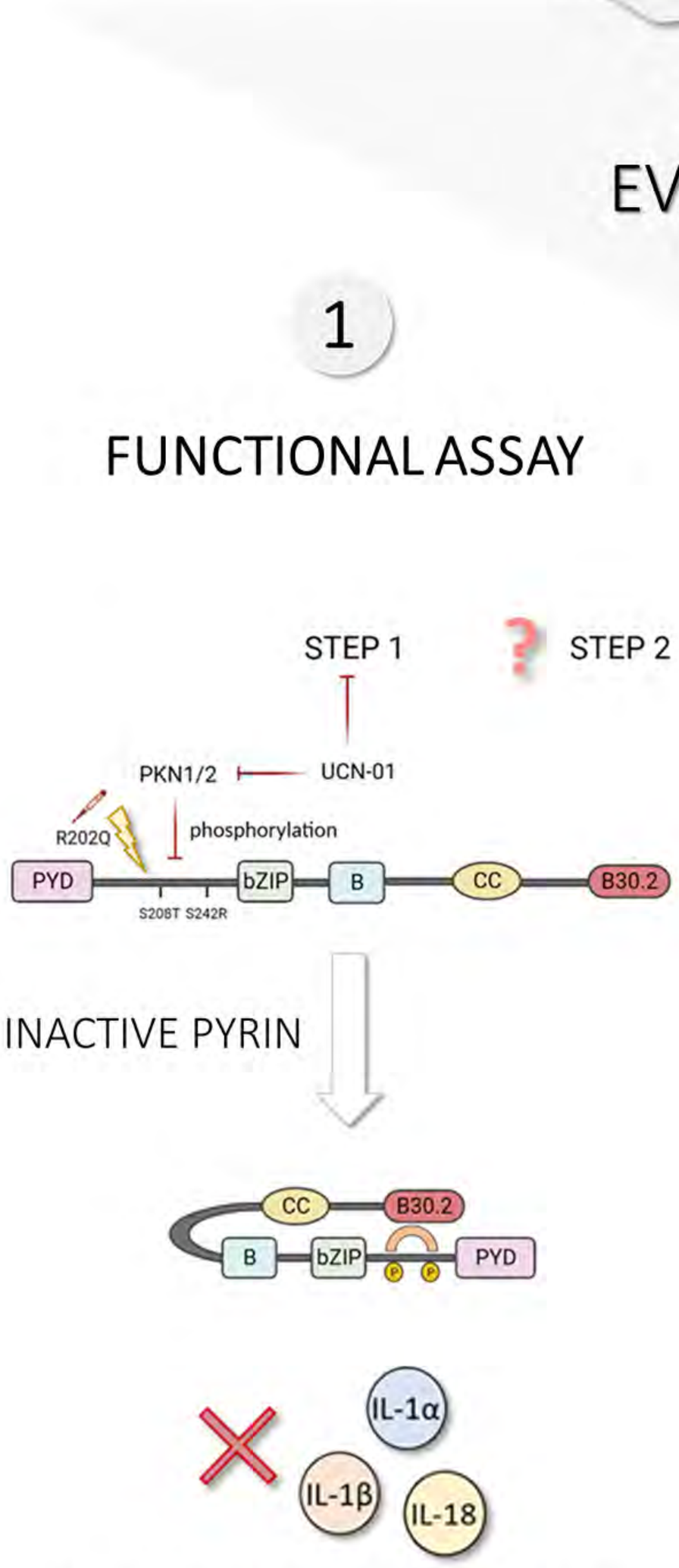
FUNCTIONAL CHARACTERIZATION OF PYRIN IN PATIENTS WITH R202Q MEFV VARIATION





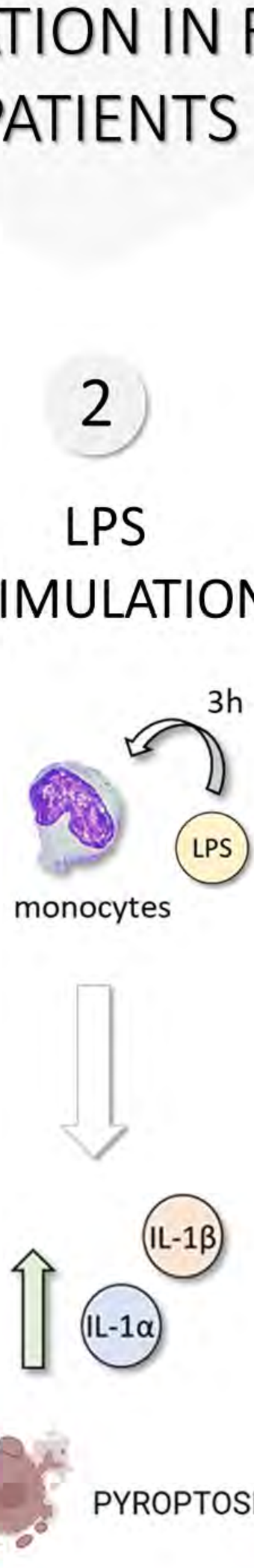
PYRIN FUNCTION EVALUATION IN R202Q PATIENTS

1 FUNCTIONAL ASSAY

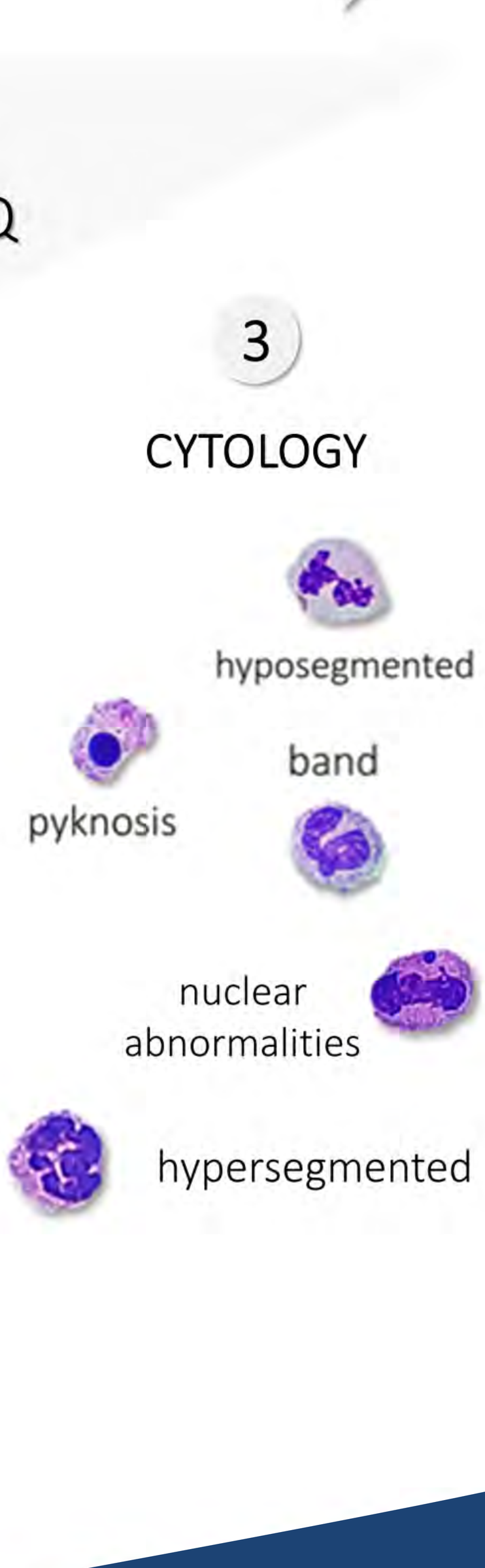


Step 2 avoid uncontrolled pyrin inflammasome activation

2 LPS STIMULATION



3 CYTOLOGY



CHIARA BAGGIO
Reumatologia

VI° CONVEGNO del DIMED
Padova, 15 novembre 2024

The impact of physical activity on cardiorespiratory fitness in people with surgically corrected congenital heart disease and different ESC 2020 competitive sports eligibility class

Francesca Battista[1,2]; Marco Vecchiato[1,2]; Barbara Mazzucato[1,2]; Gino Degano[1,2] Sara Faggian[1,2], Sara Ortolan[1,2], Giulia Quinto[1,2]; Giovanni Di Salvo[3], Daniel Neunhaeuserer[1,2]; Andrea Ermolao[1,2]

1 Sports and Exercise Medicine Division, Department of Medicine, University of Padova, 35128 Padova, Italy
2 Clinical Network of Sports and Exercise Medicine of the Veneto Region, 35131 Padova, Italy
3 Department of Women's and Children's Health, University of Padua.

BACKGROUND

- Almost 90% of people with Congenital heart disease (CHD) reach adulthood in developed countries, increasing the participation in sporting activities.
- ESC2020 guidelines for participation in recreational and competitive sports were published.

AIMS

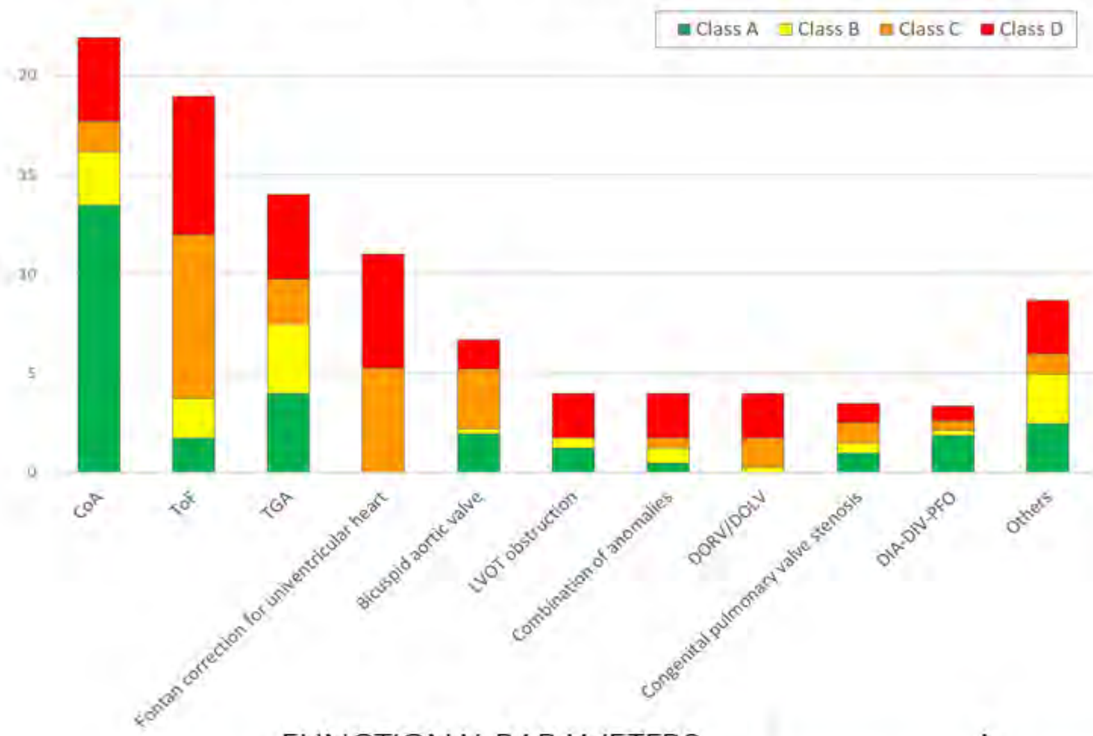
- To show the prevalence of the sport eligibility based on ESC 2020 and the level of real physical activity in a real CHD population.
- To evaluate the cardiorespiratory fitness (CRF) and the impact of training among different ESC2020 classes.

METHODS

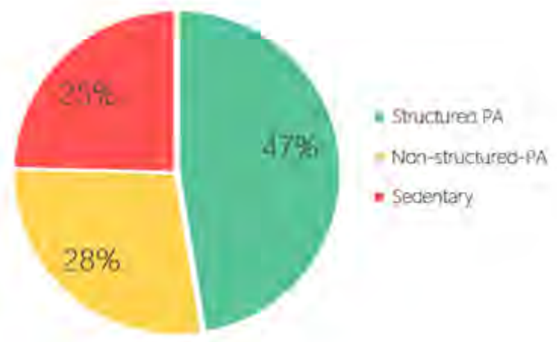
- Retrospective study on 401 patients (66% male sex) with surgically corrected CHD
- Cardiopulmonary exercise test (CPET) performed on treadmill or cycle ergometer until patients' exhaustion.
- Subjects were divided in the four ESC 2020 classes of eligibility for all competitive sports (A), all except endurance specialties (B), only for skill disciplines (C) or not eligible (D).

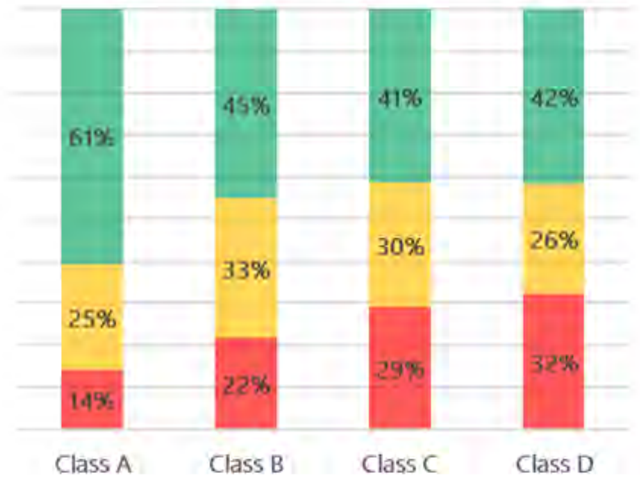
RESULTS

Prevalence of CHD and ESC 2020 eligibility classes



Prevalence of physical activity practice in the whole population and among ESC2020 classes

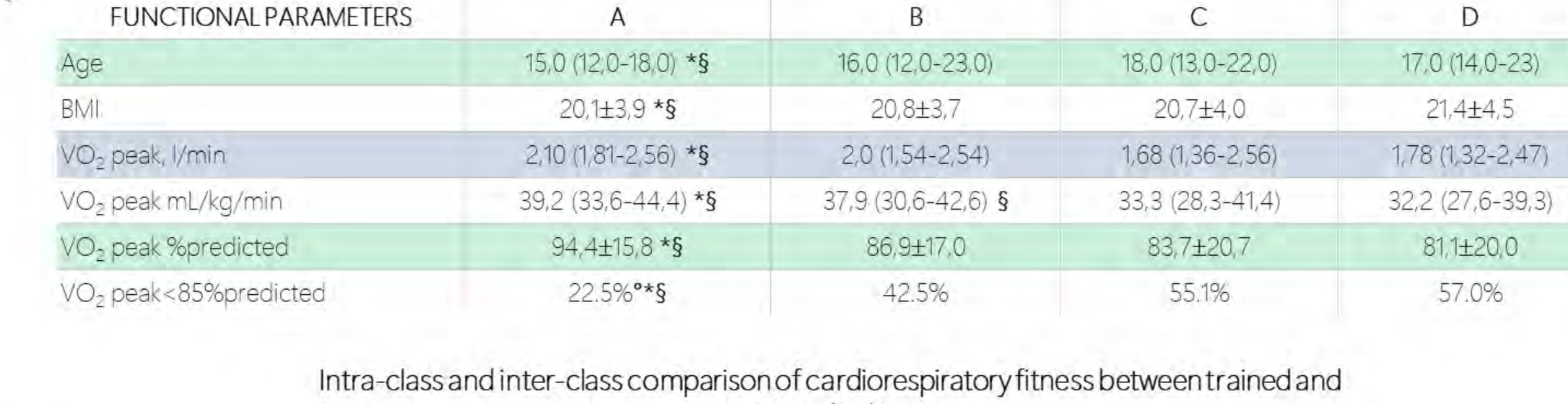




FUNCTIONAL PARAMETERS	A	B	C	D
Age	15,0 (12,0-18,0) *§	16,0 (12,0-23,0)	18,0 (13,0-22,0)	17,0 (14,0-23)
BMI	20,1±3,9 *§	20,8±3,7	20,7±4,0	21,4±4,5
VO_2 peak, l/min	2,10 (1,81-2,56) *§	2,0 (1,54-2,54)	1,68 (1,36-2,56)	1,78 (1,32-2,47)
VO_2 peak mL/kg/min	39,2 (33,6-44,4) *§	37,9 (30,6-42,6) §	33,3 (28,3-41,4)	32,2 (27,6-39,3)
VO_2 peak %predicted	94,4±15,8 *§	86,9±17,0	83,7±20,7	81,1±20,0
VO_2 peak<85%predicted	22.5%°*§	42.5%	55.1%	57.0%

°<0.05 vs.B
*<0.05 vs. C
§ <0.05 vs. D

Intra-class and inter-class comparison of cardiorespiratory fitness between trained and untrained subjects



Comparison between trainend and untrained subjects in the same ESC2020 class and between different ESC2020 classes. Comparisons among only trained and untrained subjects, as weel as the precise pvalue of all the comparisons are reported in Supplementary table 3. * p< 0.05

CONCLUSIONS

- Subjects in class A had better functional capacity.
- Exercise training (including competitive or non-competitive activities) can have a significant impact on CRF level.
- Functional evaluation and individualized exercise prescription may recruit functional "reserve" by improving the VO2peak.

ACSM EXERCISE IS MEDICINE®

FRANCESCA BATTISTA
Medicina dello Sport e dell'Esercizio

A new assay for quantitative determination of PreS1 encoded protein in hepatitis B and delta virus infected patients

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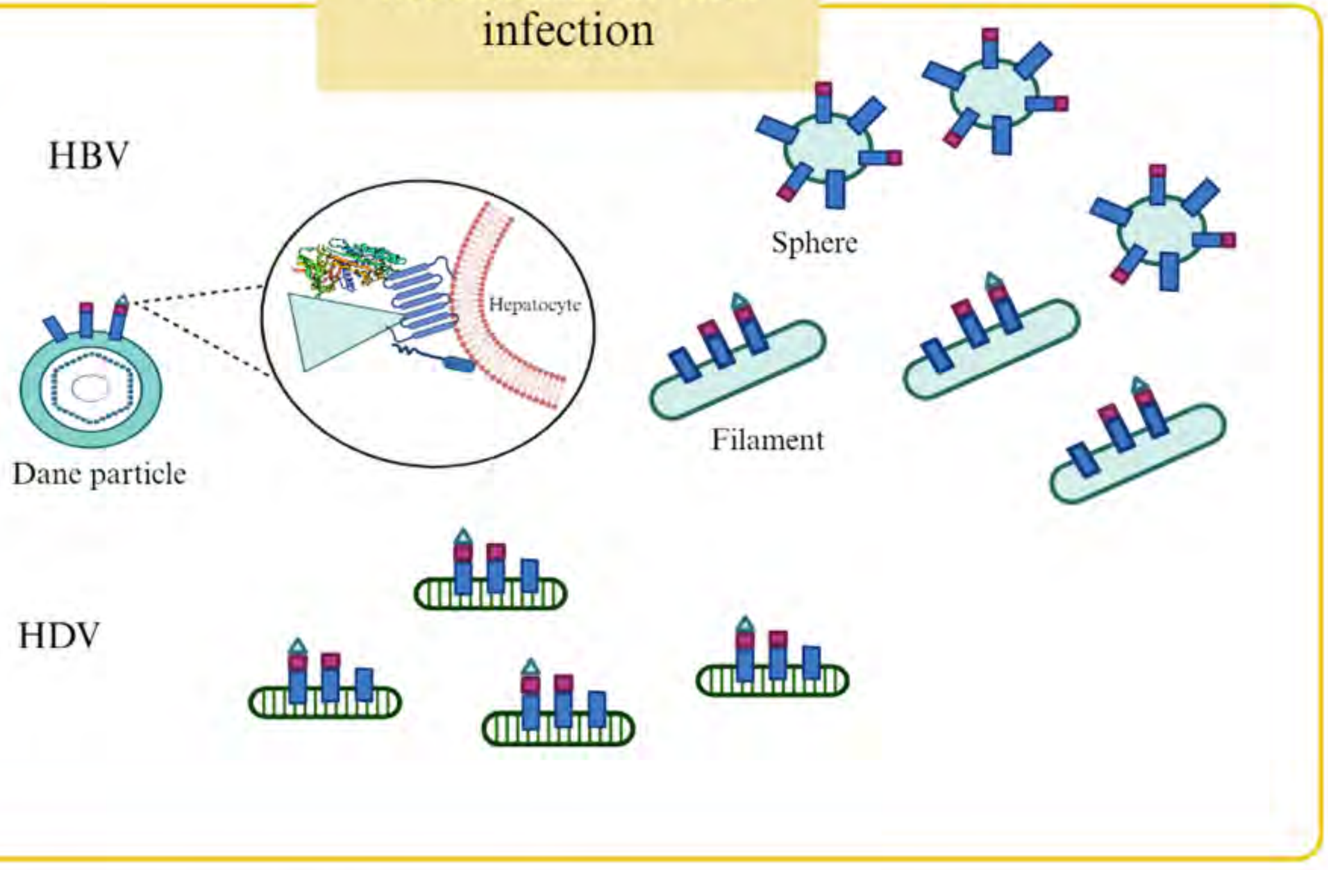
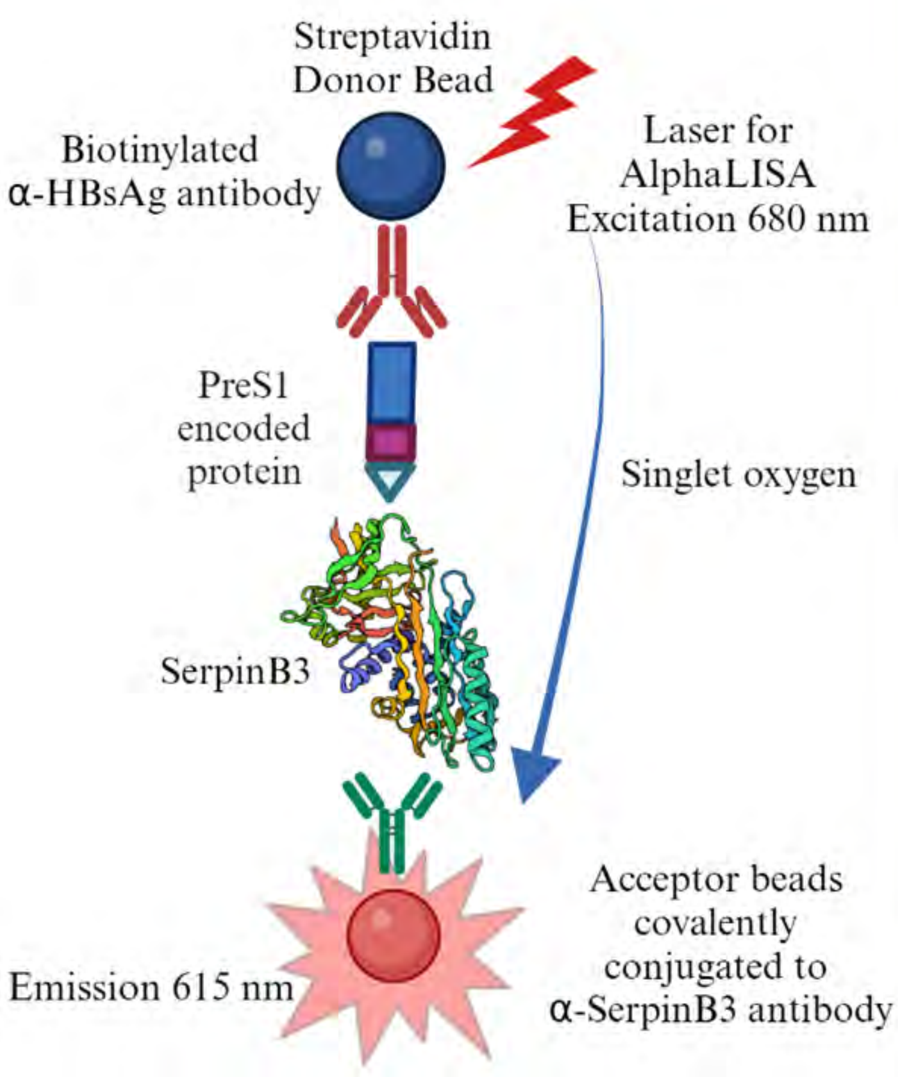
Department of Medicine, University of Padova, Via Giustiniani 2, 35128 Padova, Italy

Mechanism of viral infection

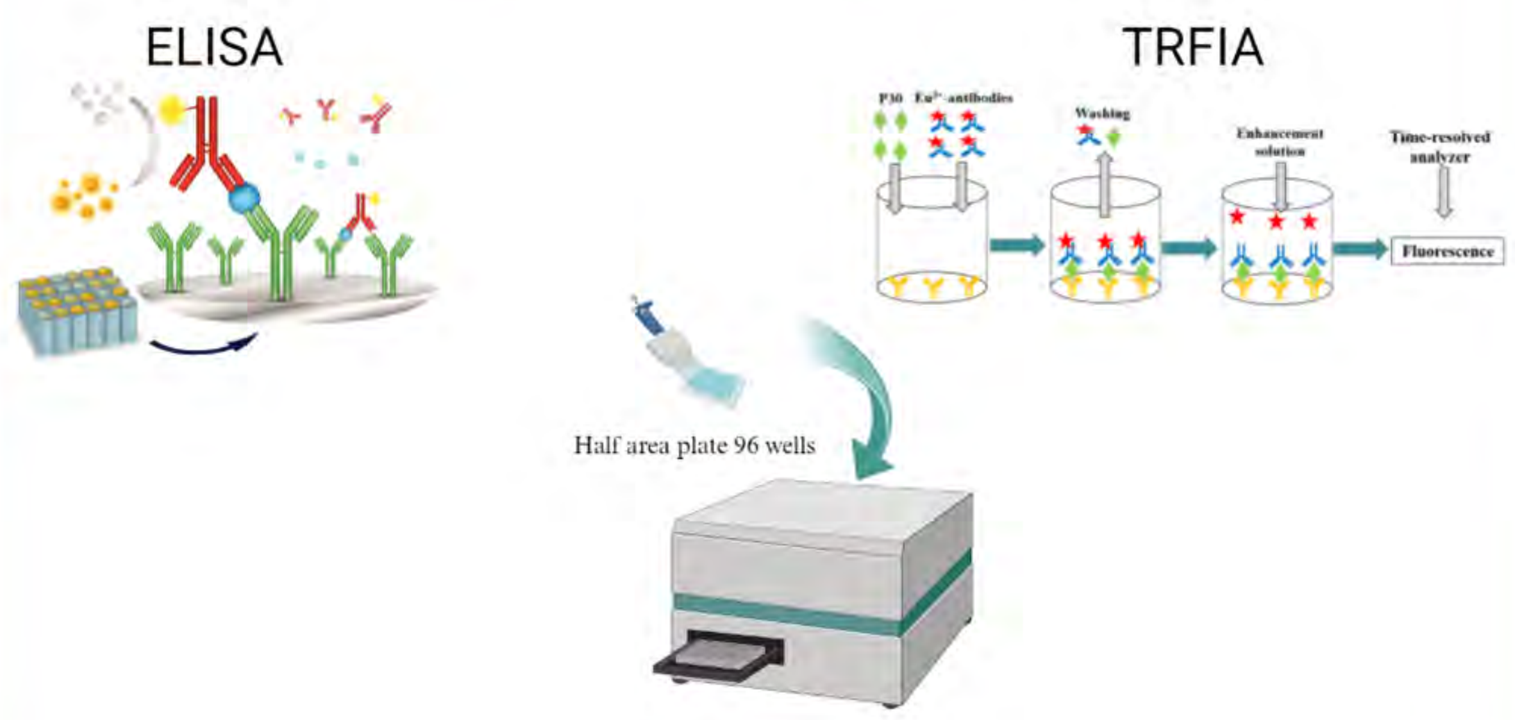


Principles of AlphaLISA assay



Screening HBV positive patients

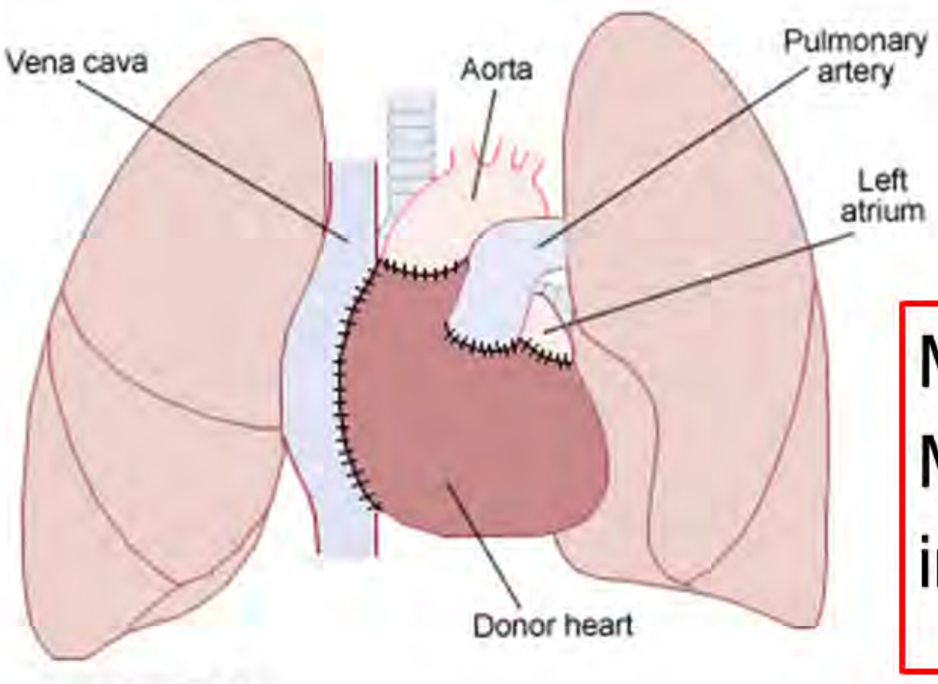
Detection methods



BioRender.com

ALESSANDRA BIASIOLO
Clinica Medica 5

Head-to-head: advanced Cardiovascular Magnetic Resonance and Endomyocardial Biopsy in heart transplantation.



Allograft rejection remains a major complication in the first year after in heart transplantation (HT).

Myocardial characterization by Cardiovascular Magnetic Resonance (CMR) is heavily entering into the follow-up of heart-transplanted (HT).

To compare CMR and Endomyocardial biopsy (EMB) findings in HT population.

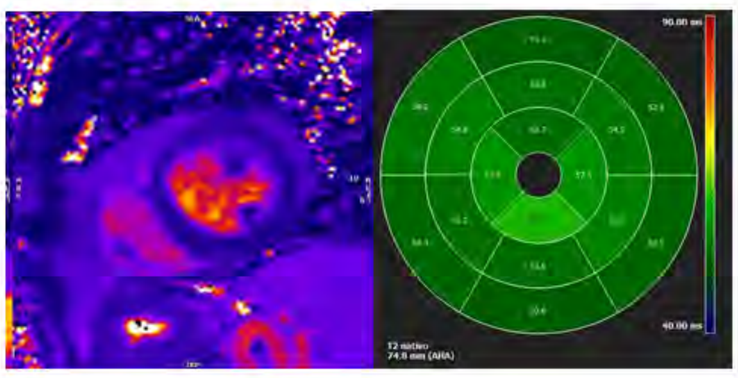
14 HT patients

CMR

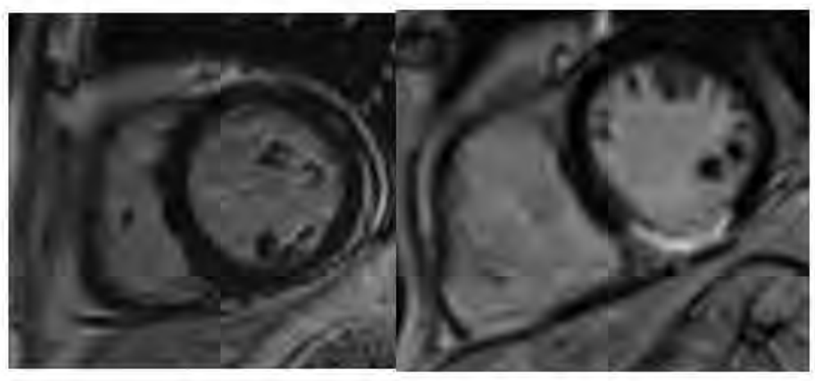
+

EMB
within 30 days form CMR

Advanced myocardial characterization sequences (**MOLLI, True-FISP**)



PSIR T1 post late MdC:
LGE assessment for fibrosis (ischemic and non-ischemic pattern)



POSITIVE FOR MYOCARDIAL EDEMA/INFLAMMATION

NEGATIVE FOR REJECTION

14 CMR of patients with heart transplantation	
Rejection	
Positive CMR (T2 mapping), N (%)	9 (64.3)
Positive EBM (inflammatory infiltration), N (%)	4 (28.6)
Positive CMR with negative EBM, N (%)	5 (35.7)
Positive EBM with negative CMR, N (%)	0
Fibrosis	
Positive CMR (LGE), N (%)	5 (35.7)
Positive EBM (fibrosis), N (%)	3 (21.4)

In five patients (35.7%) CMR provided a **diagnostic advantage** showing signs compatible with edema/inflammation based on the mapping technique, despite EBM turned out to be negative

CMR seems to be a **more sensitive non-invasive tool** for HT monitoring, compared to BEM

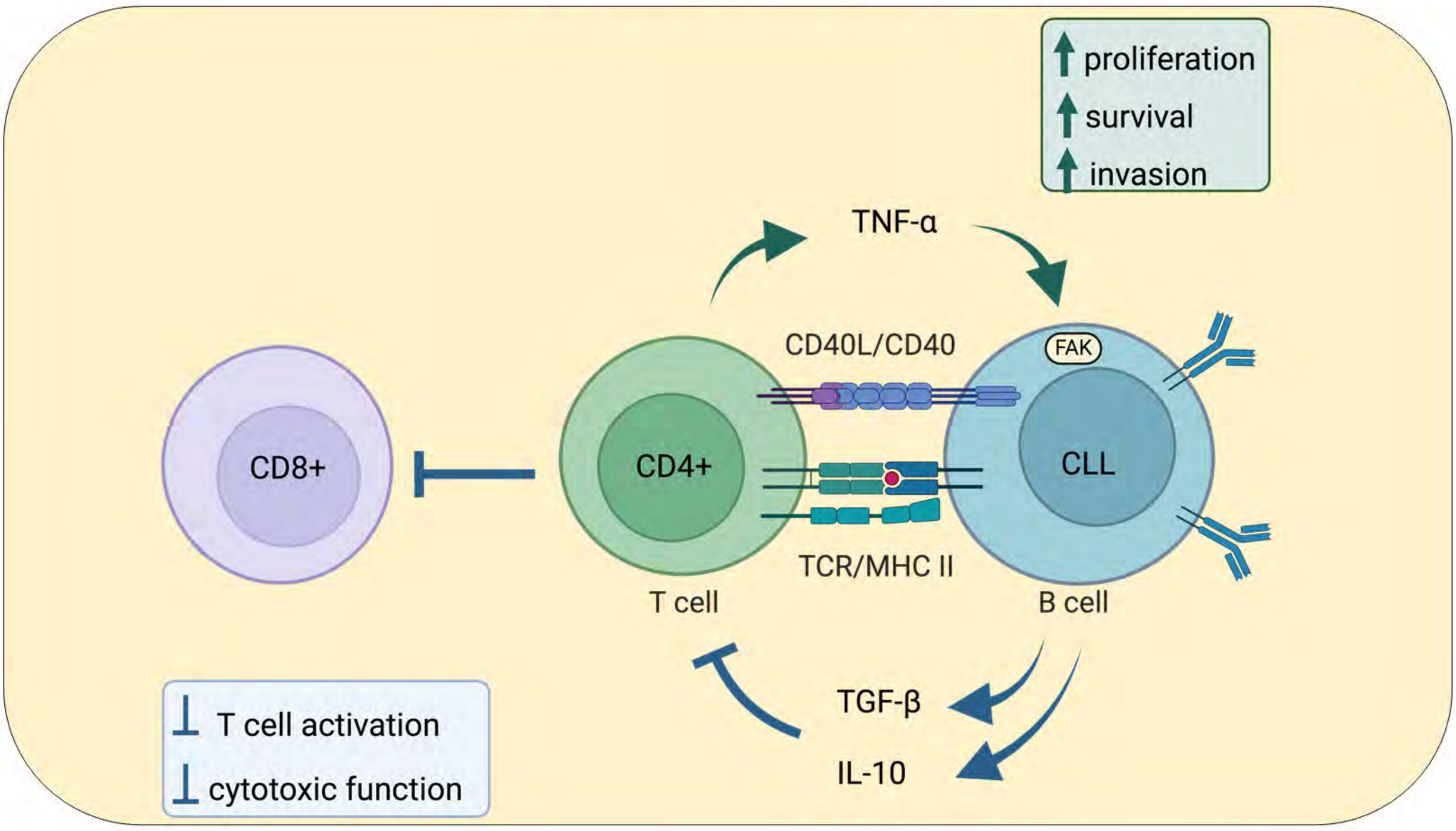
Thanks to the mapping technique CMR:

1) can identify cardiac damage even when the EMB is negative, giving the possibility to early tailor the therapy;

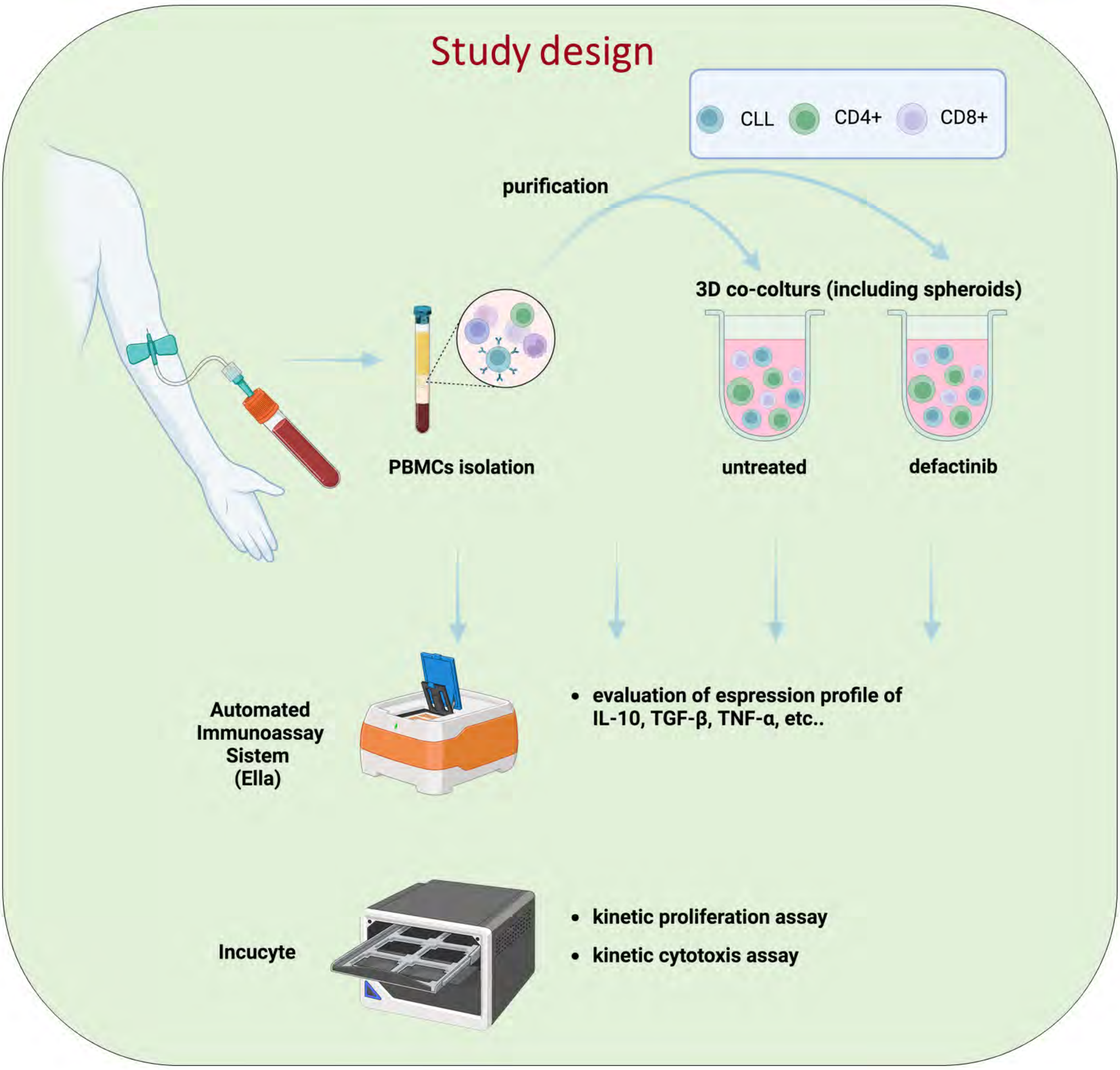
2) does not fail in detecting myocardial alterations when the EMB is positive for rejection.

GIULIO CABRELLE
Radiologia Universitaria

Investigating FAK inhibition by defactinib in CLL-TME interactions



Study design



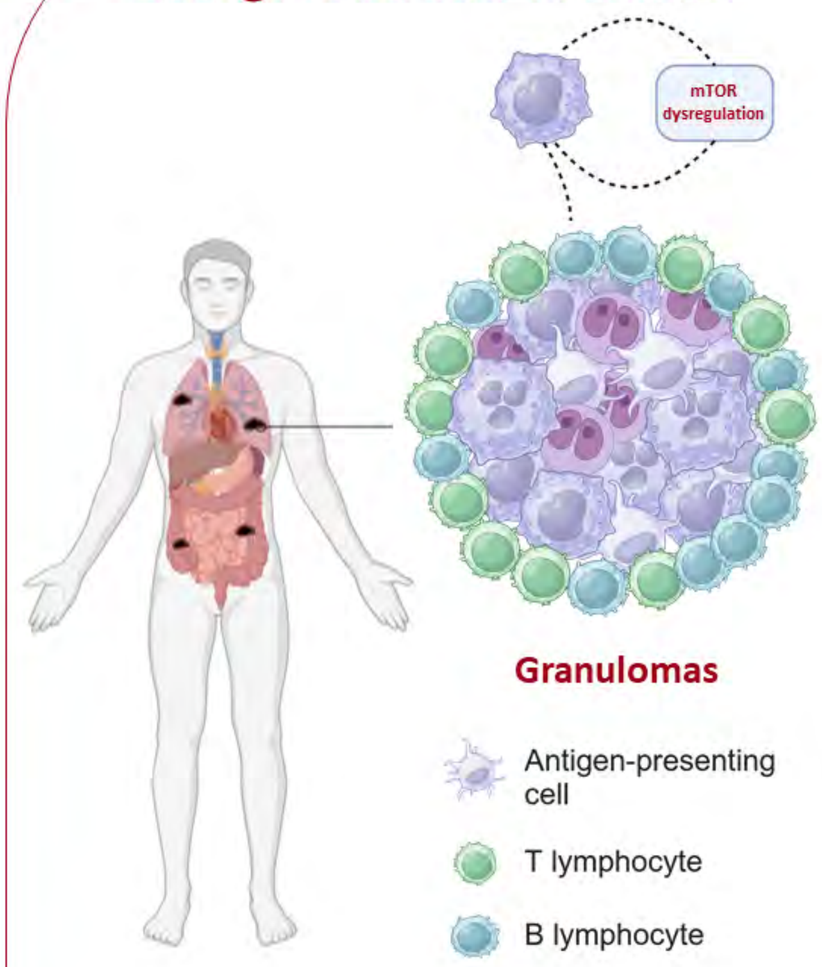
CLL (chronic lymphocytic leukemia); TME (tumor microenvironment); PBMCs (peripheral blood mononuclear cells).

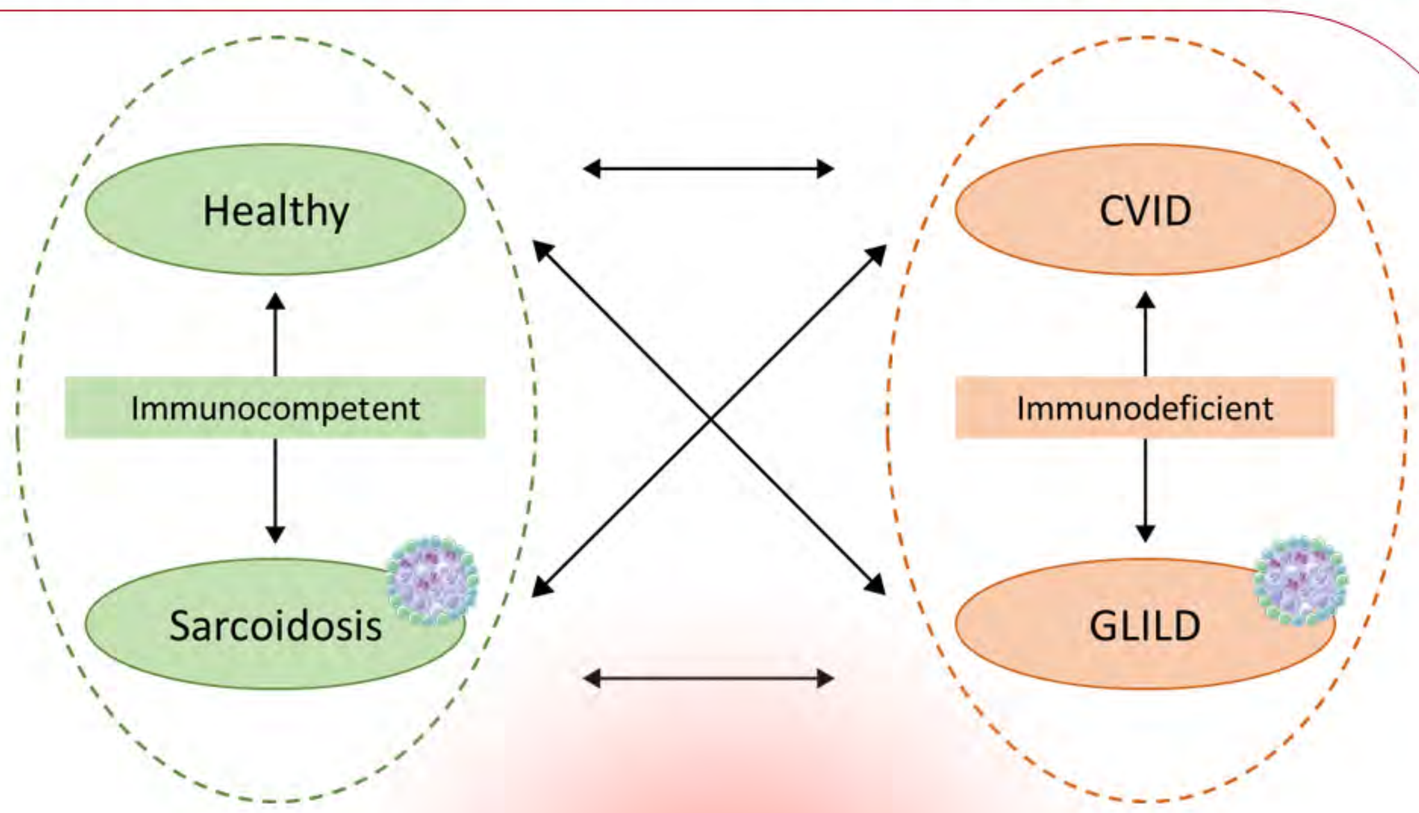
MARIA CASTRONUOVO
Laboratorio di Ematologia

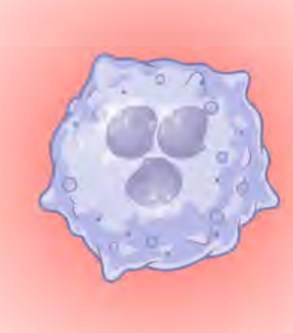
VI° CONVEGNO del DIMED
Padova, 15 novembre 2024

Will sarcoidosis help us understanding granulomatous disease in Common Variable Immunodeficiency (CVID) patients?

Background and rationale

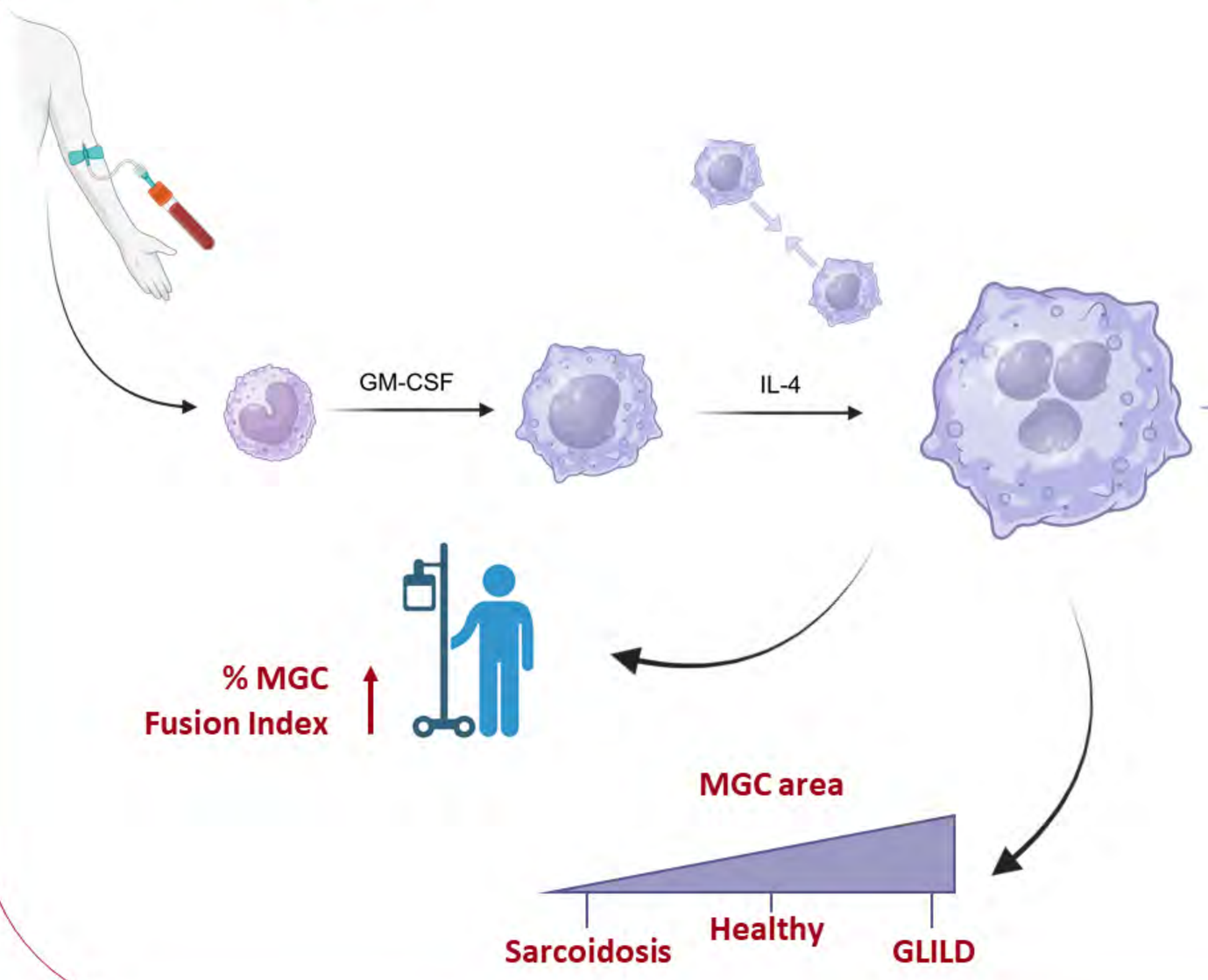


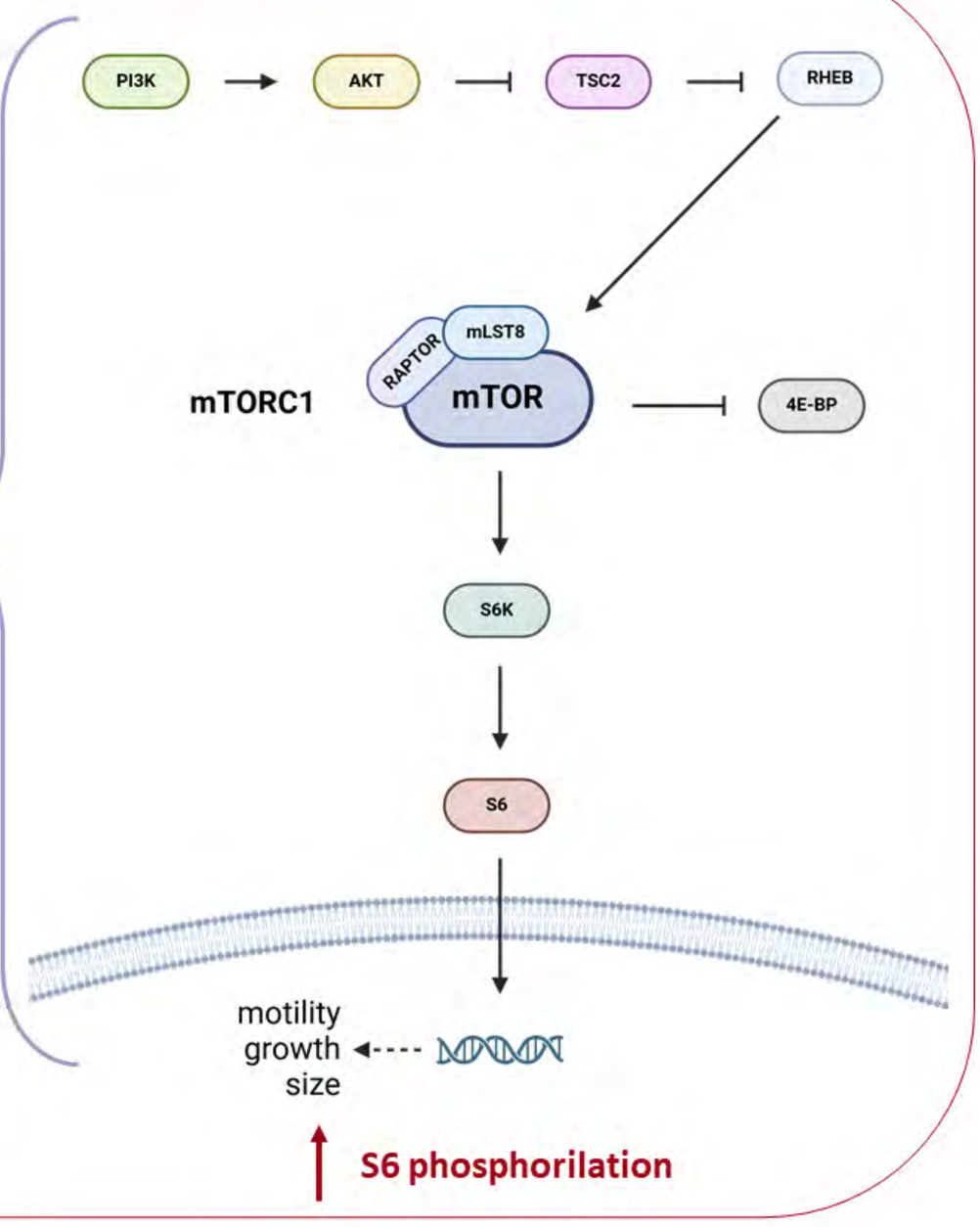




MGC role ?

Preliminary data



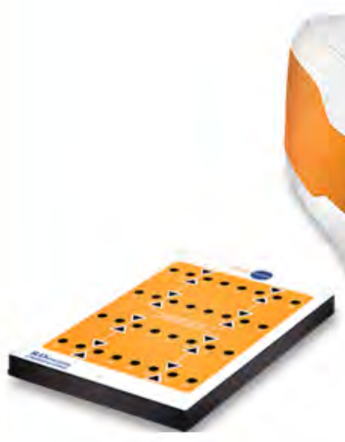


Future perspectives

Cytokines

Cell lysates

Jess Symple Western System



Protein Simple ELLA

MGC role ?

Cell fusion

Incucyte S3 Live-Cell Analysis

JESSICA CECCATO
Ematologia e Immunologia

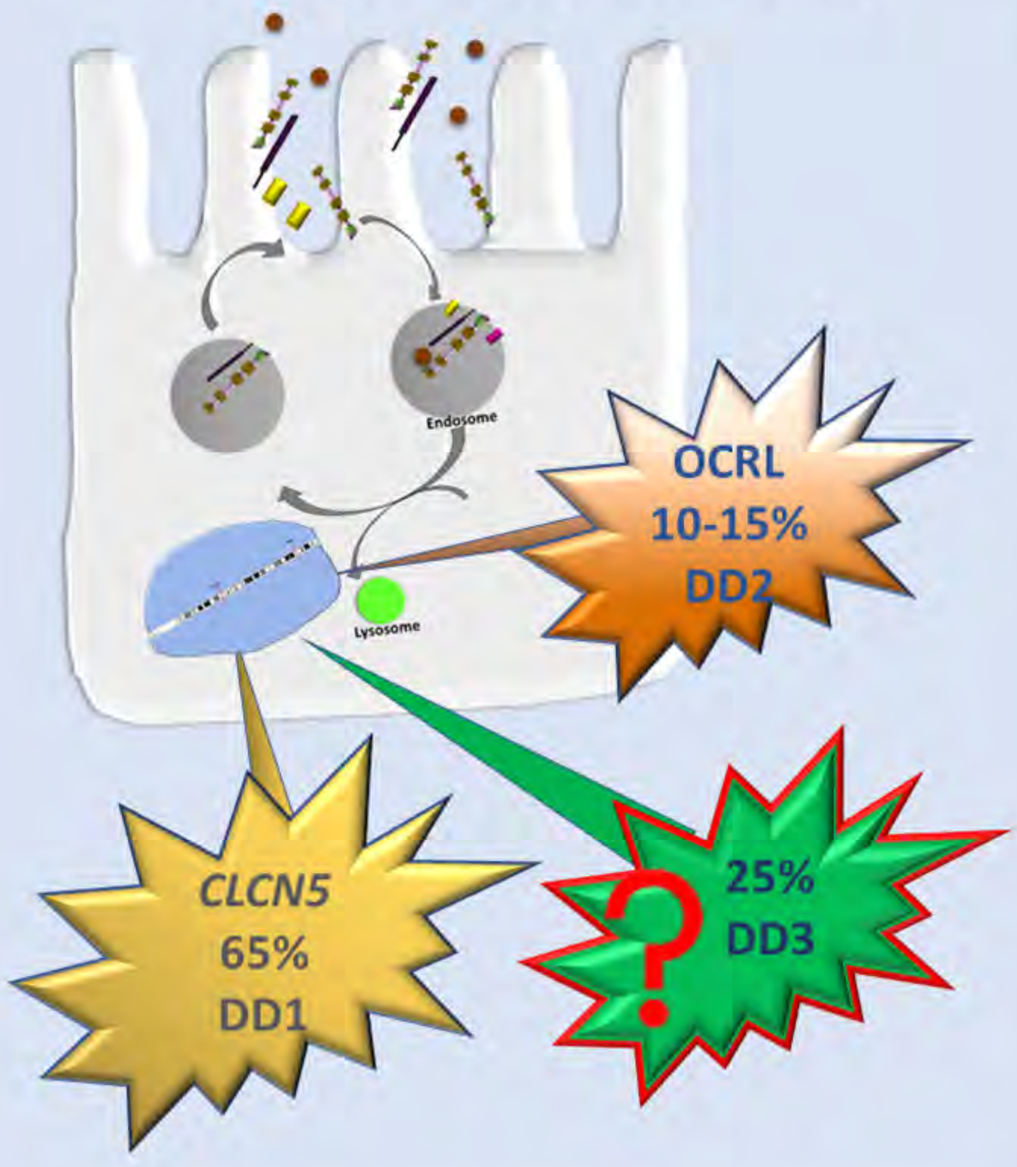
VI° CONVEGNO del DIMED
Padova, 15 novembre 2024

Identification of new pathogenic variants in Dent Disease patients with no detectable mutation in *CLCN5* and *OCRL* genes: Whole Exome Sequencing study.

Ceol M[1], Giannella A[2], Ceolotto G[3], Priante G[1], Sgro' E[1], Nalesso F[1], Anglani F[1], Calò LA[1], Del Prete D[1]

1: Kidney Histomorphology and Molecular Biology Laboratory, Nephrology Unit, Department of Medicine, 2: Department of Medicine - DIMED, University of Padua, Italy;
3: Emergency Medicine Unit, Department of Medicine - DIMED, University of Padua, Italy;

Dent disease is a rare X-linked renal tubulopathy due to *CLCN5* and *OCRL* mutations



Do DD3 represent unrecognized disease phenotype of known hereditary nephropathies?

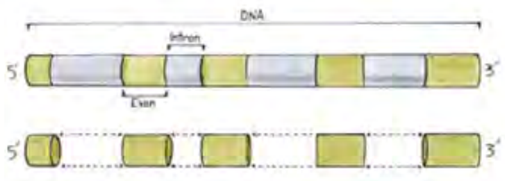
DENT DISEASE MAIN CLINICAL SIGNES

- ✓ LMWP
- ✓ Hypercalciuria
- ✓ Nephrocalcinosis
- ✓ nephrolithiasis

DD3 BLENDED PHENOTYPE

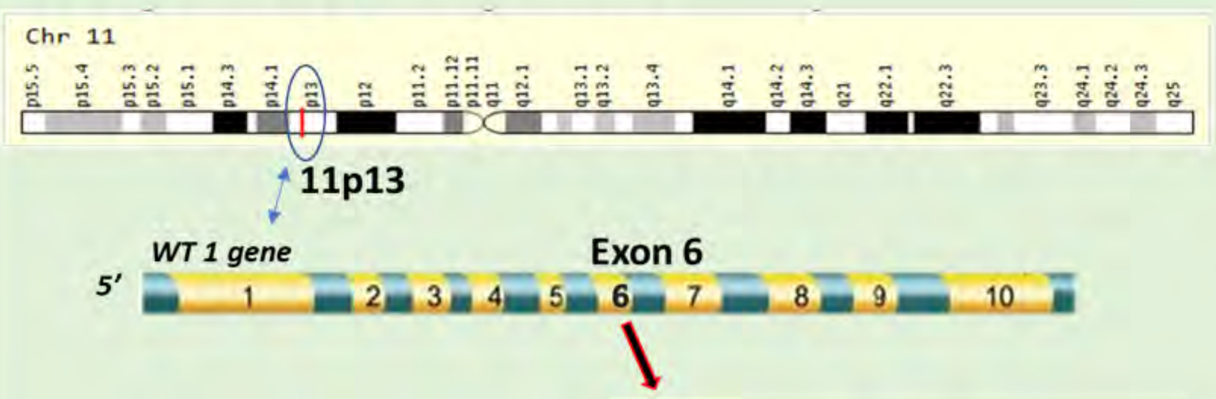
- nephrotic range proteinuria
- nephrolithiasis
- nephrocalcinosis
- LMWP.

Whole exome sequencing (WES)

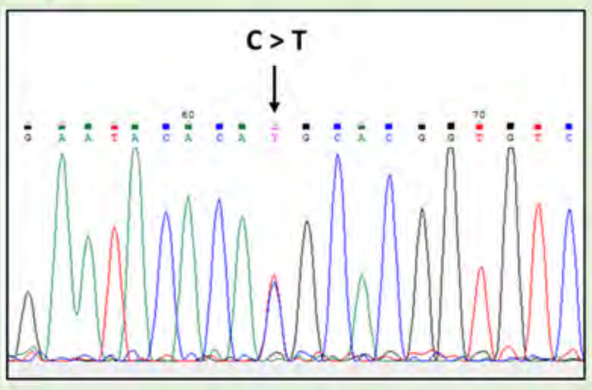


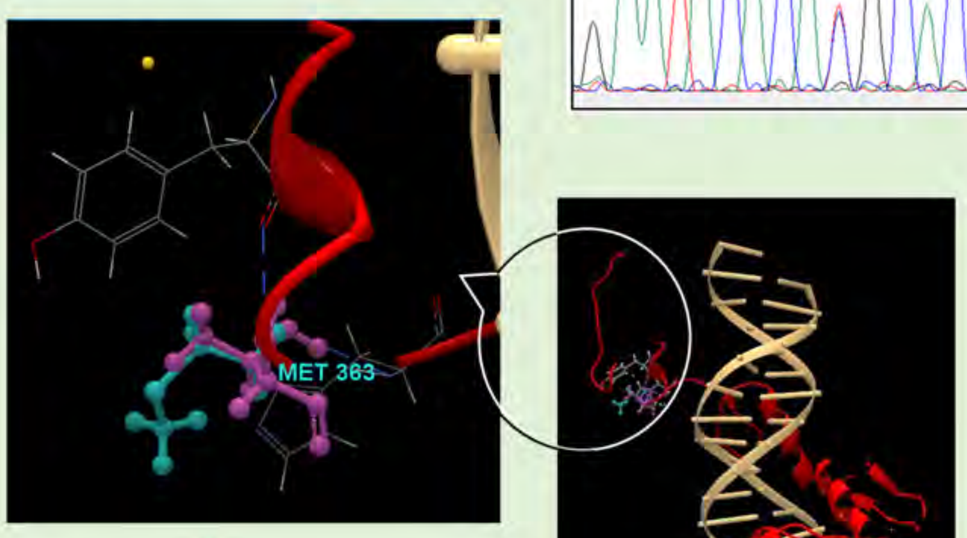
WES technology to identify novel genetic variants in disease with high degree of genetic heterogenity and blended phenotype

WT1 transcription factor

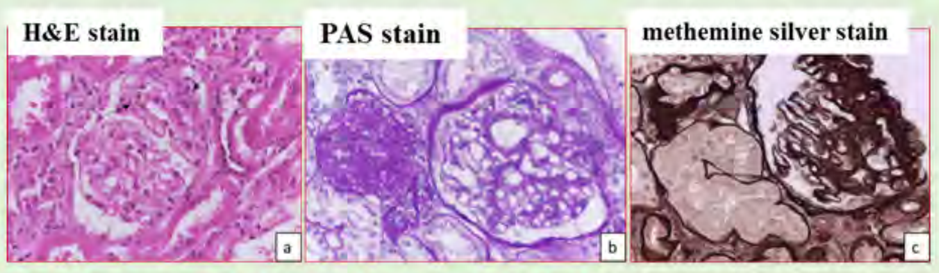


Ultra rare heterozygous missense variant (VUS) (NM_024426.6) c.1088C>T





p.Thr 363 Met

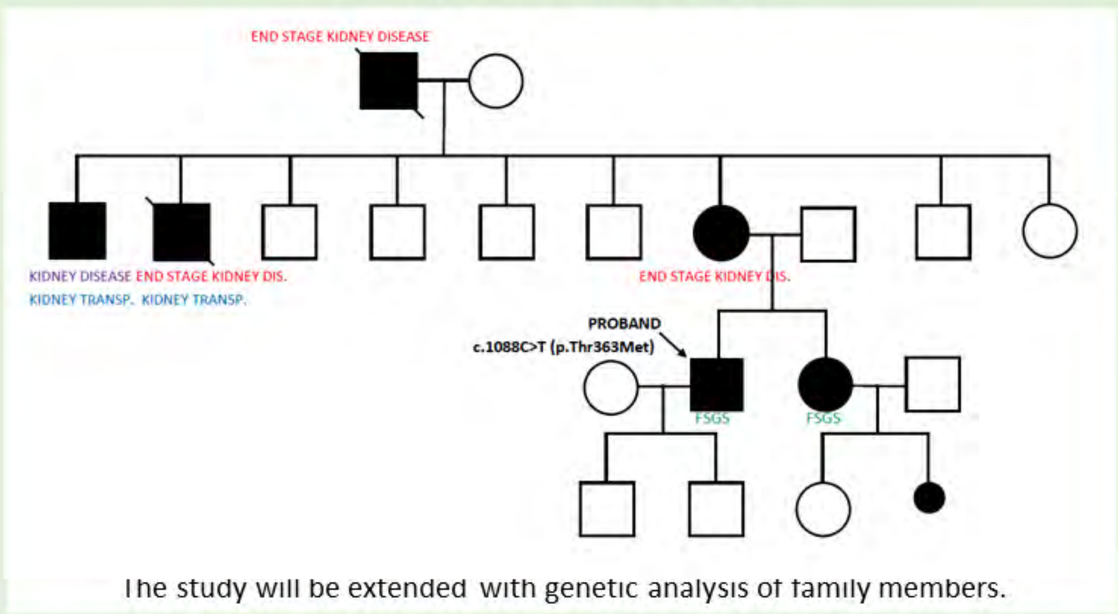


Focal segmental glomerulosclerosis (FSGS) is characterised by focal scarring of the glomerular capillary tuft and podocyte injury.

Wilms' tumour suppressor gene-1 (**WT1**) plays a critical role in kidney development and function.

A WT1 mutation is one of the most common causes of FSGS

DD3 family members also have renal impairment, End Stage Kidney Disease, Kidney Transplant and FSFG.



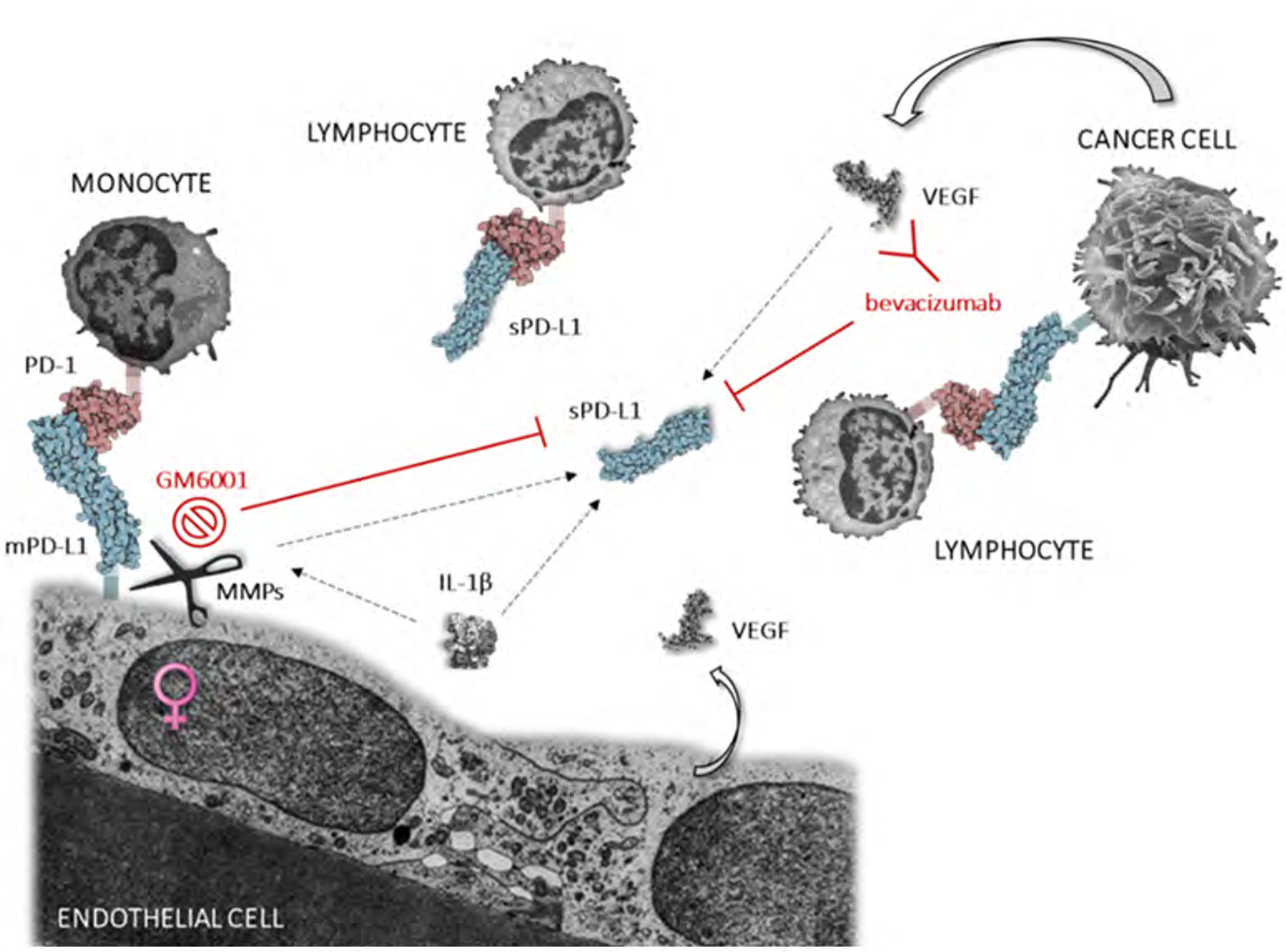
The study will be extended with genetic analysis of family members.

- WT1 autosomal dominant disorder is responsible for patient's phenotype.
- WT1 may be a good candidate for DD3 patients presenting with nephrotic syndrome and FSGS.

- The DD3 phenotype can present as an atypical phenotype or as a mixed phenotype of several known inherited nephropathies.
- From a clinical point of view, tubular abnormalities are often a diagnostic challenge. Clinical features of different tubulopathies sometimes overlap, and it is difficult to identify a classic phenotype. The WES approach may be a good strategy to identify multiple genetic defects underlying mixed phenotypes.

MONICA CEOL
Nefrologia

Sex-specific immunomodulating role of human endothelial cells



ANDREA CIGNARELLA
Farmacologia

VI° CONVEGNO del DIMED
Padova, 15 novembre 2024

Tapering of therapy in SpA: the Impact of LifesTyle and predictors of sustained remission (TILT study)

Giacomo Cozzi, Mariagrazia Lorenzin, Laura Scagnellato, Francesca Battista, Valerie Tikhonoff, Andrea Ermolao, Andrea Doria, Roberta Ramonda

Background

The tapering and discontinuation strategies of biological and targeted synthetic disease-modifying antirheumatic drugs (bDMARDs and tsDMARDs, respectively) in spondyloarthritis (SpA) are not standardized, and the recommendations provided by major international guidelines rely on expert consensus due to the absence of strong literature data. The TILT study investigates tapering strategies in SpA focusing on the impact of lifestyle changes. Tapering can reduce costs and side effects but poses a risk of disease reactivation. Non-pharmacological approaches, particularly physical exercise and diet are crucial additions to DMARD therapy.

Objective

This project aims to identify ultrasound and biohumoral predictors of flare post therapy tapering.

Primary endpoints include assessing the effects of physical activity and dietary changes on remission maintenance at 24 weeks post-tapering in SpA patients.

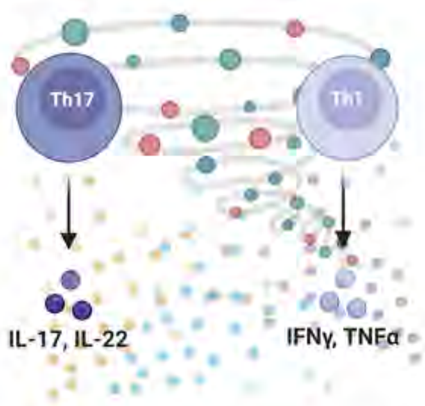
Methods

The study will recruit patients in remission for at least 12 months and will involve the collaboration of the Rheumatology, Sport and Exercise Medicine, and Nutrition units affiliated with DIMED.

A cytokine array will be performed on blood samples to identify and investigate the signaling pathways most involved in maintaining active SpA. The intervention group will undergo supervised exercise sessions and dietary consultations over 12 weeks before initiating therapy tapering. Data collection will include musculoskeletal ultrasound, clinical indices, biohumoral parameters, and anthropometric measurements.

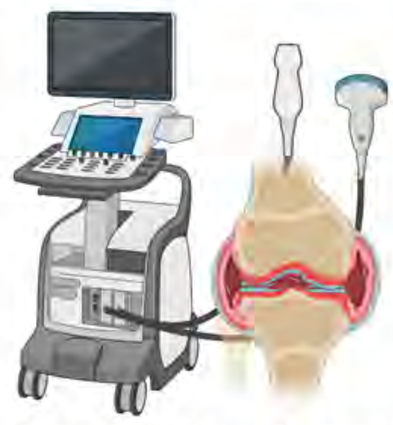
What do we expect?

Cytokine array analysis



To identify key signaling pathways

Ultrasound Predictors



To determine ultrasound predictors of maintenance of remission

Physical Activity

↑ **Maintain disease remission**

Anti-inflammatory effects

Dietary Habits

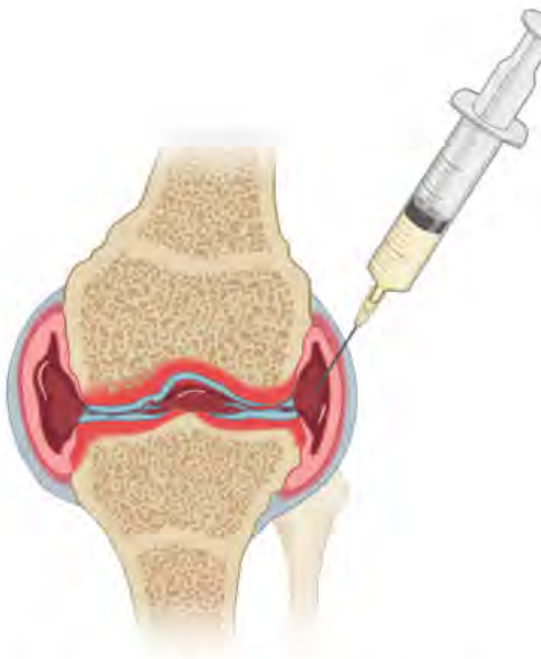
Mediterranean diet

↑ **sustained remission** ↓ **disease activity**

Lifestyle intervention could facilitate a safer reduction of therapy, thereby becoming part of international recommendations for tapering therapy in PsA

GIACOMO COZZI
Reumatologia

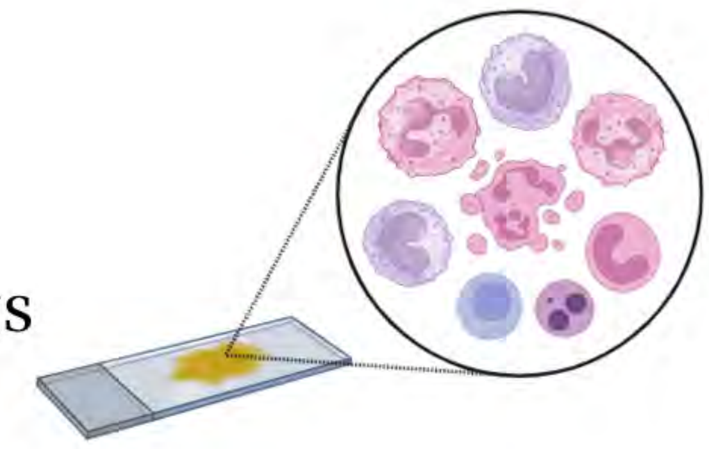
STUDY OF THE INFLAMMATORY POTENTIAL OF PATHOGENIC CRYSTALS IN VITRO AND EX VIVO



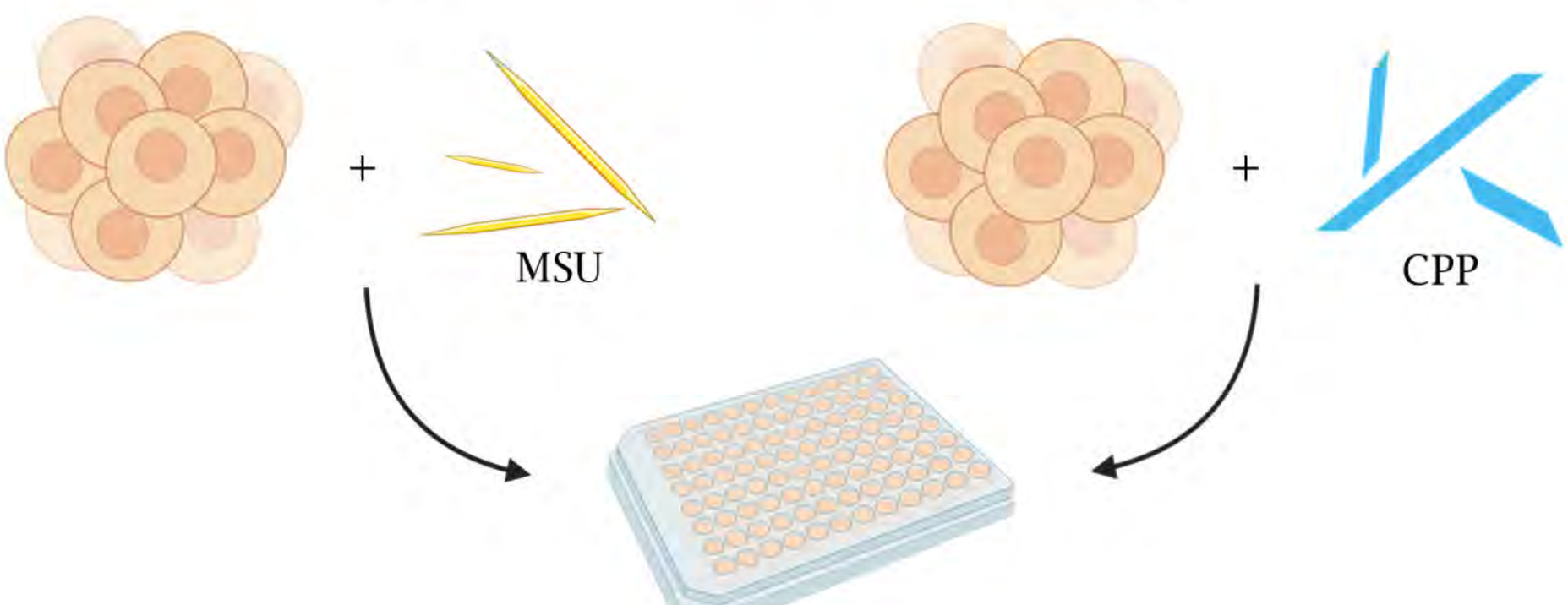
Arthrocentesis:
- Gout
- Calcium pyrophosphate deposition disease

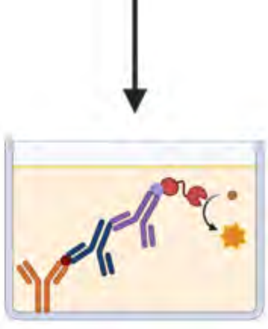
Routine analysis of synovial fluid

Identifying neutrophil subsets



In vitro stimulation of healthy monocytes



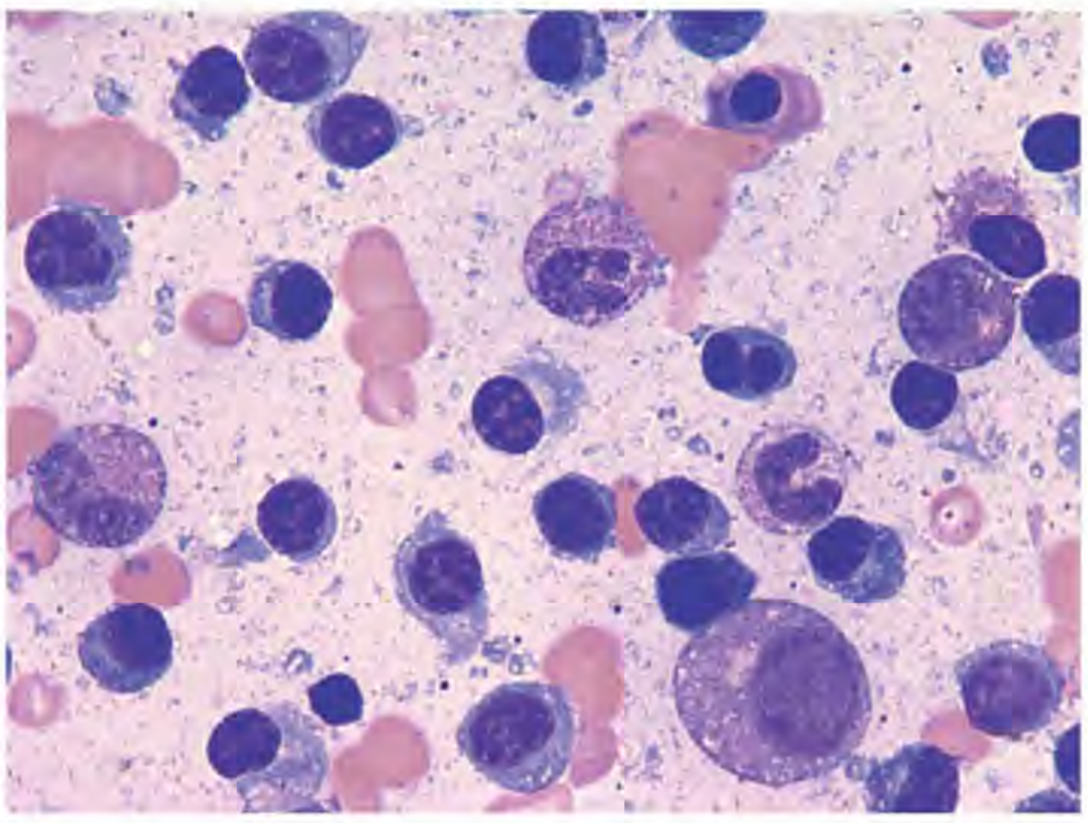


- IL-1β
- IL-6
- IL-8
- IL-10
- TGFβ
- TNFα

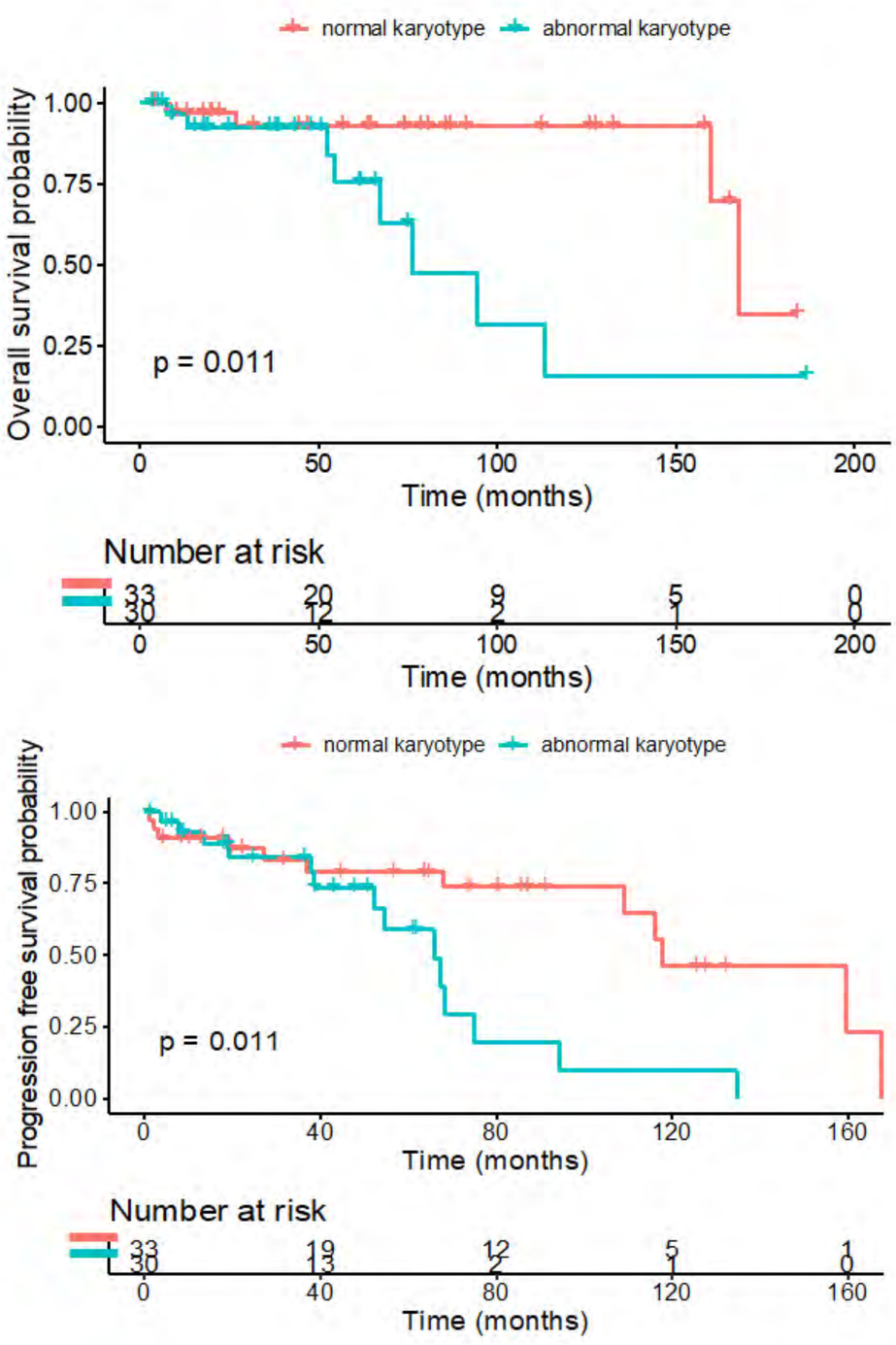
AMELIA CARMELA DAMASCO
Reumatologia

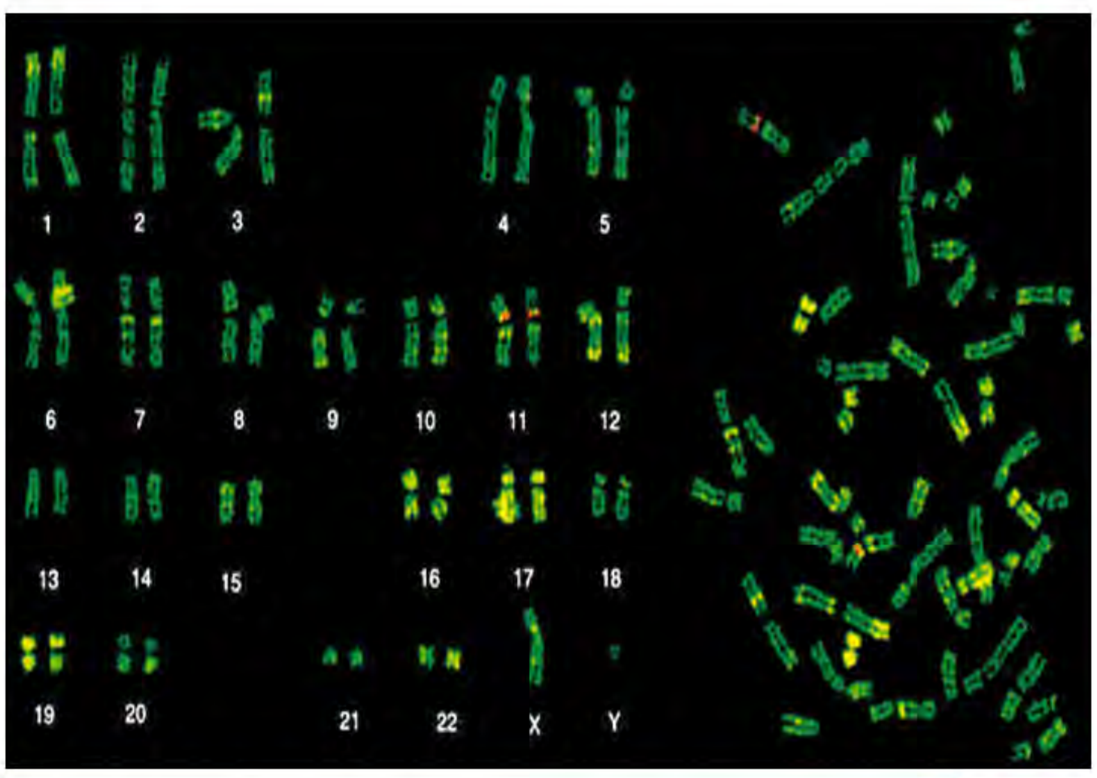
Exploring crosstalks between chromosomal abnormalities and gene mutations in Waldenstrom Macroglobulinemia: the road to new active and drugable biomarkers.

Dr. Danesin Nicolò, Hematology Unit, Department of Medicine, University of Padova, Padua, Italy



Our preliminary data showed inferior survival outcomes proportional to the increasing number of cytogenetic aberrations.[1]





PROSPECTIVE STUDY

- PRIMARY OBJECTIVE

→ Execution of chromosome banding analysis and next generation sequencing (NGS) panel of specific WM mutated genes on bone marrow samples both at diagnosis and first relapse

- SECONDARY OBJECTIVES

→ Description of new molecular cross-talks and active drugable sites

→ Overall and Progression free survival of WM patients with new molecular patterns

- SAMPLE SIZE:

→ n = 60 (both at diagnosis and/or relapse)

[1] Danesin N, Bonaldi L, Martines A et Al. Impact of the presence and number of chromosomal abnormalities on the clinical outcome in Waldenström Macroglobulinemia: a monocentric experience. Ann Hematol. 2024 Apr 30.

NICOLO' DANESIN
Ematologia

VI° CONVEGNO del DIMED
Padova, 15 novembre 2024

Simulating the administration of adapted physical exercise in patients with chronic diseases: an innovative teaching approach

F. Duregon, C. Sarri, F. Urru, G. Mormando, S. Savino, F. Battista, A. Ermolao and D. Neunhaeuserer.

Simulation:

a useful training tool in education of healthcare professionals

The aim of this project is to implement *simulation activity* as an *interactive teaching method*, within the students' internship at the Sports and Exercise Medicine Division.

Where?	How?	Target group	Teaching approach
In the hospital gym	Administering physical exercise to chronic patients with a general inflammatory state	Students of the II cycle degree course in preventive and adapted physical activity	Student-centred with practical real-world simulations, peer-to-peer education, and flipped classroom activities

Four stages:

New feedback questionnaires

created to be compared between the old and the new teaching approach.

Future perspectives:

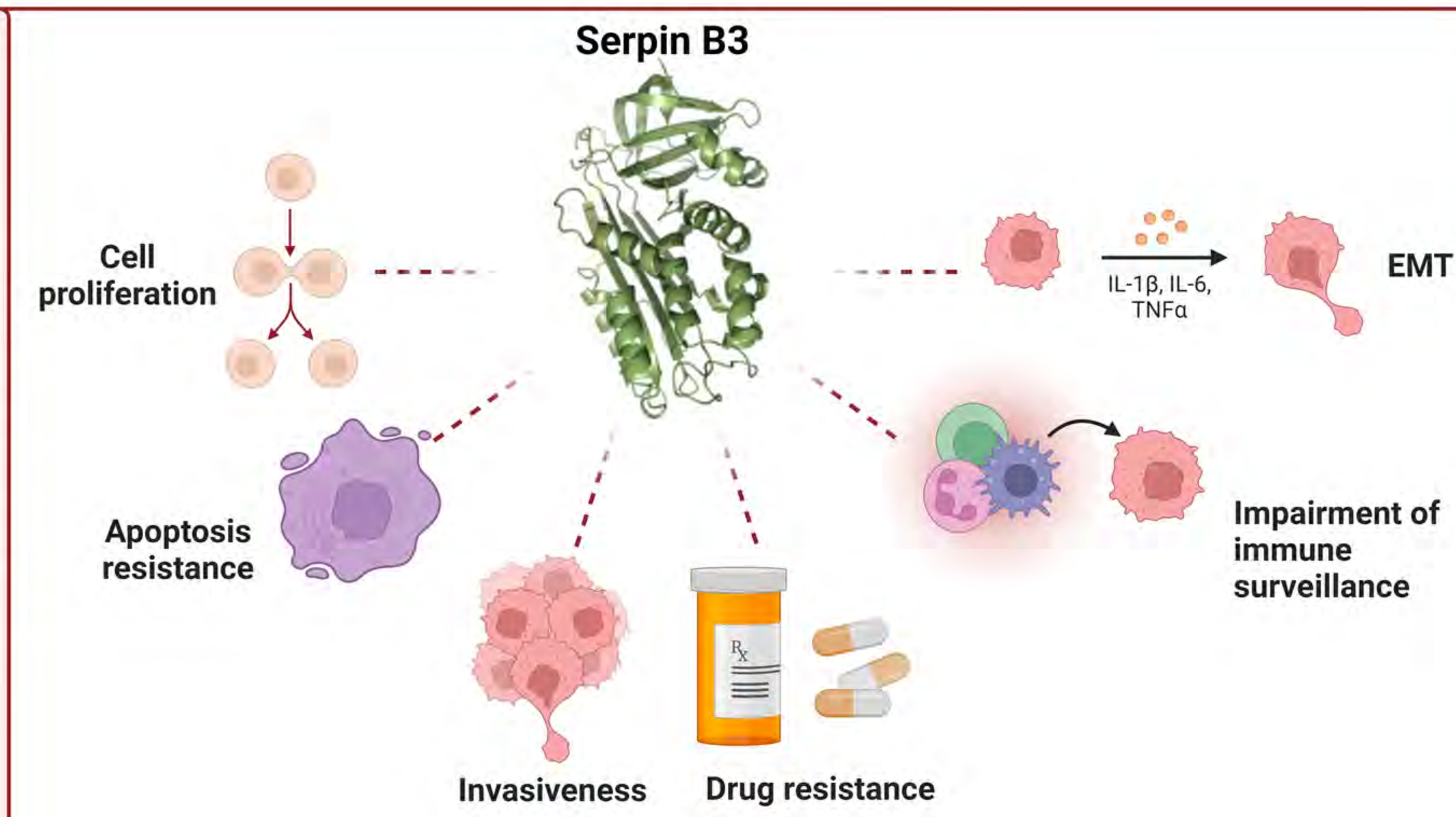
Expanding this project to multidisciplinary and inter-professional teaching with allied healthcare professionals working on lifestyle interventions for patients with chronic inflammatory diseases 🔥

➥ New proposal for funding as educational initiative of **FLAMES**, with a specific focus on inflammatory diseases.

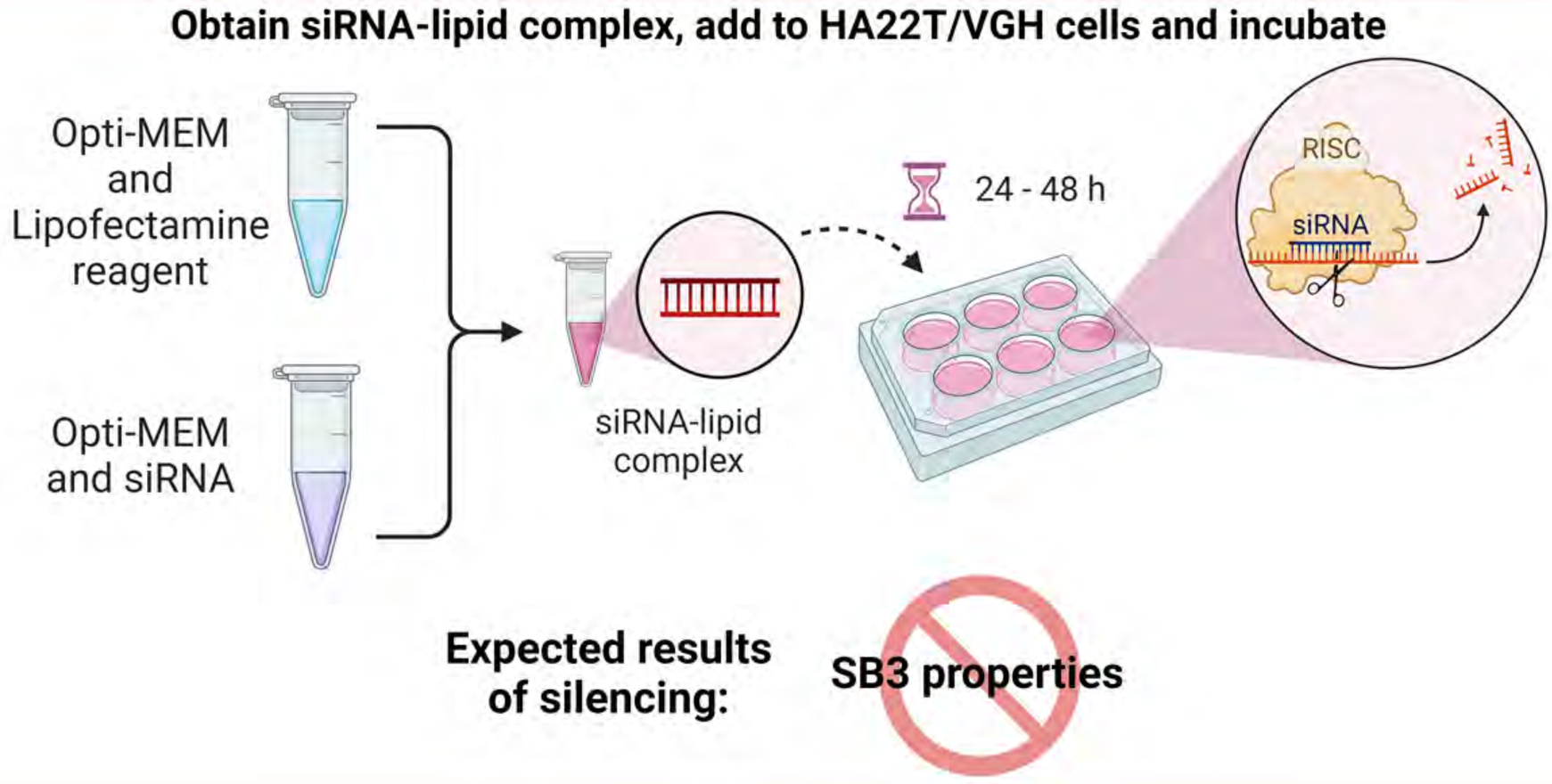
FEDERICA DUREGON
Medicina dello Sport e dell'Esercizio

Silencing of Serpin B3 as a novel approach for cancer treatment

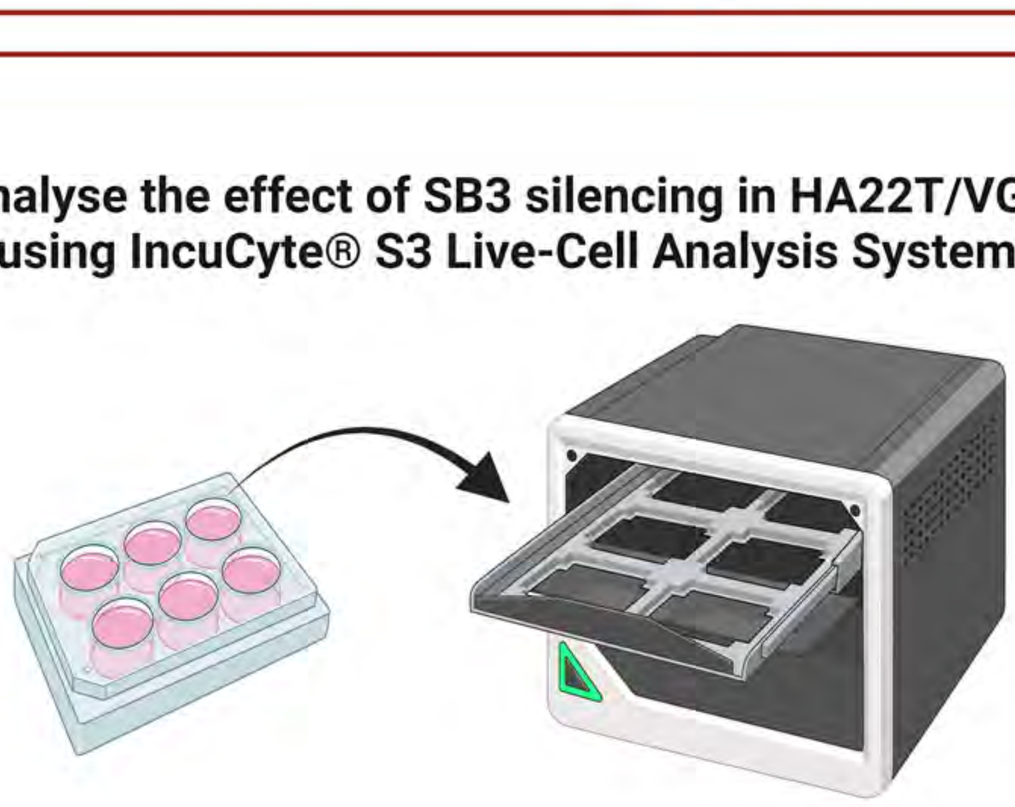
Properties of SB3



Silencing of SB3



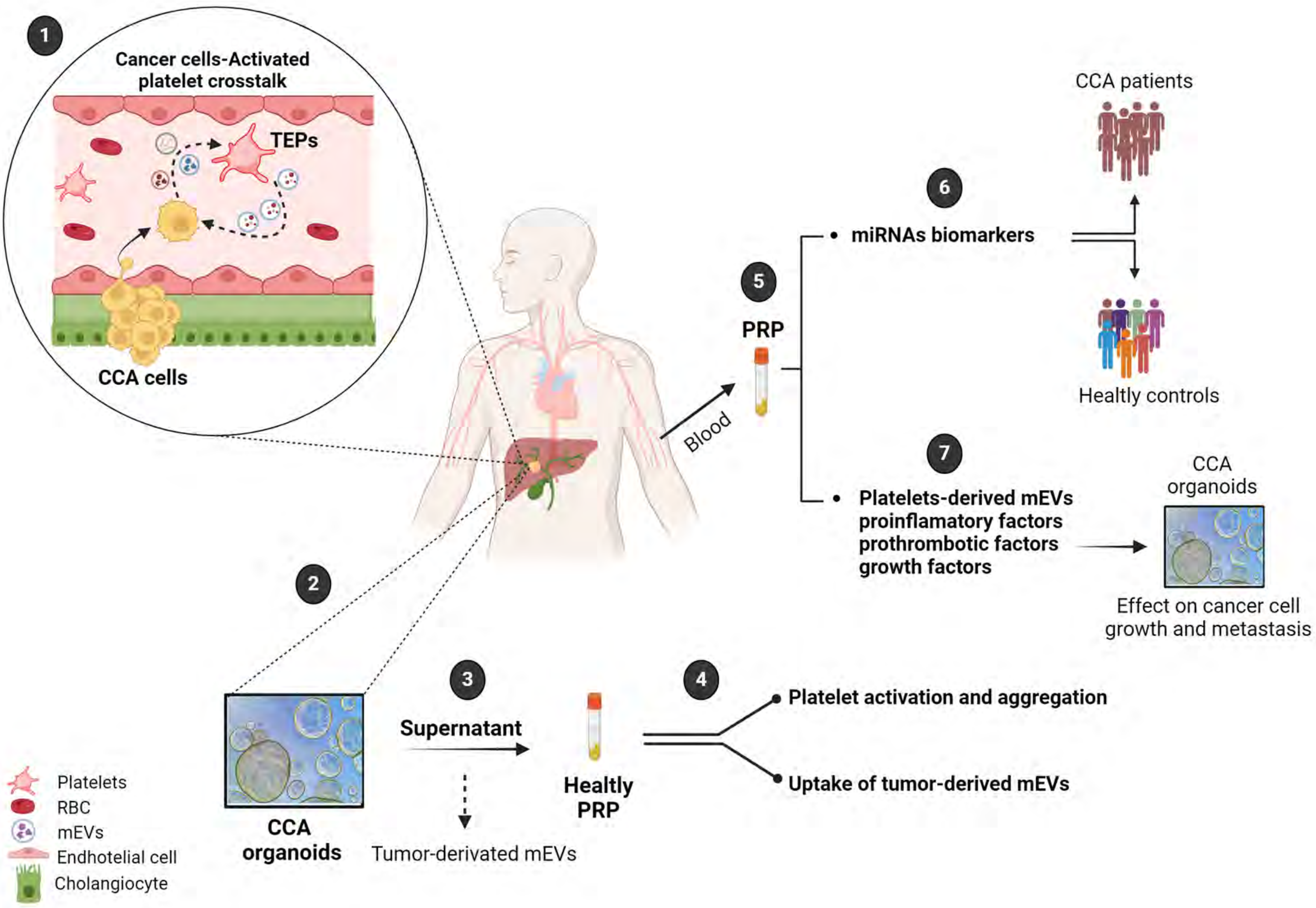
Monitoring the efficacy of SB3 silencing



Preliminary results

Valentina Favaro
U.O.C. Clinica Medica 5 – Lab. Epatologia Molecolare

VALENTINA FAVARO
Clinica Medica 5



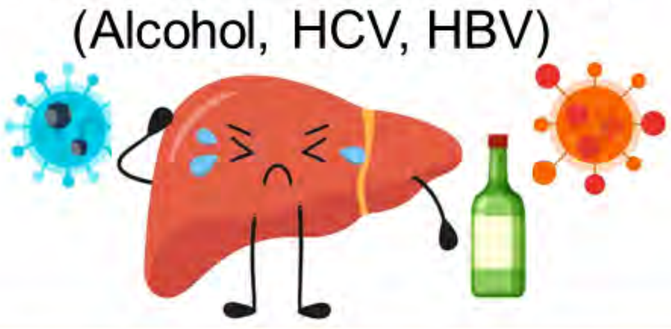
Graphical abstract. 1: Platelets are reprogrammed by tumor cell-derived signals to become in tumor-educated platelets (TEPs). **2:** Human-derivated organoids from CCA patients. **3:** Isolation of microvesicles (mEVs) from CCA organoid supernatants. **4:** Platelet treatment with CCA organoid supernatants. Evaluation of platelet activation and uptake of tumor-derived mEVs. **5:** Isolation of platelets from CCA patients. **6:** Determination of miRNA biomarkers in plasma-rich-platelet from patients with CCA and healthy individuals. **7:** Evaluation of the effect of TEP-derived microvesicles (mEVs) harboring proinflammatory, prothrombotic mediators and growth factors on cancer cell growth and metastasis.

YAHIMA FRION HERRERA
Clinica Medica 1

Validating and expanding Baveno VII criteria of recompensation in patients with decompensated cirrhosis

691 PATIENTS

CIRRHOSIS WITH CURABLE ETIOLOGY
(Alcohol, HCV, HBV)



35 months of median time follow-up

CRITERIA OF RECOMPENSATION

BAVENO VII criteria:

I. Cure/suppression/removal of cirrhosis' etiology;

II. Resolution of ascites (off diuretics), hepatic encephalopathy (off rifaximin/lactulose), and bleeding for > 12 months;

III. Stable improvement of liver function.

Expanded criteria:

Patients meeting all criteria BUT still on decompensation treatment.

Decompensated patients (n=525)

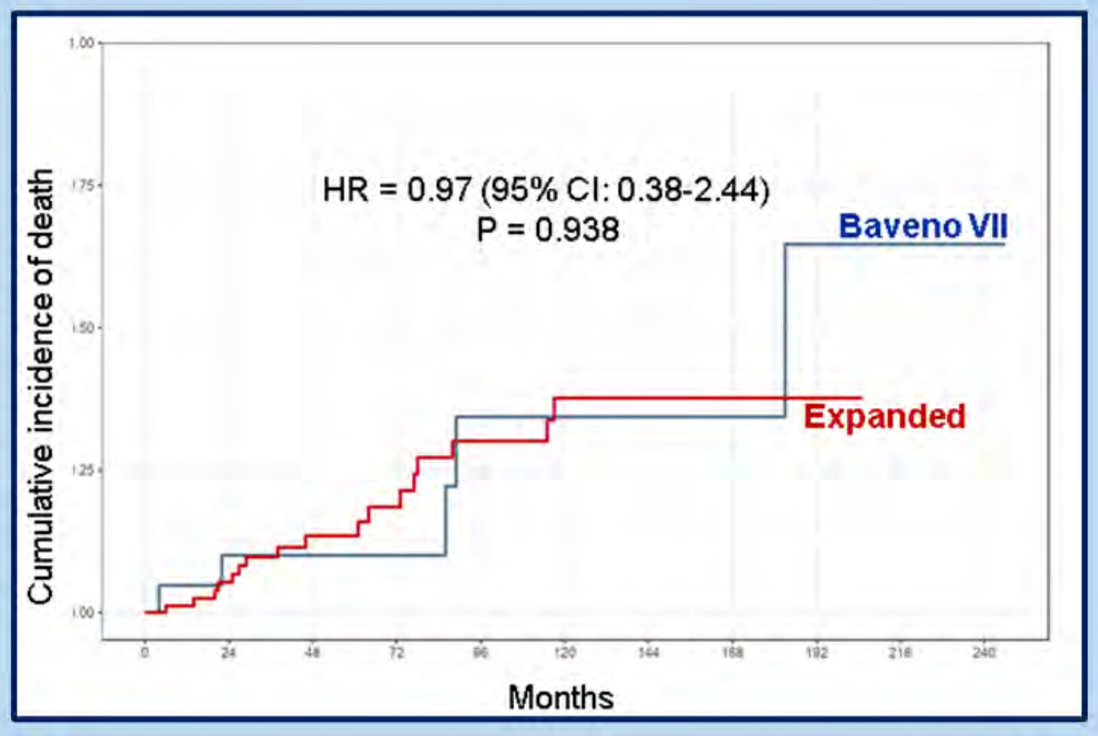
↓

Etiological treatment (n=298)

↓

Recompensation according to Baveno VII criteria (n=22; 4.2%)

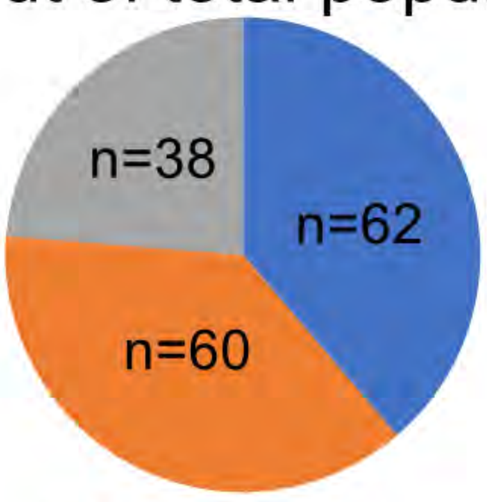
Recompensation according to expanded criteria (n=115; 21.9%)

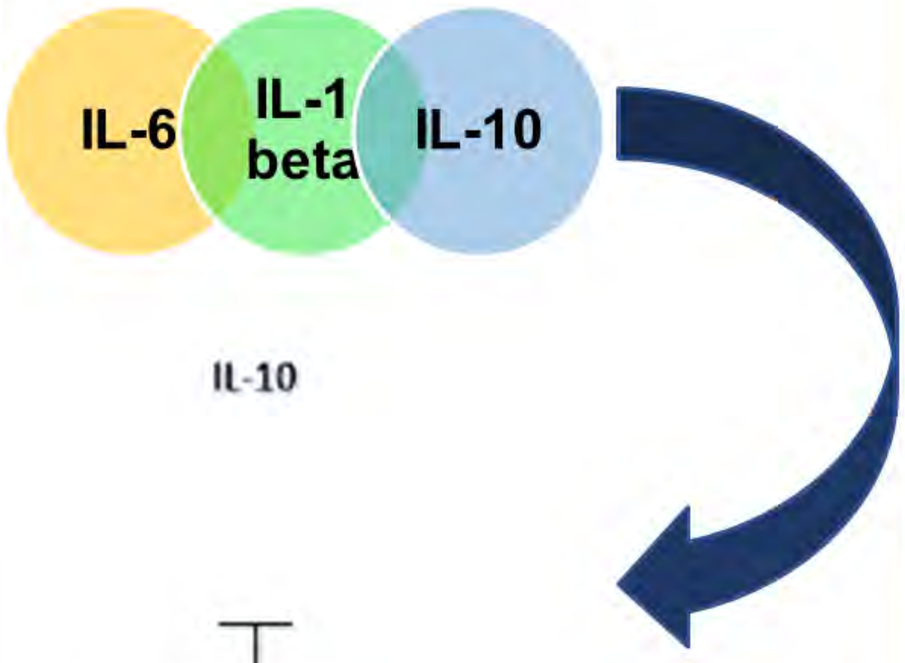


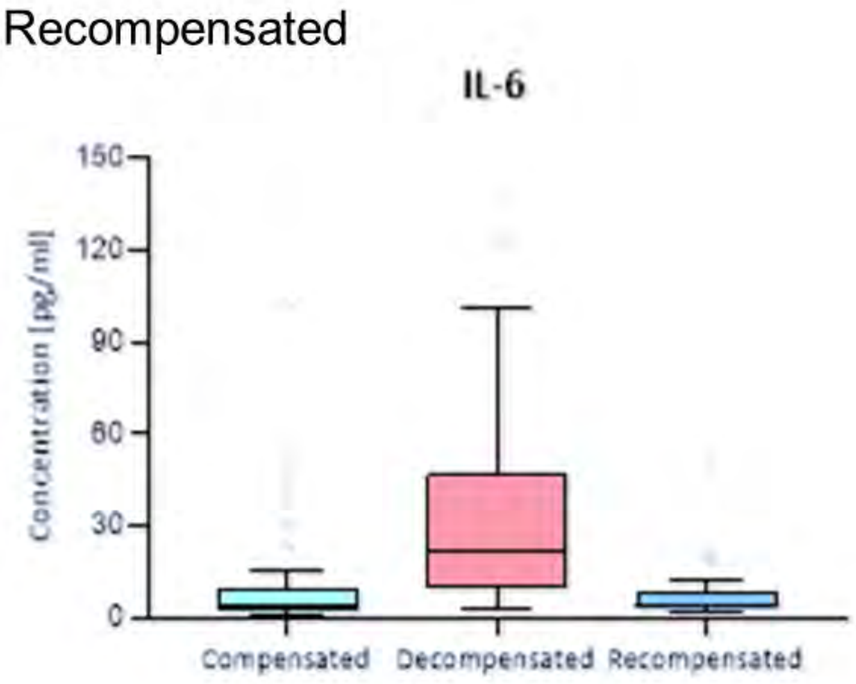
Recompensation is associated to a reduction of mortality risk

PLASMATIC LEVELS OF INFLAMMATORY CYTOKINES

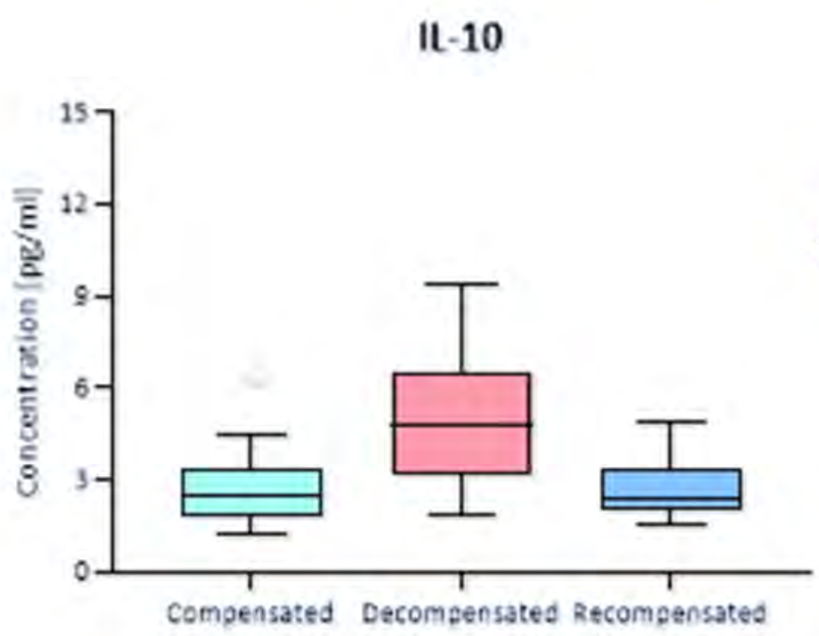
160 PATIENTS
out of total population







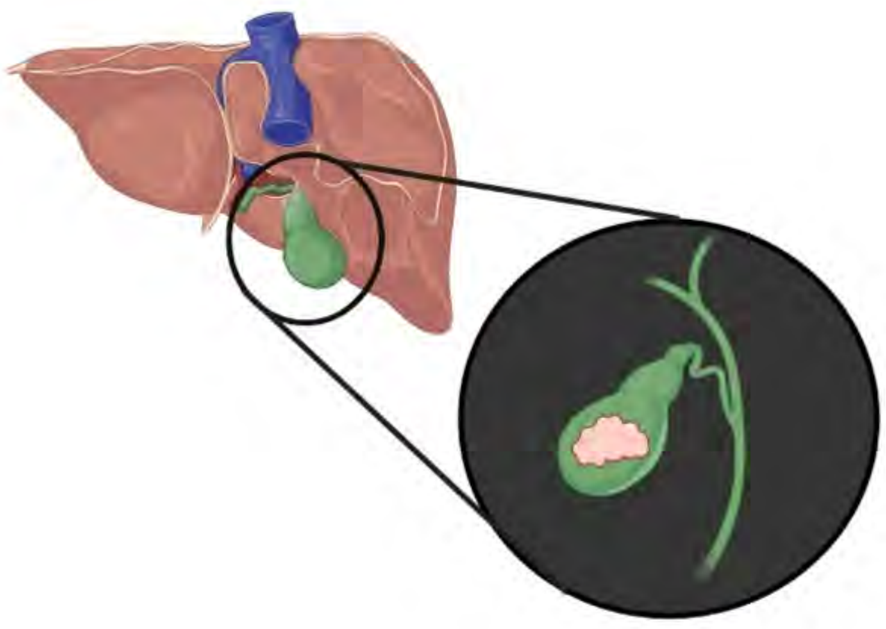
*, p<0.001 vs compensated
#, p<0.001 vs recompensated
§, p= 0.604 vs compensated



*, p<0.001 vs compensated
#, p<0.001 vs recompensated
§, p= 0.256 vs compensated

ROBERTA GAGLIARDI
Clinica Medica 5

Deciphering the molecular landscape of a rare adenosquamous gallbladder carcinoma cohort



Adenocarcinoma (>95%)

Adeno-squamous carcinoma (<5%)

25 cases from different Italian institutions

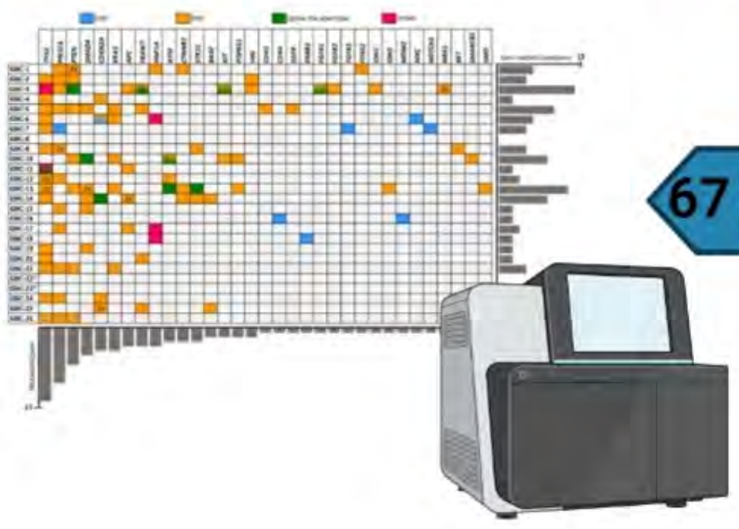
Squamous histotype cofirmation by IHC (p40, p63)

PD-L1

MMR

HER-2

IHC



67 genes

NGS

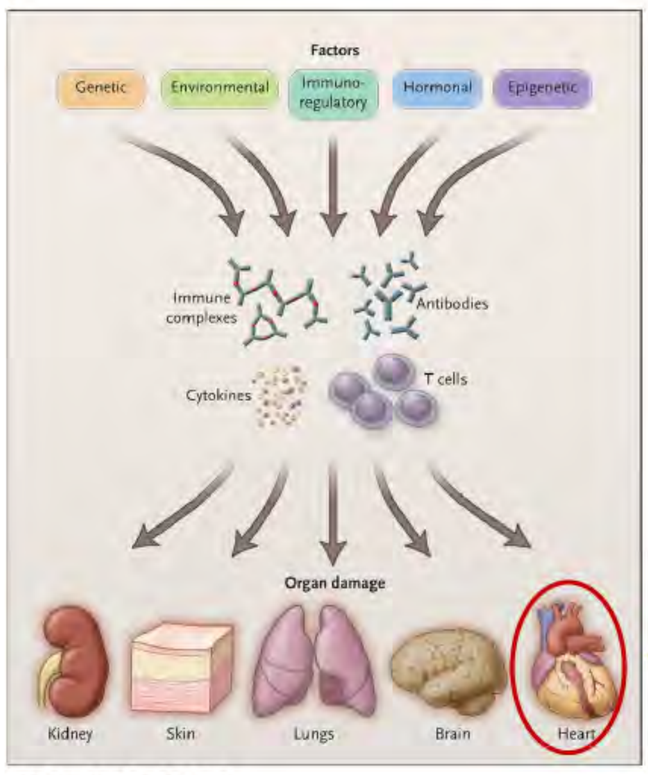
CDKN2A ATM FBXW7
APC TP53 PTEN
PIK3CA SMAD4 CTNNB1
KRAS

Aims

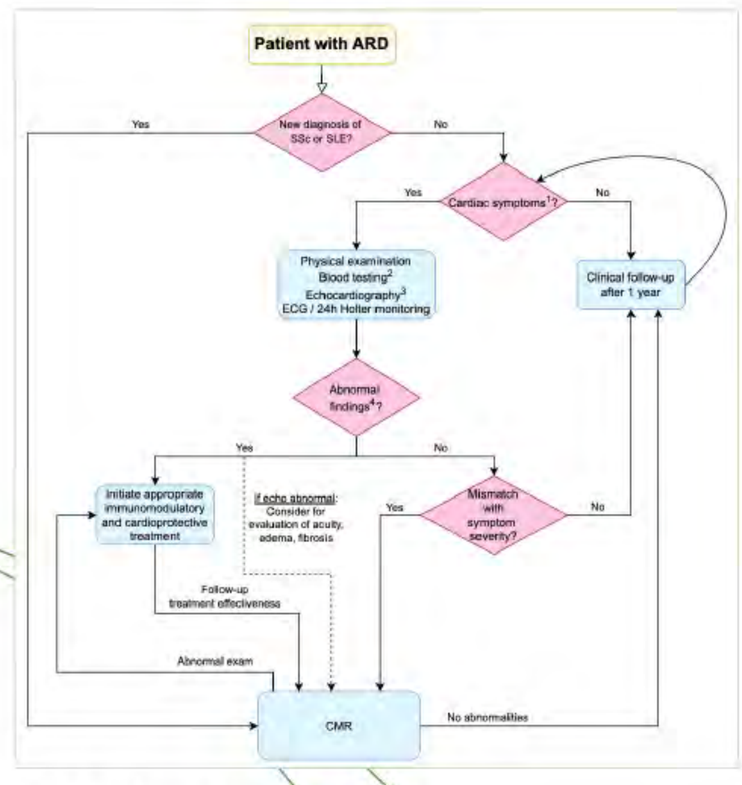
- Obtaining a molecular profilation in a robost cohort of gallbladder adenosquamous carcinomas
- Comparing the molecular biology of gallbladder adenocarcinomas vs adenosquamous carcinomas
- Identification of possible targetable genetic alterations

JESSICA GASPARELLO
Gruppo Ricerca Oncologia Molecolare

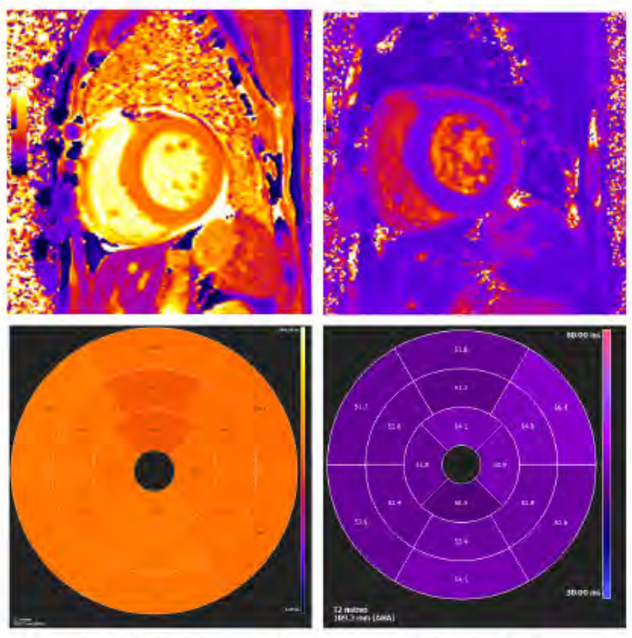
ADDITIONAL VALUE OF CARDIOVASCULAR MAGNETIC RESONANCE IN SYSTEMIC LUPUS ERYTHEMATOSUS PATIENTS EVALUATION



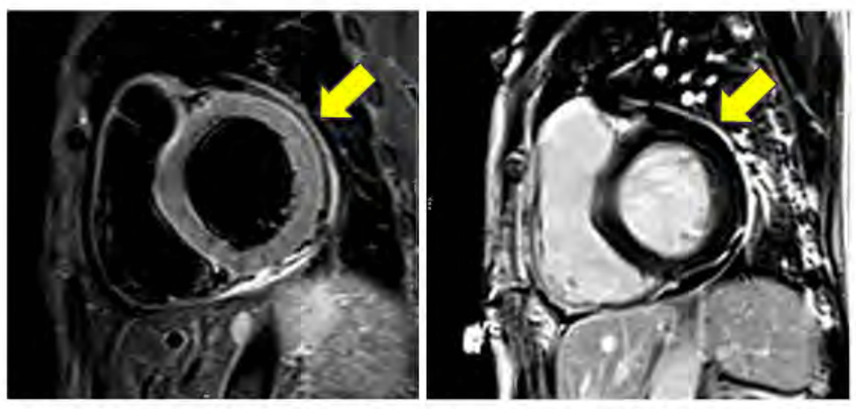
N Engl J Med 2011



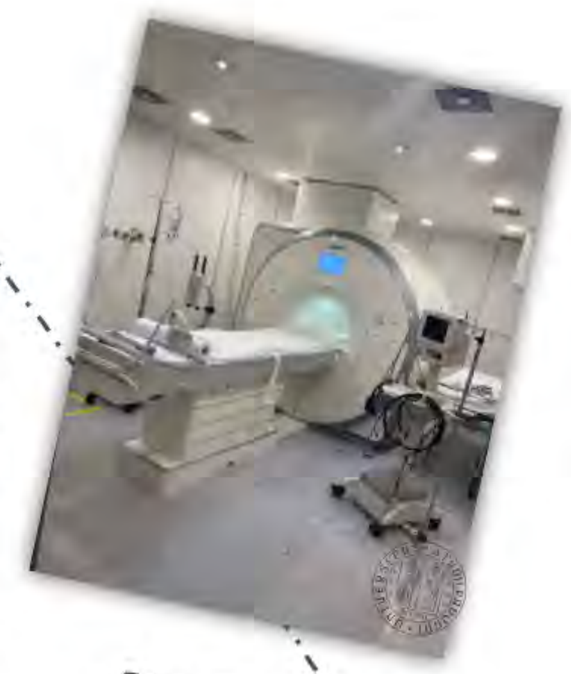
Eur H J – Cardiovasc Imaging 2022



T1 and T2 mapping showing widespread signs of miocardial inflammation in 9 (75%) patients.

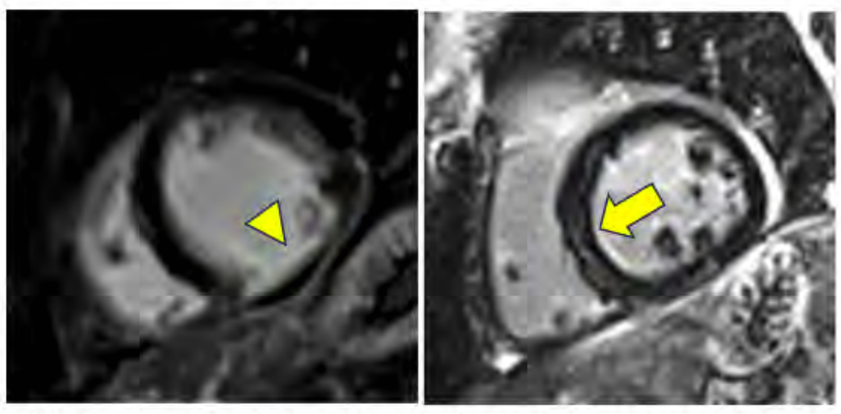


TIRM T2 and PSIR T1 sequences showing pericardial inflammation (arrows) in 5 patients (42%).

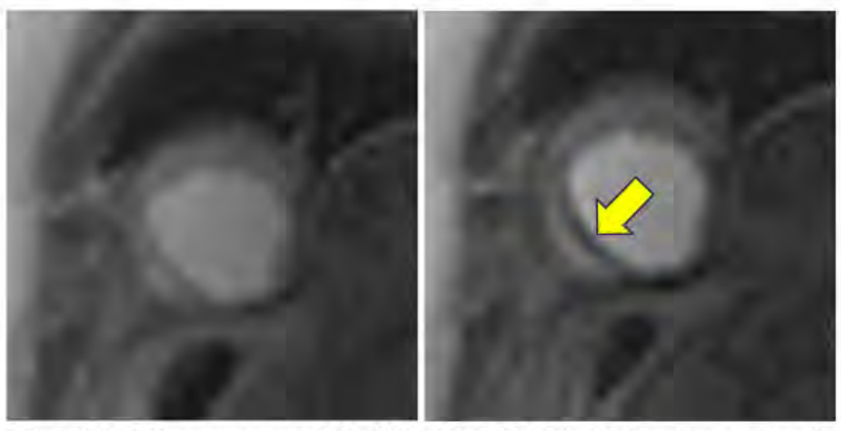


3 9

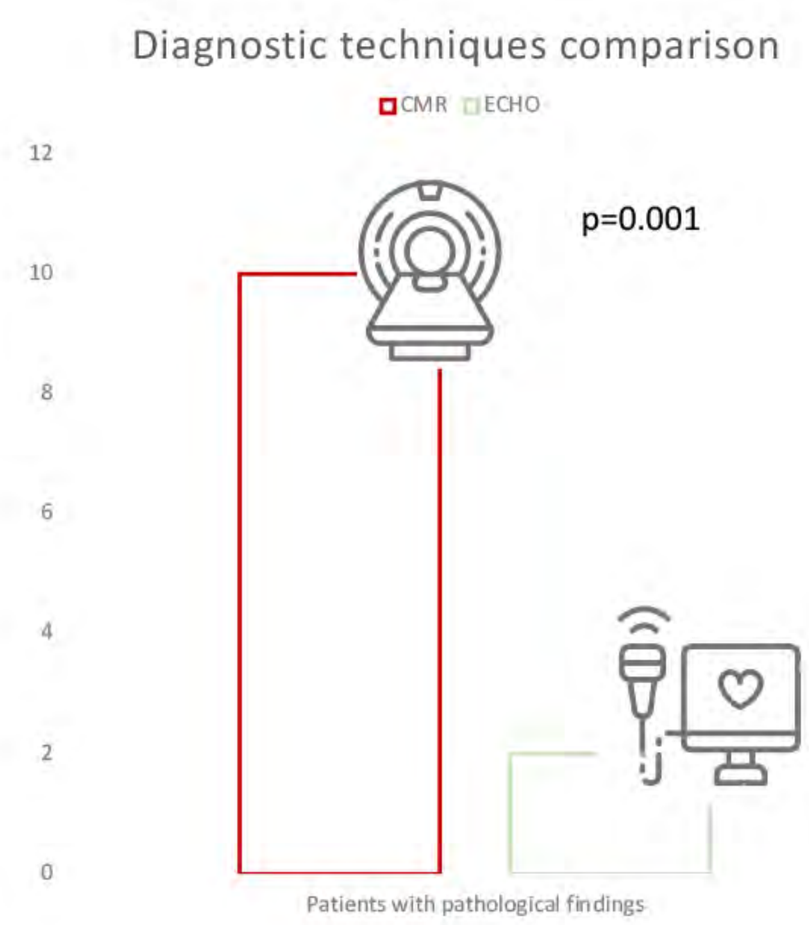
38 ± 14.8 years



PSIR T1 sequence: LGE at ischemic (arrowhead) and non-ischemic (arrow) pattern in 10 patients (83%).



Fast GRE T1 sequence: REST/STRESS PERFUSION showing reduced coronary reserve (arrow) in 2 patients (17%).



CMR allows for

- **better understanding of cardiac involvement in SLE**
- **optimization of the multidisciplinary management**
- **image-guided tailored therapy**
- **improved medical decisions making**

leading to better outcome for SLE patients.

AMANDA LUPI
Radiologia 1

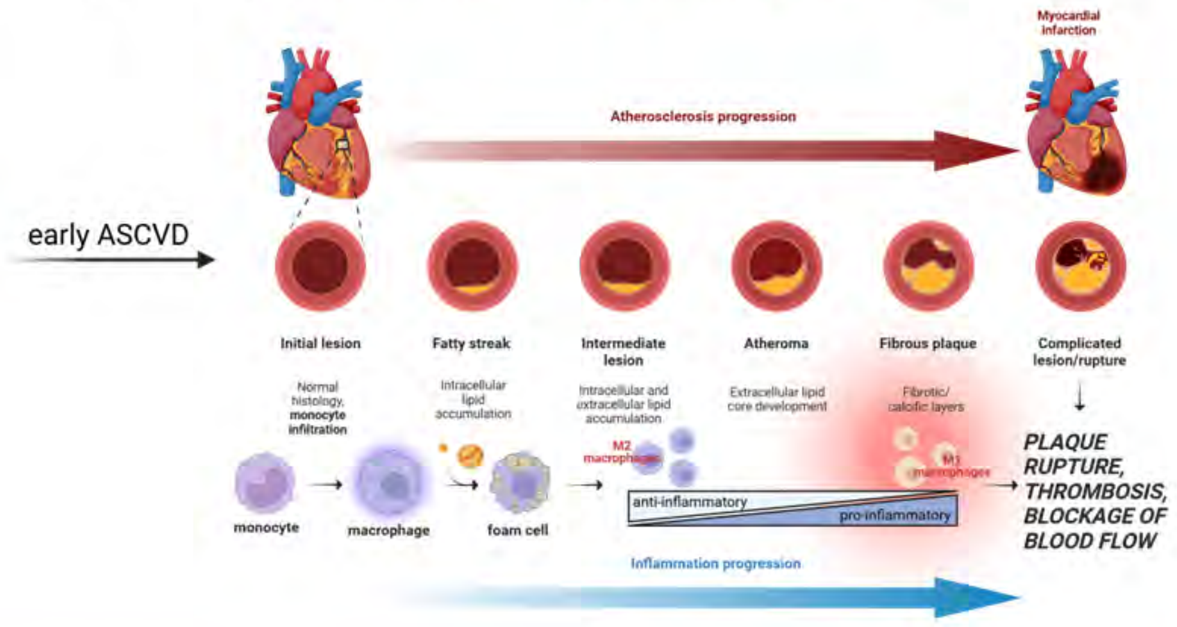
VI° CONVEGNO del DIMED
Padova, 15 novembre 2024

miRNome and proteome profiling of plasma microvesicles (MVs) isolated from patients with homozygous familial hypercholesterolemia under treatment with evinacumab

Maria Giovanna Lupo, Marta Biolo, Sabina Zambon, Alberto Zambon, Nicola Ferri

Homozygous familial hypercholesterolemia (HoFH) have extremely **out-of-range levels of plasma LDLc** that cause fatal and non fatal cardiovascular events very early in life due to the development of **atherosclerotic cardiovascular diseases** (ASCVD), a **cholesterol-driven inflammatory disease**.

Low-density lipoprotein cholesterol (LDLc) particle

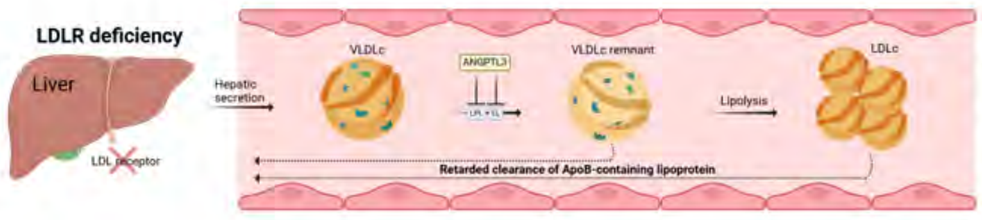


Padua University Hospital
Clinica Medica I

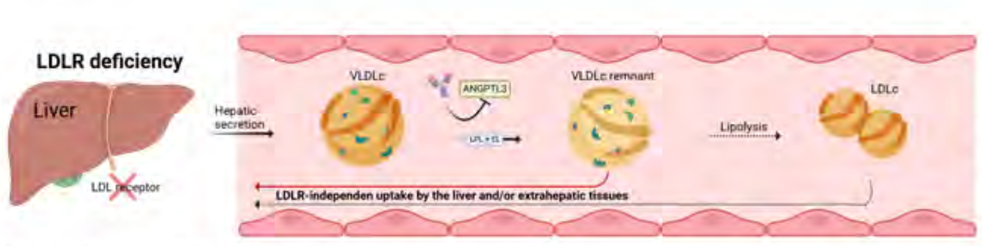
3 adults
3 pediatrics
HoFH genetically determined

→ statin
→ ezetemibe
→ lomitapide
→ PCSK9 inhibitors
→ *April 2024*
Evinacumab
ANGPTL3 inhibitor

EVINACUMAB: MECHANISM OF ACTION



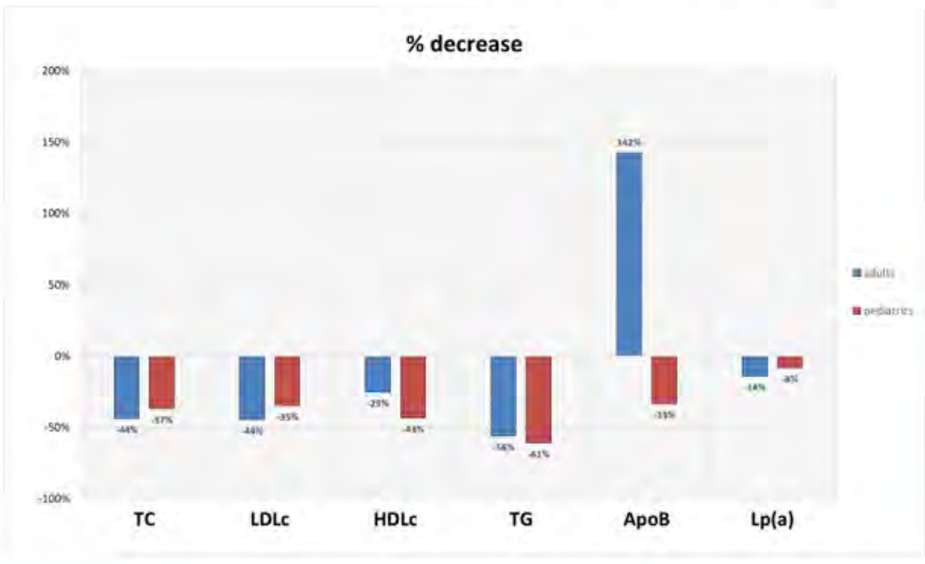
ApoB-containing lipoproteins turnover is impeded in patients with HoFH (with no or reduced LDLR activity)



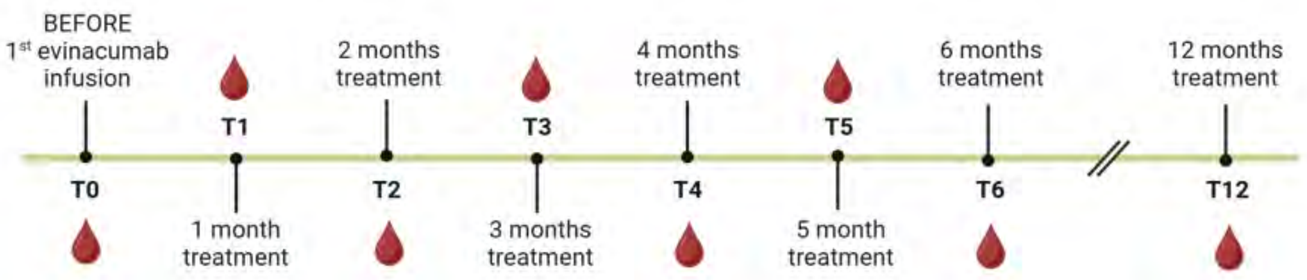
ANGPTL3 inhibition promotes VLDL remodelling and VLDL clearance without LDLR

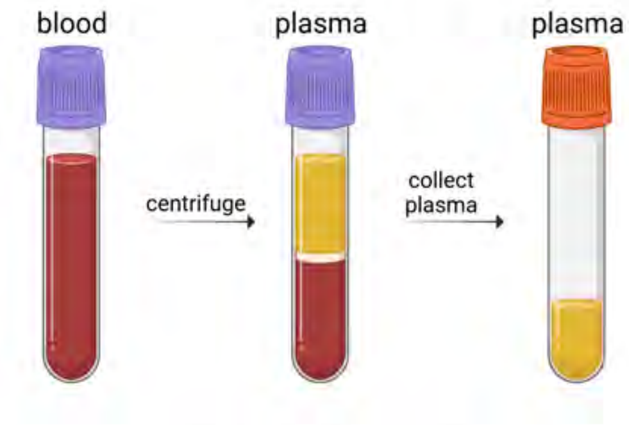
Adapted from Dingman *et al*, Clin Transl Sci. 2024;17:e13836

PRELIMINARY DATA

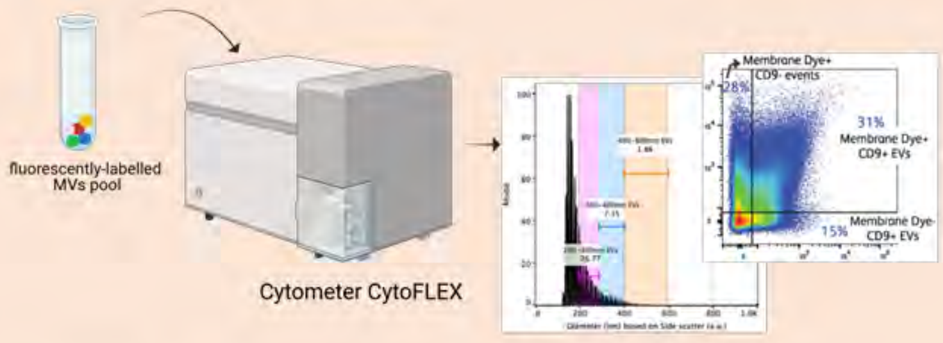


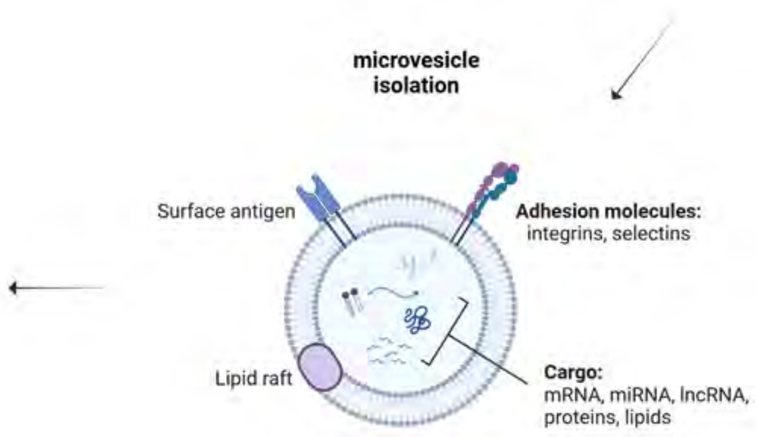
METHODS





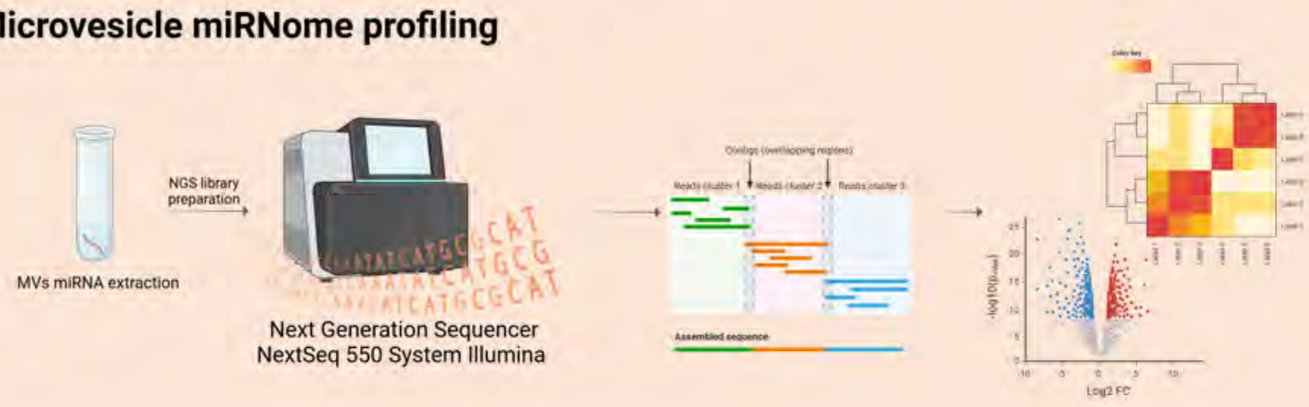
Microvesicle characterization



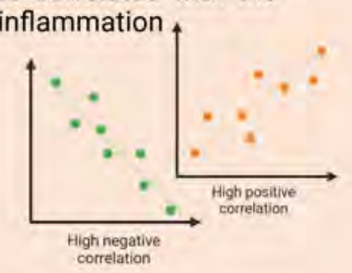


Microvesicle proteome profiling

Microvesicle miRNome profiling



T0 *vs* T3, T0 *vs* T6, T3 *vs* T6
inflammation-related differentially expressed proteins/miRNAs will be correlated with the severity of ASCVD and inflammation



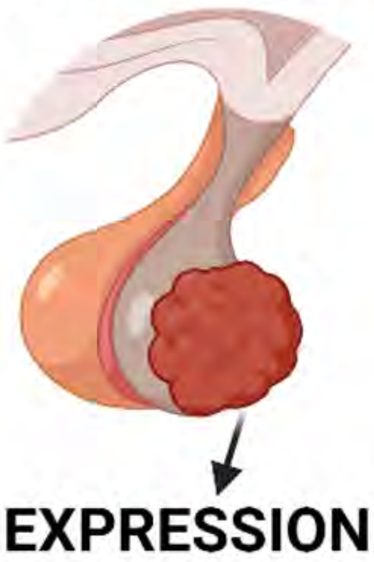
In vitro studies on endothelial cells, vascular smooth muscle cells and monocytes co-culture models challenged with LDLc and evinacumab

1. Cuchel M, *et al. Eur Heart J* 2023
2. Raal FJ, *et al. N Engl J Med* 2020
3. Beliard S, *et al. Arterioscler Thromb Vasc Biol* 2024
4. Camera M, *et al. J Am Coll Cardiol* 2020
5. D'Erasmo L, *et al. Eur J Prev Cardiol* 2024
6. Lupo MG, Ferri N. *J Cardiovasc Dev Dis* 2018
7. Suades R, *et al. Arterioscler Thromb Vasc Biol* 2019

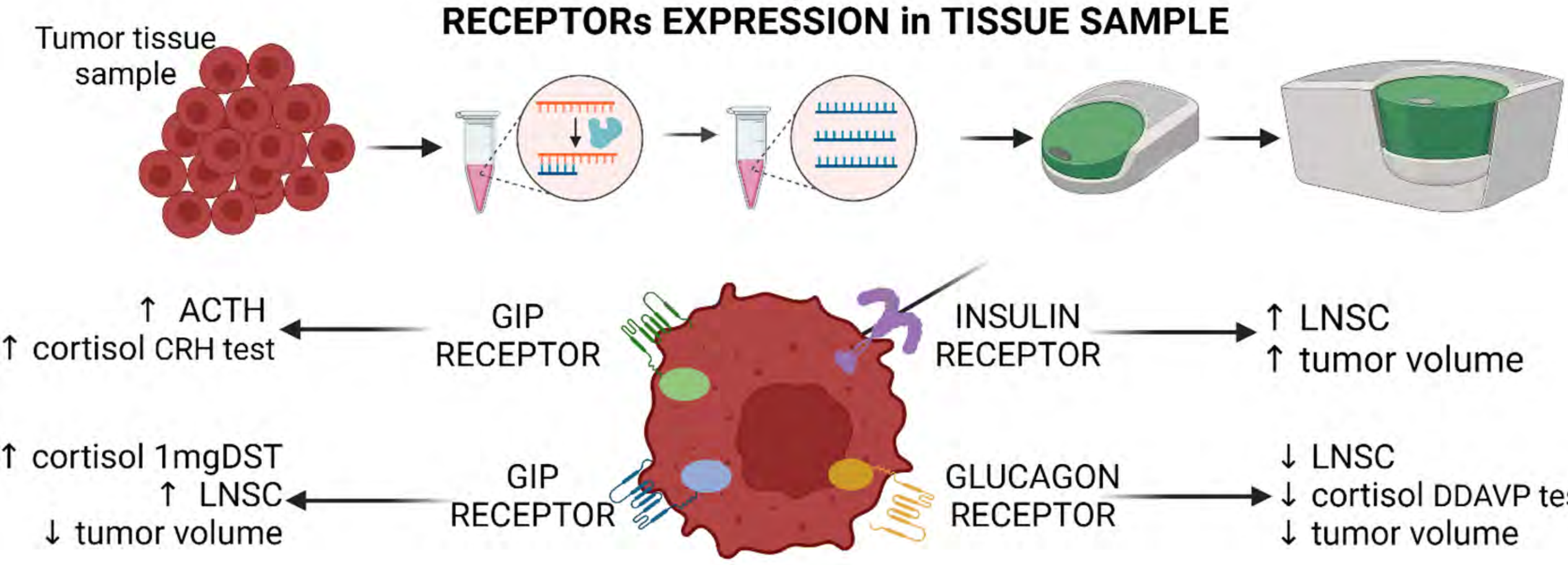
MARIA GIOVANNA LUPO
Clinica Medica 1

PRELIMINARY DATA

CORTICOTROPINOMA

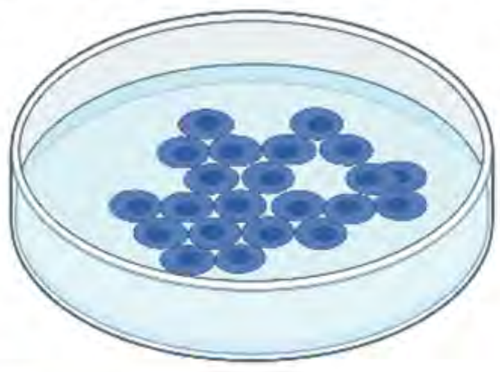


RECEPTORs EXPRESSION in TISSUE SAMPLE

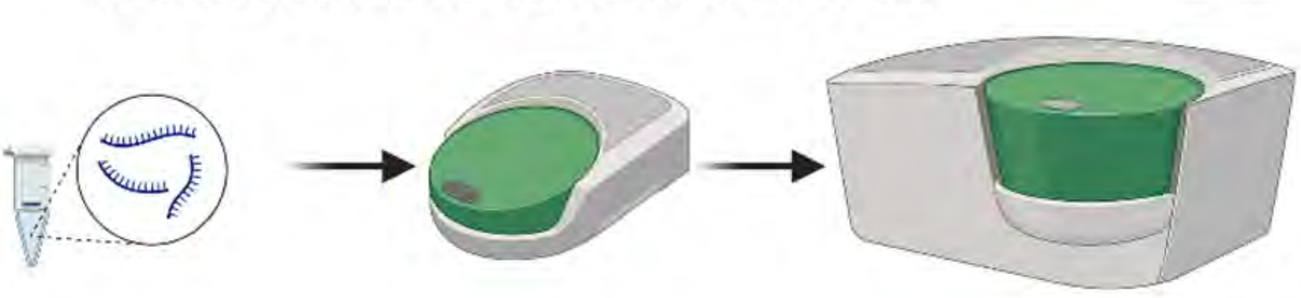


FUTURE PERSPECTIVES

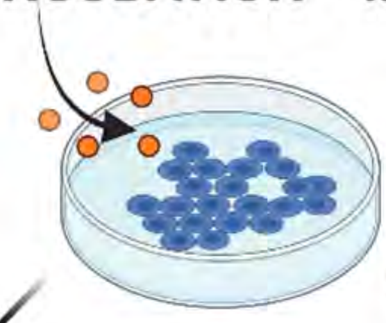
AtT-20 CELL CULTURE



INSULIN RECEPTOR EXPRESSION



INCUBATION + INSULIN

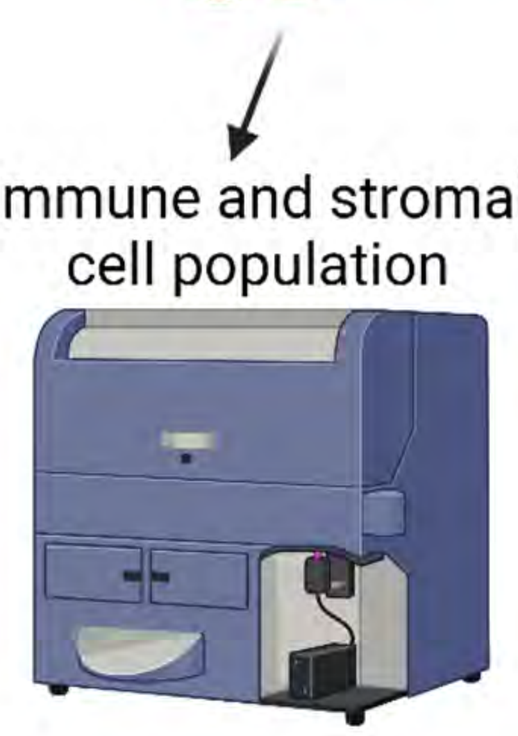


AtT-20 ACTH secretion (ELISA)

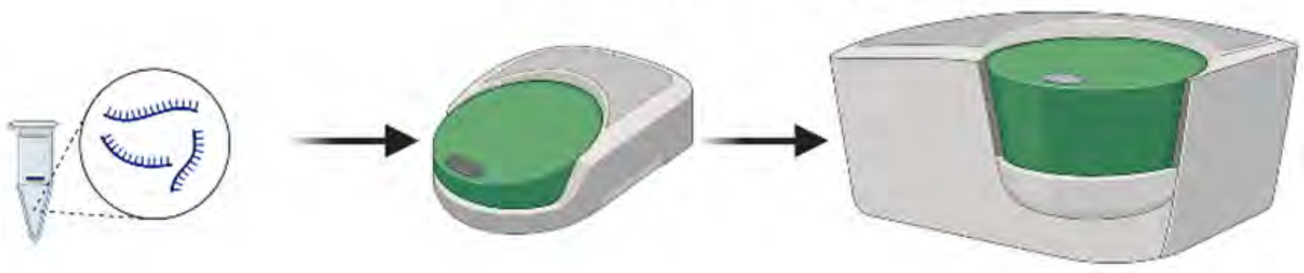
AtT-20 cell proliferation

CD TISSUE SAMPLE MICROENVIRONMENT

Immune and stromal cell population



Cytokines + chemokines + inflammation-related molecules expression



PIERLUIGI MAZZEO
Endocrinologia

Iodine status in pregnant women with chronic autoimmune thyroid disease: a monocentric study

53 I Iodine 126.90

- essential micronutrient for **thyroid function**
- source: **diet** (iodized salt, dairy products, seafood, etc)
- requirements increase by 50% during **pregnancy**

n =81

- Pregnant women
- resident in Veneto
- diagnosis of Hashimoto's thyroiditis

Median I:Cr 276 µg/g

Iodine-sufficiency (I:Cr ≥ 150 µg/g): **72.7%** ✓

Iodine-deficiency (I:Cr <150 µg/g): **27.3%** ✗

I:Cr <150 µg/g in women taking iodine supplement: 23.9%

I: Cr <150 µg/g in women not taking iodine supplement: 66.7%

Only 12.3% know about iodoprophylaxis campaign

Iodine supplement (p=0.02)

associated with higher education (p <0.0001)

Regular cheese consumption (p=0.03)

LT4 therapy (p=0.05)

Positive correlation between LT4 daily-dose per-Kg and I:Cr (p=0.057, r=0.22)

↑ Neonatal weight and ↑ neonatal TSH

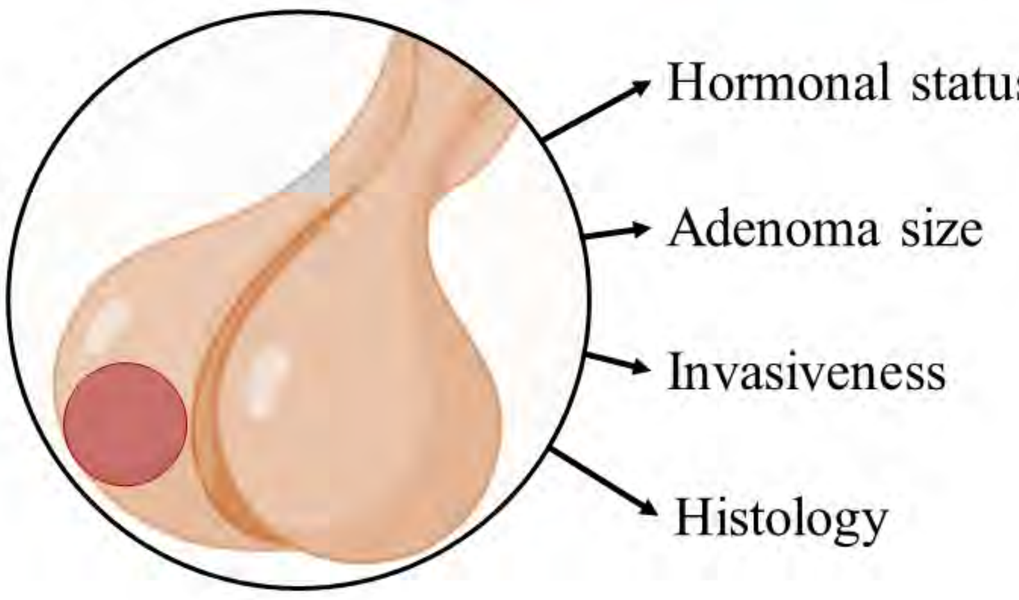
- **Pregnant women affected by Hashimoto's thyroiditis have a more than adequate iodine status.**
- **We recommend iodine supplementation in women with autoimmune thyroiditis.**
- **We suggest evaluating a personalized supplement dosage based on the pro-kg dosage of LT4 therapy.**

Giulia Messina[1], Simona Censi[1], Ilaria Piva[1], Susi Barollo[1], Loris Bertazza[1], Fiammetta Battheu[1], Cristina Clausi[1], Caterina Mian[1]
1: U.O.C. di Endocrinologia, Dipartimento di Medicina (DIMED), Università-Azienda Ospedaliera di Padova, Padova, Italia

Aiming to validate a novel grading score for pituitary adenomas

Alessandro Mondin

Background – Pituitary adenomas



Highly heterogeneous group of diseases.

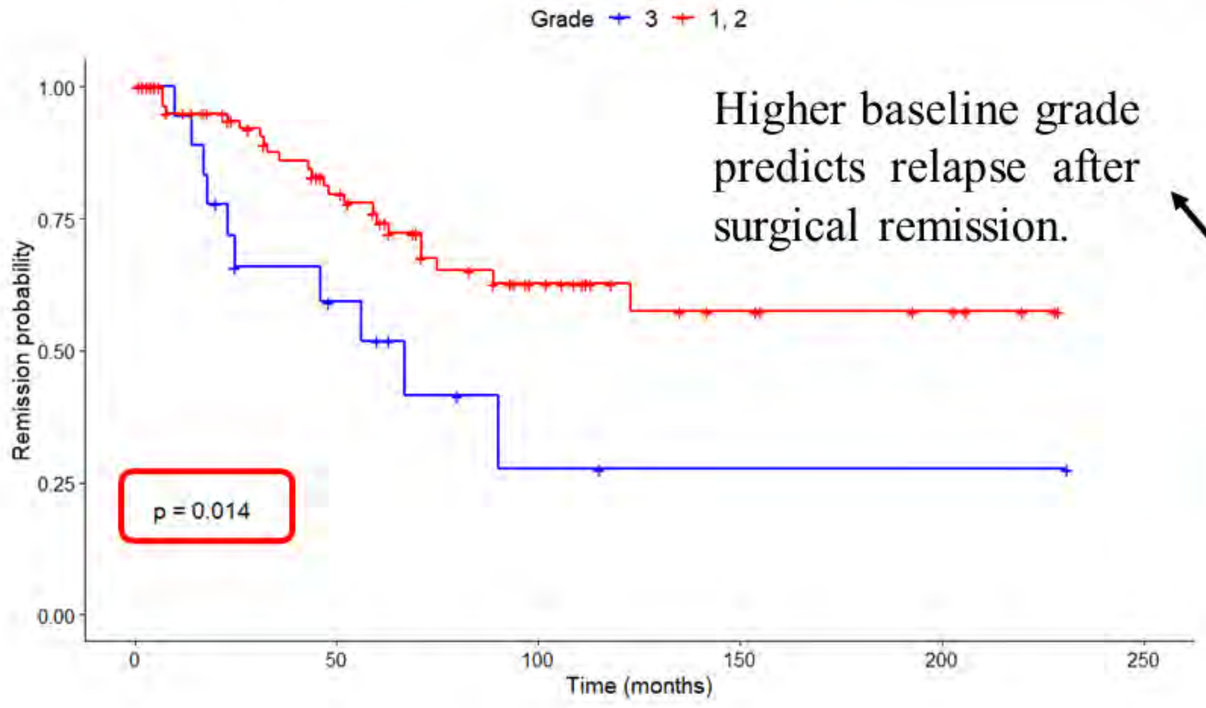
Novel grading system (Ho et al, 2024):

- From systematic review of literature;
- For day-to-day clinical practice;
- Hormonal, radiological and histological items;
- At any time, with any number of items (out of 9).

Yet to be validated.

Preliminary data – Prospective assessment of retrospective data

401 pituitary adenomas (any type) → Baseline grading → First-line treatment → Outcome and 1-year grading → Relapses or further approaches → Last follow up status



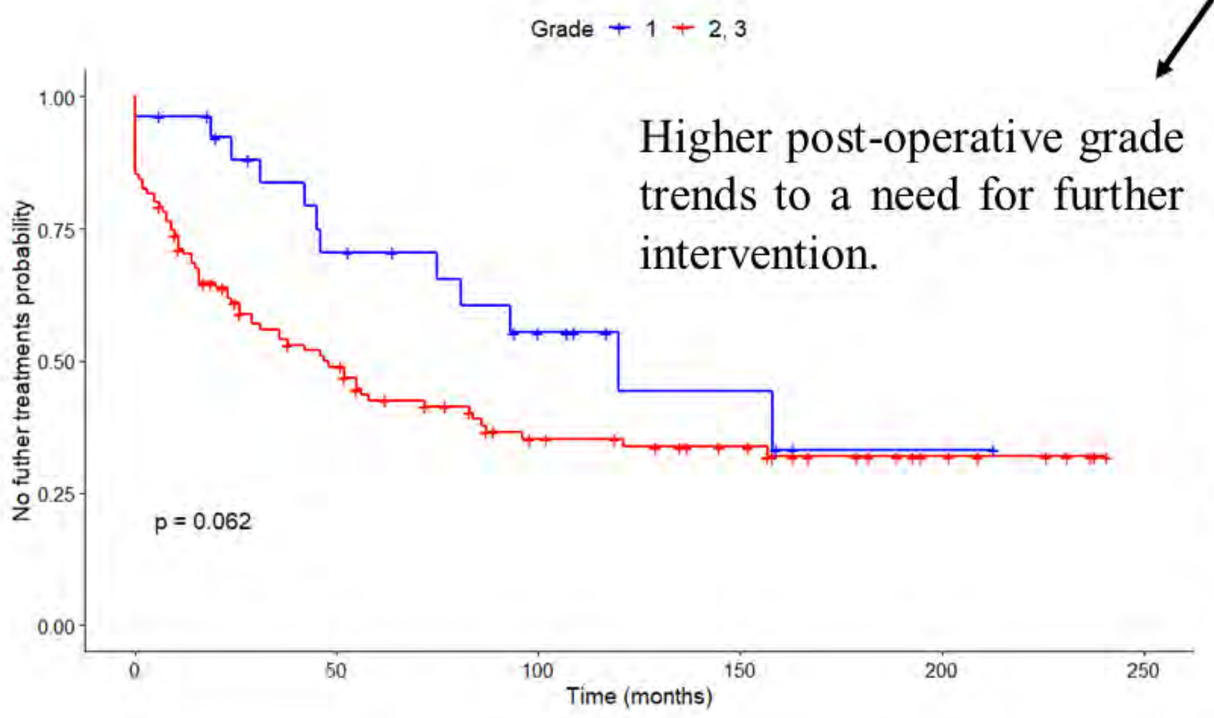
Higher baseline grade predicts relapse after surgical remission.

No significant effect on surgical outcome or 1-year hormonal control on medical treatment.

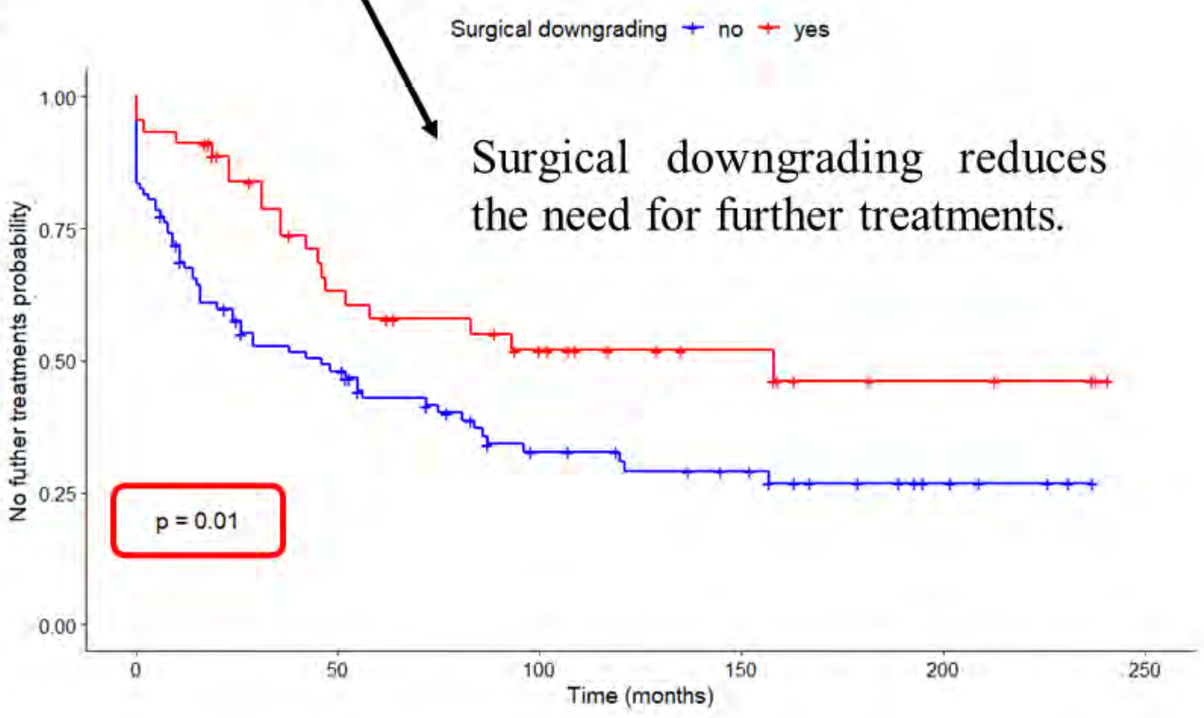
Grade

Cure after multimodal therapies is less likely for high grade disease at diagnosis (p = 0.047).

Higher remission rate after medical treatment alone in case of low baseline grade (p = 0.02)



Higher post-operative grade trends to a need for further intervention.

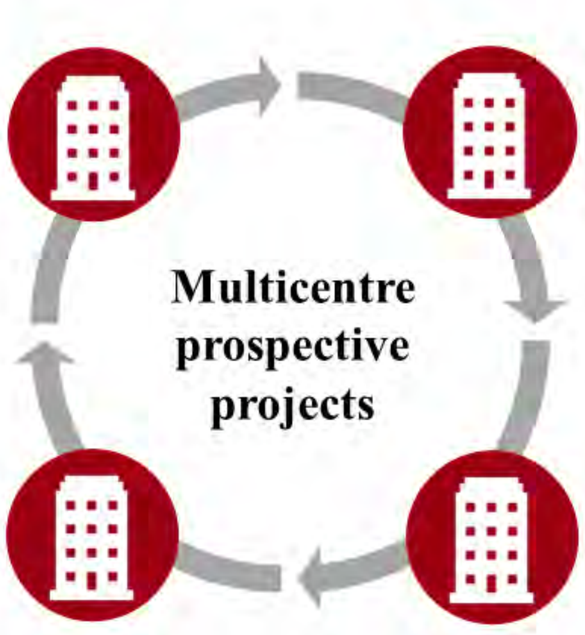


Surgical downgrading reduces the need for further treatments.

Future perspectives – Other endpoints, prospective validation cohorts

Assessing comorbidities, QoL and mortality

Evaluation for second line treatments (e.g., different drugs) and in specific subgroups (e.g., Cushing Disease only)



ALESSANDRO MONDIN
Endocrinologia

VI° CONVEGNO del DIMED
Padova, 15 novembre 2024

Genetic profiling of unprovoked venous thromboembolism patients: insights from Next-Generation Sequencing Analysis

Angela Napolitano, Chiara Simion, Alessandra Giannella, Cristiana Bulato, Lisa Gianesello, Andrea Benetti, Elena Campello, Giulio Ceolotto, Paolo Simioni
First Chair of Internal Medicine, Department of Medicine, University of Padova

SELECTION OF THE POPULATION

- Unprovoked venous thromboembolism (VTE) at a young age (18-45 years)
- Strong family history for VTE
- Tested negative for known inherited thrombophilia

60 unrelated patients (31 males, 29 females) mean age 37 years

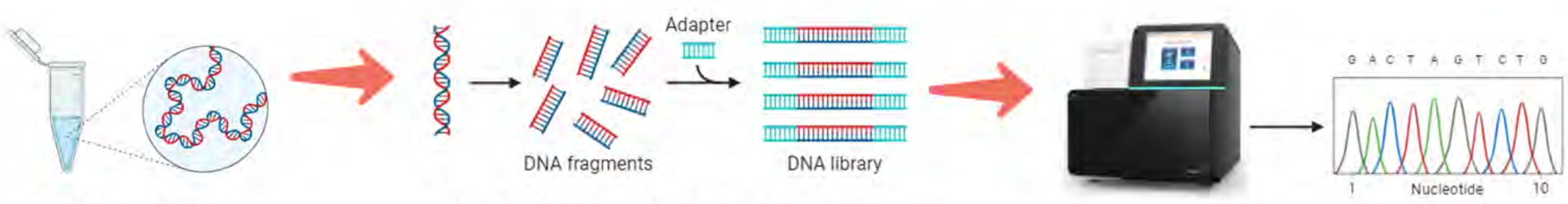
SELECTION OF POTENTIAL AFFECTED COAGULATION PROTEINS AND GENES

PROTEIN	GENE
Thrombin activatable fibrinolysis inhibitor	CPB2 (1361)
Coagulation factor X	F10 (2159)
Coagulation factor XI	F11 (2160)
Coagulation factor XII	F12 (2161)
Coagulation factor XIII A chain	F13A1 (2162)
Coagulation factor XIII B chain	F13B (2165)
Coagulation factor II, thrombin	F2 (2147)
Coagulation factor III, tissue factor	F3 (2152)
Coagulation factor V	F5 (2153)
Coagulation factor VII	F7 (2155)
Coagulation factor VIII	F8 (2157)
Coagulation Factor IX	F9 (2158)
Fibrinogen alpha chain	FGA (2243)
Fibrinogen beta chain	FGB (2244)
Fibrinogen Gamma Chain	FGG (2266)
Kallikrein B1	KLKB1 (3818)
Kininogen 1	KNG1 (3827)

PROTEIN	GENE
Tissue plasminogen activator	PLAT (5327)
Plasminogen	PLG (5340)
Protein C, inactivator of coagulation factors Va and VIIIa	PROC (5624)
Protein S	PROS1 (5627)
Protein C inhibitor	SERPINA5 (5104)
Antithrombin-III	SERPINC1 (462)
Heparin cofactor II	SERPIND1 (3053)
Endothelial Plasminogen Activator Inhibitor	SERPINE1 (5054)
Alfa 2 antiplasmina	SERPINF2 (5345)
Tissue factor pathway inhibitor	TFPI (7035)
Thrombomoduline	THBD (7056)
VWF	VWF (7450)
Alfa-2 macroglobulin	A2M
Endothelial protein C receptor	PROCR
Protena zeta	proz
Proteina zeta inhibitor	serpina10

33 genes selected

NEXT GENERATION SEQUENCING WORKFLOW



DNA extraction

Library preparation: 861 probes
xGenTM Hyb Panel, Integrated DNA Technologies

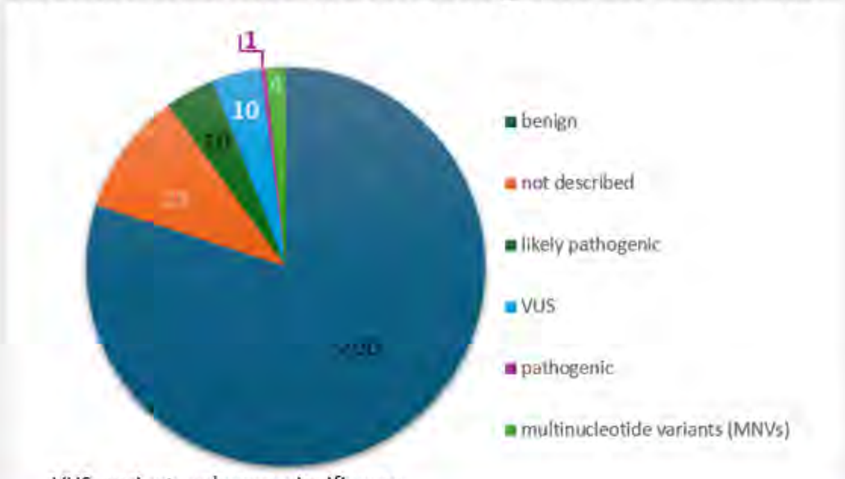
Sequencing
NextSeq550 (Illumina) platform

ANALYSIS AND RESULTS

Bioinformatic analysis
CLC genomics Workbench, Qiagen

Eleven patients shared the same multiple nucleotide variants (MNVs) on SERPIN A5, other MNVs were found on F5, F13B, and PROZ

Classification of the detected genetic variants



VUS: variants unknown significance

CONCLUSION AND FUTURE DEVELOPMENTS

We found several genetic variants whose effects are uncertain. These variants may have an impact both on prothrombotic and prohemorrhagic pathways, and may even have opposing effects.

Some of these mutations may be deleterious and linked to VTE, while others may modify the effects of pathogenic mutations on clinical expression.

Currently, we are performing protein function assays to determine how these mutations affect the function of procoagulant or anticoagulant proteins, and thus correlating these findings with the clinical phenotype.

ANGELA NAPOLITANO
Clinica Medica 1: Malattie Trombotiche ed Emorragiche

VI° CONVEGNO del DIMED
Padova, 15 novembre 2024

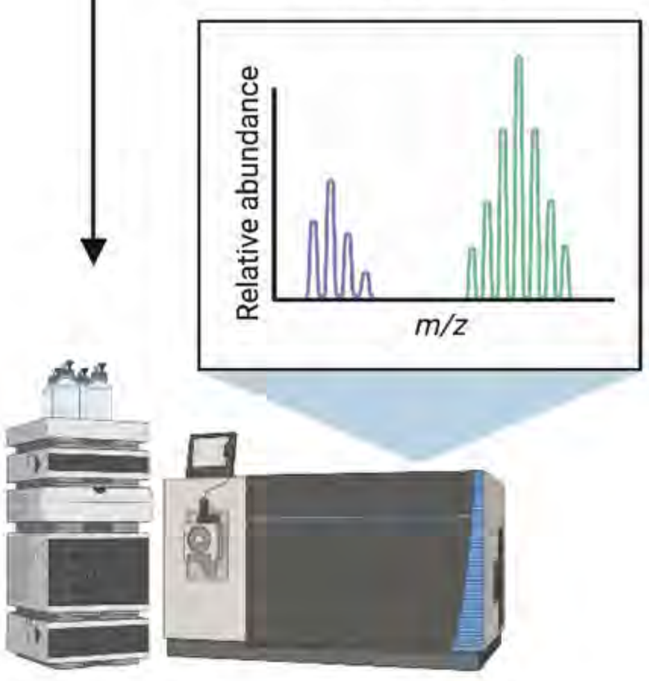
Unveiling Inflammatory Bowel Diseases (IBDs) and Colorectal Carcinoma (CRC) link: potential pathogenetic role of Basic salivary proline-rich protein 1 (PRB1) derived peptides

Nordi E., Contran N., Arrigoni G., Battisti I., Moz S., Aita A., Zingone F., Scarpa M., Pucciarelli S., Galozzi P., Basso D.

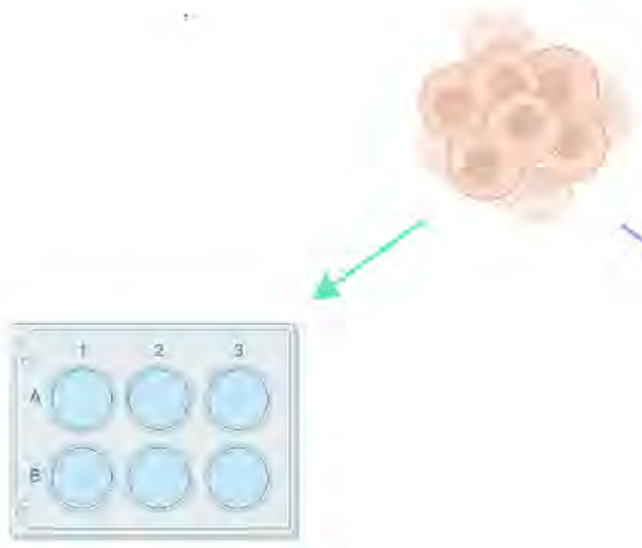
1 LTQ-Orbitrap LC-MS/MS

Fecal extract pools:
UC (*n* = 5)
CD (*n* = 5)
Controls (*n* = 6)

Basic Salivary Proline-Rich Protein 1 (PRB) 1495.66 m/z (**GQ-15**) and 1578.75 m/z (**GG-17**) peptides were detected in fecal extract from Crohn disease patients. To assess their biological activity, synthetic GQ-15 and GG-17 were used for in vitro experiments on human CRC cell lines.



2 Proliferation assay



After 24h, complete standard media were replaced with:

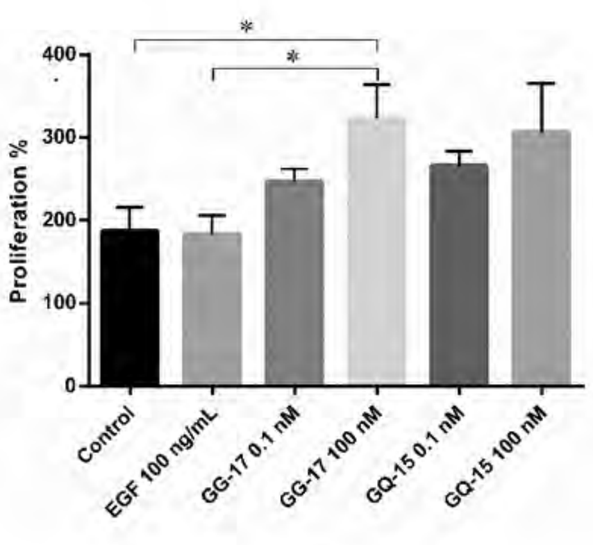
- Fresh media alone (control)
- Fresh media + EGF (100 ng/mL)
- Fresh media + 0.1 nM peptide **GQ-15**
- Fresh media + 100 nM peptide **GQ-15**
- Fresh media + 0.1 nM peptide **GG-17**
- Fresh media + 100 nM peptide **GG-17**
- Fresh media + EGF 100 ng/mL + 100 nM peptide **GQ-15**
- Fresh media + EGF 100 ng/mL + 100 nM peptide **GG-17**

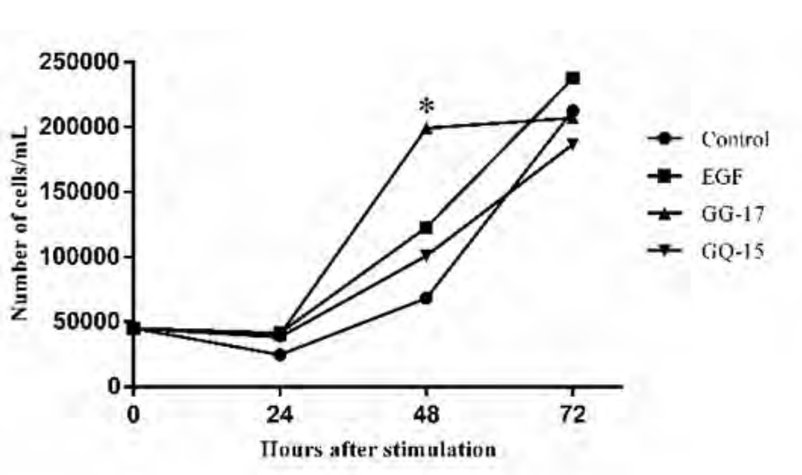
Cells were counted by optical microscope 24h, 48h, 72h after stimulation

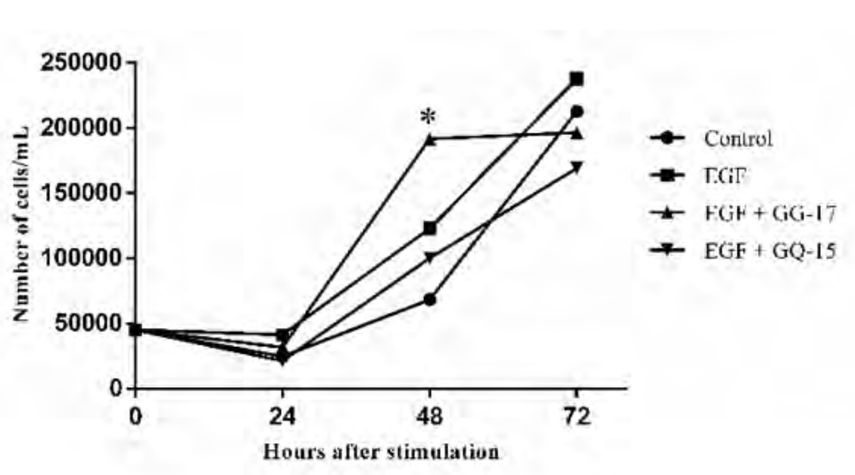
3 Signaling experiment

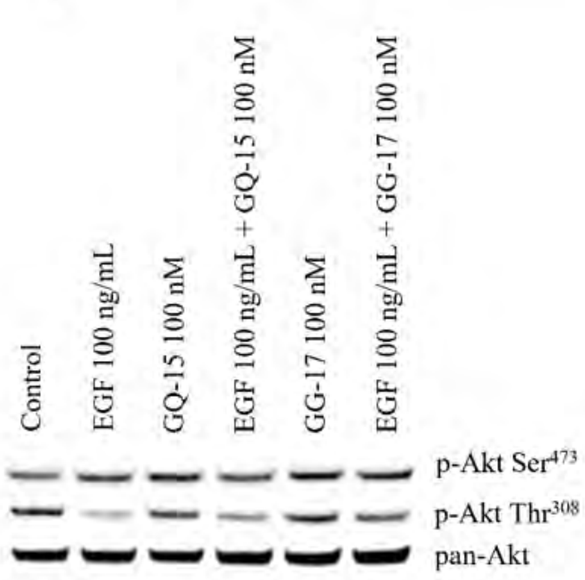
Cells were collected 24h after stimulation. Proliferation and inflammation signaling targets were evaluated by immunoblot analysis.

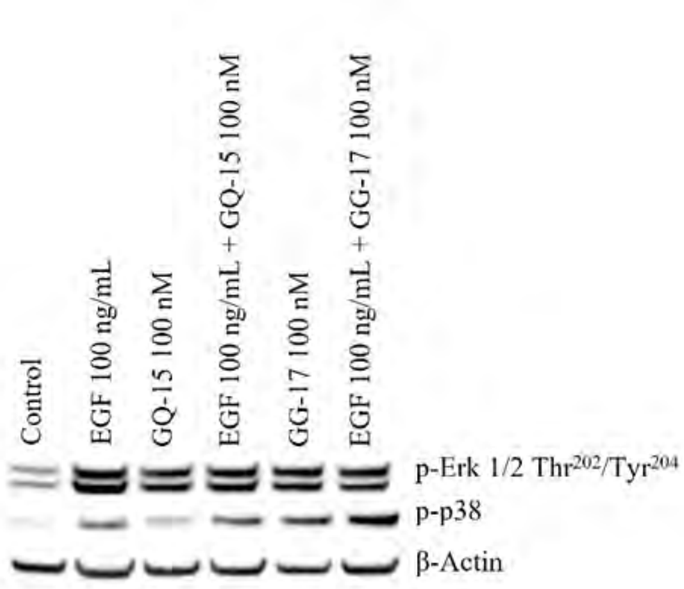
Results

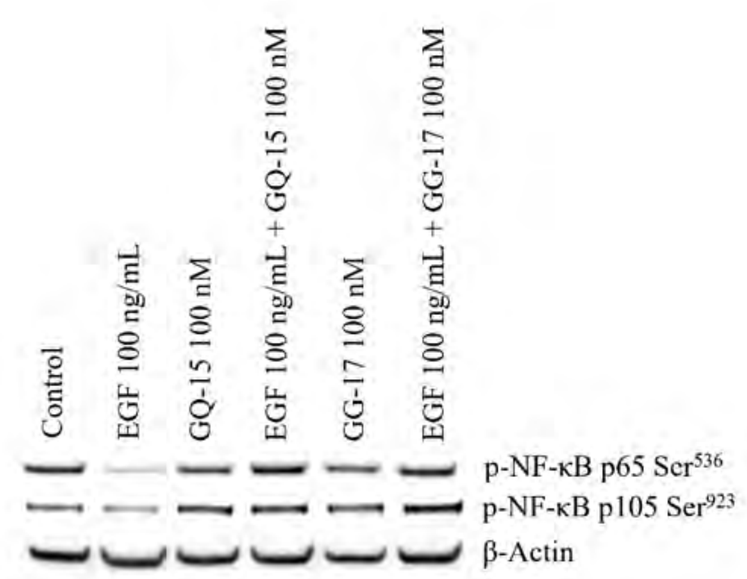












Conclusions & Future perspectives

PRB1-derived peptides accumulating in IBD stool are biologically active, able to stimulate colorectal cancer cell growth as shown in the HT-29 cell line. Both peptides increased phosphorylation of the proliferation-related targets *p-Akt*, *p-Erk 1/2* and the inflammation-related target *p-NF-κB p65* and *p105* more than EGF thus suggesting their potential pathogenetic role in linking intestinal inflammation and cancer.

To further evaluate the role of salivary PRB1-derived peptides in intestinal inflammation we intend to assess cytokines and chemokines expression patterns on cell culture media from CRC cell lines and mesenchymal stem cells isolated from colon, after peptides exposure.

EVELYN NORDI
Medicina di Laboratorio – Settore Biologia Molecolare Clinica

VI° CONVEGNO del DIMED
Padova, 15 novembre 2024

Non-canonical platelet autoantibodies induce platelet activation and release extracellular vesicles (EVs) in immune thrombocytopenia patient.

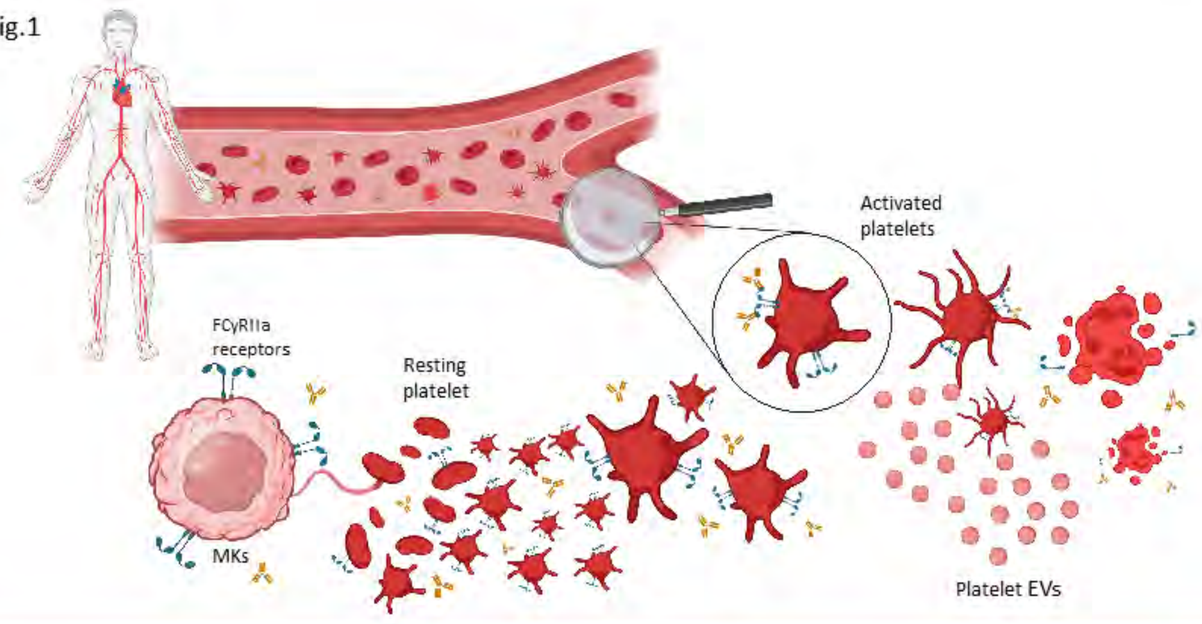
G. Nuozzi, C.M. Radu, E. Campello, S. Ferrari, S. Toffanin, C. Simion, P. Simioni.
Department of Medicine, First Chair of Internal Medicine, Padua University Hospital, Italy.

BACKGROUND AND AIM

Background: Immune thrombocytopenia (ITP) is a disease associated with autoantibodies against platelet glycoproteins causing reduced platelet count (<100×10^9/L) and bleeding predisposition. Steroids, intravenous immunoglobulin (IVIG) and TPO-receptor agonists (TPO-RAs) are first-line treatments.

Aim: Characterization of autoantibodies in a patient with severe thrombocytopenia (2-7×10^9/L) without major bleeding and refractory to corticosteroids and IVIG therapies. To evaluate the presence of pathogenic autoantibodies against platelets and/or megakaryocytes (MKs), CytoFLEX-SRT and Confocal Microscopy SP8 were used (Fig.1).

Fig.1



METHODS

- Routine monoclonal antibody immobilization of platelet antigen (MAIPA) assay to detect common platelet autoantibodies was negative.
- The presence of non-canonical platelet autoantibodies was assessed by CytoFLEX-SRT incubating platelet-rich plasma (PRP) from a healthy subject with patient platelet-poor plasma (PPP) using anti-CD41a, anti-CD62P-selectin and Annexin-V antibodies.
- Patient platelets and Peripheral Blood Mononuclear Cells (PBMCs) were isolated from blood sample. PBMCs were differentiate into megakaryocytes (MKs) to evaluate thrombopoiesis. Both platelets and MKs were incubated with patient plasma and they resulted to be extremely active.
- It is known that platelets can release extracellular vesicles (EVs) into the circulation: EVs were isolated from patient plasma to be analyzed by flow cytometry.

CytoFLEX-SRT cytometry

Confocal Microscopy SP8

Fig.1 IgG antibodies or immune complexes can activate platelets by dimerization of Fc receptor FCγRIIa receptors. Subsequently platelets release high number of platelet EVs

RESULTS

Bone marrow smear demonstrated large MKs confirming normal megakaryopoiesis according to TPO-RAs therapy. Isolated Peripheral Blood Mononuclear Cells (PBMCs) were differentiated into MKs. MKs culture demonstrated functional thrombopoiesis instead, when MKs were cultured in the presence of patient IgG demonstrated impaired function (Fig. 2). In particular, anti-IgG staining showed that the antibodies bind to MKs. Furthermore, increased expression of P-selectin in donor platelets in the presence of patient IgG suggests increased platelets activation. In addition, the ability of platelets to release extracellular vescicles (EVs) with pro-coagulant activity after activation was evaluated. CytoFLEX-SRT analysis of patient plasma EVs levels (P-selectin+calcein+AnnexinV+) were lower in comparison to healthy controls, suggesting their consumption as a compensatory mechanism to reduce bleeding.

Fig.3

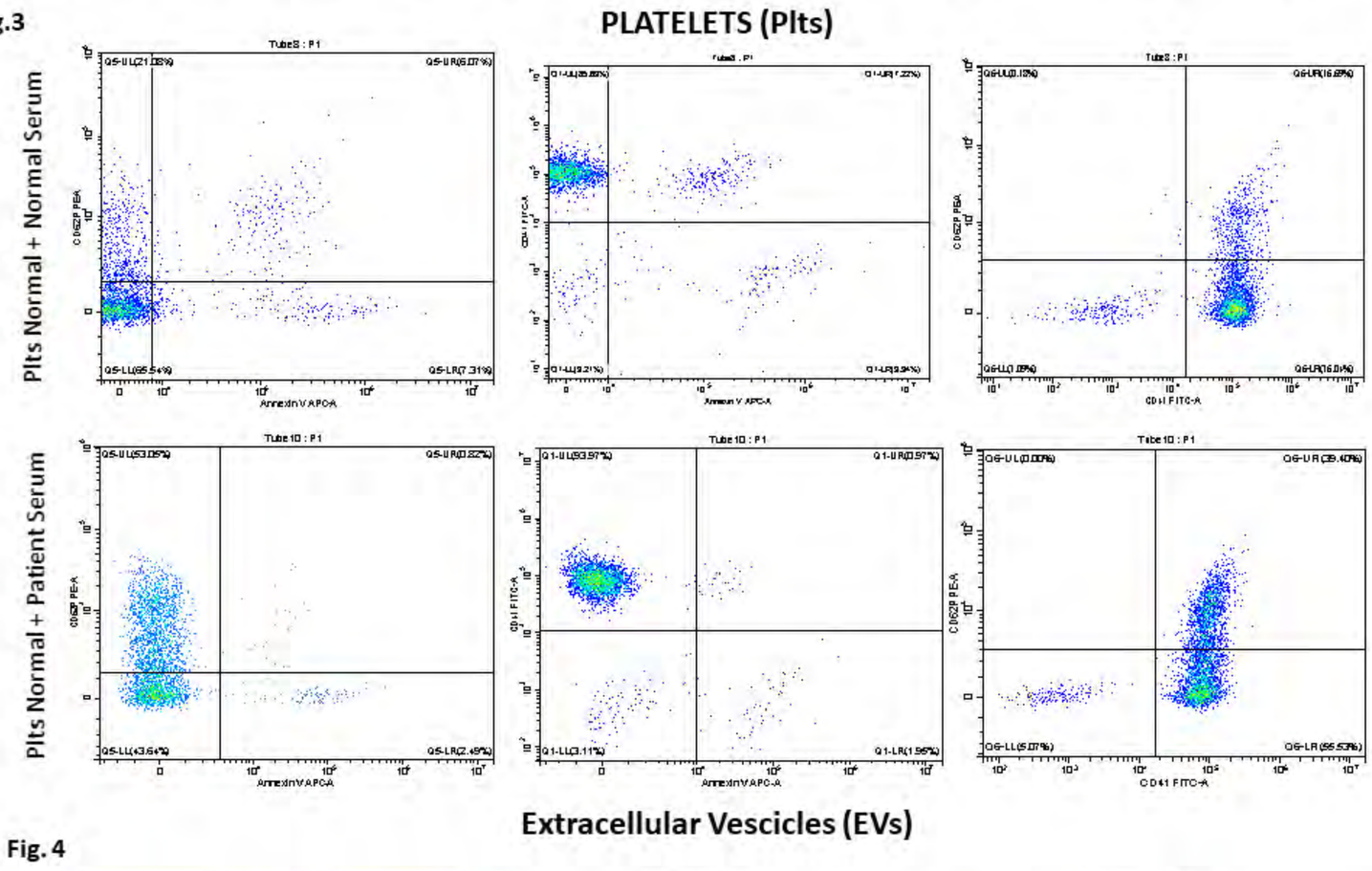


Fig. 2

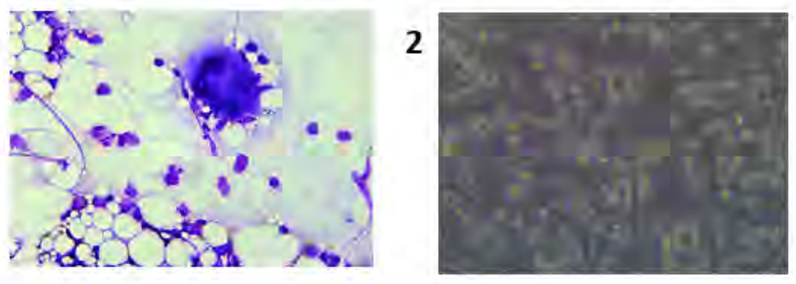


Fig. 5

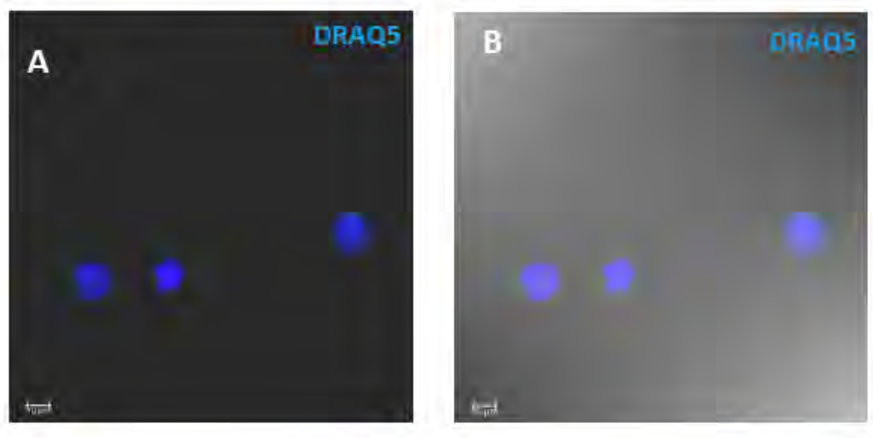


Fig. 4

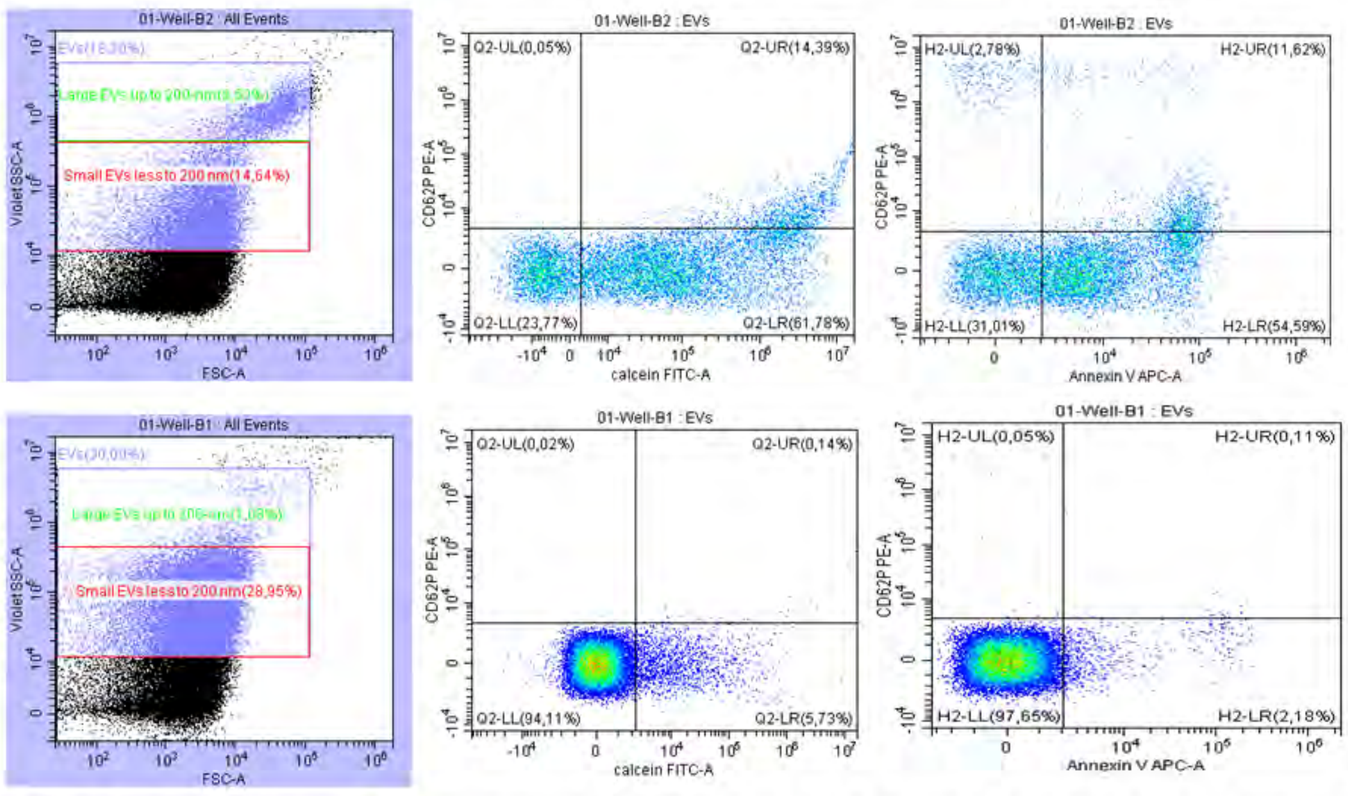


Fig. 2: 1-MKs in the blood stream; 2-PBMCs-deriverd MKs in culture. Fig.3: Acquisition and analysis of platelet flow cytometry data. Cytograms of the percentage of Annexin V and CD62P expression by mixing healthy controls platelets and patient serum. Fig. 4: Plasma analysis of Extracellular Vesicles. Fig. 5: Patient isolated MKs incubated with healthy serum do not shown IgG (A,B). Patient's isolated MKs incubated with patient serum shown IgG bind to MKs. 3D images of MK and IgG (E and F).

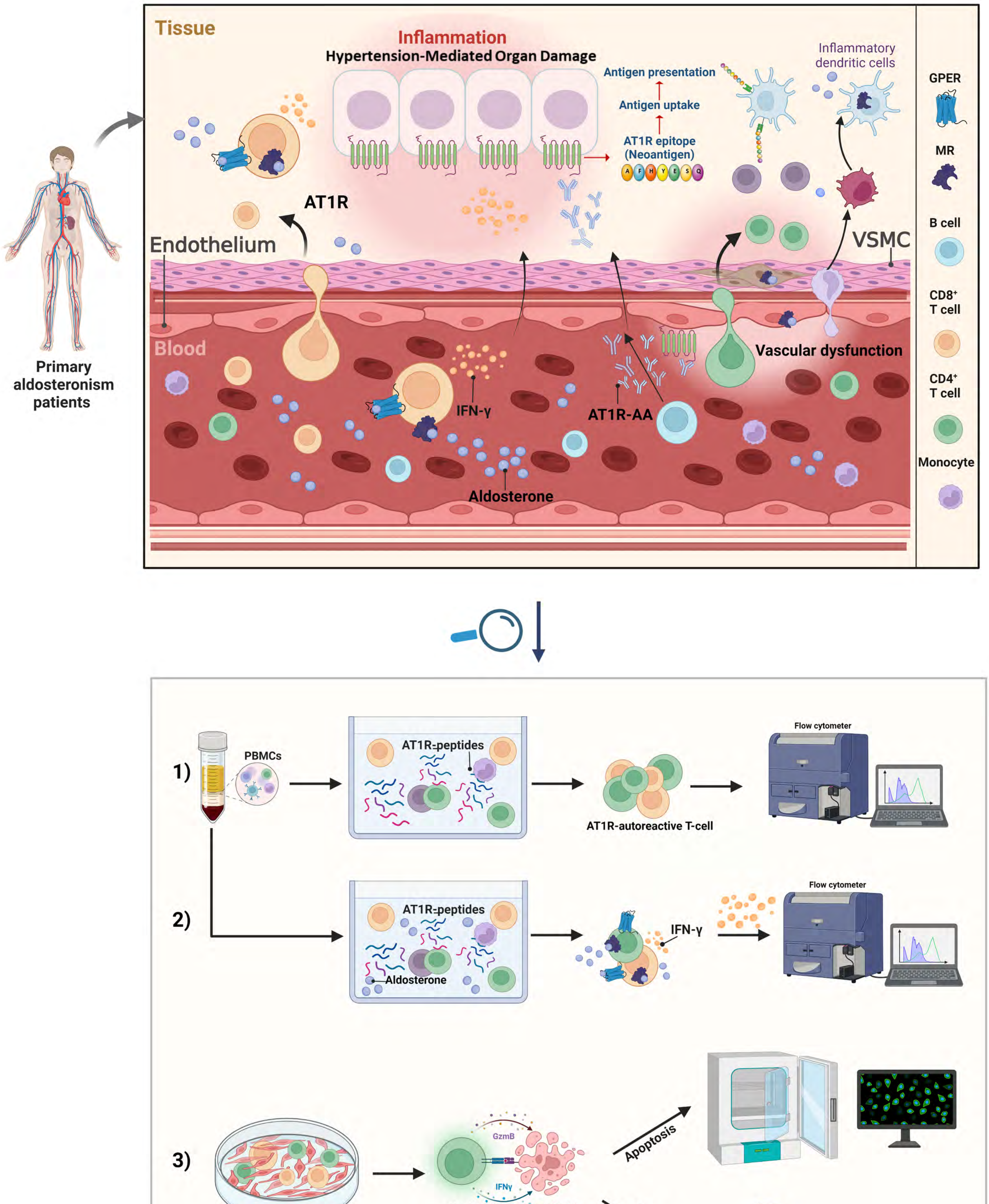
CONCLUSIONS

The results demonstrated the importance of highly sensitive instruments in the characterization of platelet autoantibodies in the diagnosis of ITP leading the possibility of targeted therapy for the patient. In particular, patient's serum showed the presence of antibodies capable of activating platelets of healthy controls as shown with Flow cytometry results. In addition, confocal microscopy demonstrated the binding of patient purified IgG to megakaryocytes. The results obtained underlying that the monitoring of platelet autoantibody levels should provide important information for assessing the patient response and prognosis in ITP.

GIORGIA NUOZZI
Malattie Trombotiche ed Emorragiche

Autoreactive T-Lymphocytes against the Angiotensin II type 1 Receptor in Salt-dependent Human Hypertension

Maria Piazza



1)AT1R-Autoreactive T-cell; 2)Aldosterone effects on AT1R-autoreactive T-cell; 3)Endothelial damage

MARIA PIAZZA
Medicina d'Urgenza OSA

VI° CONVEGNO del DIMED
Padova, 15 novembre 2024

Analysis of preanalytical variability on plasma miRNA expression profile by Next Generation Sequencing to reveal specific potential diagnostic biomarkers of myelodysplastic syndrome

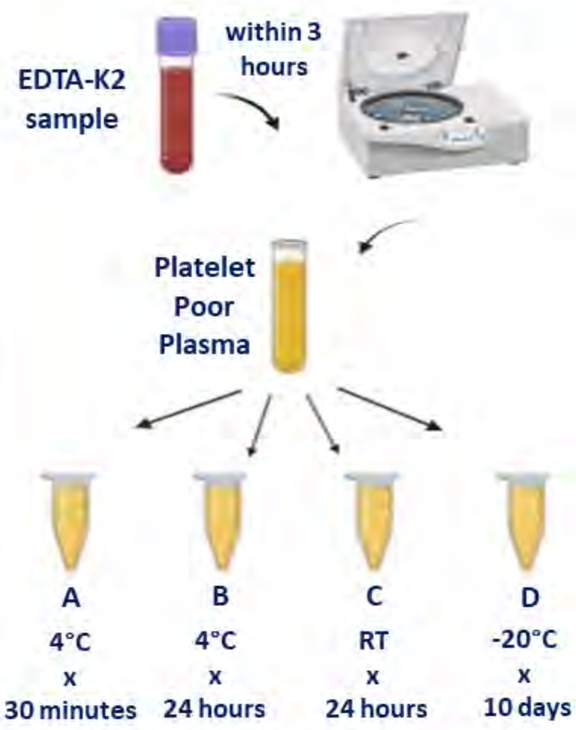
Alessandra Pinello, Alessandra Giannella, Michela Pelloso, Gianni Binotto, Andrea Serafin, Stefania Moz, Francesca Tosato, Livio Trentin, Daniela Basso, Giulio Ceolotto

Experimental design

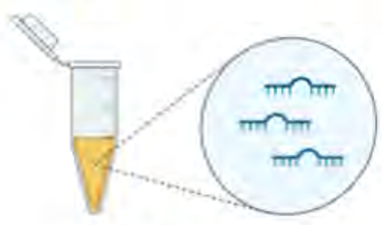
Aim

- investigate the impact of preanalytical variables in Platelet Poor Plasma samples on miRNA expression profile
- identify potential circulating miRNAs as diagnostic biomarkers of Myelodysplastic Syndrome

Preanalytical conditions



Population



NGS sequencing

NextSeq550 (Illumina)

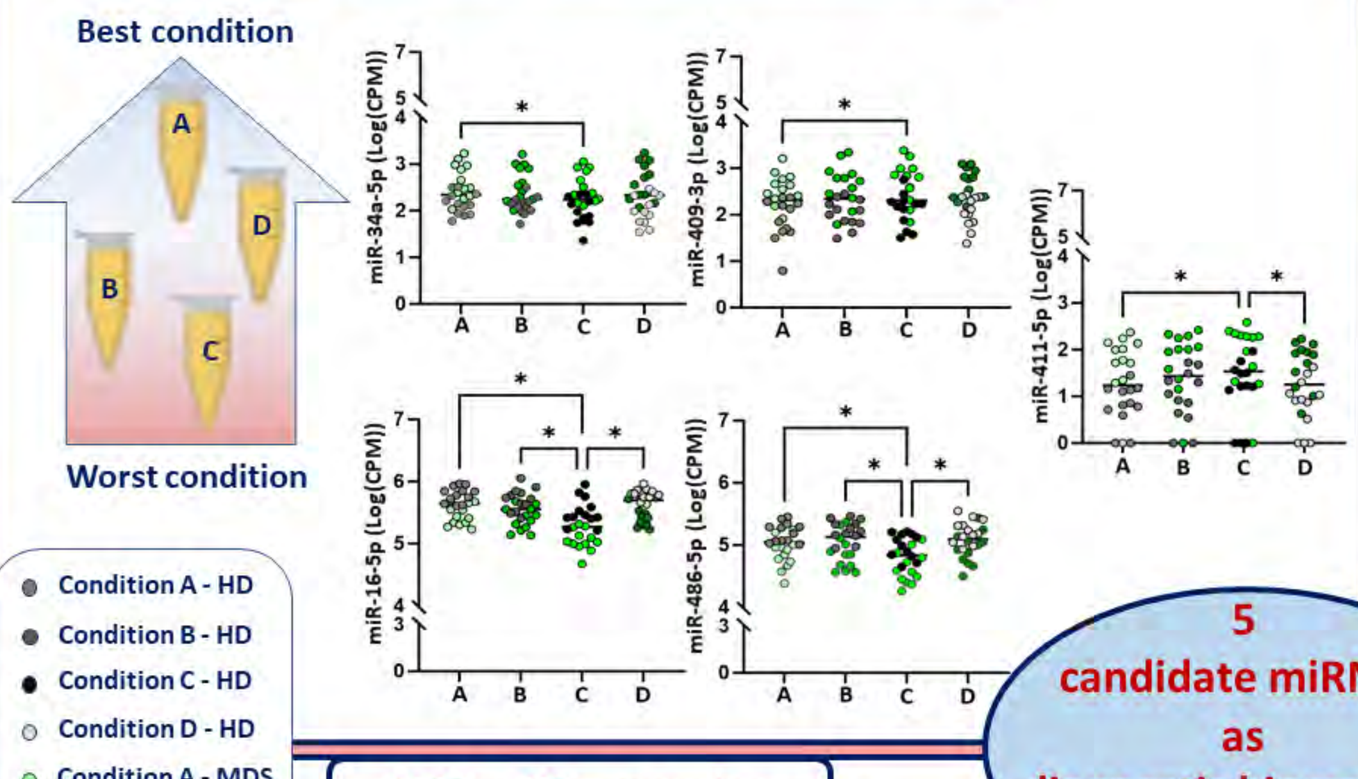
Bioinformatic Analysis

miRNA quantification
Differential expression analysis

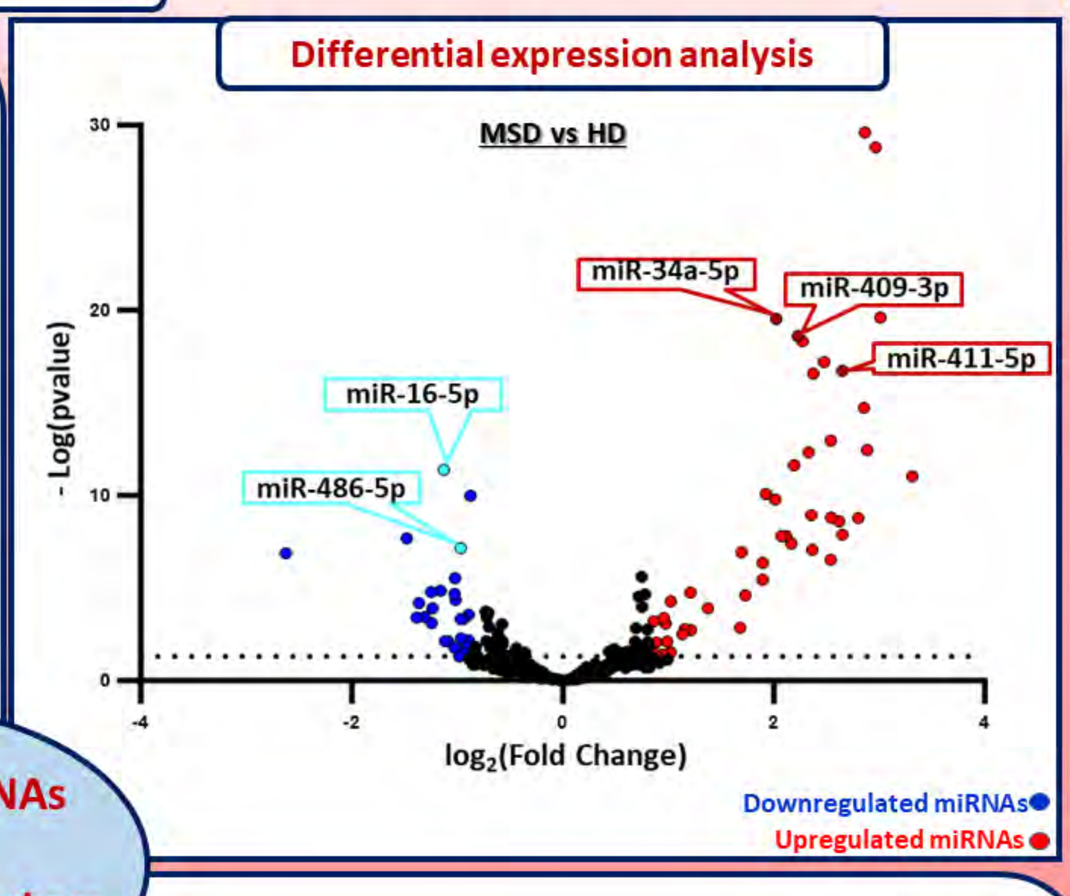
CLC Genomics (Qiagen)

Results

Preanalytical condition Analysis

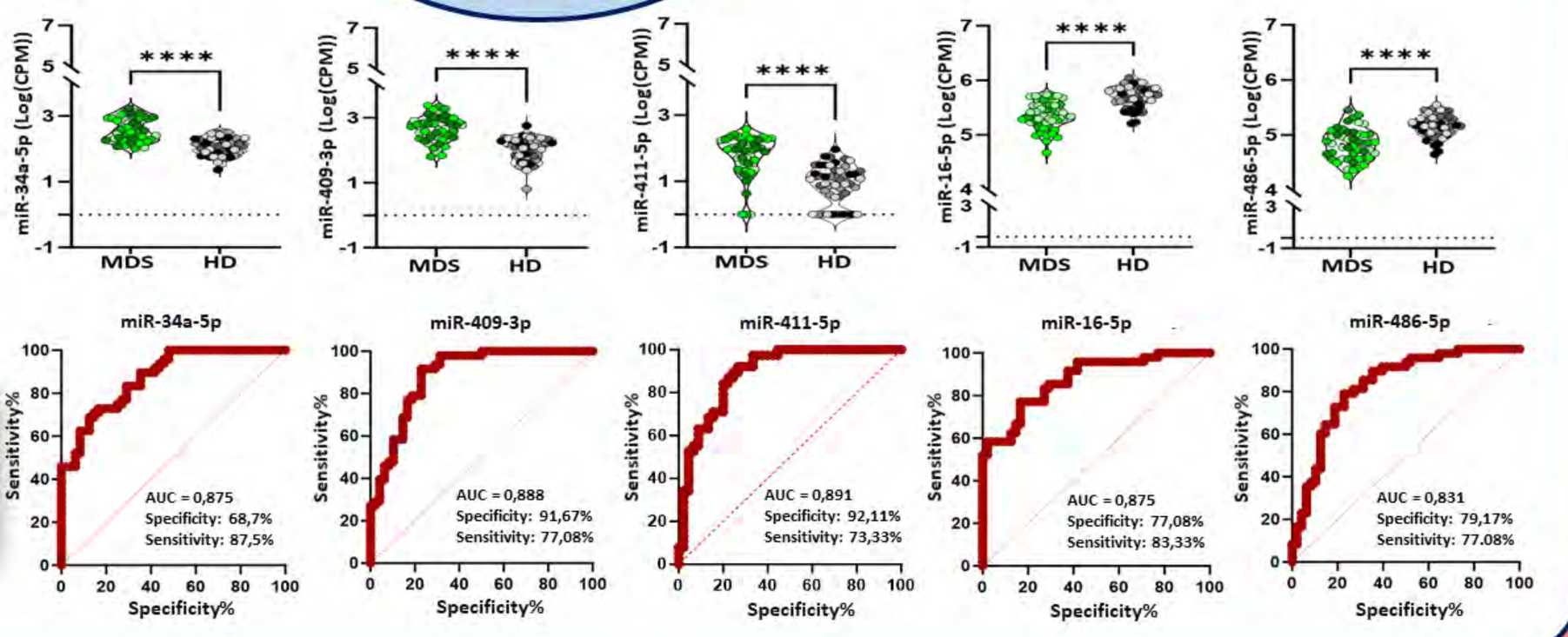


Differential expression analysis



5 candidate miRNAs as diagnostic biomarkers of MDS

MSD vs HD expression



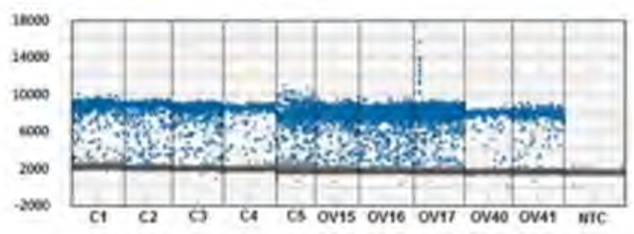
miRNA discriminatory capacity

ROC Curve Analysis

miRNA	MvsS	MAvsSA	MBvsSB	MCvsSC	MDvsSD
34a-5p	0,875	0,896	0,875	0,882	0,868
409-3p	0,888	0,892	0,861	0,840	0,910
411-5p	0,891	0,883	0,900	0,837	0,875
16-5p	0,875	0,924	0,889	0,965	0,937
486-5p	0,831	0,882	0,875	0,854	0,785

Future prospectives

- IsomiRs
- Pathway Analysis

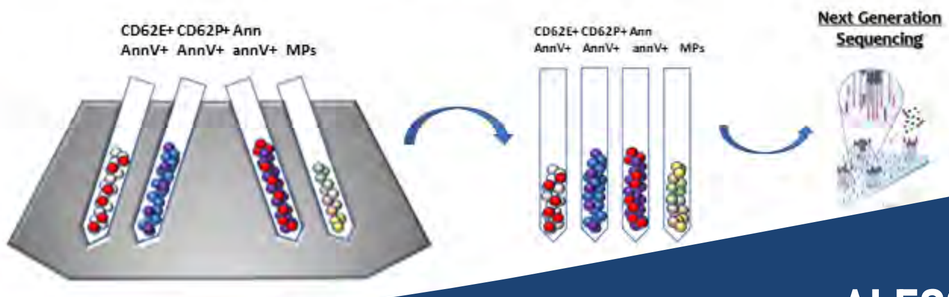


Digital Droplet PCR

- miRNA validation

- Sorted extracellular vesicles miRNA cargo

SRT- Cytoflex



ALESSANDRA PINELLO
Medicina di Laboratorio

Evaluation of the potential hepato-protective profile of SGLT2 inhibitors in animal models of genetic obesity

Domenico Pò, Angelo Di Vincenzo, Marnie Granzotto, Marika Crescenzi, Roberto Vettor, Marco Rossato

Center for the Study and the Integrated Treatment of Obesity, Department of Medicine, University Hospital of Padova

Evaluation of the potential hepato-protective profile of SGLT2 inhibitors in animal models of genetic obesity

Background

Obesity

MASLD

Metabolic-associated steatotic liver disease (MASLD)

- MASLD is the most common cause of chronic liver disease
- MASLD and obesity are intertwined: weight loss may be helpful, but the evidence about pharmacotherapy is limited to date

Aim

SGLT2 inhibitors (SGLT2i)

- A role in MASLD has been proposed, as they may decrease fat accumulation, enhance the FGF-21 pathway, and reduce fibrosis
- To test the effects of SGLT2 on the liver

B6.V-LEP ob/ob mice

C57BL/6J wilde-type

4-weeks dapagliflozin treatment

Protein and gene analysis

Analysis

Liver

Hepatic tissues analysis

- Expression of FGF-21 and markers of fibrosis such as TGF-β, α-SMA, PDGF-α and PDGF-β
- Evaluation of steatosis and hepatic triglyceride content

Results

Wilde-Type Lean Mice

- INCREASED expression of FGF-21 in the LIVER
- REDUCTION of LIVER FAT deposition
- UNAFFECTED expression of fibrotic markers

Ob/Ob Mice

- UNAFFECTED expression of FGF-21 in the LIVER
- UNAFFECTED LIVER FAT deposition
- UNAFFECTED expression of fibrotic markers

REFERENCES:
1. Kahl S, et al. Effects of empagliflozin on markers of liver steatosis and fibrosis and their relationship to cardiorenal outcomes. Diabetes Obes Metab. 24(6):1061-1071. 10.1111/dom.14670 (2022).
2. Osataphan S, et al. SGLT2 inhibition reprograms systemic metabolism via FGF21-dependent and -independent mechanisms. JCI Insight. 4(5):e123130. doi: 10.1172/jci.insight.123130 (2019).

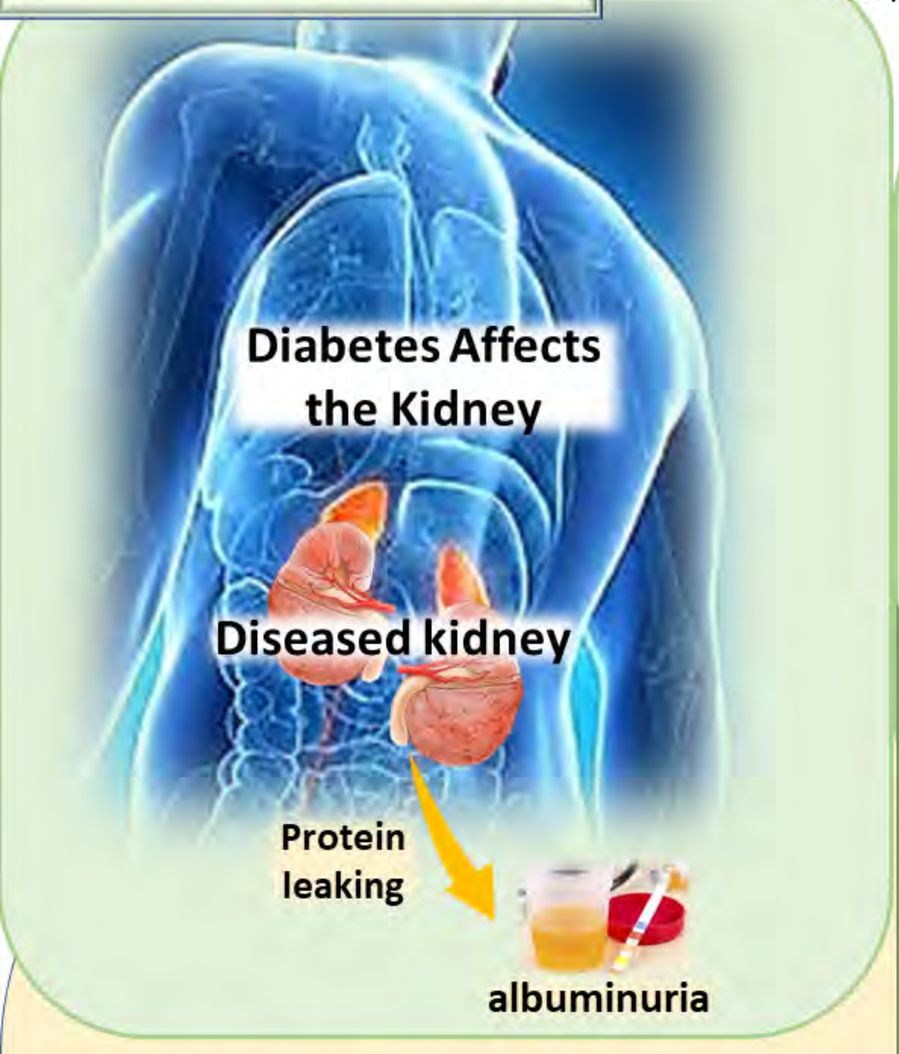
VI° CONVEGNO del DIMED
Padova, 15 novembre 2024

Diabetic kidney disease: Understanding the role of mesangial, podocytes and parietal epithelial cells in albumin handling and glomerulosclerosis

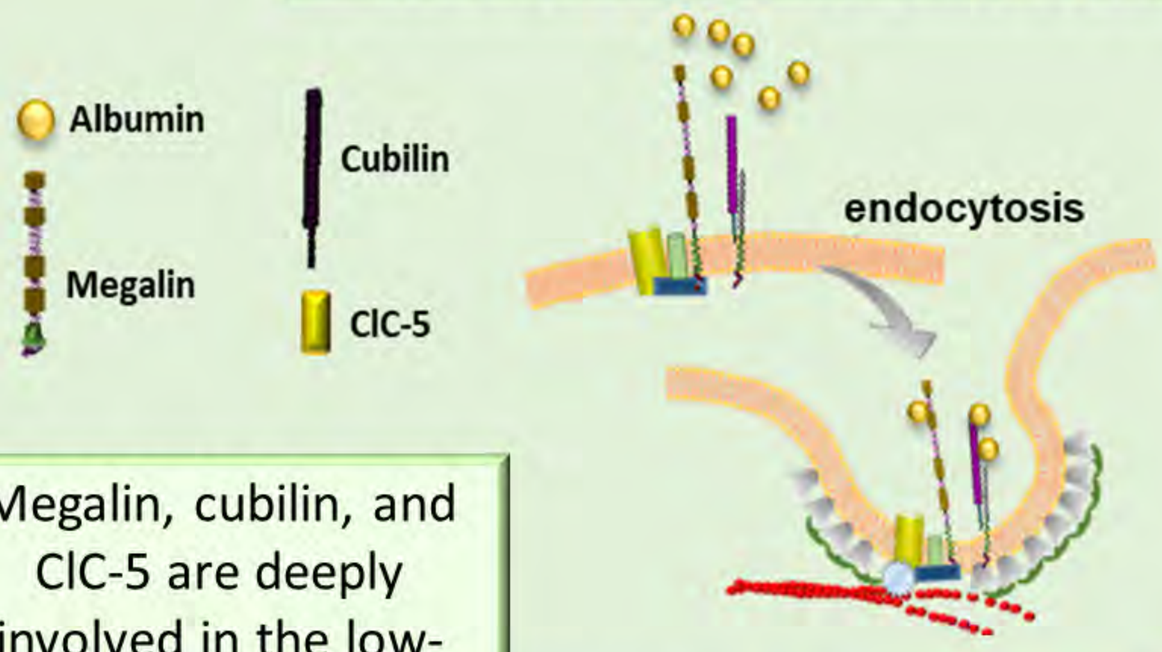
Giovanna Priante, M. Ceol, F. Nalesso, F. Anglani, L.A Calò, D. Del Prete

Kidney Histomorphology and Molecular Biology Laboratory, Nephrology Unit, Department of Medicine - DIMED, University of Padua

INTRODUCTION



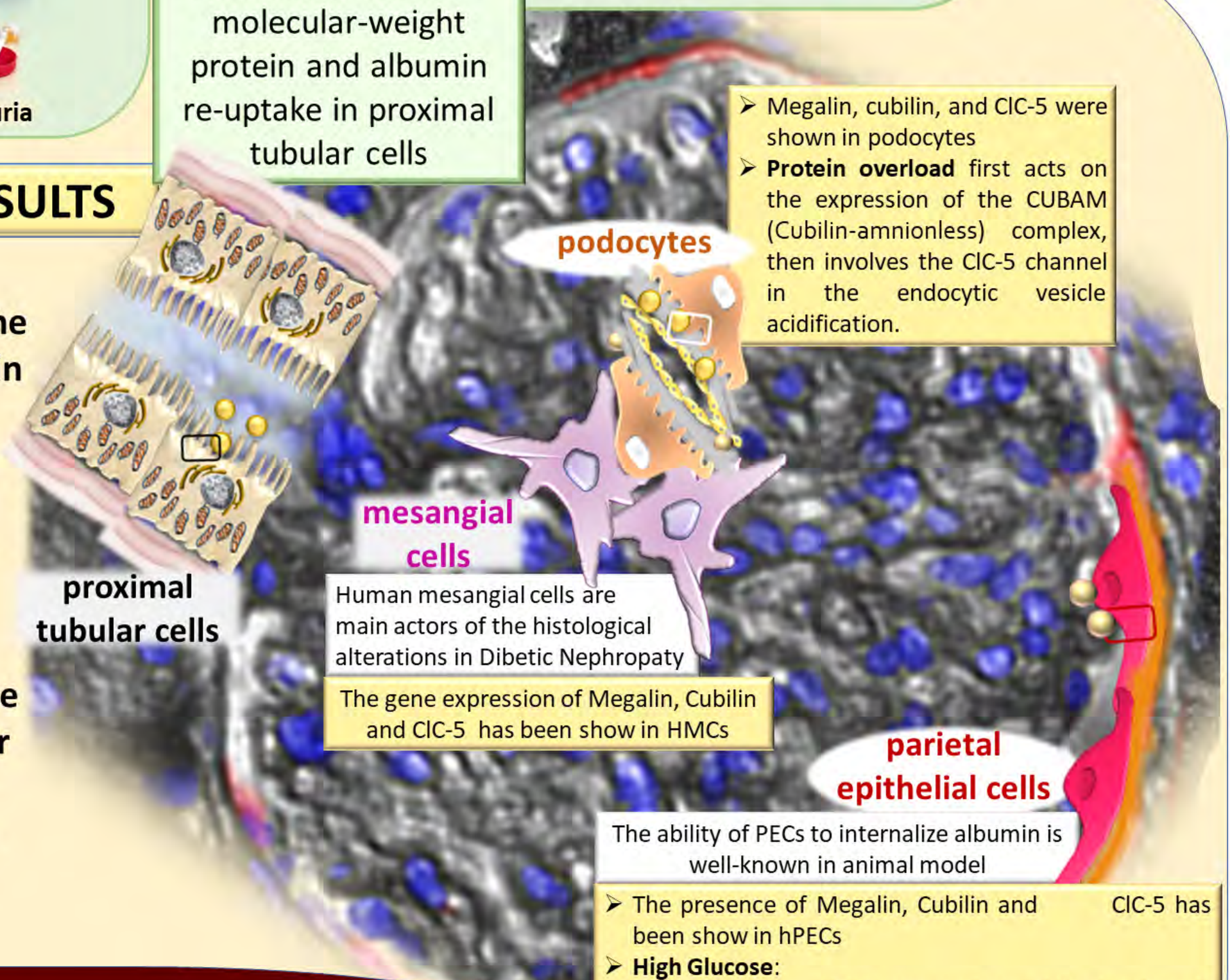
macromolecular system involved in protein uptake



Megalin, cubilin, and ClC-5 are deeply involved in the low-molecular-weight protein and albumin re-uptake in proximal tubular cells

PRELIMINARY RESULTS

We have demonstrated the presence of ClC-5, Megalin and Cubilin not only at the tubular level but also in glomerular compartment. In particular, protein overload and high glucose condition modulate their expression in human podocytes and hPECs, respectively.



- Megalin, cubilin, and ClC-5 were shown in podocytes
- **Protein overload** first acts on the expression of the CUBAM (Cubilin-amnionless) complex, then involves the ClC-5 channel in the endocytic vesicle acidification.

Human mesangial cells are main actors of the histological alterations in Dibetic Nephropaty

The gene expression of Megalin, Cubilin and ClC-5 has been show in HMCs

The ability of PECs to internalize albumin is well-known in animal model

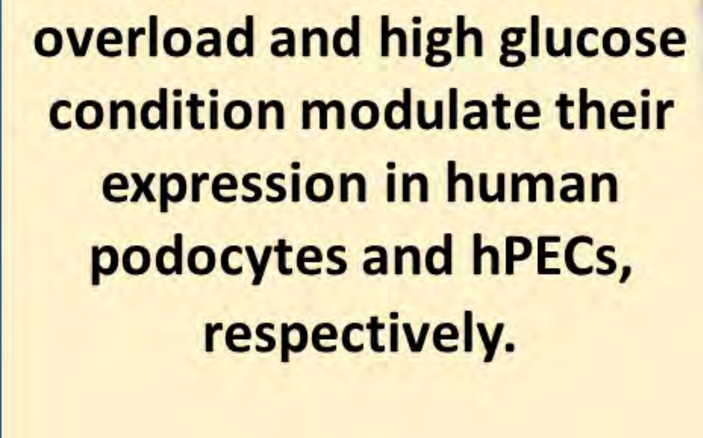
- The presence of Megalin, Cubilin and ClC-5 has been show in hPECs
- **High Glucose**:
  - downregulates CLC-5 expression while it doesn't affect megalin and cubilin expression.
  - increases of CD44 expression (marker of activation)
  - disrupts the orderly arranged stress fibers of actin cytoskeleton and we observe a marked redistribution of F-actin fibers around the nucleus
  - increases expression of both initiator caspase-9 and effector caspases -3

Based on our preliminary results, aims of our project is

To investigate in glomerular cells *in vitro* the involvement of the protein uptake machinery in the onset of diabetic milieu. Proximal tubular cells will be used as control. All cell lineages will be costimulated with high glucose condition and albumin overload.

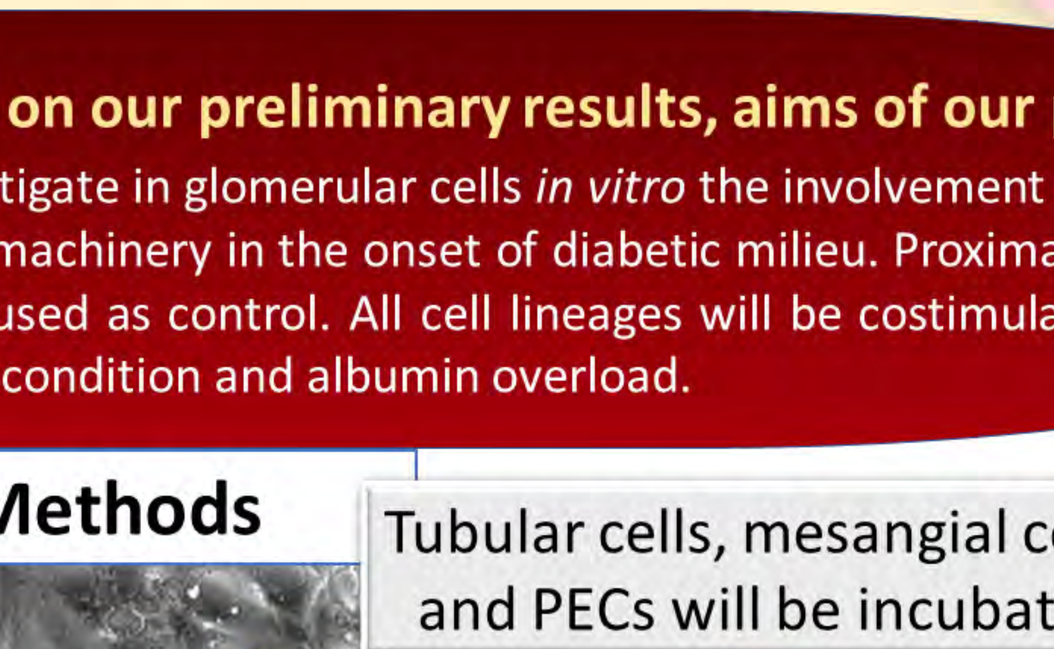
Methods

Tubular cells, mesangial cells, conditionally immortalized human Podocytes and PECs will be incubated with high glucose first and then with albumin

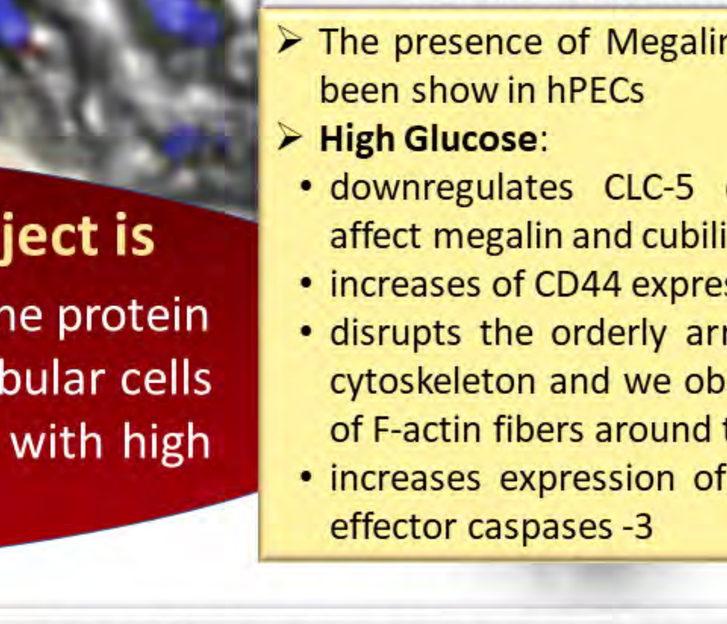


Cell morphology
Proliferation
Apoptosis
Cellular Markers analysis

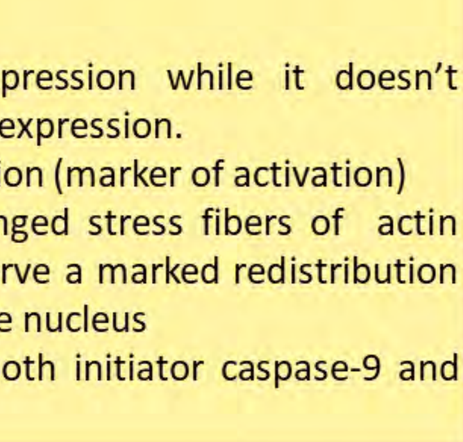
DMI6000CS-TCS SP8 fluorescence microscope, with a DFC365FX camera and subsequently analysis with LAS-AF software v.3.1.1 (Leica Microsystems)

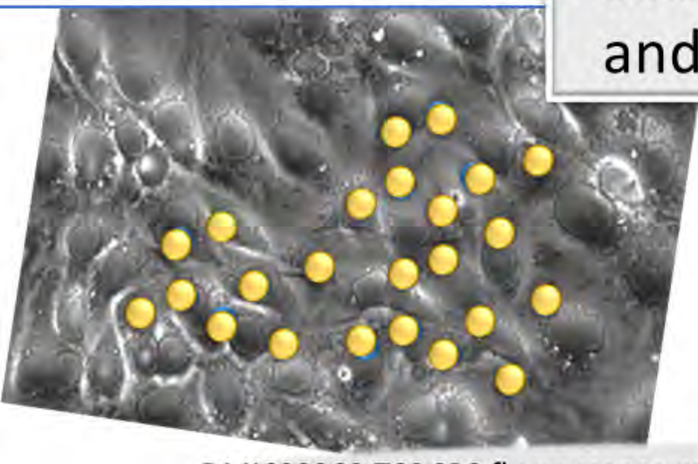


Protein analysis



Gene expression

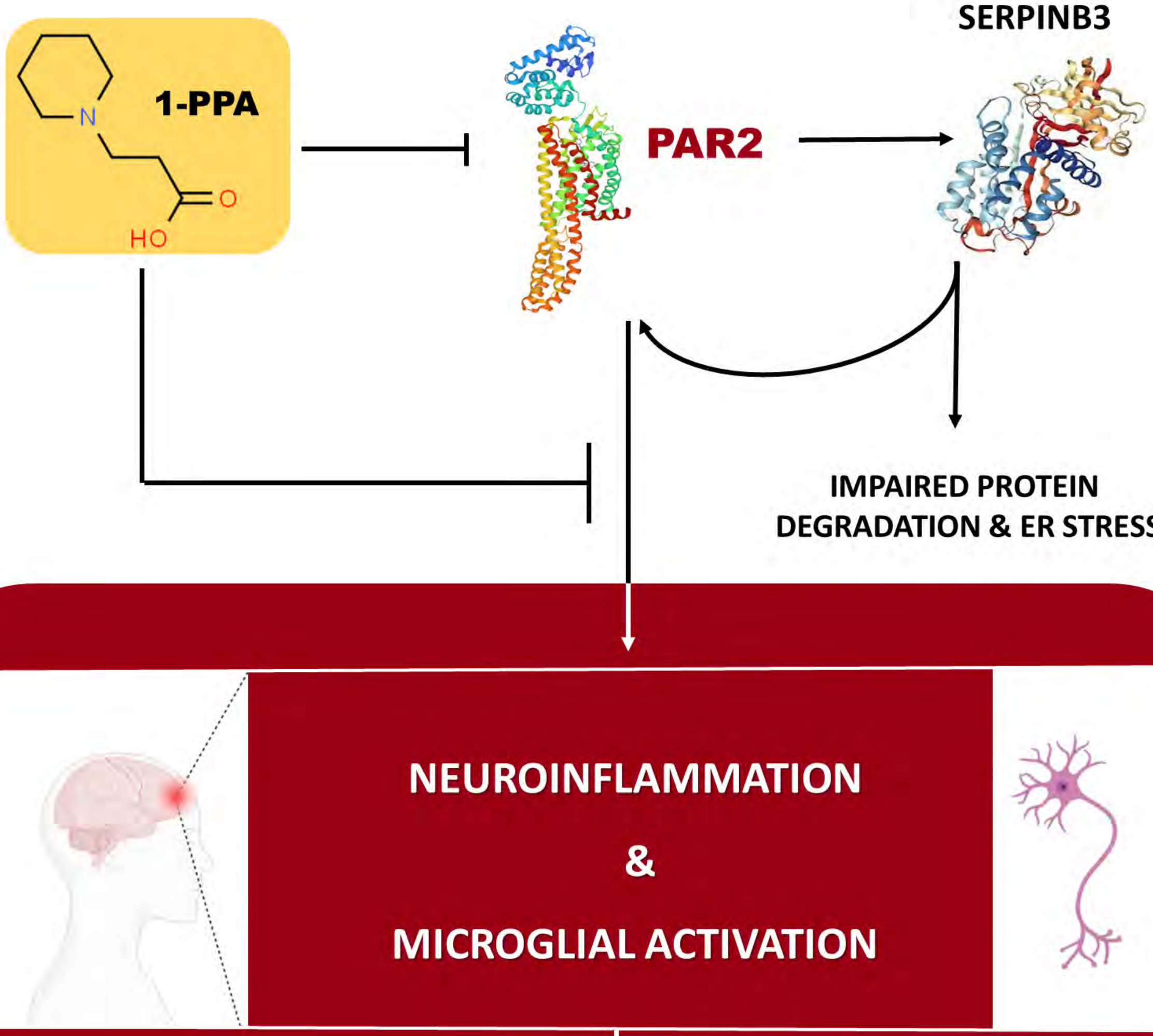




The new instruments recently acquired by DIMED allow us the possibility to best clarify the interconnection between the protein uptake machinery and glomerular damage, unveiling a new pathophysiological mechanism underlying diabetic nephropathy

GIOVANNA PRIANTE
Nefrologia

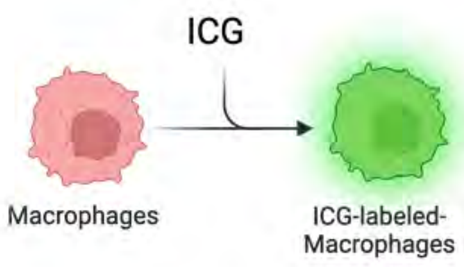
INHIBITION OF PAR2 ACTIVATION IN NEURODEGERATIVE DISEASES

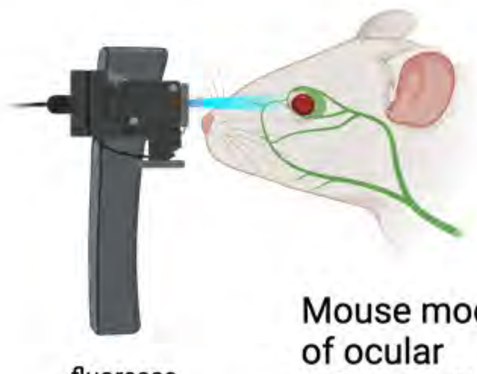


SANTINA QUARTA
Laboratorio Epatologia Molecolare

Centro per l'Ipertensione del DIMED

Indocyanine Green

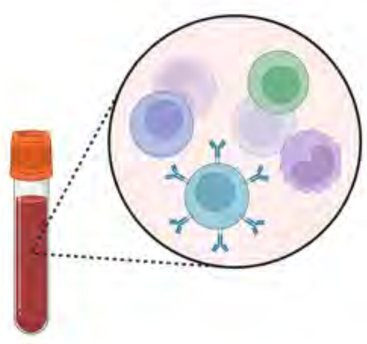


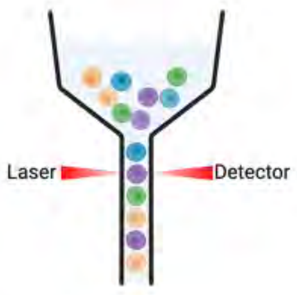


fluoresce scanning laser ophtalmoscopy

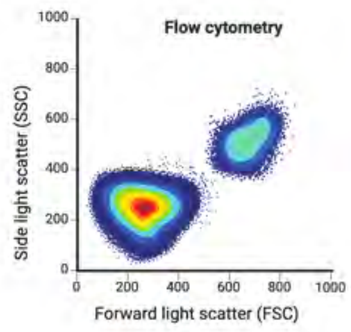
Mouse models of ocular inflammation

Successful in vivo labelling of infiltrating leukocytes [6]



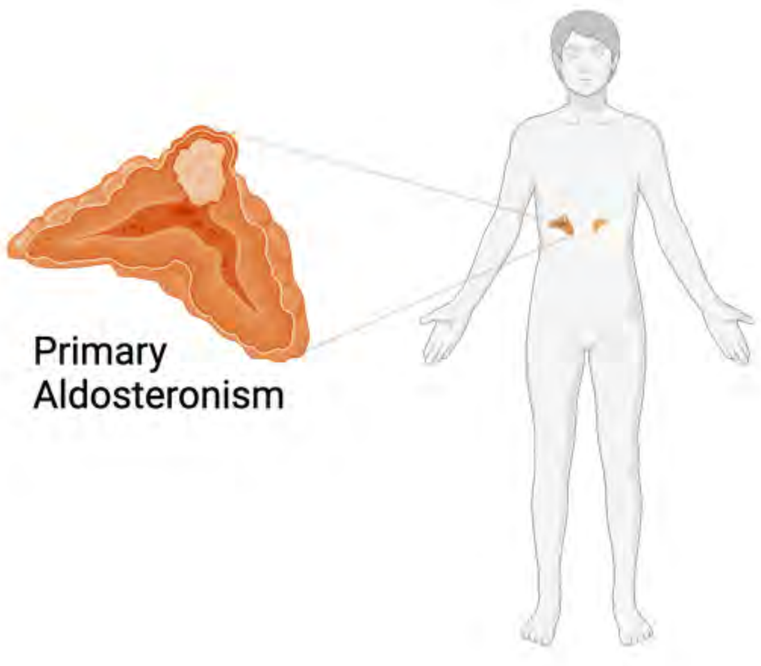


Flow cytometry

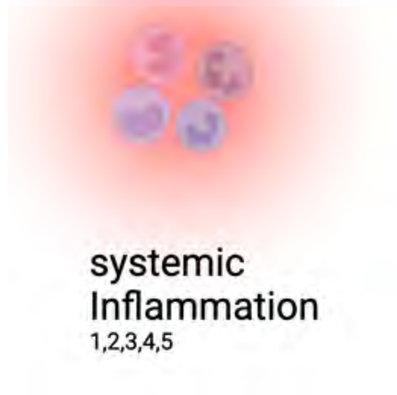


In vitro confirmation of labelling [6,9, 8]

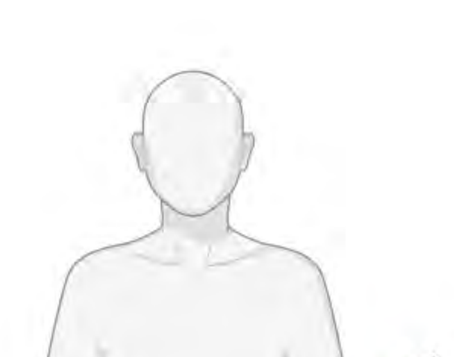
Central Serous Chorioretinopathy (CSC) or CSC like changes [6,7]



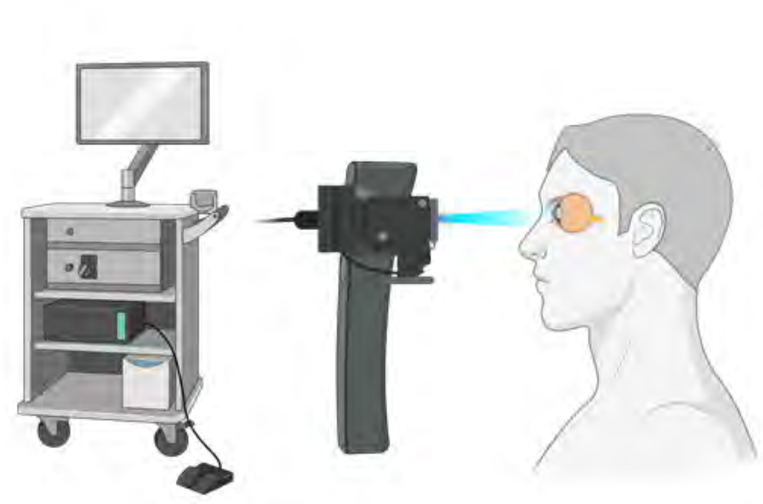
Primary Aldosteronism



systemic Inflammation
1,2,3,4,5



ICG



Spectralis HRA+OCT system (Heidelberg Engineering) for ICG Angiography

In vivo ICG mediated labelling

Next generation Flow Cytometry

in vitro confermation

FEDERICO BERNARDO ROSSI
Clinica dell'Ipertensione

VI° CONVEGNO del DIMED
Padova, 15 novembre 2024

Edoardo Ruggeri[1], Simona Censi[1], Cristina Clausi[1], Ilaria Piva[1], Elisabetta Cavedon[1], Giulia Messina[1], Jacopo Manso[1], Loris Bertazza[1], Susi Barollo[1], Chiara Sabbadin[1], Caterina Mian[1]

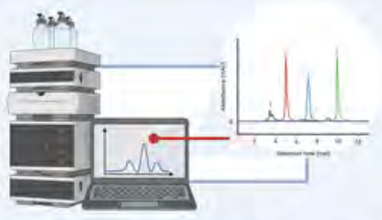
Endocrinology Unit, University of Padua, Padua, Italy.

ANTITUMORIGENIC EFFECTS OF LICORICE EXTRACT AND GLYCYRRHETINIC ACID ON PAPILLARY THYROID CANCER CULTURES

LICORICE

(Glycyrrhiza uralensis Fisch.)

Around 400 total compounds including triterpenes, chalcones, saponins, alkaloids, glycosides, phenols, tannins, and approximately 300 flavonoid.



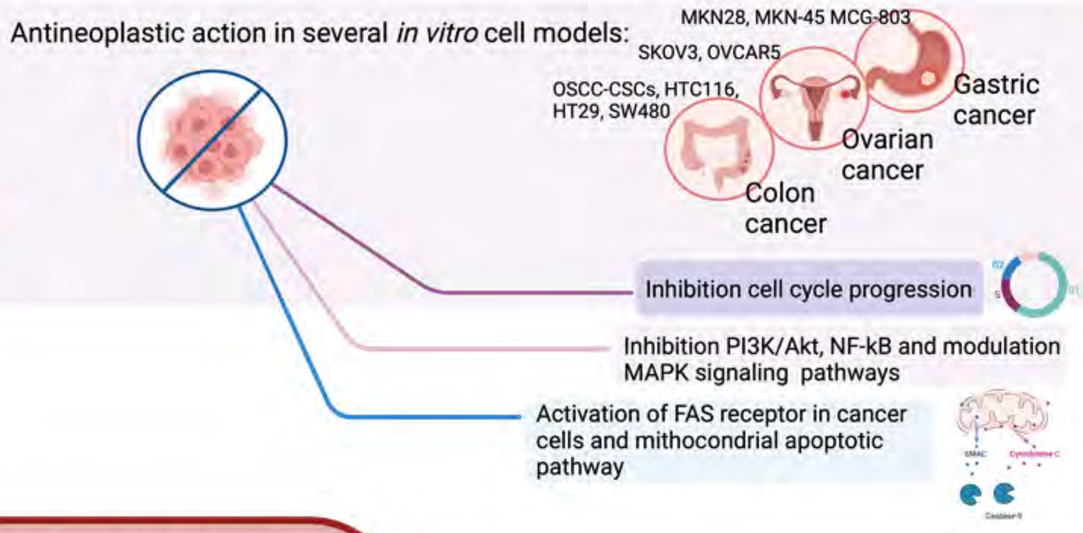
The main active phytoconstituents are:

- GLYCYRRHETINIC ACID (GA)
- GLYCYRRHIZIN; } LICORICE EXTRACT
- GLABRIDIN; } LICORICE EXTRACT
- LICOCHALCONE A. } LICORICE EXTRACT

Licorice roots extract have demonstrated efficacy in regulating respiratory functions, immunoregulation, gastroenteric protection, etc.

Antioxidant Activities:

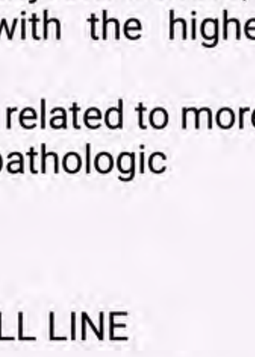
Antineoplastic action in several *in vitro* cell models:



Toxicological effects of licoriece

LICORIECE ⊥ 11β-hydrogenase type II (11ß-HSD2)

Cortisol ⇌ Cortisone

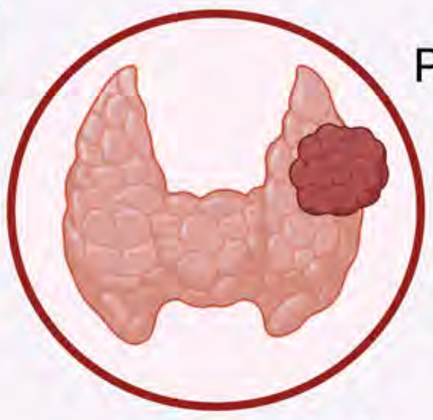


EXCESSIVE LICORIECE CONSUPTION RESULTS IN:

- hypernatremia,
- hypokalemia,
- blood pressure increasment,
- cadiovascular impeirments

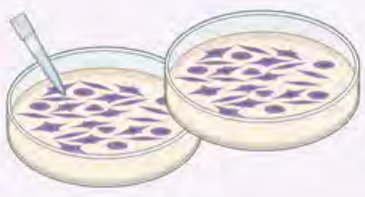
AIM

Potential role of licorice extract to improve treatment in papillary thyroid cancer (PTC): an in vitro study.



PTC:

- The most frequent thyroid cancer;
- malignant tumor with the highest growth rate;
- $BRAF^{V600E}$ mutation related to more aggressive clinicopathologic features



K1 CELL LINE

BCPAP CELL LINE

Glabridin + AGONIST ACTIVITY → Estrogen receptor (ER)

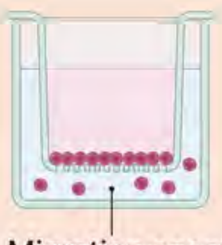
GA + AGONIST ACTIVITY → Mineralcorticoid receptor (MR)

Both the Receptors are expressed by PTC cell line

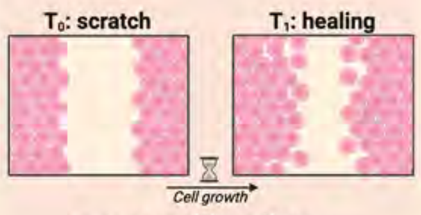
workflow

- Treatment evaluation of Licorice extract and GA using PTC cell lines

- MTT test and EC50

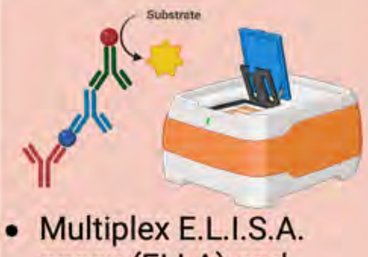


- Migration assay



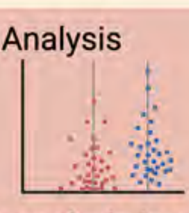
- wound healing assay

- Checking targets expression/ modulation using microscopy, qPCR and western blotting

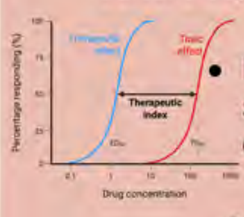


- Multiplex E.L.I.S.A. assay (ELLA) and cytokines identification (e.g.,TNF-α, IL-1β, IL-6, IL-8, IP-10, MCP-1, MIP-1α MIP-1β, and sE-Selectin)
- Receptor-Ligand study between Licorice extract and GA with MR and ER (e.g., IP, siRNA/ receptor transfection)

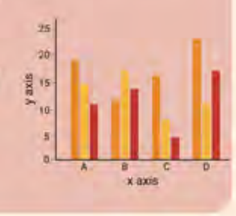
Data Analysis



- Cytokine analysis in cell culture supernatant



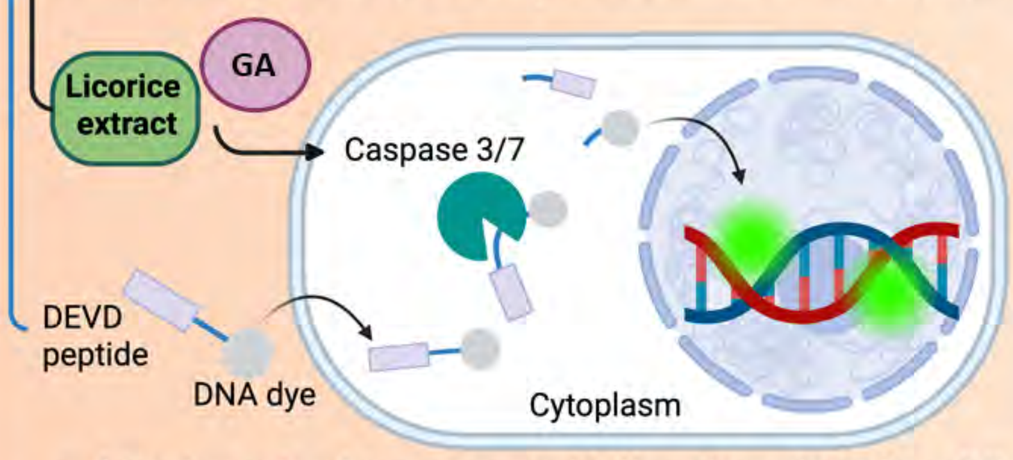
- EC50, synergism calculation

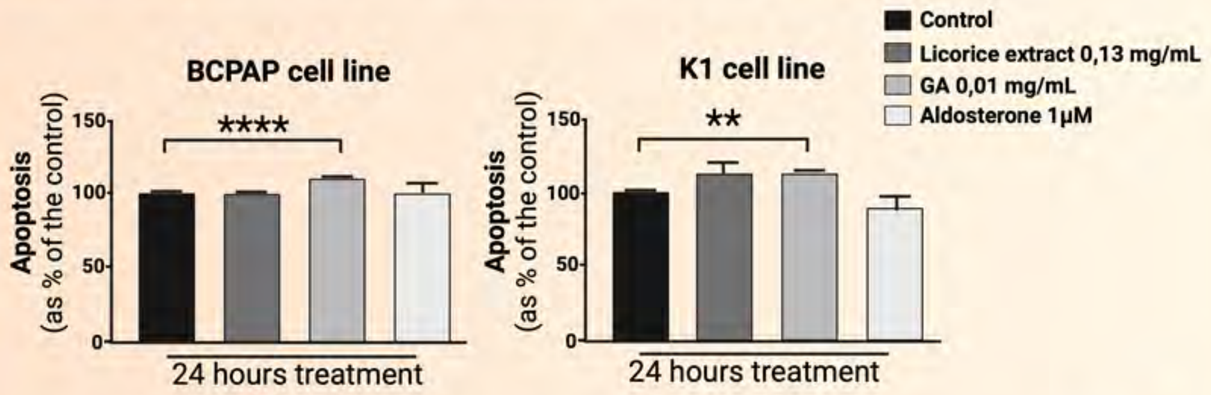


- viability

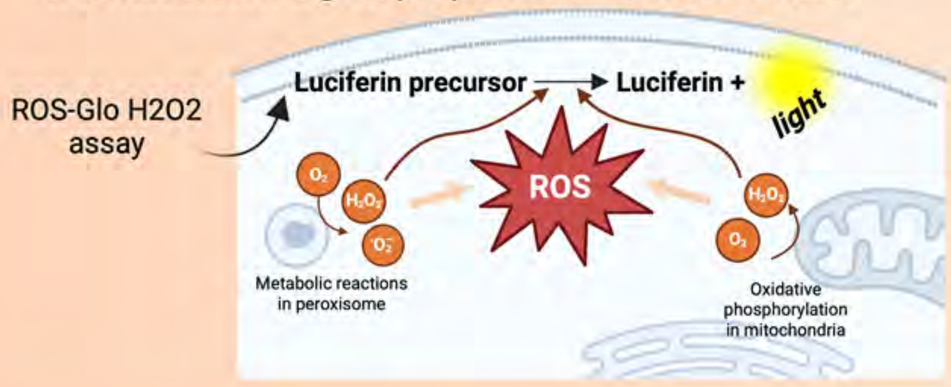
preliminary data

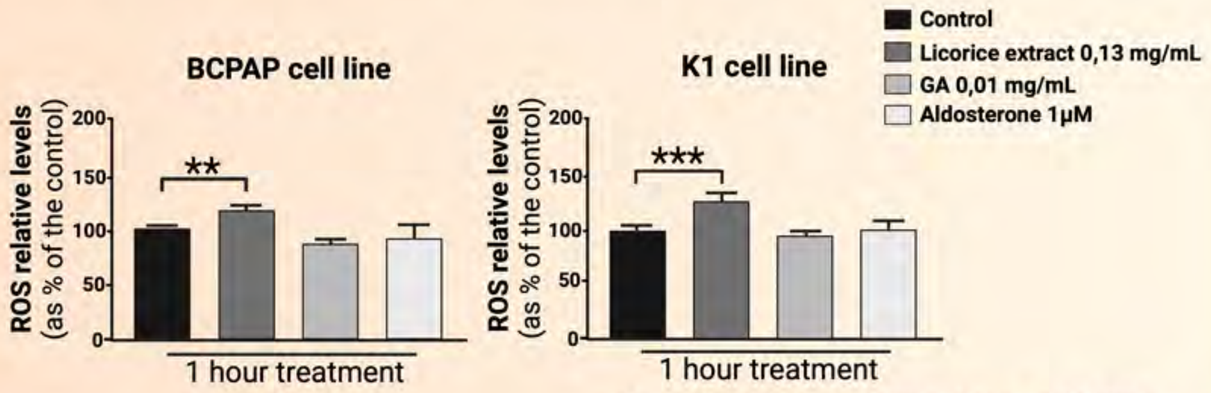
- Licorice extract and GA induce *in vitro* apoptosis through caspase dependent recruitment.
- The Caspase-Glo 3/7 Assay is a luminescent test that measures caspase-3 and -7 activation for detecting apoptosis.





- Licorice extract increases oxidative stress in both cell lines. The ROS-Glo H2O2 assay detected reactive oxygen species (ROS) via a luminescent signal proportional to H2O2 levels.





**=p<0.01; ***= p<0.001 ; ****= p<0.0001.

CONCLUSION / FUTURE PROSPECTIVE:

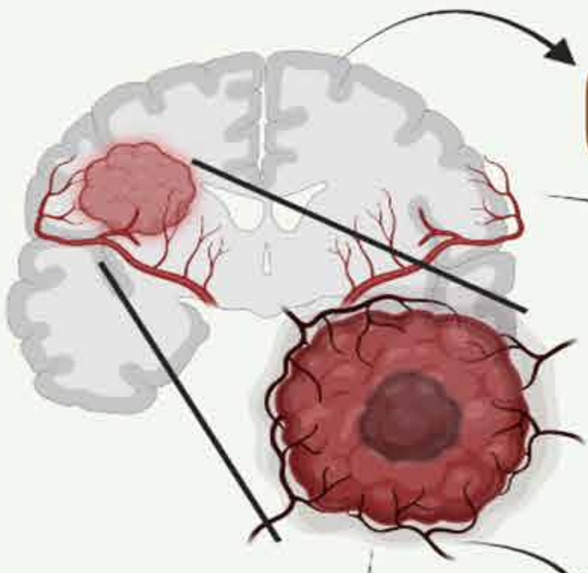
- LICORICE EXTRACT AND GA DISTINCTLY ESCALATED APOPTOSIS, WHILE LICORICE EXTRACT INCREASED INTRACELLULAR OXIDATIVE STRESS LEVELS IN BOTH PTC CELL CULTURES.
- STUDY OF THE POTENTIAL IMPLICATIONS OF THE AGONIST EFFECT ON ER AND MR IN PTC CELL LINE MODELS.
- VALIDATION OF THE INFLAMMATORY SPECTRUM PRODUCED BY TUMOR LINES AND INVESTIGATION OF THE EFFECTS ON CYTOKINE AND CHEMOKINE PRODUCTION FOLLOWING THE ADMINISTRATION OF GA AND LICORICE EXTRACT.

EDOARDO RUGGERI
Endocrinologia

Novel therapeutic strategy for glioblastoma through PAR2 inhibition

Mariagrazia Ruvoletto
Department of Medicine- University of Padua

Glioblastoma

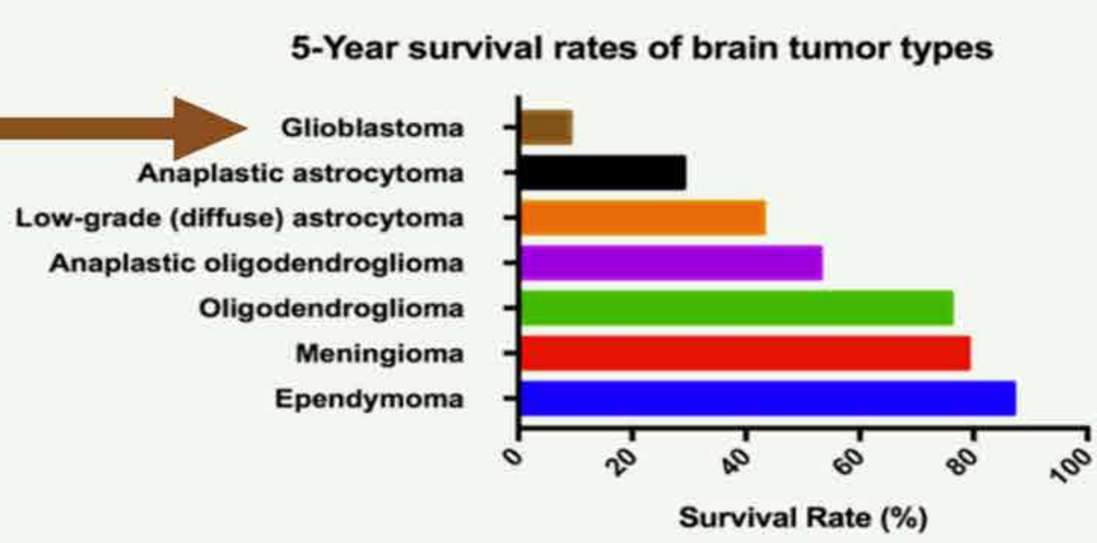


Low Overall Survival

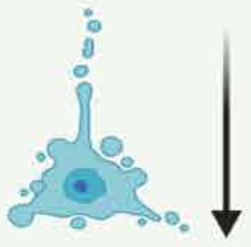
High recurrence

Lack of effeective therapy

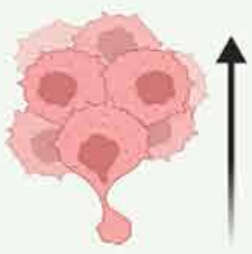
Resistance to chemotherapy



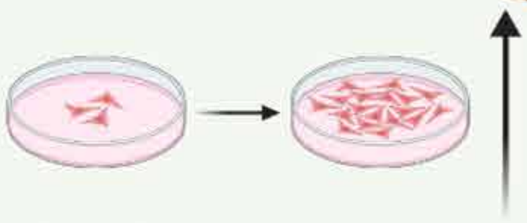
High PAR2 expression



Apoptosis



Migration Invasion

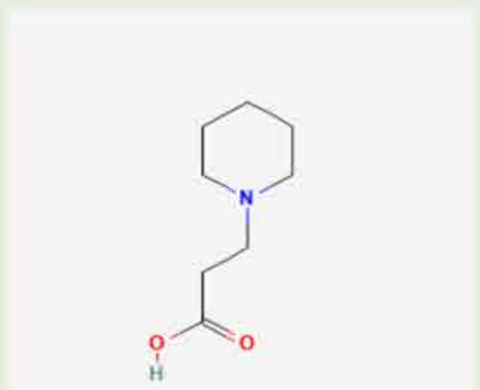


Proliferation

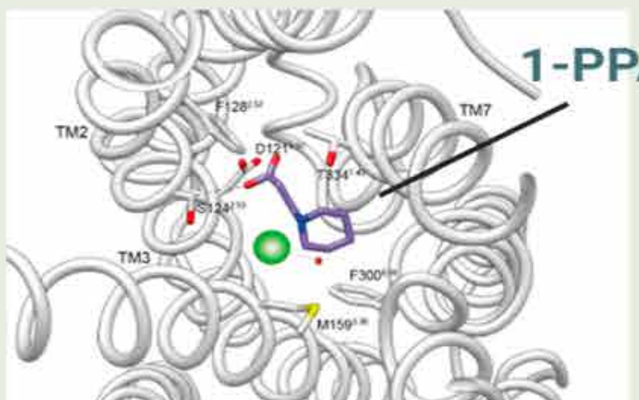
Mechanism of PAR2 inhibition

PAR2

+



1-Piperidine Propionic Acid (1-PPA)



PAR2-1-PPA Complex

Proliferation

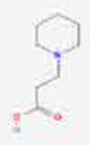
Apoptosis

Migration invasion

Glioblastoma cell lines

+

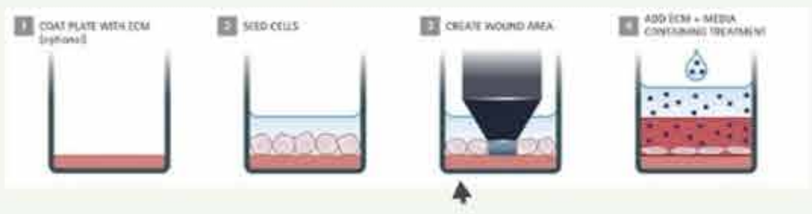
1-PPA



Procedure

Incucyte S3

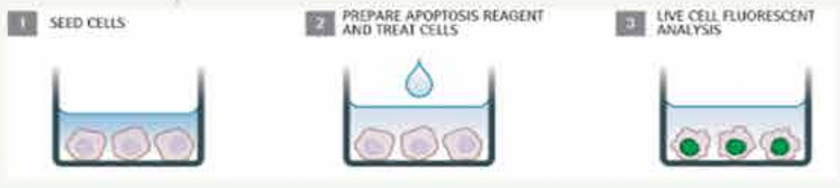
by Scratch wound cell migration and invasion assay



?

apoptosis assay by Fluorescent detection of caspase3/7

?



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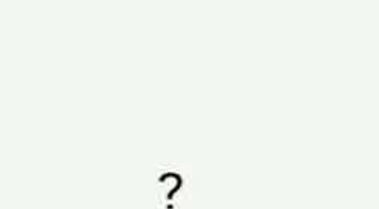


?

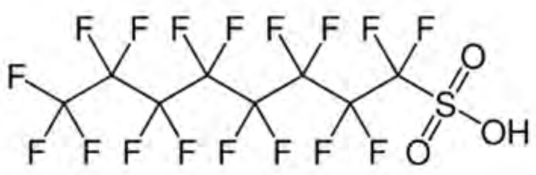
by Label free proliferation assay

MARIAGRAZIA RUVOLETTO
Clinica Medica 5

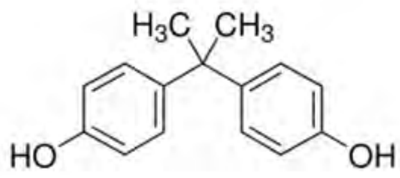
Developmental and Reproductive Effects of Endocrine Disruptors: role of single and combined subtoxic dosage

ENDOCRINE DISRUPTORS

PFOS
(3 ug/kg)



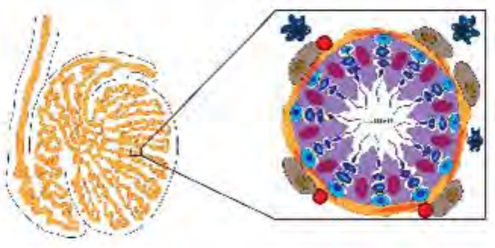
BPA
(4 ug/kg)



Experimental group:

- Group I – control (no EDs)
- Group II – BPA exposure
- Group III – PFOS exposure
- Group IV – BPA/PFOS mixture exposure

In vitro - preliminary results

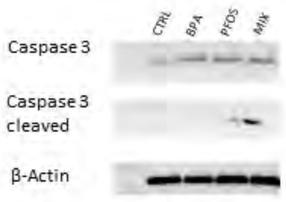


Sertoli cell

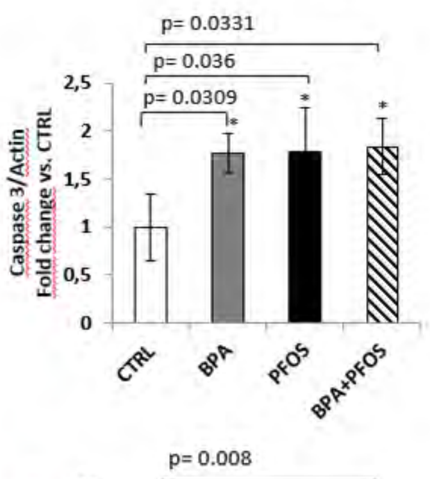
Sertoli pre-pubertal murine cell line (TM4)

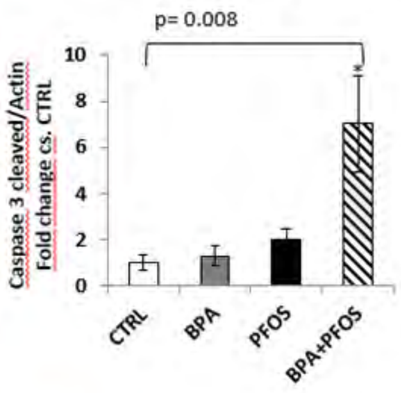
24 h exposure

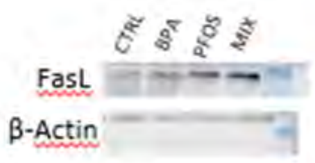
Apoptotic markers

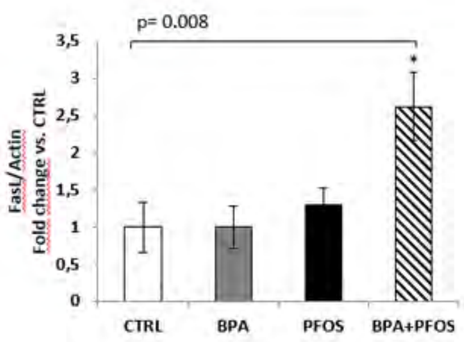


NF-kB NUCLEAR PROTEIN









In vivo - study design

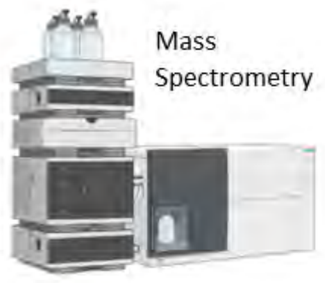
3 sets of tretments

GD 14.5 — PND 2 — PND 14

4 time point of sacrifice

GD 14.5 — PND 2 — PND 14 — PND 90

BPA and PFOS quantification in organs and blood samples



Mass Spectrometry

RT-PCR

JESS – automated Western blot system

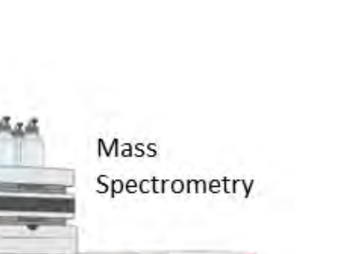
1° Testis

Analysis of genes and proteins involved in the hormonal and spermatogenetic function of the testis

2° Testis

Organ inclusion

IH/IF analysis



IVA SABOVIC
Andrologia e Medicina della Riproduzione

VI° CONVEGNO del DIMED
Padova, 15 novembre 2024

EVALUATION OF A NEW SOMATOSTATIN RECEPTOR ANALOG IN MODULATING HORMONE SECRETION AND CELL PROLIFERATION

E. Santillo[1], S. Avallone[2], D. Regazzo[1], P. Simioni[1] and M. Barbot[1]
1 – Department of Medicine – DIMED, Padova, Italy
2 – Department of Biology – DIBIO, Padova, Italy

PRELIMINARY STUDIES

Surgery of ACTH- and GH- pituitary adenomas

→ Primary cell culture

→

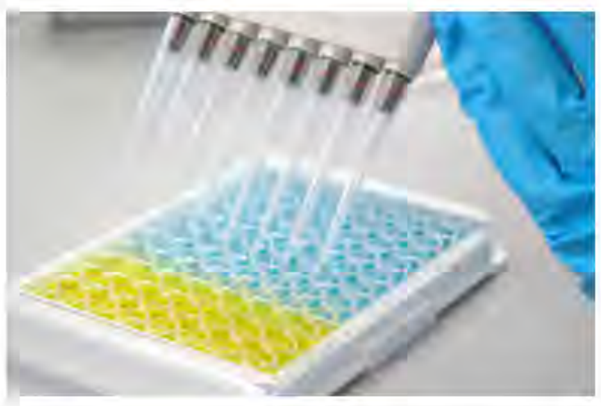
Test ELISA

GH and ACTH secretion

NOW

Cell line culture

AtT-20 GH3



↓ Hormone's secretion

↓ Proliferation and apoptosys

NEXT STEP

Cell line culture → RNA extraction → DNAse treatment and reverse transcription → qPCR

ERICA SANTILLO
Endocrinologia

VI° CONVEGNO del DIMED
Padova, 15 novembre 2024

THE TRANSITION CLINIC IN RHEUMATOLOGY: OUTCOMES OF JUVENILE IDIOPATHIC ARTHRITIS IN ADULTHOOD

Laura Scagnellato, Alessandra Meneghel, Giacomo Cozzi, Mariagrazia Lorenzin, Francesco Zulian, Paola Galozzi, Chiara Giraudo, Andrea Doria, Roberta Ramonda

JUVENILE IDIOPATHIC ARTHRITIS (JIA) IN PILLS

- ❑ The most common chronic inflammatory musculoskeletal disease in children.
- ❑ It can present as systemic vs non-systemic disease
  - **Systemic disease** = alter ego of Adult Onset Still Disease
  - Non – systemic can be **oligoarticular ANA positive/negative, polyarticular, rheumatoid-factor positive, psoriatic, enthesitis-related** = very heterogeneous and possibly erosive
- ❑ Can be complicated by **chronic anterior uveitis** that is asymptomatic in early phases and leads to blindness unless untreated.
- ❑ Age of onset of the most common form (oligoJIA): 2-6 years old = **long disease duration by the age of 18**
  - **Best case: no damage but life-long treatment**
  - **Worst case: joint and eye damage + life long treatment**
- ❑ Non – systemic disease can be **treated** with systemic or local glucocorticoids, conventional DMARDs (methotrexate) and biologic DMARDs (adalimumab, etanercept, golimumab, tocilizumab, abatacept, and more recently in some cases with secukinumab and baricitinib). But **sometimes is not enough (intolerance, resistance)**

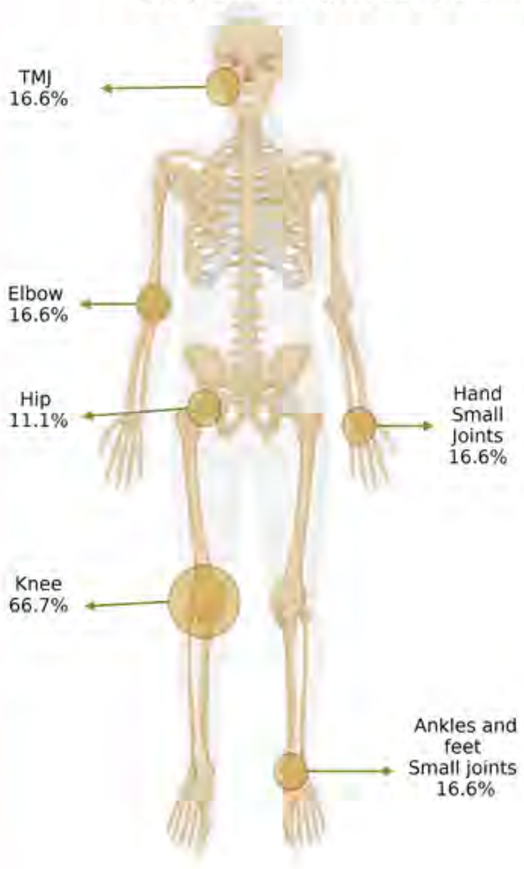
WHAT HAVE WE BEEN DOING?

➢ **NEW MONTHLY TRANSITION CLINIC SINCE October 2023** for handing over patients non systemic JIA patients aged 18 or older.

- ✓ **Each** patient is discussed previously (**holistic approach**: *family environment, treatment adherence, comorbidities*) → easier for clinicians
- ✓ Dedicated nurse to aid the entrance in the adults' world → the patient has a **clear and higher quality healthcare pathway** ahead

➢ **ASSESSMENT OF THE RISK OF RELAPSE AFTER TRANSITION**

- ✓ 50 patients, most patients are oJIA



- ✓ A **third** of young adults with **o/p JIA** suffer an arthritis **relapse within the first three years after transitioning** to adult care
- ✓ The major risk factor for disease relapse within this timeframe was **MONOARTHRITIS**

Link to the paper!

➢ **«HOLISTIC» ASSESSMENT OF DAMAGE AT TRANSITION**

27 patients recruited Oct 2023 – June 2024

Prospective PROs 0-12 months

27 patients
Pain, impairment&limitations
Lifestyle: sports; comorbidities
Education and career

Baseline MRI of the most involved joint

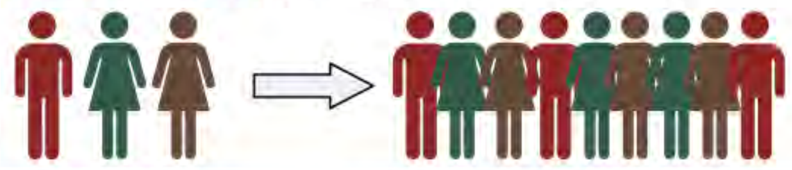
22 patients performed MRI
- 14 knee, 4 hand, 3 ankle, 2 elbow
  - ❖ 55% bone oedema
  - ❖ 55% effusion
  - ❖ 44% cartilage damage

Gynecological evaluation

15 patients
- ❑ Transvaginal US examination; PCOS 31%
- ❑ Ovarian reserve: anti-mullerian hormone (AMH) dosage

WHAT IS IN OUR PLANS?

➢ **COHORT ENLARGMENT** for higher stastical power



ONGOING RECRUITMENT

➢ **ASSESSMENT OF THE RISK OF RELAPSE AFTER bDMARDs TAPERING**

- ✓ Quantification of absolute risk
- ✓ Identification of clinical risk factors
- ✓ Identification of musculoskeletal ultrasound risk factors

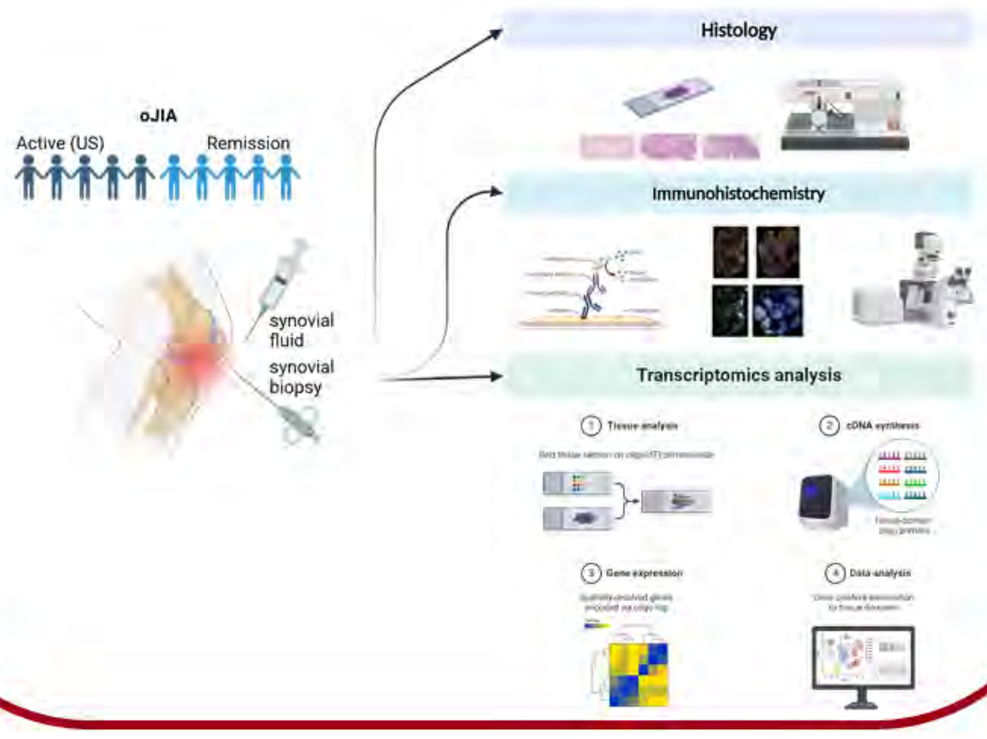
ONGOING RECRUITMENT

➢ **DEEP IMMUNOPHENOTYPING**

- ✓ Assessment of the link between an **immunological genetic panel** and disease aggressiveness (=MRI damage)

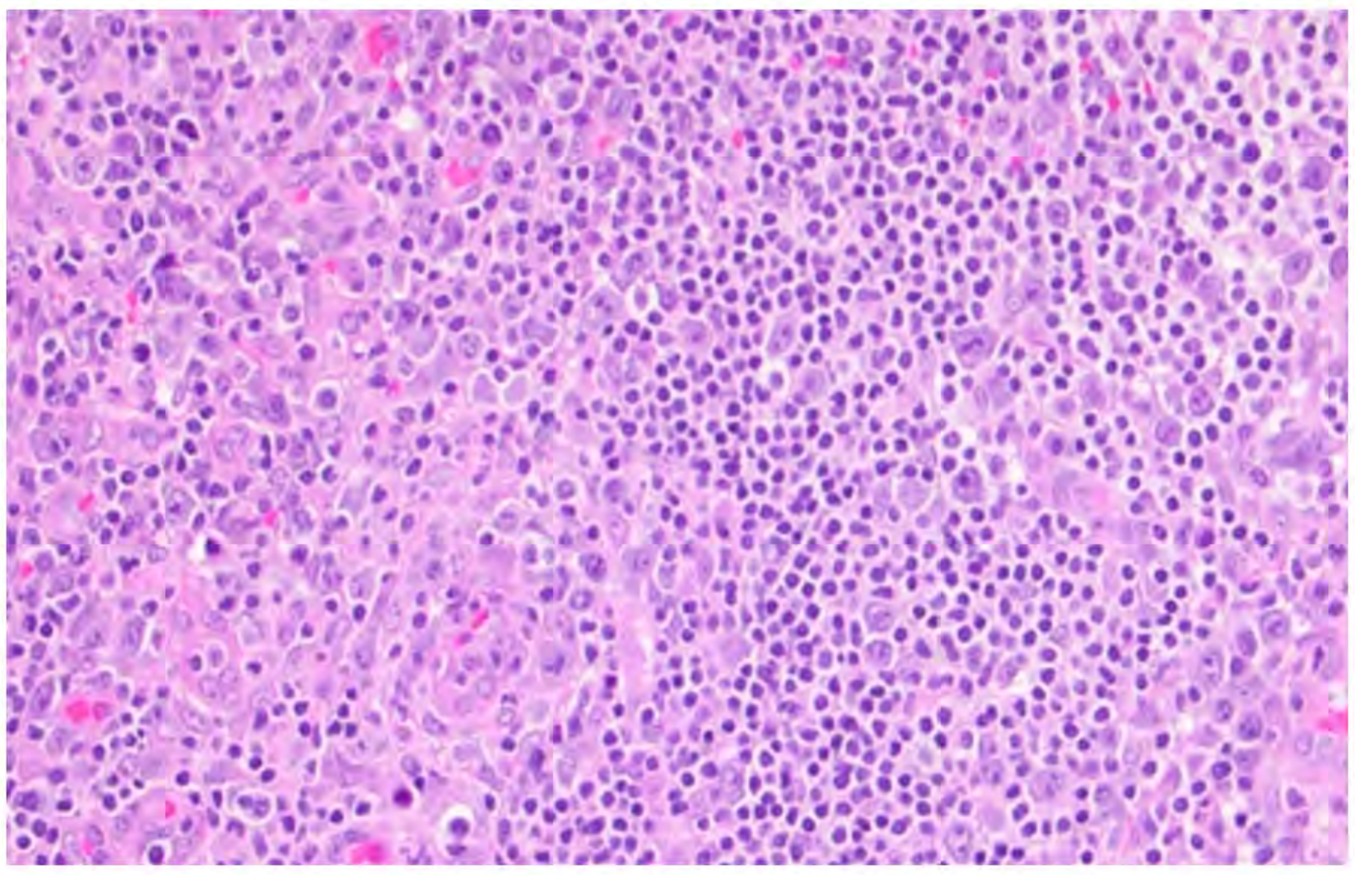
ONGOING SAMPLE COLLECTION

- ✓ **Tissue transcriptomics**
  - Synovial biopsy at disease relapse (project n°=5) & synovial fluid (SF) collection
  - Synovial biopsy after long term remission prior to therapeutic tapering (n°=5) & SF collection
    - ❖ Role of **resident macrophages**



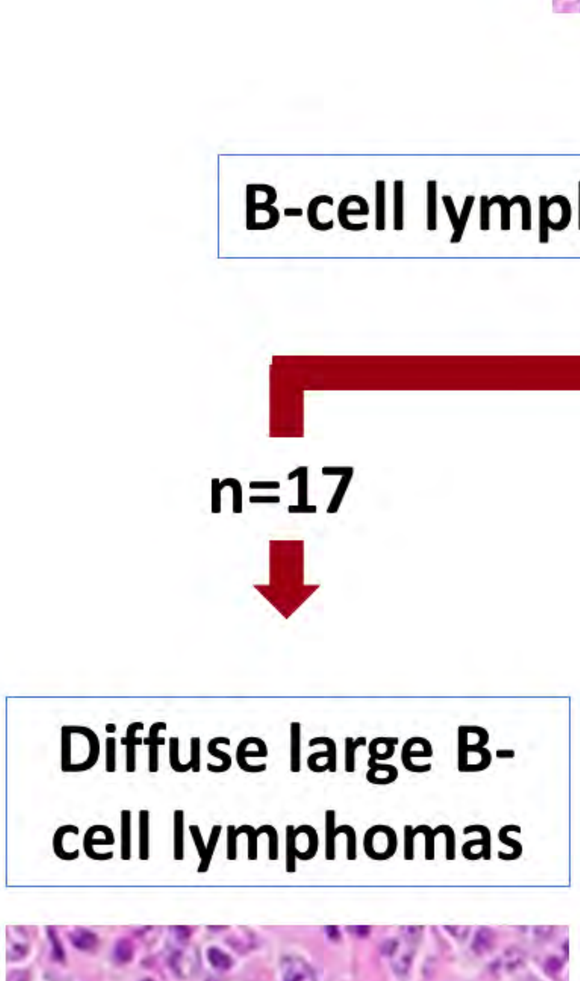
LAURA SCAGNELLATO
Reumatologia

Angioimmunoblastic T-cell lymphoma (AITL)

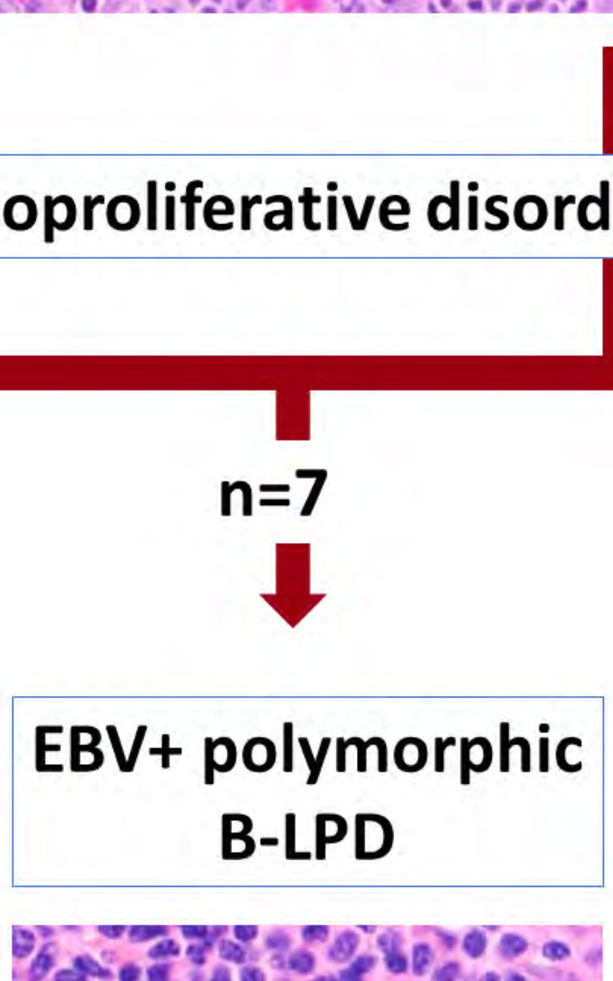


B-cell lymphoproliferative disorders (B-LPD) arising in AITL (n=32)

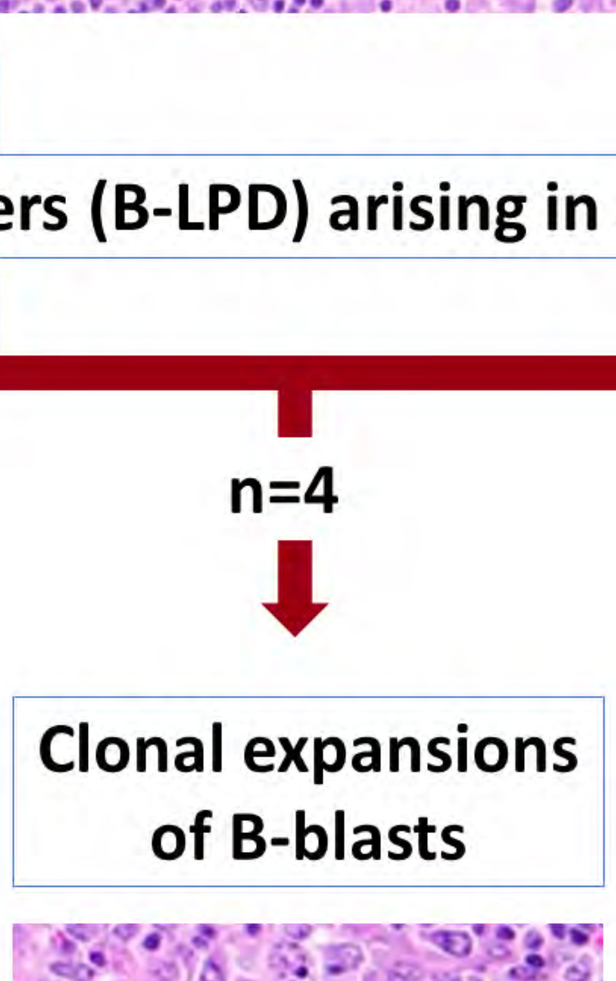
n=17 — Diffuse large B-cell lymphomas



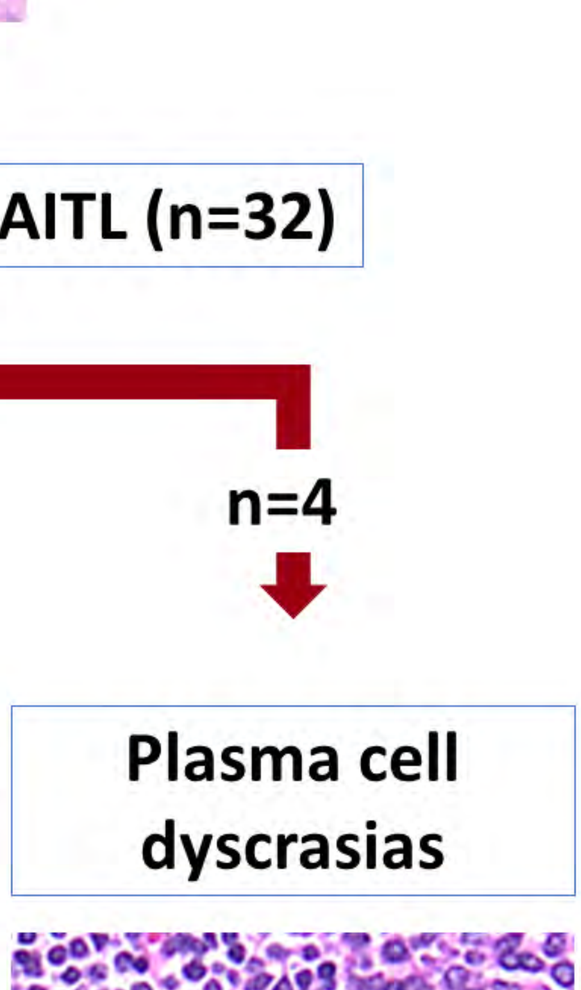
n=7 — EBV+ polymorphic B-LPD



n=4 — Clonal expansions of B-blasts



n=4 — Plasma cell dyscrasias



Mutational landscape of B-LPDs arising in AITL (n=12)

Diffuse large B-cell lymphomas	EBV+ polymorphic B-LPD	Clonal expansions of B-blasts	Plasma cell dyscrasias
TET2= 4/7 RHOA= 1/7	TET2= 1/2 RHOA= 1/2	TET2= 1/1 IDH1= 1/1	TET2= 1/2 DNMT3A= 1/2 IDH2= 1/2

FEDERICO SCARMOZZINO
Anatomia Patologica

VI° CONVEGNO del DIMED
Padova, 15 novembre 2024

Impact of Clonal Hematopoiesis of Indeterminate Potential (CHIP) in patients who received Autologous Stem Cell Transplantation

Dott. A. Serafin
Medico Specializzando U.O.C. Ematologia

BACKGROUND

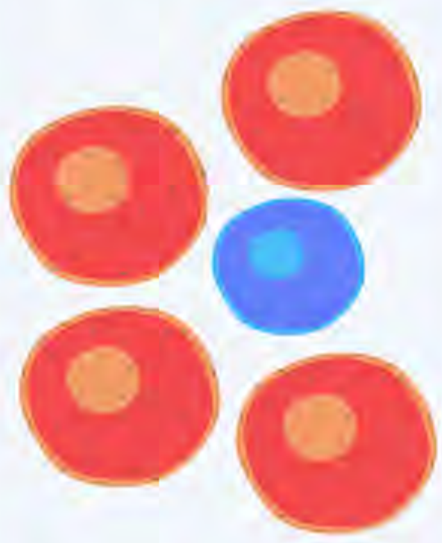
Healthy HSC

CHIP HSC

Driver mutation
(DNMT3A, TET2, JAK2, ASXL1)

Ageing
Chronic inflammation

CHIP progression



Chemotherapy
Radiation
Lifestyle dieases
Autologous HSCT

Myeloid neoplasm (MN, i.e. Acute Myeloid Leukemia or Myelodisplastic syndrome) and occasionally lymphoid neoplasms

Increased risk for cardiovascular diseases (CVD)

TET2 and JAK2 CHIP are associated with chronic liver disease (CLD)

Increased VTE in JAK2-CHIP

Development of solid cancer and immune disorders

METHODS and OBJECTIVES

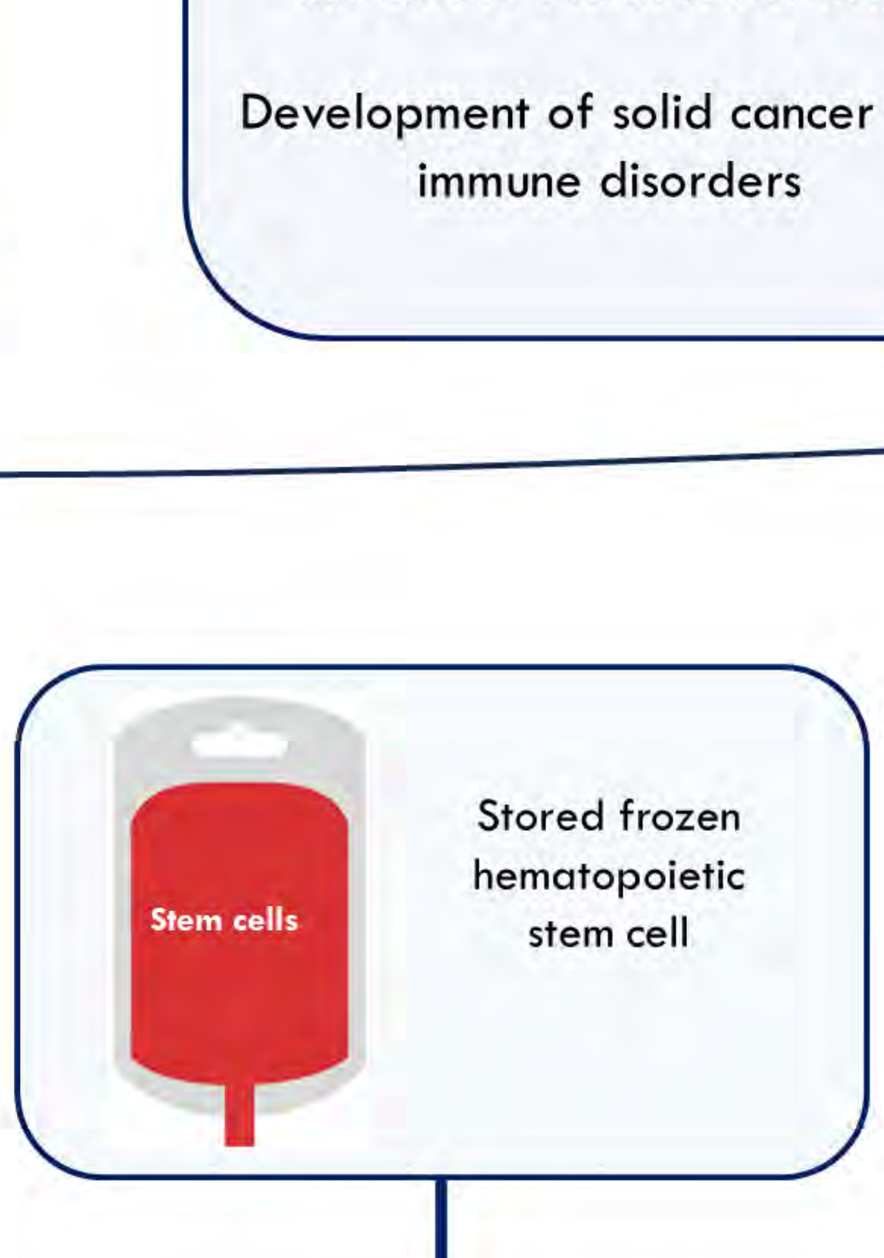
RETROSPECTIVE STUDY

Primary end-points:

1. Cumulative incidence of CHIP at baseline.

Secondary end-point:

1. Cumulative incidence of secondary Myeloid Neoplasms (MN) after autologous stem cell transplantation;
2. Clinical risk factors for therapy-related CHIP pre-HSCT and for sMN post-HSCT;
3. Cumulative incidence of CHIP-related CVD, CLD, VTE;
4. Death-rate due to haematological and non-haematological causes;
5. Correlation of chip with markers of inflammation;
6. Correlation between CHIP and bone marrow lymphocyte subsets.



References:

[1] Awada, H., Gurnari, C., Visconte, V. *et al.* Clonal hematopoiesis–derived therapy-related myeloid neoplasms after autologous hematopoietic stem cell transplant for lymphoid and non-lymphoid disorders. *Leukemia* 38, 1266–1274 (2024). https://doi.org/10.1038/s41375-024-02258-y

[2] Jaiswal, S., Libby, P. Clonal haematopoiesis: connecting ageing and inflammation in cardiovascular disease. *Nat Rev Cardiol* 17, 137–144 (2020). https://doi.org/10.1038/s41569-019-0247-5

ANDREA SERAFIN
Ematologia

WHAT WE KNOW

Hepatic steatosis ≥5%

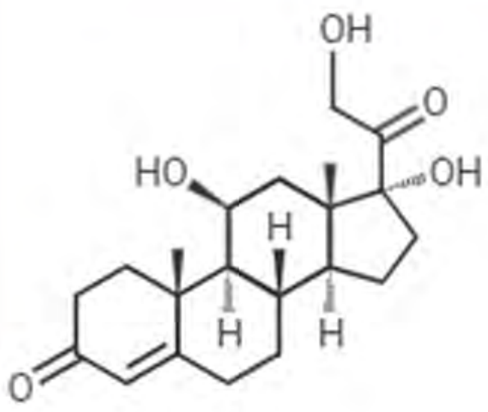
Type 2 diabetes

Overweight /obesity

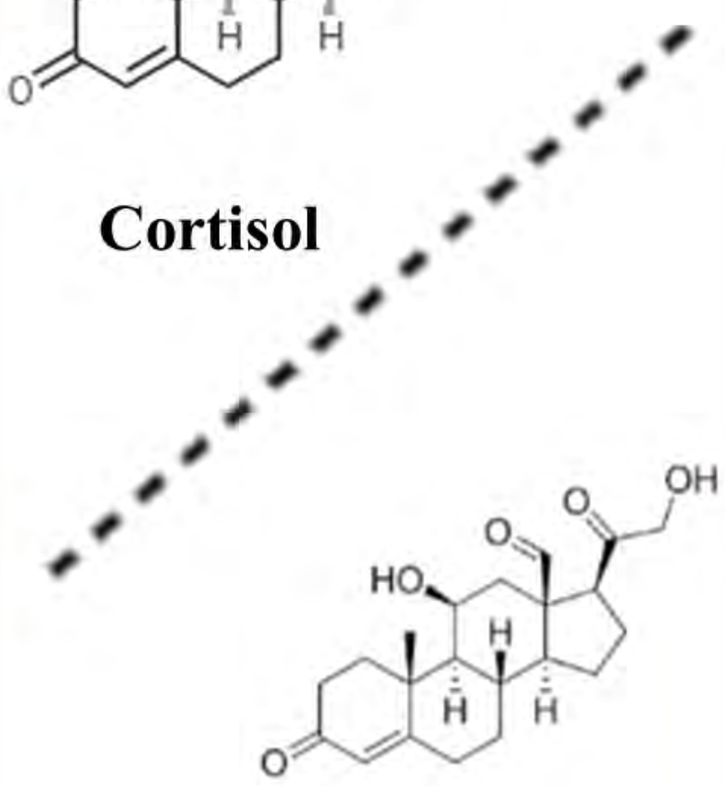
Metabolic complications

Metabolic
Associated
Fatty
Liver
Disease

WHAT WE DISCOVERED



Cortisol



Aldosterone

Hepatic steatosis ≥5%

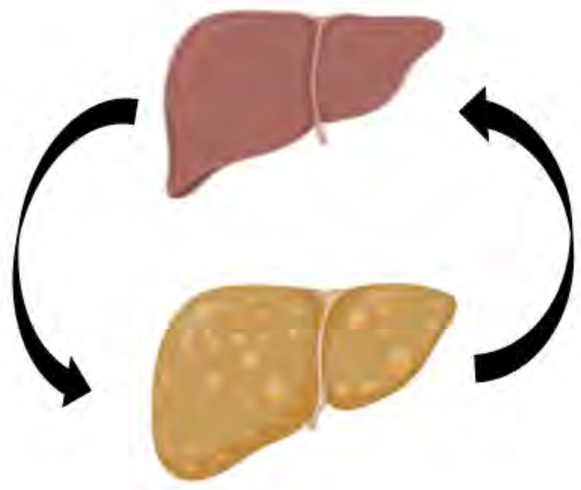
Type 2 diabetes

Overweight /obesity

Aldosterone /cortisol excess

Endocrine
Associated
Fatty
Liver
Disease

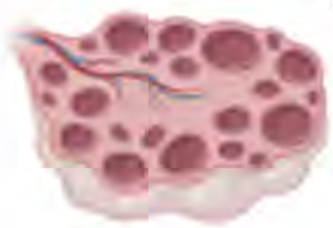
THE FUTURE of EAFLD



Pheochromocytoma

catecholamines

Polycystic
Ovary
Syndrome



aldosterone/ androgens

Diabetes
mellitus

Obesity

cortisol

IRENE TIZIANEL
Endocrinologia

VI° CONVEGNO del DIMED
Padova, 15 novembre 2024

Reticulated platelet pro-coagulant role in patients with cirrhosis

S. Toffanin[1], C.M. Radu[1], E. Campello[1], C. Bulato[1], G. Nuozzi[1], A. Zanetto[2], M. Senzolo[2], P. Burra[2], P. Simioni[1].

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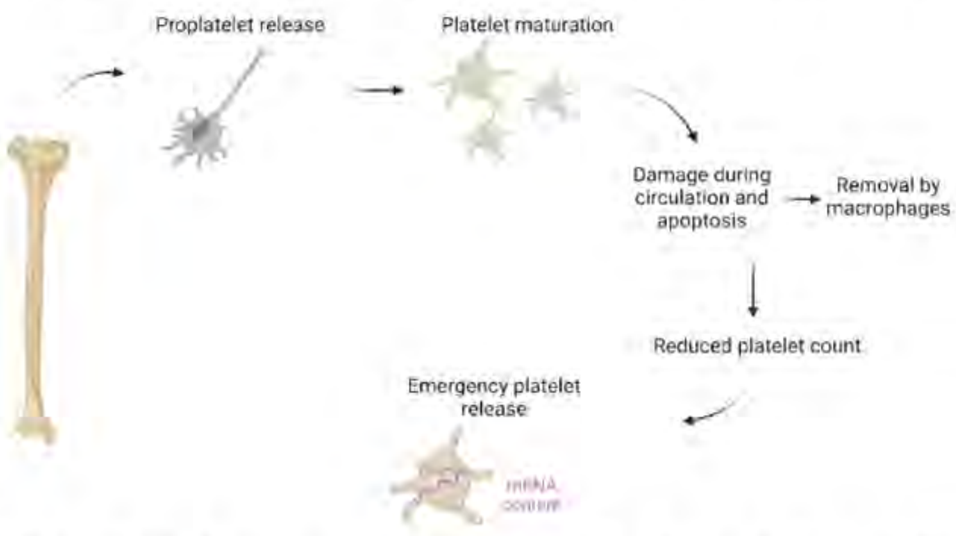
BACKGROUND AND AIM

Cirrhosis is a chronic liver disease characterized by:

- a reduced synthesis of hepatic-dependent coagulation factors;
- a decreased platelet count.

In patients without liver disease, these alterations lead to a pro-haemorrhagic state, whereas in those with cirrhosis thrombotic events may occur.

Reticulated platelets (RePLTs) are bigger and more reactive emergency circulating platelets, characterized by an high content of RNA, higher levels of surface activation markers and directly released by megakaryocytes to contrast peripheral platelet destruction, which is a major mechanisms responsible for thrombocytopenia in cirrhosis.



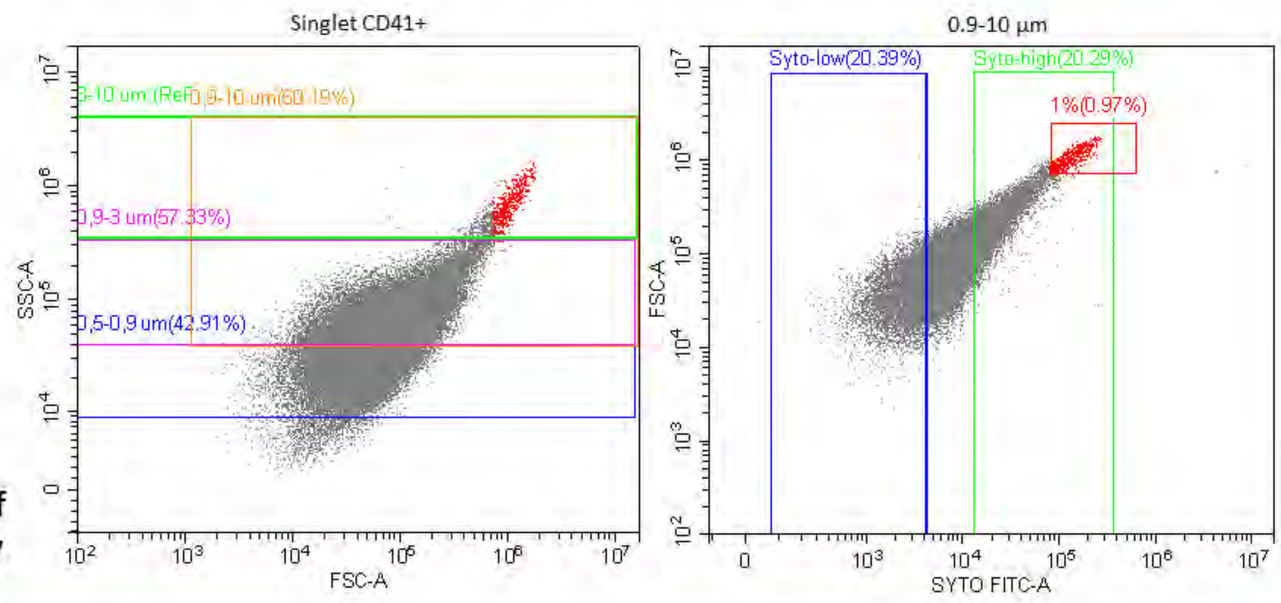
The aim of this study focuses on improving the knowledge of the role of RePLTs in the hemostatic balance in patients with liver cirrhosis by comparing surface antigenic properties with CytoFLEX SRT.

METHODS

Platelet rich plasma from cirrhotic patients of different severity (n=25) and healthy subjects (n=14) was collected for CytoFLEX-SRT analysis. Anti-CD41 and SYTO-13 positivity defines RePLTs between 3-10 µm beads diameter.

CytoFLEX SRT

Based on SYTO-13 positivity, three populations were defined: SYTO-13-low, mature platelets; SYTO-13-high, RePLTS and 1% of the most SYTO-13-high positive population. Additionally, the expression of platelet activation markers, Annexin V (phosphatidylserine) and anti-CD62P (P-selectin), was evaluated both with flow cytometer and TCS SP8 confocal microscopy.



RESULTS

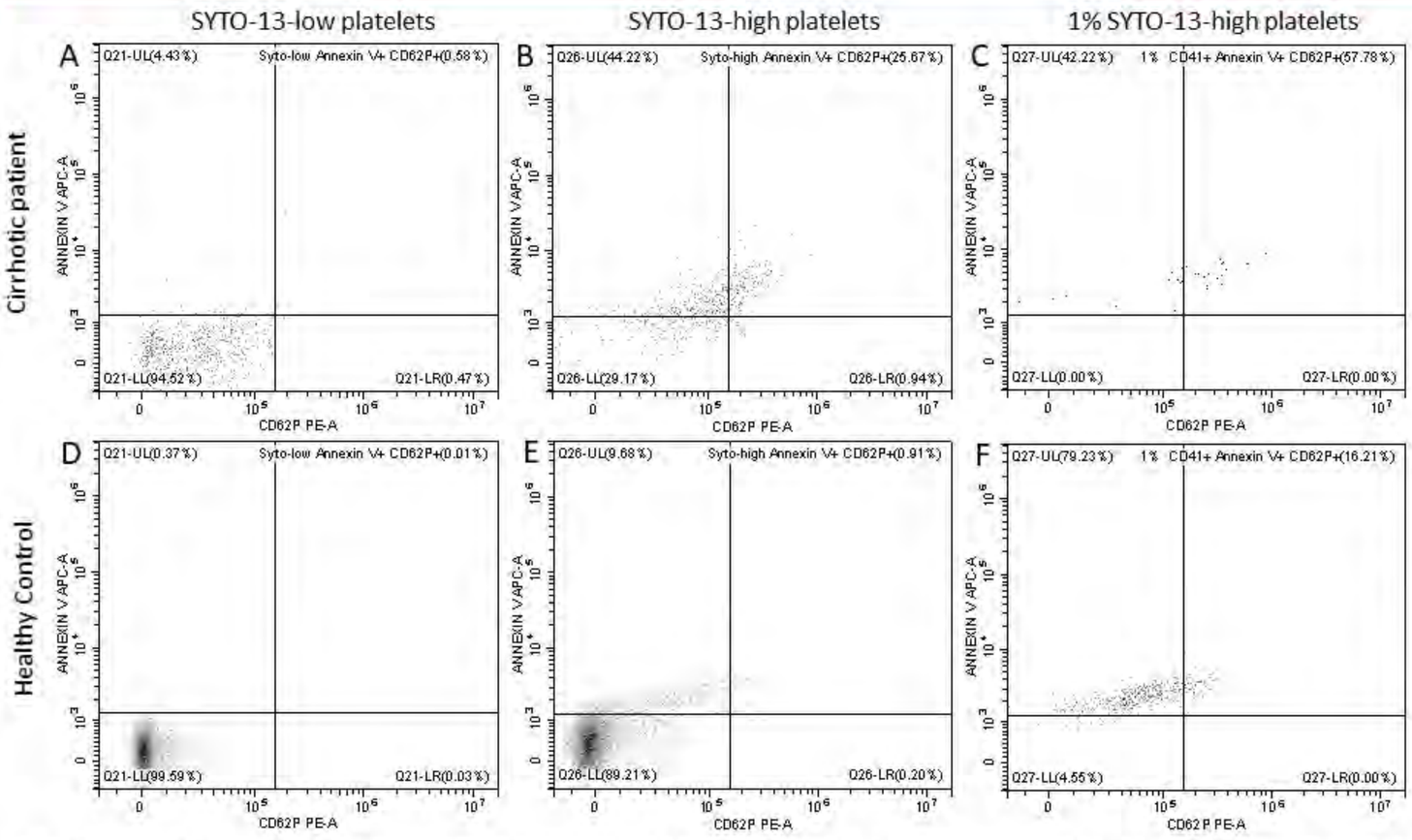


Fig1. Examples of cytograms of the percentage of Annexin V and CD62P expression in a cirrhotic patient (A-C) and in an healthy subject (D-F) comparing SYTO-13-low platelets (A,D), SYTO-13-high platelets (B,E) and 1% of the most SYTO-13-high positive (C,F).

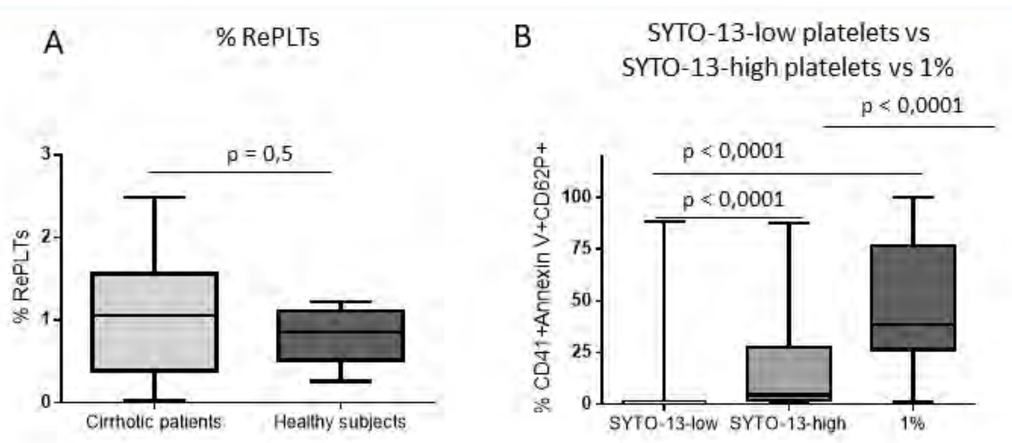


Fig2. RePLTs analysis in cirrhotic patients vs healthy subjects. (A) Comparison of RePLT percentage between cirrhotic patients and healthy controls. (B) Comparison of marker expression (Annexin V and CD62P) between SYTO-13-low platelets, SYTO-13-high platelets and the subpopulation 1% of SYTO-13-high platelets.

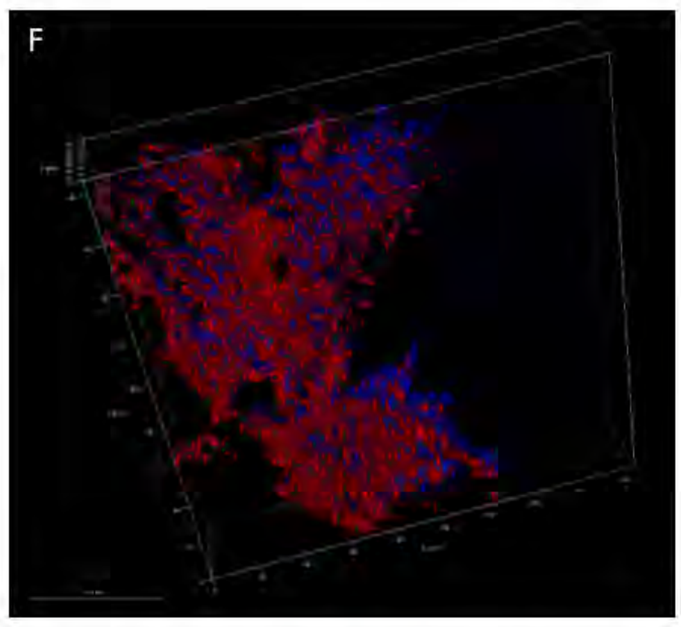


Fig3. Images were acquired with TCS SP8 confocal microscope, 63x/1.4 oil immersion lens, using a DFC365FX camera and processed using the Leica Application Suite (LAS-AF) 3.1.1. software (Leica Microsystems). A) AO represents green fluorescence; B) P-Selectin, red fluorescence; C) Annexin V, blue fluorescence; D) Overlay of the three fluorescence; E) DIC image; F) 3D confocal image of a blank thrombus, CD41 is represented as blue fluorescence; CD62P, red fluorescence; SYTO-13, green fluorescence. Scale bar of (A-E)10 µM and (F) 50 µM.
Abbreviation: DIC: differential interference contrast; AO: Acridine orange, similar to SYTO-13.

CONCLUSIONS

- The number of RePLTs is higher in cirrhosis than in healthy controls;
- in both cirrhotic patients and healthy subjects, RePLTs expressed more platelet activation markers than mature platelets and RePLTs with the highest amount of RNA are the most activated;
- in cirrhosis, RePLTs are more activated than in healthy controls, suggesting a hyperactive state.

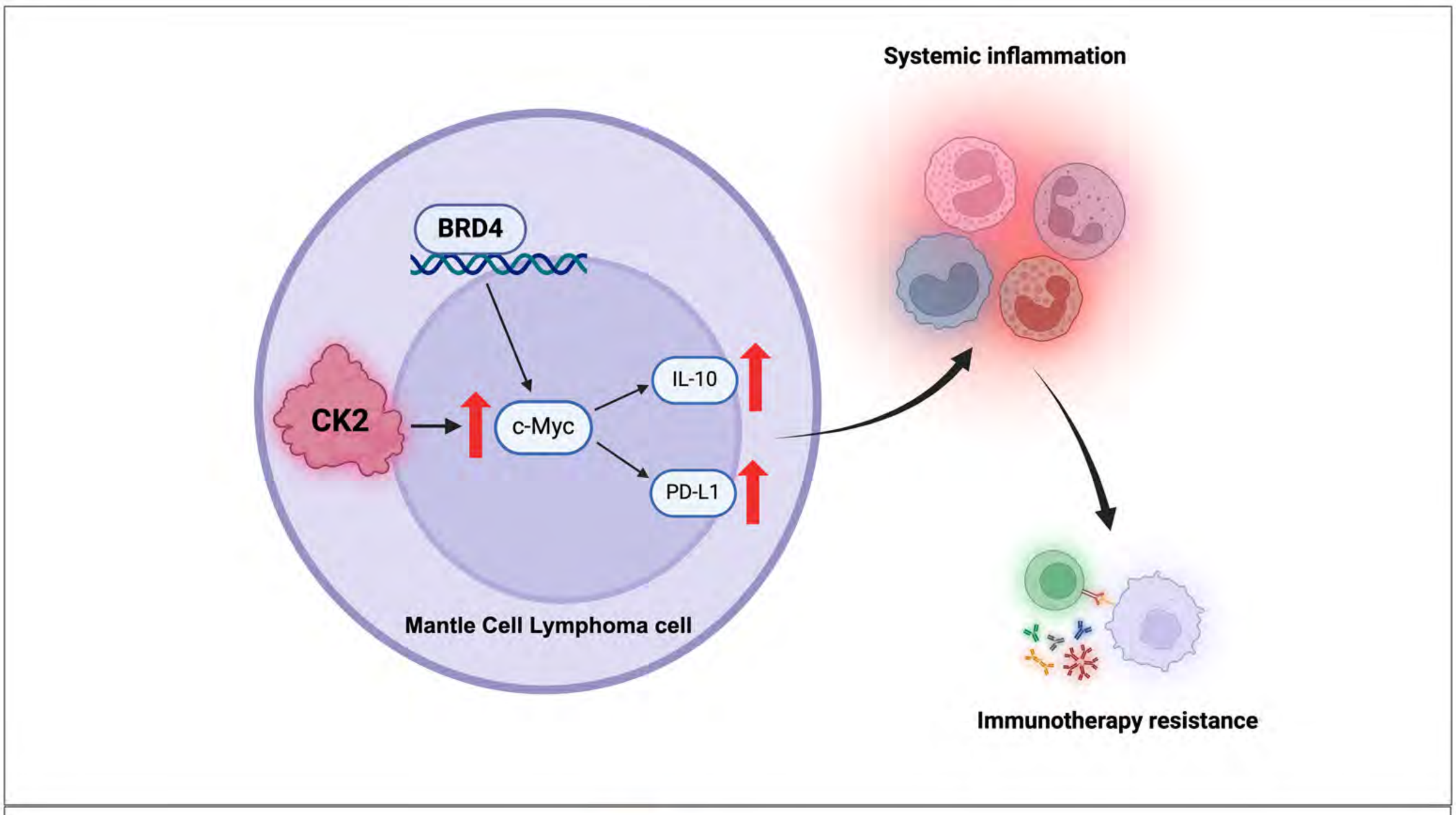
Finally, a better understanding of RePLTs properties may improve the knowledge of cirrhotic coagulopathy, leading to a better identification of patients at higher risk of thrombosis and disease progression.

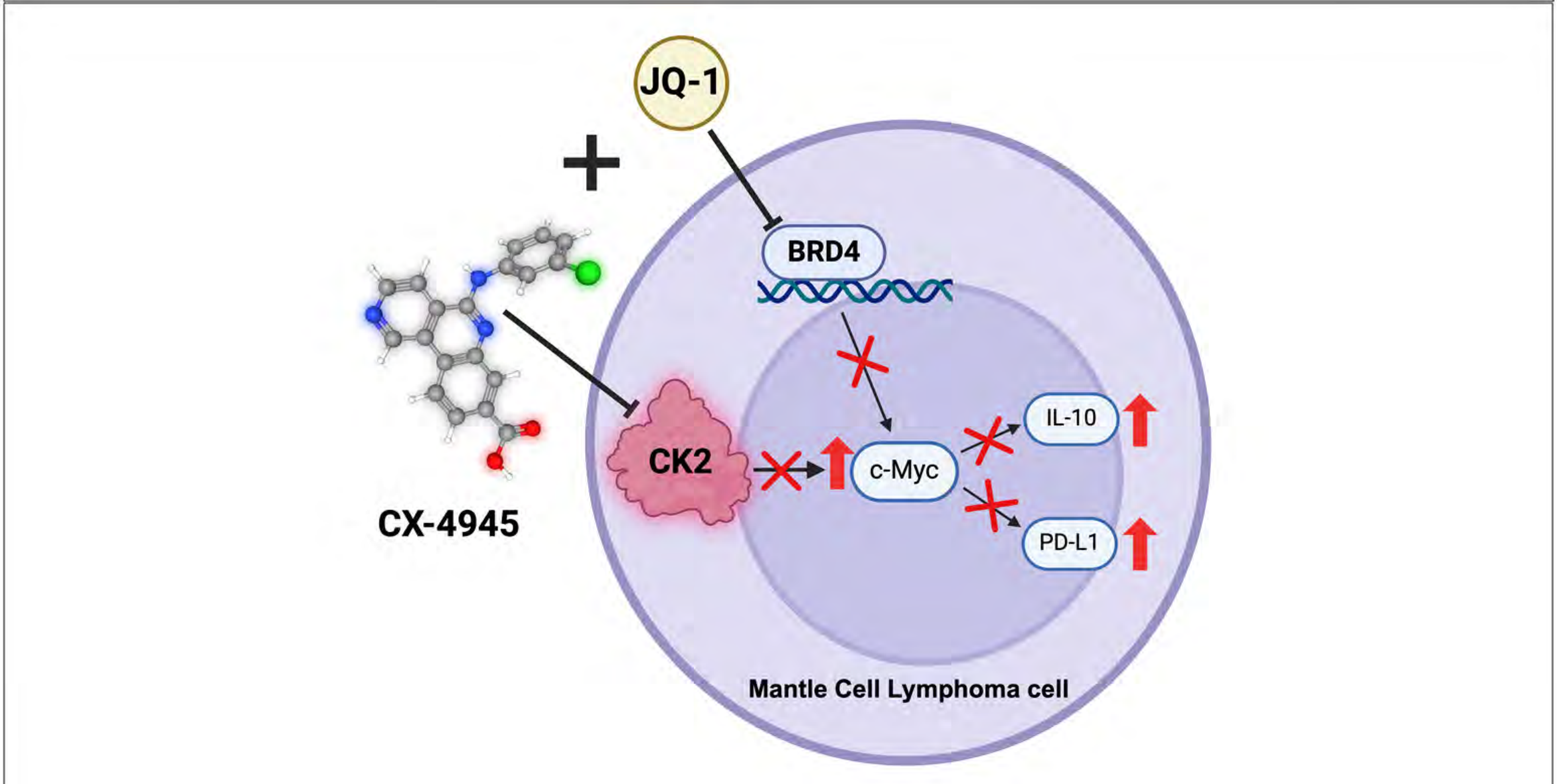
FUTURE DEVELOPMENT

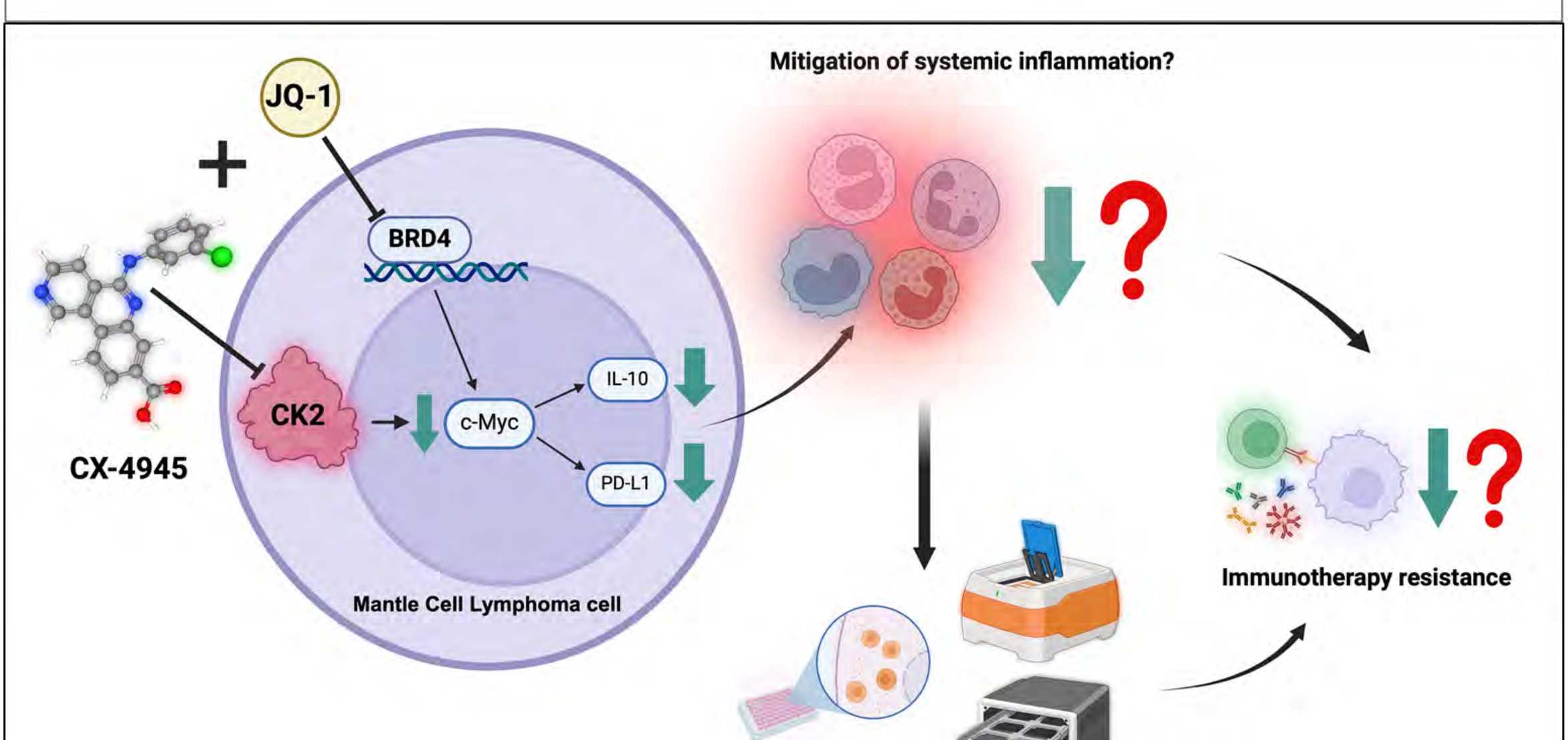
Fig5. Sorting between SYTO-13-low (mature platelets) and SYTO-13-high (RePLTs) in healthy subjects. (A) Examples of cytograms of platelets before sorting. (B) SYTO-13-low platelets and (C) SYTO-13-high platelets after sorting. Comparison of platelet activation marker expression (Annexin V and CD62P) between (D) SYTO-13-low platelets and (E) SYTO-13-high platelets after sorting following stimulation with thrombin receptor activating peptide (TRAP).

SERENA TOFFANIN
Clinica Medica 1: Malattie Trombotiche ed Emorragiche

Targeting PROTEIN KINASE CK2 and BET PROTEINS to Reduce Inflammation and Improve MCL Treatment Outcome







JUAIRIA UDDIN DEKHA
Ematologia

VI° CONVEGNO del DIMED
Padova, 15 novembre 2024

Tutor
Prof. Andrea Ermolao
Sports and Exercise Medicine Division
Department of Medicine
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Cardiovascular risk and hiking activity: do we need personalized guidance?

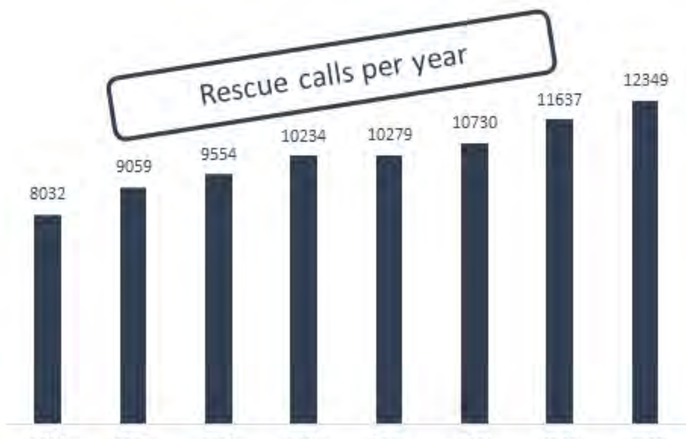
Marco Vecchiato, MD – PhD Candidate in Clinical and Experimental Sciences - Sports and Exercise Medicine Division, Department of Medicine

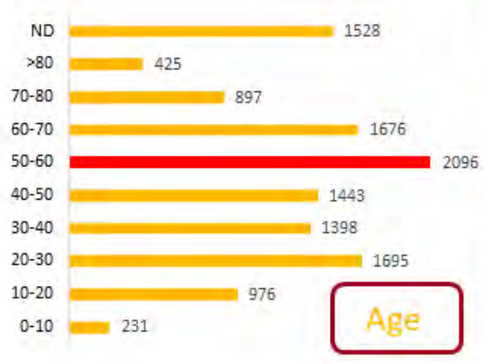
Introduction

Hiking is the most popular outdoor activity. It requires considerable physical effort and cardiorespiratory demands depending on trail characteristics as well as environmental shifts, including changes in temperature, humidity, and partial oxygen pressure.

Despite most hiking excursions are well tolerated and incident-free, this outdoor activity ranks first in requiring rescue operations. While most hiking accidents are non-fatal, sudden cardiac death represents the leading cause of death among males over 34 years of age during excursions.

Mountain signage and dedicated websites usually offer no information regarding the required physical effort, and when present, this is generic, not suitable for everyone.





*Data provided by CNSAS

Aim

The purpose of this study is to objectively investigate the required exercise intensity needed for hiking a trail classified as "easy" by mountain signage and to understand which individual factors and trail/environment characteristics may affect the associated cardiorespiratory and metabolic response.

Methods

A group of 72 participants (65% male; mean age 43.5 years) underwent a baseline outpatient maximal cardiopulmonary exercise test with subsequent standardised and monitored outdoor hiking on a trail labelled as "easy" by mountain signage.

OUTPATIENT TEST

Hospital setting

Sports and Exercise Medicine Division

Medical screening, CV risk score assessment and maximal CPET

SCORE2
VO_2 peak
VT1 and VT2
HR peak
VE peak

Within 1 week

OUTDOOR TEST

Hiking trail

Parco Regionale dei Colli Euganei

Monitored outdoor test hiking with portable gas analyzer

Hiking time
VO_2 hike
HR hike
VE hike
Exercise intensity

Location: Sentiero del Monte Grande - N° 14, Teolo (PD), Parco Regionale dei Colli Euganei

Starting and ending point

Halfway point

Survey points of perceived fatigue and saturation

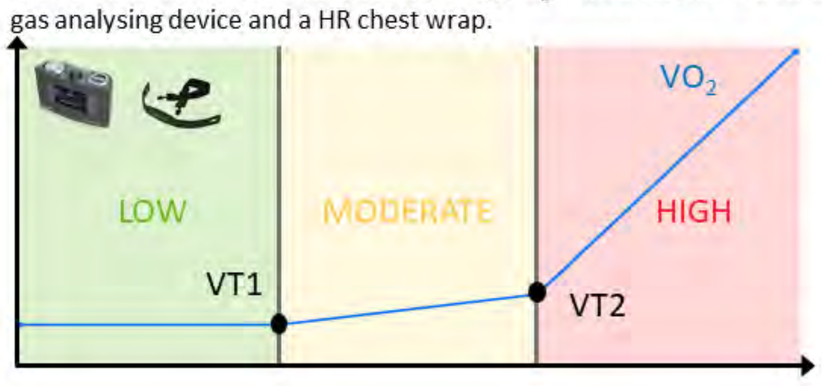
Length: 8.2 km

Height difference: 300 m

Suggested time: 120 min

Technical difficulty: easy

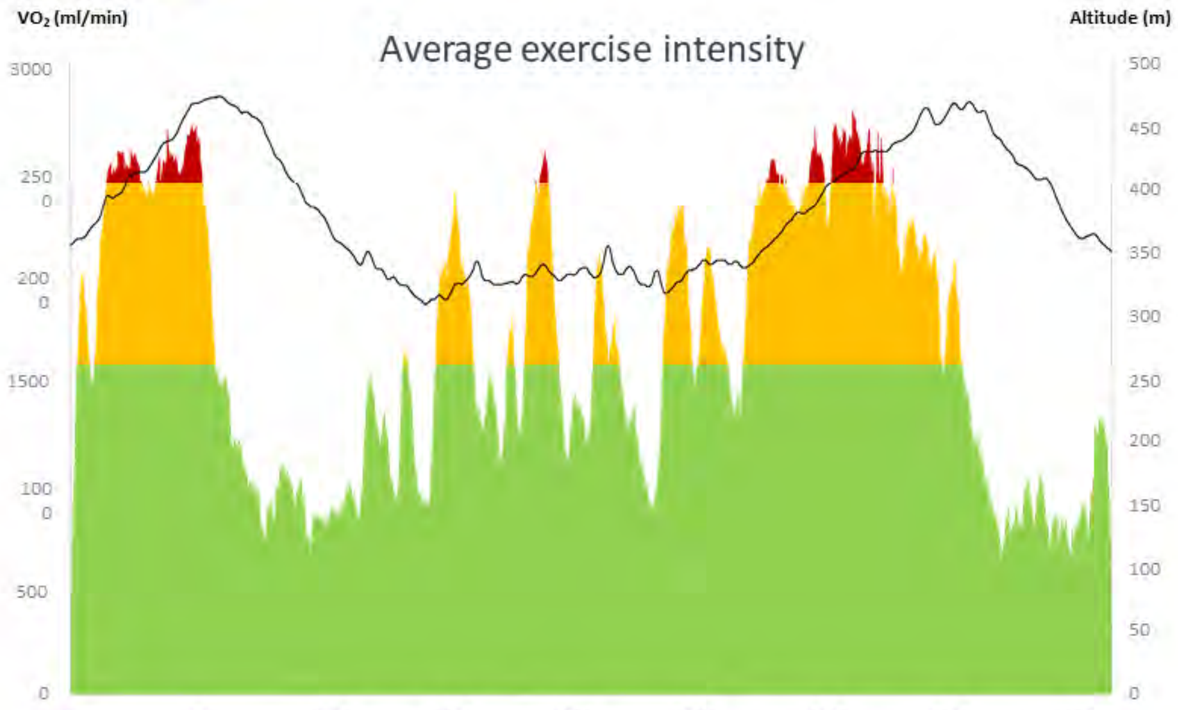
Exercise intensity was obtained comparing VO_2 measured with a wearable gas analysing device and a HR chest wrap.



Results

Participants were faster than expected (97 ± 11 min vs 120 min), with higher perceived exertion in participants with higher cardiovascular risk scores.

82% LOW
16% MODERATE
2 % HIGH



Conclusion

- The exercise intensity required for hiking can vary significantly between individuals, suggesting that one-size-fits-all hiking recommendations might not be suitable, especially for those with higher cardiovascular risk.

- Personalized hiking indications that consider individuals' cardiorespiratory fitness and health status with trail characteristics are needed to prevent potential cardiovascular adverse events and maximize the health benefits of mountain hiking.

MARCO VECCHIATO
Medicina dello Sport e dell'Esercizio

VI° CONVEGNO del DIMED
Padova, 15 novembre 2024

Tutor
Prof. Andrea Ermolao
Sports and Exercise Medicine Division
Department of Medicine
University of Padova

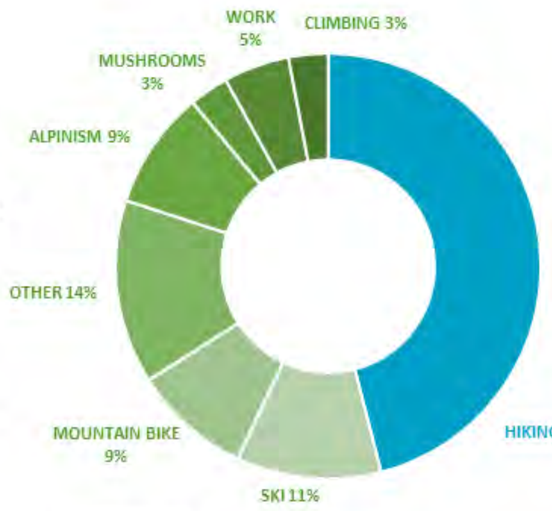
MOVE

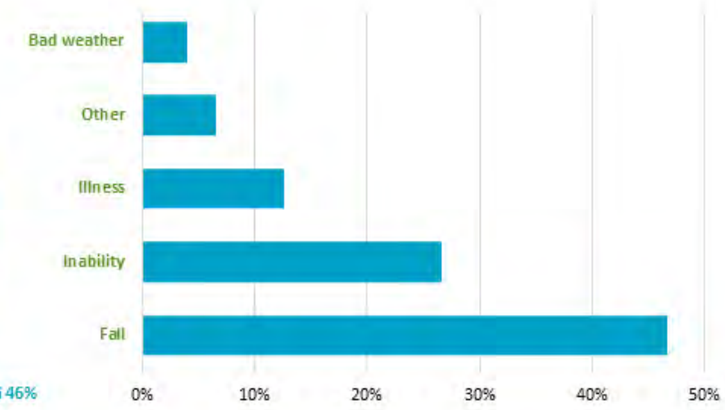
Software for the customization of outdoor activities

Marco Vecchiato, MD – PhD Candidate in Clinical and Experimental Sciences - Sports and Exercise Medicine Division, Department of Medicine

The problem

In recent years, there has been an increase in rescue calls from hikers and cycle tourists. More and more people are engaging in outdoor activities without adequate physical or technical preparation, basing their excursion on times or advice indicated by common signage or websites. While this information is accurate, it remains generic as it is estimated based on average values, thus it is not suitable for everyone.



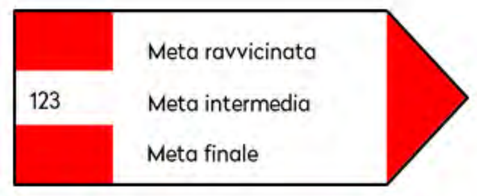


State of the art

There is currently no tool available on the market that could help reducing rescue calls, by providing the hiker with more accurate and individualized safety recommendations.

Aim

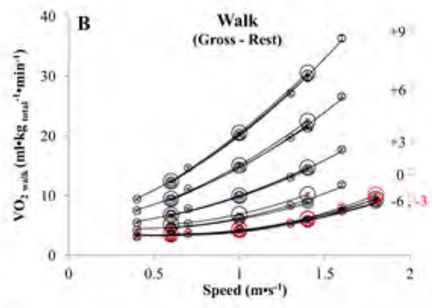
To create a tool providing indications of personalized hiking times, physical effort and caloric consumption considering the specific physical characteristics of the trail and the individual in every possible aspect.

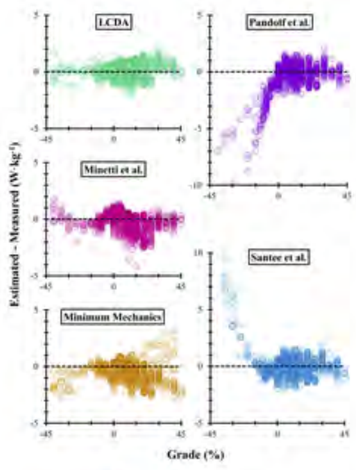


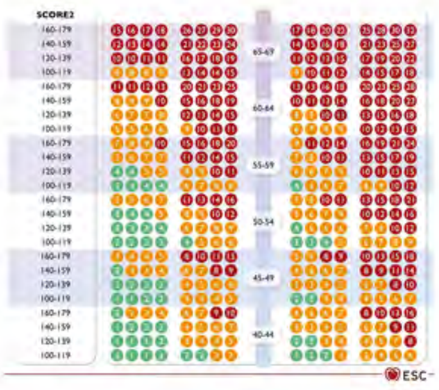
Algorithm development

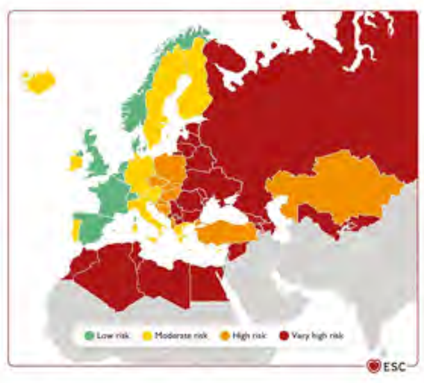
Development of an algorithm that combines oxygen consumption estimation equations, equations for uphill and downhill hiking and calculation of cardiovascular risk.

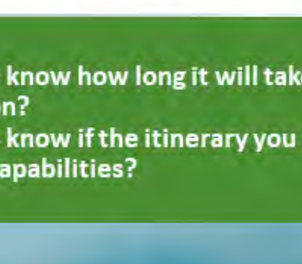
Model	Formula
LCDA	$EE = 1.44 + 1.94S^{0.43} + 0.24S^{4} + 0.34SG(1 - 1.05^{1 - 1.1G + 3S})$
Minetti et al. (10)	$Cw = 280.5i^{5} - 58.7i^{4} - 76.8i^{3} + 51.9i^{2} + 19.6i + 2.5$
Minimum Mechanics (12)	If $G \geq 0$, $\dot{V}O_{2\text{-walk}} = \dot{V}O_{2\text{-rest}} + 0.32G + 3.28 + (1 + 0.19G)2.66S^{2}$ If $G < 0$, $\dot{V}O_{2\text{-walk}} = \dot{V}O_{2\text{-rest}} + 0.73(3.28 + 2.66S^{2})$
Pandolf et al. (1)	$EE = 1.5M + 2(M + L)(\frac{L}{M})^{2} + \eta(M + L)(1.5S^{2} + 0.35SG)$
Santee et al. (11)*	$EE = \eta\left[\frac{(SG(M + L))}{3.5} - \left(\frac{(M + L)(G + 6)^{2}}{M}\right) + 25S^{2}\right]$

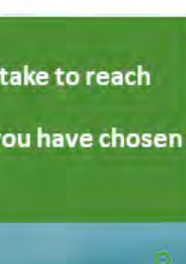












Patent application for Italy

Date: 24.01.2024
Number: 102021000026513

Algorithm validation

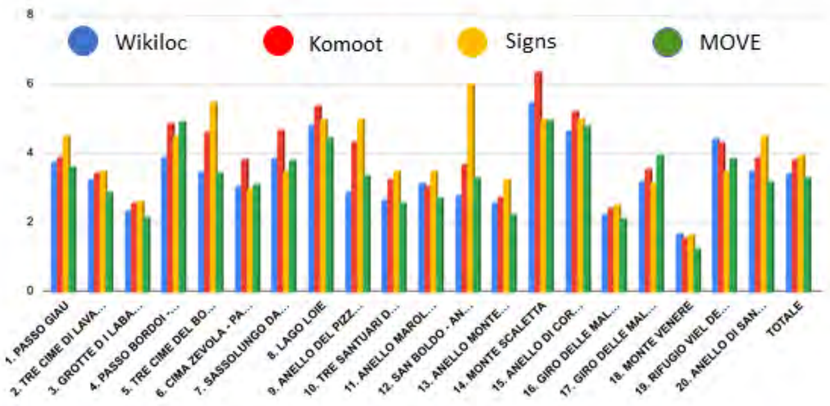
Two validation studies were conducted:

1. Digital study comparing hiking times uploaded by users on a free web platform and different hiking time estimation methods
2. Field study comparing the hiking times of real users with estimated hiking time by MOVE

1) 530 real users

wikiloc

20 ring trails

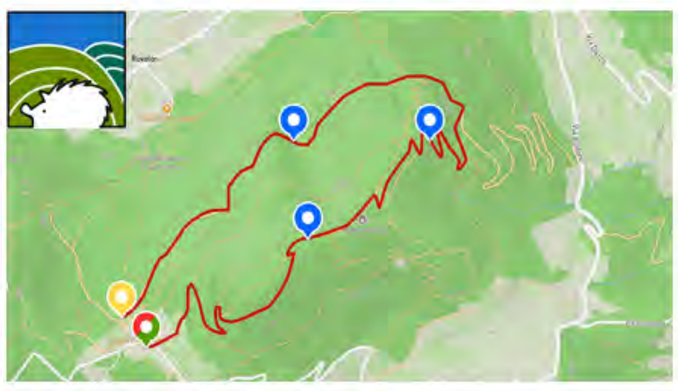


MOVE estimates real hiking times most accurately

2) MOVE in SPACE

ESA Kick-Start Thematic Call Winner

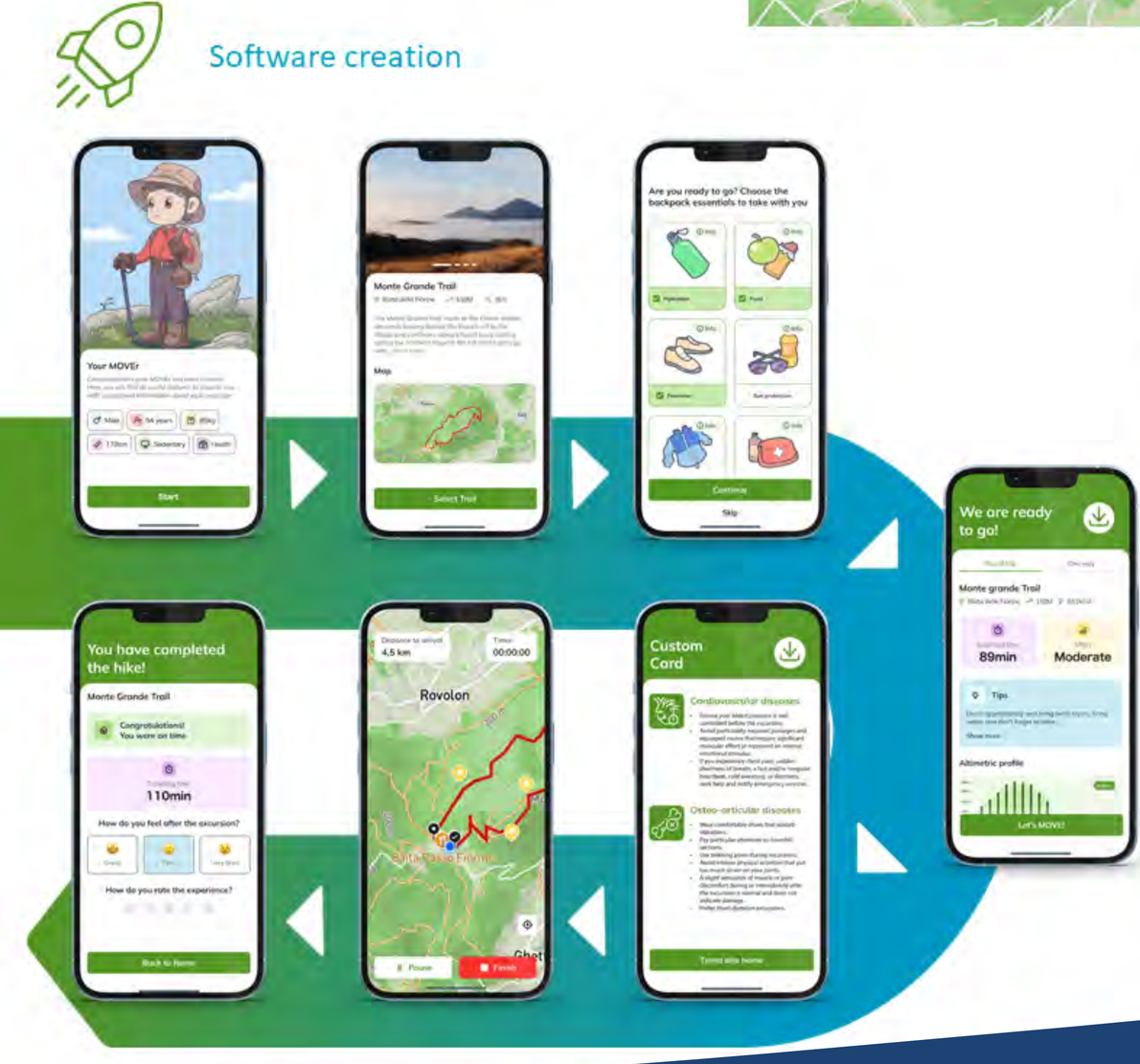
esa European Space Agency



72 healthy participants and patients with chronic diseases were monitored with wearable gas analyzer and a HR chest wrap during the same trail.

Δ Hiking time = + 7 ± 3 min

Software creation



MOVE is the first outdoor activities application that offers personalized guidance to safely approach your excursion.

By analyzing the information provided by the user (such as anthropometric data, physical activity level, CV risk factors, and health status) through the creation of an avatar called MOVEr, and the physical characteristics of the chosen itinerary, MOVE can provide personalized estimates of travel time, the required physical effort, along with specific advice and useful information to enhance the user's safety during the activity.

MOVE

Download on the App Store

GET IT ON Google Play

Future

SPIN-OFF UNIVERSITÀ DI PADOVA

www.moveproject.it
info@moveproject.it

MARCO VECCHIATO
Medicina dello Sport e dell'Esercizio

VI° CONVEGNO del DIMED
Padova, 15 novembre 2024

SARS-CoV-2 infected liver pericytes stimulate vWF expression by portal endothelial cells and an angiogenic response in the pulmonary endothelium

Venturin C, Frión-Herrera Y, Cadamuro M, Radu CM, Calistri A, Parolin C, Fabris L, Simioni P

BACKGROUND AND AIM

Severe SARS-CoV-2 infection can lead to the formation of systemic microthrombosis with alterations in the vascular component of several organs, including liver. In the liver, the involvement of the portal vasculature may be associated with the development of intrapulmonary vascular dilatations and worsening of hypoxemia, featuring the hepatopulmonary syndrome. Previous studies indicate that, in COVID-19, alterations in the pulmonary circulation with persistent hypoxemia were observed.

We hypothesized that liver pericytes, mesenchymal cells intimately associated with the endothelium, infected by SARS-CoV-2 induce a pro-coagulant phenotype sustaining an angiogenic response of the pulmonary circulation.

METHODS

1. Immunofluorescence of liver specimens from COVID-19 autopsies (n=16) and controls (n=17) was used to study SARS-CoV-2 spike (SP) or nuclear (NP) proteins expression with von Willebrand Factor (vWF) and PDGFR-β (pericyte marker), later we quantified the expression levels of viral SP/NP in endothelial cells and pericytes.

2. Pericytes were infected with SARS-CoV-2, the collected supernatant was used to infect human umbilical vein endothelial cells (HUVEC) and human pulmonary microvascular endothelial cells (HPMEC) to assess vWF expression.

3. The tubulisation of HPMEC was assessed upon vWF treatment with Angiogenesis Analyser software patch for ImageJ following treatment with the recombinant human vWF protein.

RESULTS

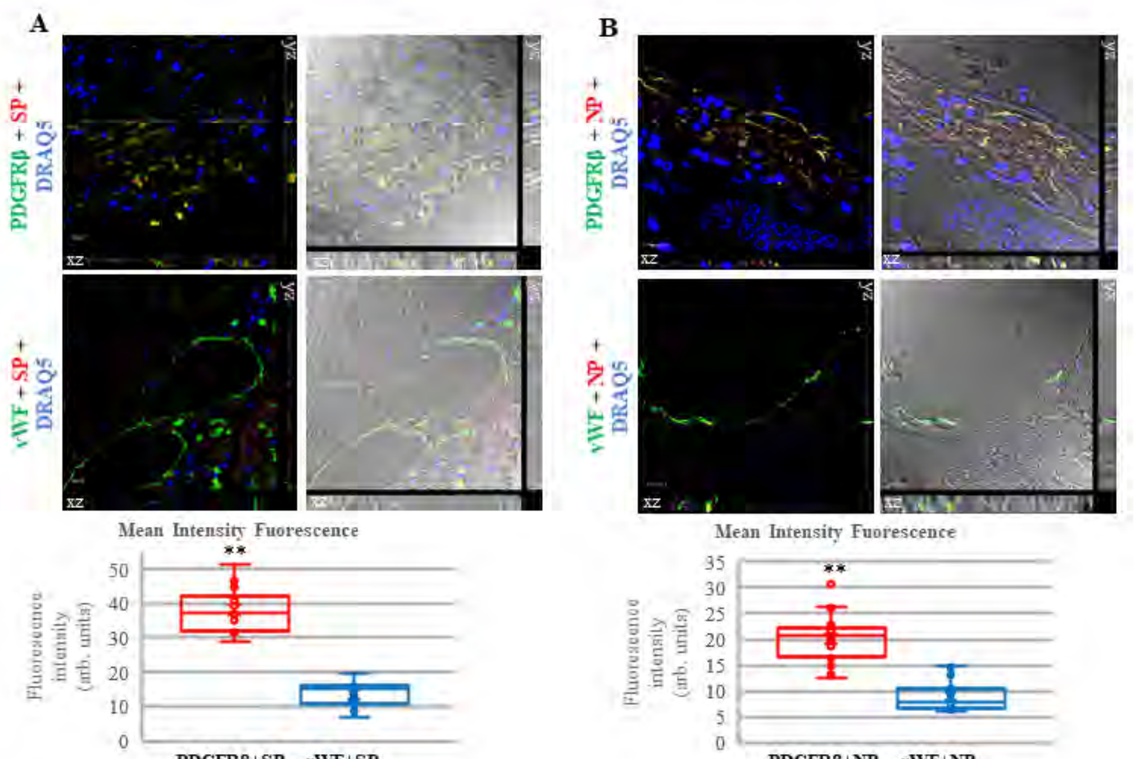


Figure 1. Expression of viral spike protein (SP) and nucleoprotein (NP) is predominant in pericytes of portal microvasculature in COVID-19/IPVD and intravacuolar virus-like particles are present in pericytes. Representative immunofluorescence (IF) images of tissue specimens from COVID-19/IPVD liver with tissue morphology visualization by differential interference contrast (DIC, gray image). SP (A) and NP (B) (red) and PDGFR-β (green, pericyte marker) show intense co-expression (merged image, yellow), which was not observed in SP/NP (red) and vWF (green, endothelial marker). Orthogonal projection, x/z and y/z planes, clearly demonstrates the co-expression of SP/NP with either cellular markers (overlay images xz and yz axis). In the below-sided plot, mean intensity fluorescence of SP and NP was quantified in tissue sections from COVID-19/IPVD livers, showing significantly higher values in PDGFR-β+ (SP, 37.20±8.85; NP, 20.46±3.58) than vWF+ cells (SP, 16.54±3.03; NP, 9.65±2.09). SP/NP++PDGFR-β+; SP/NP++vWF+, both n=16 patients; **p<0.001, two-tailed Wilcoxon ranksum test (A, B).

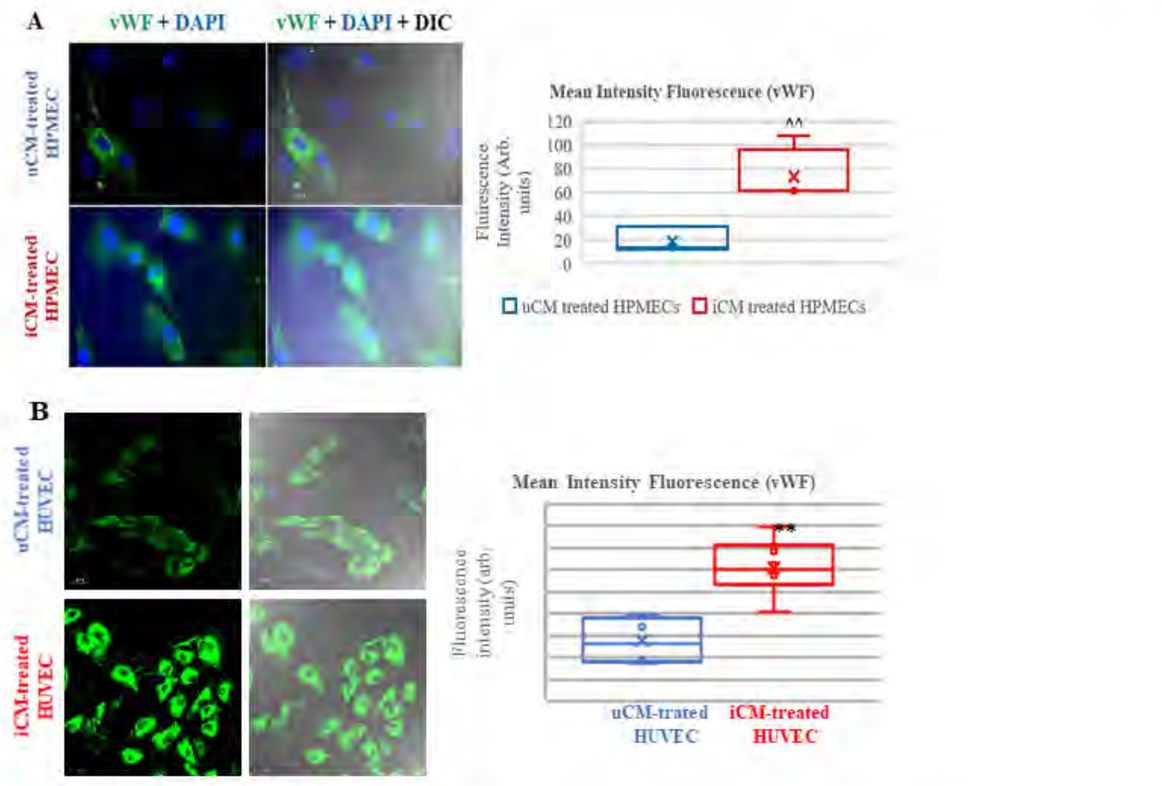


Figure 2. HPMEC and HUVEC treatment with conditioned medium from pericytes infected with SARS-CoV-2 up-regulates expression of vWF. Treatment with conditioned medium (CM) harvested from infected pericytes in BSL-3 conditions (iCM), stimulates a significant increase in vWF expression by cultured HPMECs compared with HPMECs treated with uninfected CM (uCM) (A), evaluated as mean intensity of fluorescence. IF analysis of vWF in cultured HUVECs, with and without exposure to conditioned medium (CM) harvested from either uninfected (uCM) or infected (iCM) pericytes, reveals that iCM-treated HUVECs show a significant and uniform increased expression of vWF (green) compared with uCM-treated HUVEC, which mostly express very low levels of vWF (white arrow). In the right-sided graph, quantification of fluorescent intensity shows significantly higher values of vWF expression in HUVEC treated with iCM (60.99±12.67 vs 27.57±11.06). uCM-treated HUVEC, n=4, iCM-treated HUVEC, n=6, **p<0.01 vs uCM using two-tailed t test (B)

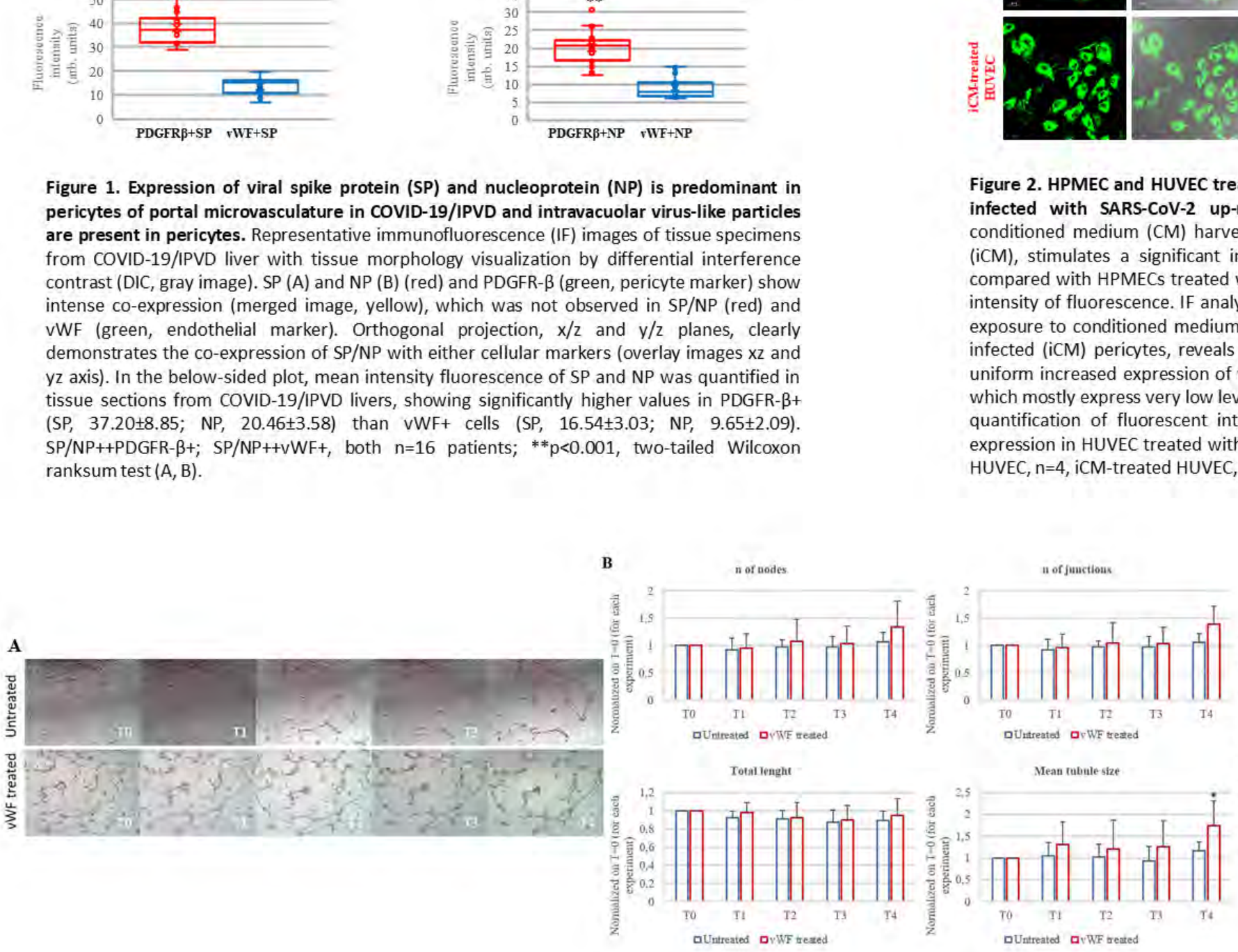


Figure 3. *In-vitro* vWF supplementation increases HPMEC tubule size. Representative micrographs of 3D-cultured HPMEC with and without vWF exposure are given at the different time points (from T0 to T4) below the graphs (A). Original magnification: 4x. Treatment of 3D tubular cultures of HPMECs with vWF (4µg/ml) for 4 hours (T0 to T4) induces, at T4, a significant enlargement of about 1.5x, of the mean tubule size as respect to untreated controls at the same time point, whereas other parameters of angiogenesis (number of nodes and junctions, and total length of tubule) are unaffected (B).

CONCLUSIONS

SARS-CoV-2 infection in liver leads to a local procoagulant response by up-regulating vWF in endothelial cells contributing to portal microthrombosis. Moreover, vWF induced an increase in the tubule expansion of HPMEC, consistent with a vessel lumen widening effect relevant to intrapulmonary vascular dilations found in COVID-19 patients with severe hypoxemia. Thus, vWF released by portal endothelial cells may behave as a putative molecular effector linking hepatic lesions with pulmonary vascular perturbations in hepatopulmonary syndrome.

FUTURE DEVELOPMENT

Hepatic pericytes, mesenchymal cells intimately associated with the endothelium, have not been considered as a therapeutic target for arresting pro-thrombotic events, but future research aimed at targeting pericytes could provide innovative tools to combat microvascular complications following SARS-CoV-2 infection. Furthermore, it is possible that the mechanisms described are involved in causing portal obliterative venopathy, one of the most neglected and underdiagnosed hepatic vascular diseases. Our aim is to take this study further by translating these findings into liver vascular diseases characterised by portal vein microthrombosis.

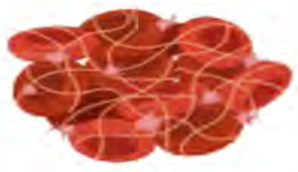
CAMILLA VENTURIN
Clinica Medica 1: Malattie Trombotiche ed Emorragiche

What we know

Cardiovascular risk

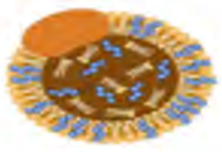
Hypertension

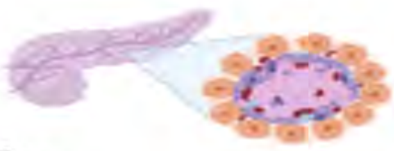
Thrombotic risk



Glucocorticoids excess

Lipid metabolism impairment





Glucose metabolism impairment

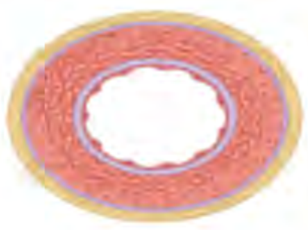
Fragility fractures in 11-76% of Cushing patients

What we discovered

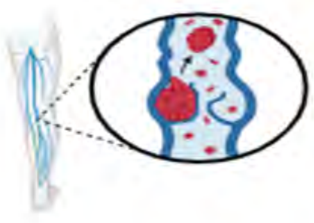
Cushing's syndrome

Fragility fractures

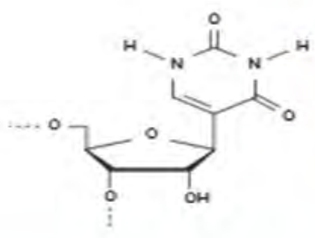
Atherosclerosis



Intima-media thickness



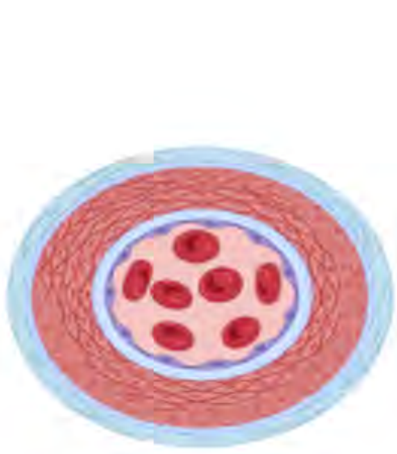
Venous thromboembolism



Uric acid

What we want to know

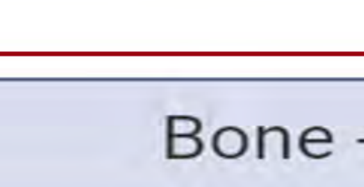
Bone - Vascular Axis



Inflammation

RANK/RANKl/OPG axis

Wnt -beta-catenin pathway



Aldosterone excess ?

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Evaluation of Oncogenic Risk and Cumulative Effective Dose from Radiological Investigations in Intensive Care Unit Patients. Variability between Risk Models

Aim

To compare oncogenic risk related to radiation exposure according to different risk models in intensive care unit (ICU) patients.

Methods

This was an IRB-approved observational retrospective study. One hundred-and-fifty patients (45 F; 105 M, mean age 63.2 ± 27 years) [Tab 1] admitted to intensive care multivisceral transplant unit (44 patients) or cardiac surgery unit (106 patients), who underwent x-ray radiological examinations between april and june 2023, were included. For each patient the cumulative effective dose (ED) during one single hospital admission was calculated [Tab 2] and the oncogenic risk, based on Additional Oncogenic Risk (AOR) corresponding to Effective Dose /0.1 × Lifetime Attributable Risk (LAR), for all cancer and leukemia were estimated according to biological effects of ionizing radiation seventh report (BEIR VII), Radiation Risk Assessment Tool (RadRAT), International Commission on Radiological Protection (ICRP) 103, and US Environmental Protection Agency (EPA) risk models.

Results

All cancer risk was lower according to ICRP 103 *vs* BEIR VII *vs* RadRAT *vs* US EPA (median ± IQR, 20 ± 105 *vs* 40 ± 226 *vs* 47.5 ± 346 *vs* 41.88± 254, $P < 0.001$), while it was higher in male *vs* female patients according to both BEIR VII (median ± IQR, 58.6 ± 279 *vs* 18.6 ± 112, $P < 0.001$), RadRAT (77.2 ± 456 *vs* 24.2± 130, $P < 0.001$), ICRP 103 (20 ± 121 *vs* 15.94 ± 64, $P < 0.001$) and US EPA (69.40 ± 329 *vs* 20.59 ±125, $P < 0.001$). Leukemia risk was higher in US EPA *vs* BEIR VII vs RadRAT *vs* ICRP 103 *vs* (median ± IQR, 7.75 ± 41 *vs* 7.6 ± 37 *vs* 7.27 ± 42 *vs* 1.8 ± 13, $P < 0.001$), while it was higher in male *vs* female patients according to both BEIR VII (male *vs* female median ± IQR, 9.5 ± 52 *vs* 2.12 ± 11, $P < 0.001$), RadRAT (9.94 ± 50 *vs* 2.03 ± 12, $P < 0.001$), ICRP 103 (2.17± 18 *vs* ± 0.92 ± 4, $P < 0.001$), and US EPA (9.83 ± 51 *vs* 2.34 ± 12, $P < 0.001$) [Tab 3, Fig 1-2].

Patients	
Total (n°)	150
Male (n°; %)	105 (70%)
Females (n°; %)	45 (30%)
Age	
Male (Mean ± SD)	63.3 ± 14
Female (Mean ± SD)	64.3 ± 13
ICU Departments	
MTU (n°; %)	44 (29%)
CSU-(n°; %)	106 (71%)
Hospitalization period (days)	
Male (Mean ± SD)	36 ±27
Female (Mean ± SD)	24 ± 43
MTU (Mean ± SD)	36 ± 33
CSU (Mean ± SD)	31 ± 42
Radiological Investigation	
Total (n°)	2847
X-rays (n°; %)	2473 (87%)
CT (n°; %)	270 (9%)
Interventional Procedures (n°; %)	49 (2%)
Angiographic Procedures (n°; %)	57 (2%)

Table 1: Patient characteristics and radiological investigations. MTU, multivisceral transplant unit; CSU, cardiac surgery unit.

Cumulative effective Dose (mSv)	n°	Mean ± SD
Total	150	42.34 ± 70
Male	45	22 ± 41
Female	105	51 ± 77

Table 2: Cumulative effective dose (ED) (mSv) comparison between the total population, sex, and ICU Departments. MTU, multivisceral transplant unit; CSU, cardiac surgery unit.

	BEIR VII	RadRAT	ICRP 103	US EPA
AOR All cancer*°	40 ± 226	47.5 ± 346	20 ± 105	41.88 ± 254
AOR Leukemia*	7.6 ± 37	7.27 ± 42	1.8 ± 13	7.75 ± 41
AOR All cancer male*	58.6 ± 279	77.2 ± 456	20 ± 121	69.40 ± 329
AOR All cancer female*	18.6 ± 112	24.2 ± 130	15.94 ± 64	20.59 ± 125
AOR Leukemia male*	9.5 ± 52	9.94 ± 50	2.17 ± 18	9.83 ± 51
AOR Leukemia female*	2.12 ± 11	2.03 ± 12	0.92 ±4	2.34 ± 12

Table 3: Comparison of All-cancer risk and Leukemia-risk model between BEIR VII, RadRAT, ICRP 103 and US EPA

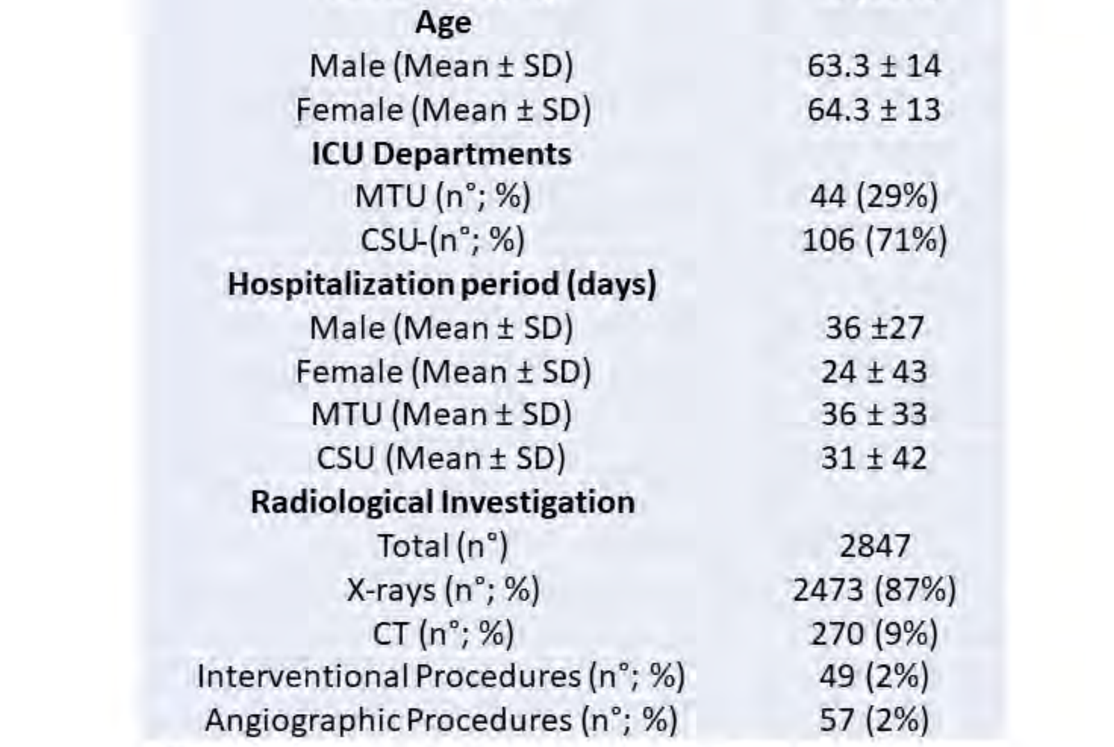

Figure 1: Comparison between Additional oncogenic risk (AOR) /100.000 between to BEIR VII, RadRAT, ICRP 103, and U.S. EPA Risk models. *(median ± IQR) ° p value (< 0.001)

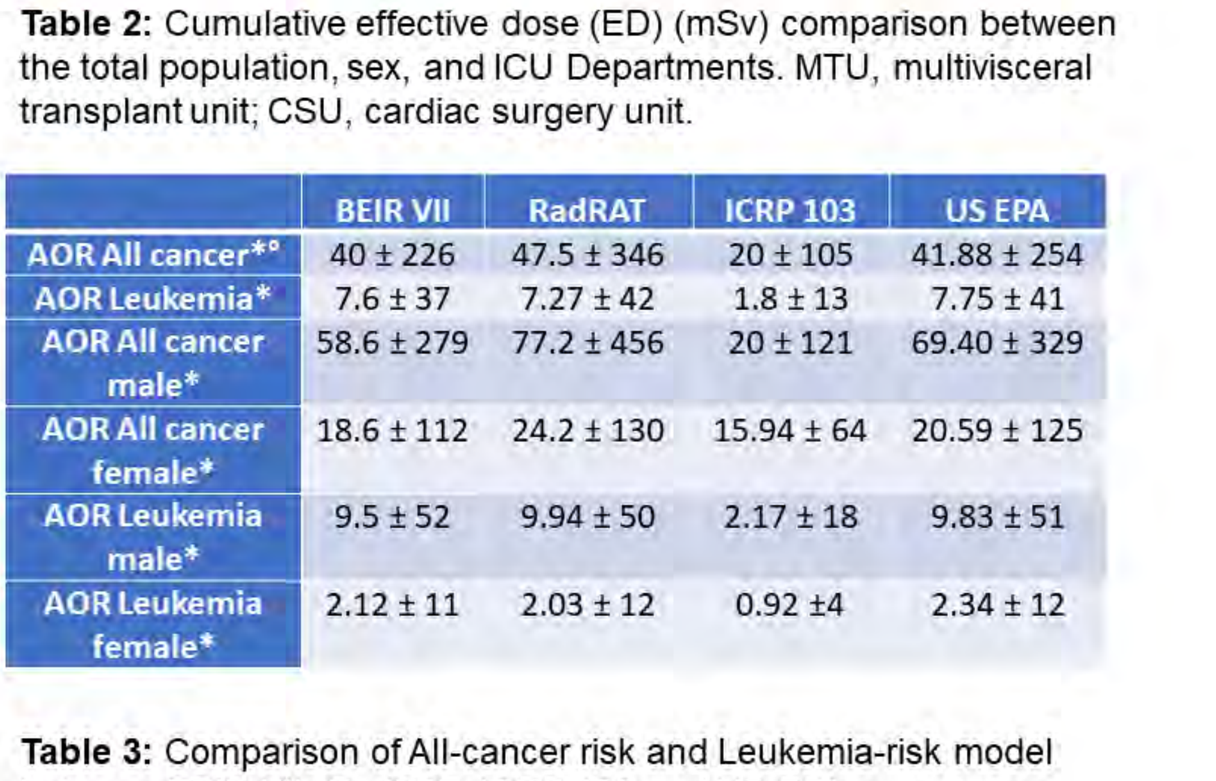

Figure 2: Comparison between Additional oncogenic risk (AOR) /100.000 between to BEIR VII, RadRAT, ICRP 103, and U.S. EPA Risk models divided by sex.*(median ± IQR) ° p value (< 0.001

Conclusions

ICRP 103 risk model estimated a lower radiation-induced cancers risk for all cancer risk, while US EPA estimated an increased radiation- induced leukemia risk compared to BEIR VII, RadRAT, and ICRP 103.

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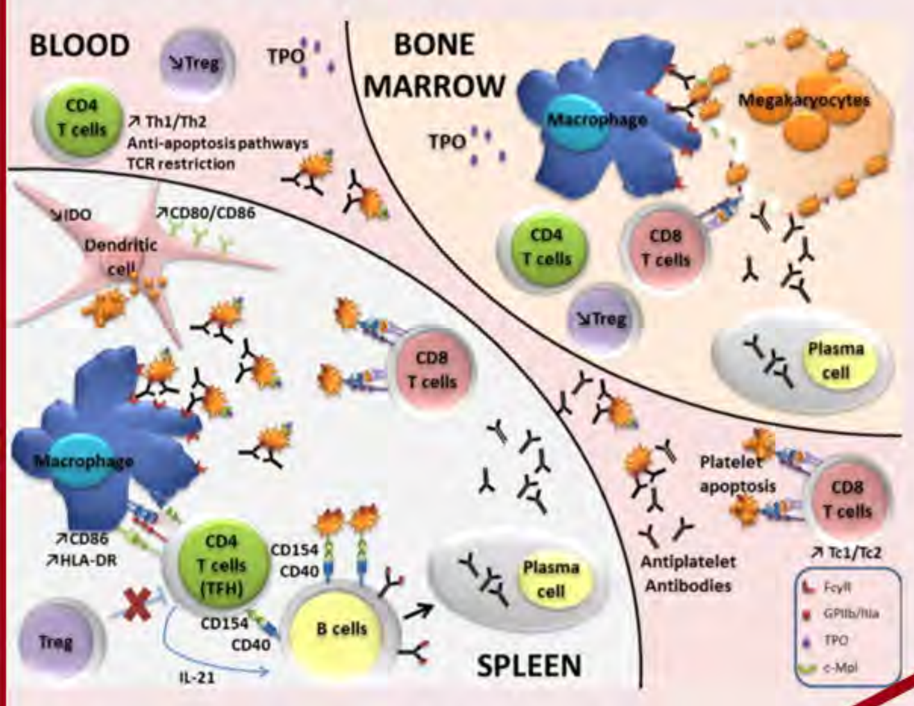
VI° CONVEGNO del DIMED
Padova, 15 novembre 2024

Comprehensive Profiling of T Cell Compartment in Immune Thrombocytopenia (ITP): A Multi-Modal Approach

Dott. S. Zoletto
Scuola di Specializzazione di Ematologia

BACKGROUND.



ITP: an inflammatory symphony

STUDY AIM.

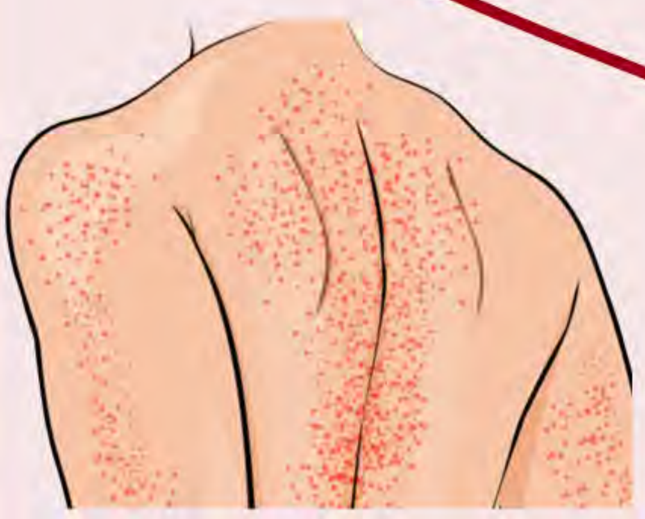
- **Immunophenotypic and transcriptomic patterns of T lymphocytes in ITP**

METHODS.

- 2-year **observational prospective study**
- 50 ITP patients and 25 healthy volunteers
- **Flow cytometric analysis of T-cells**
- **T-cell receptor (TCR) clonality assessment**
- **Single Cell RNA Sequencing** (selected cohort)

PRELIMINARY RESULTS.

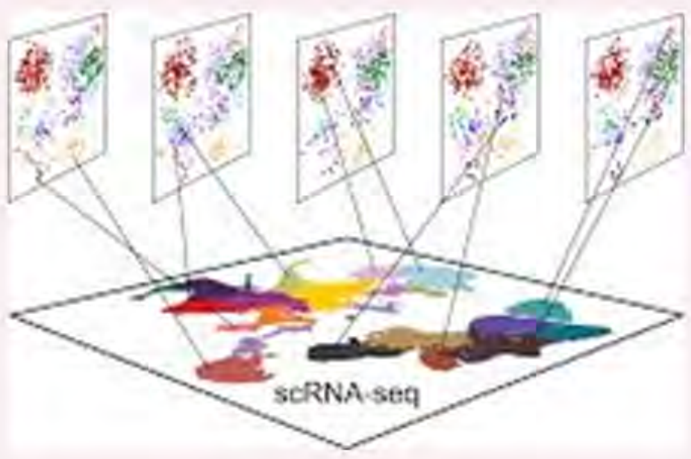
- **Increased CD8+** T-cells **and CD8** terminally differentiated effector CD8+ (**TEMRA**) **in ITP** patients
- **Increased CD4+/CD8+ central memory T-cells in secondary** versus primary **ITP**
- **Reduced CD4+/CD8+ naive T-cells in splenectomized** patients



The final note: hemorrhagic syndrome

NEXT STEPS.

1. I**ncrease** patient and control **cohort sizes** (ongoing study)
2. Gather **TCR clonality** information
3. Analyze **transcriptomic data**
4. Evaluate T-cell subset fluctuations during treatment
5. Characterize aberrant populations from transcriptomic analyses
6. Possibly **identify** new diagnostic and prognostic **biomarkers**



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[2] Amna Malik, Anwar A. Sayed, *et al.*; The role of CD8+ T-cell clones in immune thrombocytopenia. Blood 2023; 141 (20): 2417–2429. doi: https://doi.org/10.1182/blood.2022018380.
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